A Phase I-IIa trial to assess the safety and antitumor activity of autologous CD44v6 CAR T-cells in acute myeloid leukemia and multiple myeloma expressing CD44v6

Compound:	MLM-CAR44.1 T-cells
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Signature:	
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Synopsis of the Protocol

Title	A Phase I-IIa trial to assess the safety and antitumor activity of autologous CD44v6 CAR T-cells in acute myeloid leukemia and multiple myeloma expressing CD44v6
Version	C
Sponsor	MolMed S.p.A.
Indication	Acute Myeloid Leukemia and Multiple Myeloma
Objectives	**Primary objectives of Phase I** 1. To determine the maximum tolerated dose (MTD) and recommended phase IIa dose of MLM-CAR44.1 T-cells in patients with relapsed/refractory acute myeloid leukemia (AML) or multiple myeloma (MM) expressing CD44v6. 2. To evaluate the overall safety of treatment with MLM-CAR44.1 T-cells. 3. To monitor for the absence of replication-competent retrovirus (RCR) **Secondary objectives of Phase I** 1. To evaluate hematological response to MLM-CAR44.1 T-cells in AML and MM. 2. To characterize the in vivo pharmacokinetic profile (engraftment, persistence, trafficking) of MLM-CAR44.1 T-cells. 3. To assess suicide gene activation and elimination of transduced cells through administration of ganciclovir in case of cytokine release syndrome (CRS) or other MLM-CAR44.1 T-cell related toxicities. 4. To conduct an ancillary study, including but not limited to the characterization of the cellular composition of circulating LNGFR+ (CAR T-cells) and LNGFR- cells, and the monitoring of the patient's immune status through analyses of peripheral blood mononuclear cells (PBMC). 5. To set-up a validation process among the participating centers for the CD44v6 marking of tumor cells (AML and MM). **Primary objective of phase IIa** To evaluate hematological response to MLM-CAR44.1 T-cells in AML and MM. **Secondary objectives of Phase IIa** 1. To evaluate the antitumor activity of MLM-CAR44.1 T-cells 2. To characterize the in vivo pharmacokinetic profile (engraftment, persistence, trafficking) of MLM-CAR44.1 T-cells. 3. To evaluate the feasibility of treatment with MLM-CAR44.1 T-cells 4. To evaluate the safety of MLM-CAR44.1 T-cells. 5. To conduct an ancillary study, including but not limited to the characterization of the cellular composition of circulating LNGFR+ (CAR T-cells) and LNGFR- cells, and the monitoring of the patient's immune status through analyses of PBMCs.

	Exploratory objectives of Phase IIa To evaluate minimal residual disease
Study design	This is a seamless Phase I/IIa, open-label, multicenter clinical trial that combines Phase I dose escalation based on toxicity with Phase IIa dose expansion based on antitumor activity. **Phase I dose escalation** A Bayesian Optimal Interval (BOIN) design with cohorts of 3 patients for each indication (AML and MM) will be used to single out the MTD based on a target toxicity rate of 30% and an interval of 25% - 35%. The first AML cohort and first MM cohort will be made up of adult patients and will be treated with the lowest dose of autologous MLM-CAR44.1 T-cells (0.5 x 10E6/Kg). The first three enrolled patients (regardless of indication) will be treated one at a time at intervals of 36 days to evaluate toxicity. These three patients will undergo lymphodepleting chemotherapy prior to CAR T-cell infusion only once the previously enrolled patient has completed the 30-day follow-up after CART T-cell infusion. The remaining patients of the first cohorts will be treated without staggering once the first three enrolled patients have completed follow-up and the dose has proven to be safe. After all patients of the first AML cohort or first MM cohort have completed the 30-day safety monitoring period, the decision on whether to escalate to the next dose will be made together with a Data and Safety Monitoring Board (DSMB) based on the rate of dose-limiting toxicities (DLT) and the decision rules presented in the Table below. The second and then third dose will be administered to cohorts of new patients as outlined for dose 1 with an escalation process that continues until the MTD or the maximum sample size of 15 patients for each indication has been reached. The duration of the evaluation period for each patient (30 days) is based on previous studies with similar cellular products, in which severe cytokine release syndrome (CRS) (main DLT for the study) was observed for up to 7-15 days after infusion. 0.5 x 10E6/Kg, 1 x 10E6/Kg and 2 x 10E6/Kg are the three doses of autologous MLM-CAR44.1 T-cells for this study. The recommended Phase IIa dose corresponds to the MTD. If no DLT is reported, the dose will be the maximum dose investigated (2 x 10E6/kg). The BOIN design is a novel Bayesian dose-finding method that minimizes the decision errors of dose assignment (for example the chance of exposing patients to overly toxic doses) and thus safeguards patient ethics and safety by allowing decisions to be made at any time during the study, even when one of the patients in the cohort is not evaluable. This design has performance superior to the traditional 3 + 3 design but shares with it the fact that it can be easily implemented, differently from the well-known continual reassessment method (CRM), the gold standard of Phase I dose finding designs. The BOIN design yields an average performance comparable with CRM in selecting the MTD but has a lower risk of assigning patients to sub therapeutic or overly toxic doses.

Transition rules for each dose

Cumulative Number of Patients

Number of patients with at least one DLT	3	6	9	12	15
0	E	E	E	E	E
1	S	E	E	E	E
2	D	S	E	E	E
3	DU	D	S	S	E
4		DU	D	S	S
5		DU	DU	D	S
6		DU	DU	D	D
7			DU	DU	D
8			DU	DU	DU
9			DU	DU	DU
10				DU	DU
11				DU	DU
12				DU	DU
13					DU
14					DU
15					DU

E: Escalate to the next higher dose; **S**: Stay at the same dose; **D**: De-escalate to the previous lower dose; **DU**: De-escalate to the previous lower dose and the current dose will never be used again in the trial (safety rule).

Phase IIa dose expansion

The activity of the MTD will be assessed on 14 patients of each indication (AML and MM separately) using Simon's two-stage design. These cohorts will include the patients enrolled in Phase I treated with the MTD, and in the case of AML, also children (except for Czech Republic (CZ) site).

In the first stage, the hematologic response to treatment of the first 6 patients with each indication will be evaluated, and if no response is observed the study will be terminated. Otherwise, the study will proceed to stage two and the remaining 8 subjects will be enrolled.

Patients with either indication will be followed up for 24 months to evaluate the antitumor activity, safety and feasibility of treatment with MLM-CAR44.1 T-cells.

Transition from Phase I to Phase II will be managed through the submission of a substantial amendment to the protocol, which will be evaluated in consultancy with the Data Safety Monitoring Board (DSMB).

Number of patients

The study will be conducted in 5 sites in Italy, Spain, Germany and Czech Republic.
During Phase I, the study will enroll up to 15 adult patients with AML and up to 15 adult patients with MM. Phase IIa of the study will evaluate newly enrolled patients, including children with AML, and patients who were treated at the MTD during Phase I for a combined maximum of 14 patients per indication.

The trial will include pediatric patients (except for CZ site) with AML (<18 years old) only in Phase IIa and if all the following criteria have been met by adult patients in Phase I:

1. No SAEs related to lymphocyte apheresis.
2. No immediate SAEs related to MLM-CAR44.1 T-cell infusion (within 48 hours of infusion).

	3. No early SAEs related to infusion (within 30 days). 4. Evidence of MLM-CAR44.1 T-cell engraftment. Should one or more of these criteria not be met, the DSMB (including a pediatrician) will evaluate the risk/possible benefit profile of study treatment and give recommendations on whether to include pediatric patients. The protocol will be amended to classify eligible pediatric patients according to age groups.
Study population	The study population is made up of patients with relapsed/refractory AML or MM expressing CD44v6. **Inclusion criteria** 1. Written informed consent before any study-related procedure. 2. Adults and children a) Adults 18 to 75 years old with AML or MM or 18 to 63 years old for CZ site b) Children 1 to 17 years old with AML, only in Phase IIa, except for CZ site 3. Confirmed diagnosis of AML or MM as follows: a) **AML**: Primary or secondary AML (any subtype except acute promyelocytic leukemia) according to World Health Organization (WHO) classification b) **MM with measurable disease** defined at least one of the following: • Serum M protein ≥5 g/dL (≥5 g/L); • Urine M protein ≥100 mg/24 hours; • abnormal serum free light chain (sFLC) assay: FLC ratio <0.26 or >1.65 and/or involved Serum free light chain >=100 mg/L 4. Patients with relapse or refractory disease: a) **AML** patients must be unlikely to benefit from cytotoxic chemotherapy as follows: • Leukemia refractory to at least 2 induction attempts (use of a hypomethylating agent for at least 4 cycles can be considered a line of treatment). • Leukemia in relapse within 1 year following complete response (CR) after at least 2 induction attempts (use of a hypomethylating agent for at least 4 cycles can be considered a first line of treatment). • High-risk leukemia in adults according to 2017 European LeukemiaNet (ELN) in first relapse after a hypomethylating agent or a cycle containing cytarabine at a dose ≥1g/sqm a day (e.g. FLAG-IDA), except for FLT3-mutated AML. • High-risk leukemia in children as defined by the Italian Association of Pediatric Hematology and Oncology (AIEOP). b) Patients with **MM** must have a relapse or refractory disease after at least 4 different prior treatments in 3 treatment lines, or 4 treatments in 2 treatment lines in case of early relapsing patients (relapse in less than 1.5 years). Treatments include: • Proteasome inhibitor

- High-dose alkylating agent if patient less than 70 years old
- Immunomodulatory drug (IMID)
- A monoclonal antibody (i.e. anti CD38 monoclonal antibody)

5. Positive CD44v6 expression on tumor cells by flow cytometry.
6. Eastern Cooperative Oncology Group (ECOG) performance status 0-2.
7. Life expectancy of at least 12 weeks.
8. Adequate organ function:
   a) Alanine aminotransferase (ALT) level within 2.5 times the institutional upper limit of normal (ULN).
   b) aspartate aminotransferase (AST) level ≤ 2.5 x ULN.
   c) Total bilirubin level ≤ 1.5 x ULN, or ≤ 2.5 x ULN in case of history of Gilbert's disease.
   d) Serum creatinine ≤ 2.0 mg/dL or a calculated or measured creatinine clearance ≥ 45mL/min.
   e) Corrected Diffusing Capacity of Carbon Monoxide (DLCO) (via Dinakara Equation) or FEV1 of ≥ 66% without dyspnea on slight activity after hemoglobin correction.
   f) Left ventricular ejection fraction > 45%.
9. Recovery from toxicities of clinical consequence attributed to previous chemotherapy to CTCAE v5.0 Grade 1 (i.e., certain toxicities such as alopecia will not be considered in this category).
10. Ability to comply with study procedures, including hospitalization and protocol-specified acquisition of blood and/or bone marrow specimens.
11. Willing to be followed up long-term (15 years) as required by health authorities for cell and gene therapy products
12. Women of childbearing potential must have test negative for pregnancy at enrolment and during the study and agree to use an effective method of contraception.

Exclusion criteria

1. History of or candidate for allogeneic stem cell transplantation.
2. Cardiovascular, pulmonary, renal, and hepatic functions that in the judgment of the investigator are insufficient to undergo investigational CAR T-cell therapy.
3. Any history of or suspected current autoimmune disorders (apart from vitiligo, resolved childhood atopic dermatitis, Graves' disease clinically controlled).
4. History of rheumatologic disorders requiring specific treatment at any time in the patient's medical history.
5. Second primary malignancy that requires active therapy. Adjuvant hormonal therapy is allowed.
6. Known or suspected central nervous system (CNS) leukemia.
7. Presence or history of myeloid sarcoma or any extramedullary mass.
8. Any medical or psychiatric condition that may limit compliance or increase safety risks, such as:
   a) Active uncontrolled infection (including, but not limited to viral,

bacterial, fungal, or mycobacterial infection).

b) Patients with known multiple antibiotic resistant infections in their clinical history.

c) Known human immunodeficiency virus infection, active or chronic hepatitis B or C infection.

d) Grade 3 or 4 bleeding.

e) Uncontrolled hypertension (systolic pressure > 180 mm Hg or diastolic pressure > 100 mm Hg).

f) Clinically significant arrhythmia, clinically significant baseline QTcF, or QTcF > 480 msec.

g) Unstable angina.

h) Myocardial infarction within 6 months prior to study entry.

i) Clinically significant heart disease (e.g. CHF NYHA III or IV, unstable coronary artery disease, myocardial infarction < 6 mo. prior to study entry).

j) Pregnancy or breast feeding.

k) Major surgery or trauma within 4 weeks before enrollment.

l) Dementia or altered mental status that would preclude sufficient understanding to provide informed consent.

Once all the above eligibility criteria are confirmed, a lymphocyte apheresis product of non-mobilized cells must be received and accepted by the manufacturing site. Note: the lymphocyte apheresis product will not be shipped or assessed for acceptance by the manufacturing site until documented confirmation of all other inclusion/exclusion criteria have been satisfied.

Prior to lymphocyte apheresis (week -9 to -7), the following criteria must be met:

1. Peripheral blast count ≤ 20,000/mm^3 (AML).
2. No treatment with any other investigational agent in the previous 4 weeks.
3. No treatment with an immunostimulatory agent (IMIDs are allowed) or any cell therapy in the previous 30 days.
4. Negative to the following tests: HCV (Antibody, NAT), HIV 1-2 (p24, AB, Ag and NAT), total Ig Treponema Pallidum (if positive perform specific test), Australia HBsAg, total anti HB core Ab (if positive perform HBV DNA NAT), mycoplasma (PCR or IgM) and HTLV I-II.

Prior to lymphodepleting chemotherapy and MLM-CAR44.1 T-cell infusion (day -5 to day -3), the following criteria must be met:

1. Evidence of active disease at the beginning of lympho-depleting chemotherapy.
2. The following medications are excluded and should not be administered concomitantly or following lymphodepleting chemotherapy:
   a) Monoclonal antibodies in the 8 weeks prior to MLM-CAR44.1 T-cell infusion are prohibited.
   b) Salvage chemotherapy (e.g. clofarabine, cytosine arabinoside > 100 mg/m^2, anthracyclines, cyclophosphamide, proteasome inhibitors, IMIDs) must be stopped > 2 weeks prior to lymphodepleting chemotherapy

	c) Granulocyte colony stimulating or granulocyte-macrophage colony stimulating factor in the 2 weeks prior to study CAR T-cell administration. d) Immunosuppressant medications in the 2 weeks prior to CAR T-cell administration. e) Cytosine arabinoside < 100 mg/m^2/day must be stopped > 1 week prior to MLM-CAR44.1 T-cell infusion. f) Therapeutic systemic doses of steroids must be stopped > 72 hours prior to product infusion. However, < 12 mg/m^2/day hydrocortisone or equivalent are allowed as physiological replacement doses of steroids. g) Hydroxyurea must be stopped > 72 hours prior to MLM-CAR44.1 T-cell infusion. 3. No cardiovascular, pulmonary, renal, and hepatic functions that in the judgment of the investigator are insufficient to undergo investigational CAR T-cell therapy. 4. Female patients of childbearing potential must have a negative pregnancy test within 24 hours prior to starting lymphodepleting therapy
Study procedures	**Screening and Enrolment (Week -10 to Week -8)** After providing written informed consent, patients with AML or MM will be screened, and those compliant with inclusion/exclusion criteria including CD44v6 expression on tumor cells will be enrolled in the study. Children with AML will be enrolled in Phase IIa only, except for CZ site In Phase I, the first three enrolled patients (regardless of indication) for each dose will be treated one at a time at intervals of at last 36 days. These three patients will be enrolled accordingly once the previously enrolled patient has been approved for lymphodepleting chemotherapy. The remaining patients for each dose will be enrolled once the dose has proven to be safe in the first three enrolled patients. **Pretreatment and treatment** • Lymphocyte apheresis (Week -9 to Week-7) The first step in manufacturing CAR T-cells is to collect autologous lymphocytes by apheresis. Patients will undergo non-stimulated lymphocyte apheresis at least 7 weeks prior to MLM-CAR44.1 T-cell infusion to allow cell production, quality and release controls, and completion of lymphodepleting chemotherapy. For an adequate cell collection, apheresis will take place before lymphodepleting chemotherapy. Patients may be required to undergo multiple apheresis procedures so a sufficient number of CD3+ cells (approximately 7 x 10E8) or, if not possible, a sufficient number of WBC (approximately 10 x 10E9) can be collected for the preparation of the investigational product. • Lymphodepleting chemotherapy (Day -5 to Day -3) Lymphodepleting chemotherapy prior to T-cell infusion has been shown to favor T-cell engraftment and proliferation. This preconditioning creates space for the expansion of infused cells, limits the competition for homeostatic gamma chain cytokines IL-7 and IL-15, depletes regulatory T cells, and activates the innate immune system. Patients will undergo lymphodepleting chemotherapy with cyclophosphamide (500 mg/m^2) and fludarabine (30 mg/m^2) daily from day -5 to day -3.

	• Treatment with MLM-CAR44.1 T-cell (Day 0) Patients will receive one dose of MLM-CAR44.1 T-cells during a single infusion. **Follow-up** After being infused with MLM-CAR44.1 T-cells, patients will be monitored closely in the hematology or pediatric ward for at least 15 days and then followed up twice weekly through Month 1, weekly for the second month, every two weeks for the third month, monthly until Month 6 and then every 3 months until Month 24. **Long-term follow-up** A long-term follow-up is planned for assessing any potential delayed toxicity and lasting anti-tumor effects of MLM-CAR44.1 T-cells. Patients will continue to be followed for 15 years after MLM-CAR44.1 T-cell infusion as per health authority guidelines.
Product	Frozen autologous T lymphocytes genetically modified ex vivo with a γ retroviral vector to express a mutated form of the Herpes Simplex Virus Thymidine Kinase (HSV-TKmut2) and the CAR CD44v6ΔNL genes; formulated in freezing medium (7% HSA/10% DMSO in saline solution) at the final concentration of 1-10x10E6 cells/ml. 0.5 x 10E6/Kg, 1 x 10E6/Kg and 2 x 10E6/Kg are the three doses of autologous MLM-CAR44.1 T-cells for this study
Comparator	Not Applicable
Endpoints	**Phase I** <u>Primary endpoints - safety</u> 1. MTD established through BOIN design and the following DLTs occurring within 30 days following CAR T-cell infusion: a. Grade 4 CRS b. Grade 3 or higher CRS not responsive to therapy with steroids and or tocilizumab/siltuximab within 24 hours c. Grade 3 or higher toxicity at least possibly related to treatment with MLM-CAR44.1 T-cells excluding hematological toxicities d. Skin toxicities: o NCI-CTC grade 3 or higher erythroderma (generalized exfoliative dermatitis) o Stevens-Johnson Syndrome o Toxic Epidermal Necrolysis (TEN) o Any other grade 3 or higher skin toxicity histologically confirmed as being related to CAR T-cell treatment e. Grade 3 or higher neurotoxicity 2. Type, frequency and severity of adverse events and monitoring of systemic reactions (fever, tachycardia, nausea and vomiting, joint pain, skin rash) for 30 days following CAR T-cell infusion. 3. Absence of replication competent retrovirus (RCR) monitored by DNA PCR for the Galv gene 3,6, 12 and 24 months after infusion and then yearly as defined in the long-term follow-up <u>Secondary endpoints</u> 1. Hematologic disease response

a. AML: complete response (CR), incomplete response (CRi) and partial response (PR) according to ELN criteria 1 and 2 months following MLM-CAR44.1 T-cell infusion.
b. MM: overall response rate (ORR): stringent complete response (sCR), CR, very good partial response (VGPR) and partial response (PR) according to IMWG criteria 1 and 3 months following T-cell infusion

2. Levels of circulating MLM-CAR44.1 T-cells by flow cytometry.
3. Suicide gene activation and elimination of transduced cells through administration of ganciclovir in case of cytokine release syndrome or other significant MLM-CAR44.1 T-cell related toxicities.
4. To set-up a validation process among the participating centers for the CD44v6 marking of tumor cells (AML and MM).

Phase IIa

Efficacy

Primary endpoint – hematologic disease response

- AML: CR, CRi and PR rate as per ELN criteria, 2 months after MLM-CAR44.1 T-cell infusion.
- MM: ORR: (sCR, CR, VGPR, and PR) as per IMWG criteria, 3 months after T-cell infusion.

Secondary endpoints

1. Hematologic disease response:
   a. AML: CR, CRi and PR rate as per ELN criteria, 1 month and 6 months after MLM-CAR44.1 T-cell infusion. Morphologic leukemia-free state (MLFS) 1, 2 and 6 months after MLM-CAR44.1 T-cell infusion.
   b. MM: ORR (sCR, VGPR and PR) as per IMWG criteria, 1,2 and 6 months after T-cell infusion.
2. Overall Survival (OS) at 2 years: time from MLM-CAR44.1 T-cell infusion to death due to any cause.
3. Disease-Free Survival (DFR) in AML patients: time from achievement of response to relapse or death due to any cause during response.
4. Event Free Survival (EFS) in AML patients: time from the date of MLM-CAR44.1 T-cell infusion to the date of the earliest of the following: last follow-up, resistance, relapse or death due to any cause.
5. Progression Free Survival (PFS) in MM patients at 2 years: time from MLM-CAR44.1 T-cell infusion to progression/relapse or death due to any cause.
6. Duration of Remission (DOR) in MM patients: time from achievement of response to relapse or death due to any cause.

Exploratory endpoint

Minimal Residual Disease:

- AML: proportion of patients with a molecular CR, 2 months after infusion.
- MM: proportion of patients with a molecular CR, 3 months after infusion.

Pharmacokinetics

Levels of circulating MLM-CAR44.1 T-cells by flow cytometry.

	Feasibility 1. Percentage of eligible patients for each indication after initial screening who undergo both lymphocyte apheresis and CAR T-cell infusion. 2. Percentage of patients who are eligible for lymphocyte apheresis and are then effectively infused with CAR T-cells. **Safety** 1. Adverse events (any grade) and monitoring of systemic reactions (fever, tachycardia, nausea and vomiting, joint pain, skin rash). 2. Absence of RCR monitored by DNA PCR for the Galv gene 3, 6, 12 and 24 months after infusion and then yearly as defined in the long-term follow-up. 3. Absence of abnormal MLM-CAR44.1 T-cell clonal expansion monitored by clinical and laboratory surveillance, as well as by TCR-Vbeta repertoire study 6 months after infusion. 4. Suicide gene activation and elimination of transduced cells through administration of ganciclovir in case of cytokine release syndrome or other significant MLM-CAR44.1 T-cell related toxicities.
Pharmacokinetics/Pharmacodynamics	**Pharmacokinetics:** Levels of circulating MLM-CAR44.1 T-cells by flow cytometry
Quality of life	Not Applicable
Statistical analysis	**Phase I** <u>Primary endpoints – safety</u> a. The proportion of patients with DLTs occurring within one month from CAR T infusion will be calculated for each dose level along with the 95% exact Clopper-Pearson confidence intervals. b. The incidence of adverse events and of systemic reactions for 30 days following CAR T-cell infusion will be tabulated by MedDRA preferred term, system organ class and severity. c. The proportion of patients with absence of replication competent retrovirus monitored by DNA PCR at 3, 6, 12 and 24 months after infusion of MLM-CAR44.1 T-cells will be calculated for each dose level. The corresponding 95% exact Clopper-Pearson confidence intervals will also be calculated. <u>Secondary endpoints</u> a. Hematologic disease response for AML The percentages of patients either in CR, Cri or PR (ELN criteria) at 1 and 2 months following MLM-CAR44.1 T-cell infusion will be estimated at each dose level and the 95% exact Clopper-Pearson confidence intervals calculated. b. Hematologic disease response for MM The overall response rate (ORR) is defined as the proportion of patients in: stringent complete response (sCR), CR, very good partial response (VGPR) and partial response (PR) (IMWG criteria). The percentages of overall response at 1 and 3 months following MLM-CAR44.1 T-cell infusion will be estimated at each dose level and the 95% exact Clopper-Pearson confidence intervals calculated. c. Suicide gene activation The percentage of patients for whom activation of suicide gene was

needed will be calculated for each dose level along with the corresponding 95% exact Clopper-Pearson confidence intervals.

The levels of transduced cells in patients treated with ganciclovir will be listed and described at each dose level.

d. In vivo pharmacokinetic profile
The pharmacokinetic analysis will be provided in the statistical analysis plan.

Phase IIa

Primary endpoints

a. Hematologic disease response for AML at 2 months
The percentages of patients in CR, Cri and PR (ELN criteria) at 2 months following MLM-CAR44.1 T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

b. Hematologic disease response for MM at 3 months
ORR: the percentages of overall response at 3 months following MLM-CAR44.1 T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

Secondary/exploratory endpoints:

Efficacy

a. CR, Cri, PR and morphologic leukemia-free state rates as per ELN criteria, 1 month and 6 months after MLM-CAR44.1 T-cell infusion (AML) will be summarized and presented with 95% confidence intervals.

b. ORR (sCR, VGPR and PR) as per IMWG criteria, 1, 2 and 6 months and 6 months after T-cell infusion (MM) will be summarized and presented with 95% confidence intervals.

c. OS, DFS (AML patients), EFS (AML patients) DoR (MM patients), and PFS (MM patients) will be estimated using the Kaplan-Meier product-limit method. Median and two-sided 95% confidence intervals for median values will be computed and Kaplan-Meier plots will be presented.

d. Minimal Residual Disease for AML: The percentages of patients with a molecular CR at 2 months for AML and at 3 months for MM will be estimated and the 95% exact Clopper-Pearson confidence intervals calculated.

Feasibility

a. The percentages of eligible patients who undergo both lymphocyte apheresis and CAR T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

b. The percentages of eligible patients effectively infused with CAR T-cells after undergoing lymphocyte apheresis will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

Safety

a. SAEs, treatment-emergent AEs and systemic reactions will be summarized for each dose level by system organ class and/or preferred term and severity by means of frequency tables.

	b. The proportion of patients with absence of replication competent retrovirus monitored by DNA PCR at 3, 6 and 12 and 24 months after infusion of MLM-CAR44.1 T-cells will be calculated. The corresponding 95% exact Clopper-Pearson confidence intervals calculated. c. The proportion of patients with absence of abnormal MLM-CAR44.1 T-cell clonal expansion monitored by clinical and laboratory surveillance, as well as by TCR-Vbeta repertoire study will be calculated 6 months after infusion. The corresponding 95% exact Clopper-Pearson confidence intervals will also be calculated. d. The percentage of patients for whom activation of suicide gene was needed will be calculated along with the corresponding 95% exact Clopper-Pearson confidence intervals. The levels of transduced cells in patients treated with ganciclovir will be listed and described. <u>Pharmacokinetic profile</u> The pharmacokinetic analysis will be provided in the statistical analysis plan. <u>Ancillary study</u> The ancillary study will be provided in the statistical analysis plan <u>Validation process for the CD44v6 marking of tumor cells (AML and MM)</u> The analysis will be provided in the statistical analysis plan
Study duration	48 months (the end of study is defined as the last patient's last visit (LPLV), which is the last patient's Month 24 evaluation, or the time of premature withdrawal

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List of Abbreviations

ACT	Adoptive Cell Therapy
AIEOP	Italian Association of Pediatric Hematology and Oncology
ALC	Absolute Lymphocyte Count
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
ASCT	Allogeneic Stem Cell Transplantation
BBB	Blood-Brain Barrier
BCMA	B-Cell Maturation Antigen
BOIN	Bayesian Optimal Interval
CAR	Chimeric Antigen Receptor
CEA	Carcinoembryonic Antigen
CMML	Chronic Myelomonocytic Leukemia
CR	Complete Response
CRA	Clinical Research Associate
Cri	Incomplete response
CRS	Cytokine Release Syndrome
CZ	Czech Republic
DFS	Disease-Free Survival
DLCO	Corrected Diffusing Capacity of Carbon Monoxide
DLT	Dose Limiting Toxicity
DOR	Duration of Response
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
FEV1	Forced Expiratory Volume in 1 second
FLC	Free Light Chain
Galv	Gibbon ape leukemia virus
GCV	Ganciclovir
GvHD	Graft versus Host Disease
GvT	Graft versus Tumor
HRQoL	Health-Related Quality of Life
HSC	Hematopoietic Stem Cells
HSV-TK	Herpes Simplex Virus-Thymidine Kinase
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IFE	Immunofixation Electrophoresis
IMID	Immune Modulating Drug
IRB	Institutional Review Board
JMML	Juvenile Myelomonocytic Leukemia
LFS	Leukemia Free Survival
LNGFR	Low Affinity Nerve Growth Factor Receptor
LPLV	Las Patient Last Visit
LSC	Leukemia Stem Cells
Mab	Monoclonal Antibodies
MLFS	Morphologic Leukemia-Free State
MM	Multiple Myeloma
MNC	Mononuclear Cell
MRD	Minimal Residual Disease

MTD	Maximum Tolerated Dose
NCI-CTC	National Cancer Institute Common Terminology Criteria
ORR	Overall Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PEP	Protein Electrophoresis
PFS	Progression Free Survival
PR	Partial Response
RCR	Replication Competent Retrovirus
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
ScFV	Single-chain Variable Fragment
sCR	Stringent Complete Response
SD	Stable Disease
SFLC	Serum Free light chain protein assessment
SIFE	Serum Immunofixation
SJS	Stevens-Johnson Syndrome
SPEP	Serum Protein Electrophoresis
STP	Soft Tissue Plasmacytoma
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAA	Tumor Associated Antigen
TLS	Tumor Lysis Syndrome
TEN	Toxic Epidermal Necrolysis (TEN)
UAR	Unexpected Adverse Reaction
ULN	Upper Limit of Normal
UIFE	Urine Immunofixation
UPEP	Urine Protein Electrophoresis
USM	Urgent Safety Measures
VCV	Valganciclovir
VGPR	Very Good Partial Response
WHO	World Health Organization

1 INTRODUCTION

1.1 Background

1.1.1 Acute Myeloid Leukemia

Relapsed or refractory acute myeloid leukemia (AML) poses a serious challenge to current therapeutic strategies. Of patients who are fit enough to receive standard induction therapy, about 60-80% of younger adults and 40-50% of older adults achieve a complete remission, leaving a substantial population of patients who are refractory to initial induction therapy (*Mangan et al., 2011*). An additional large population of patients have disease relapses after achieving first complete response (CR). In a study of 1,069 patients achieving a first CR at MD Anderson Cancer Center between 1991 and 2003 without undergoing Hematopoietic Stem Cell Transplantation (HSCT), the probability of relapse-free survival at 3 years was only 29% (*Yanada et al., 2007*). This has been attributed to leukemia stem cells (LSCs), which hide in endosteal and sinusoidal niches in the bone marrow and evade immune surveillance through the outgrowth of poorly immunogenic tumor cell variants, known as immune selection, and/or through disruption of the immune system. A robust innate immune system is therefore mandatory to avoid relapses by targeting chemoresistant malignant cells (*Nwajei F et al., 2013*).

Especially in fit patients that suffer of intermediate or high-risk AML and achieve CR after induction therapy, HSCT remains the most effective long-term treatment, yielding cure in 50–60% of patients (*Cormelissen et al., 2015; Passweg et al., 2016*). Nevertheless, several patients never become eligible for transplant because of co-morbidities, failure to reach a CR or lack of a suitable donor. Furthermore, available data suggest that HSCT may not be the most effective strategy to eradicate minimal residual disease (MRD) and that novel agents such as molecularly targeted drugs (FLT3 or IDH inhibitors) or monoclonal antibody–based agents including antibody-drug conjugates and bispecific antibodies, and, potentially, checkpoint inhibitors and CAR T-cells, may improve therapeutic strategies to eradicate persistent minimal residual disease remaining after cytotoxic regimens or can be used to “bridge” to transplant (*Medlinger et al., 2016*).

1.1.2 Multiple Myeloma

Multiple myeloma is the second most commonly diagnosed hematological neoplasm, with an incidence rate of 6.2 per 100,000 individuals (*Seigel et al., 2016*). In 2016, there were an estimated 30,330 newly diagnosed cases in the United States, accounting for ~1.8% of all new cancer cases (*Howlader et al., 2016*).

Traditional treatment regimens for newly diagnosed MM date back to the 1960s, with melphalan plus prednisone as the first-line therapy along with combinations of vincristine, doxorubicin and dexamethasone. By the mid-1990s, HSCT became common but primarily for younger patients with adequate kidney function. Over the past decades, one of the major advances in the treatment regimen of patients with MM has been the introduction of novel therapies, including immune-modulating drugs (lenalidomide) and proteasome inhibitors such as bortezomib. Many of these novel therapies are currently recommended as initial treatment in newly diagnosed patients irrespective of HSCT eligibility. In the last few years, the therapeutic landscape of MM has improved even further with the approvals of pomalidomide, another immune-modulating drug, the monoclonal antibodies daratumumab and elotuzumab, as well as

the new-generation proteasome inhibitors carfilzomib and ixazomib. The addition of these novel drugs has improved both progression free (up to ~30 months) and median overall survival (~4.7 years) (*Warren et al., 2013; San Miguel et al., 2008*). There is thus agreement that eligible patients in second or later relapse should be considered for trial participation (*Sonneveld et al. 2017*).

1.1.3 Chimeric Antigen Receptor (CAR) T-Cells

T cells are known to play a major role in immune surveillance and tumor eradication. Over the past 25 years, cancer research has been focused on developing technologies that generate, educate, and/or enhance T cells against tumors, and at the same time make these innovative treatments clinically and economically feasible.

Adoptive Cell Therapy (ACT) is a form of immunotherapy that has demonstrated promising results for blood cancers and may have potential against other types of cancers as well. In contrast with checkpoint inhibitors, i.e. antibodies that block inhibitory molecules on T cells and thus unleash T-cell–mediated antitumor activity, ACT creates a productive immune response: through several techniques, autologous T cells are harvested, engineered to recognize tumor antigens, and returned to the patient to specifically target tumor cells.

Chimeric Antigen Receptor (CAR) T-cells are engineered by combining an antibody-derived single-chain variable fragment (scFv) to T-cell intracellular signaling domains. These T cells recognize cell surface antigens in a non–MHC-restricted manner and thus do not depend on antigen processing and presentation. The first-generation CARs consisted of an scFv linked to the CD3 intracellular signaling domain. To improve persistence and proliferation of infused T cells, second- and third-generation CARs have been developed that incorporate the intracellular domains of one or multiple co-stimulatory molecules such as CD28, OX40 and 4-1BB within the endodomain (*Houot R et al., 2015*). CAR T-cells have also been designed to express multiple CARs that recognize several tumor antigens and stimulate production of cytokines and interleukins when bound to their antigen.

Of the current trials investigating CAR T-cell therapy, 133 involve hematological malignancies and 78 solid tumors. Seventeen different CAR antigens are under investigation for tumors of the hematopoietic and lymphoid system. The most frequently targeted antigen is CD19, with 56 ongoing and eight non-active trials. Even more antigens (22) are being investigated for the treatment of solid tumors. Previous trials focused on carcinoembryonic antigen targeting colorectal cancer, breast cancer, gastric cancer, adenocarcinoma as well as liver metastases. Ongoing trials target mesothelin, ErbB2/Her2, GD2 (neuroblastoma or sarcoma), or GPC3 (hepatocellular carcinoma) (*Hartmann et al., 2017*).

Promising results have been obtained in multiple myeloma with B-Cell Maturation Antigen (BCMA) CAR T-cells. Twenty-one (21) MM patients with ≥ 3 prior regimens including a proteasome inhibitor and an immunomodulatory agent or who are double refractory and have ≥ 50% BCMA expression on MM were treated. No dose-limiting toxicity was observed and cytokine release syndrome (CRS) was readily manageable. Overall response rate (ORR) was 89% with all patients receiving ≥150 x10^6 CAR. Minimal residual disease (MRD) negative status was seen in all MRD-evaluable patient samples (N=4). No patients treated with ≥ 150 x10^6 CAR had disease progression with time from infusion between 8 and 54 weeks (*Berdeja Jesus G et al, 2017, ASH Abstract N 740*).

On July 1, 2014, the FDA granted 'breakthrough therapy' designation to CTL019, the anti-CD19 CAR T-cell therapy developed at the University of Pennsylvania. CTL019 is the first personalized cellular therapy for cancer to be so designated, and 25 years after the first publication describing genetic redirection of T cells to a surface antigen of choice.

Two CAR T-cell treatments are currently approved by the FDA and CHMP. Tisagenlecleucel (Kymriah™) is a CD19-directed genetically modified autologous T cell immunotherapy indicated for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse. The second, Axicabtagene ciloleucel (Yescarta™), is a CD19-directed CAR T-cell therapy for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma.

Approval of Tisagenlecleucel was based on the ELIANA trial, a single-arm, open-label, multicenter, global, phase II study of CTL019 in pediatric/young adult patients with CD19+ R/R B-cell ALL with ≥ 5% bone marrow lymphoblasts by morphology. As of November 2016, 88 patients were enrolled. There were 7 (8%) manufacturing failures, 9 (10%) patients were not infused due to death or adverse events (AEs), and 4 patients (5%) were pending infusion at the time of data cutoff. Following lymphodepleting chemotherapy in most patients, 68 patients were infused with a single dose of CTL019, with a median study follow-up of 6.4 months. Median age was 12 y (range, 3-23 y) and 59% of patients had prior allogeneic stem cell transplant (allo-SCT). Five infused patients had not reached 3 months of follow-up. Among 63 evaluable patients, 52 (83% [95% CI, 71%-91%]) achieved CR/CRi within 3 months of CTL019 infusion, all of whom had minimal residual disease–negative marrow. The relapse-free probability at 6 months after remission onset was 75% (95% CI, 57%-87%; median DOR not reached). The probability of survival was 89% (95% CI, 77%-94%) at 6 months and 79% (95% CI, 63%-89%) at 12 months. Seven patients (13% of responders) proceeded to alloSCT within 6 months while in remission. Cytokine release syndrome (CRS) occurred in 78% of patients (21% grade 3; 27% grade 4); no CRS-associated deaths occurred. The most common grade 3/4 nonhematologic AEs (>15%) other than CRS were hypotension (22%), hypoxia (18%), and increased aspartate aminotransferase (16%). The incidence of serious AEs within 8 weeks of infusion was 69%. Fifteen percent of patients experienced grade 3 neuropsychiatric AEs, with no grade 4 events and no cerebral edema reported. Grade 3/4 neutropenia with high (>38.3˚C) fever occurred in 60% of patients. Two patients died within 30 days of infusion (ALL progression, n=1; cerebral hemorrhage, n=1), and 9 patients died >30 days after infusion (ALL relapse/progression, n=6; HHV-6 encephalitis, pneumonia, systemic mycosis, n=1 each). CTL019 expansion in vivo correlated with CRS severity, and persistence of CTL019 along with B-cell aplasia in peripheral blood was observed for ≥1 year in some responders (*Buechner et al., 2017*).

Maude et al. conducted a Phase 2, single-cohort, 25-center, global study of tisagenlecleucel in pediatric and young adult patients with CD19+ relapsed or refractory B-cell ALL. The primary endpoint was the overall remission rate (the rate of complete remission or complete remission with incomplete hematologic recovery) within 3 months. Seventy-five (75) patients received an infusion of tisagenlecleucel and were evaluated for efficacy. The overall remission rate within 3 months was 81%, with all patients who had a response to treatment found to be negative for

minimal residual disease, as assessed by flow cytometry. The rates of event-free survival and overall survival were 73% (95% confidence interval [CI], 60 to 82) and 90% (95% CI, 81 to 95), respectively, at 6 months and 50% (95% CI, 35 to 64) and 76% (95% CI, 63 to 86) at 12 months. The median duration of remission was not reached. Persistence of tisagenlecleucel in the blood was observed for as long as 20 months. Grade 3 or 4 adverse events that were suspected of being related to tisagenlecleucel occurred in 73% of patients. The cytokine release syndrome occurred in 77% of patients, 48% of whom received tocilizumab as treatment. Neurologic events occurred in 40% of patients and were managed with supportive care, and no case of cerebral edema was reported (*Maude et al., 2018*).

Approval of Axicabtagene ciloleucel was based on a multicenter, Phase 2 trial that enrolled 111 patients with diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, or transformed follicular lymphoma who had refractory disease despite undergoing recommended prior therapy. Patients received a target dose of 2×10^6 anti-CD19 CAR T cells per kilogram of body weight after receiving a conditioning regimen of low-dose cyclophosphamide and fludarabine. The primary endpoint was the rate of objective response (calculated as the combined rates of complete response and partial response). Secondary end points included overall survival, safety, and biomarker assessments. Among the 111 patients who were enrolled, axicel was successfully manufactured for 110 (99%) and administered to 101 (91%). The objective response rate was 82%, and the complete response rate was 54%. With a median follow-up of 15.4 months, 42% of the patients continued to have a response, with 40% continuing to have a complete response. The overall rate of survival at 18 months was 52%. The most common adverse events of grade 3 or higher during treatment were neutropenia (in 78% of the patients), anemia (in 43%), and thrombocytopenia (in 38%). Grade 3 or higher CRS and neurologic events occurred in 13% and 28% of the patients, respectively. Three of the patients died during treatment. Higher CAR T-cell levels in blood were associated with response (Neelapu et al., 2017).

1.1.4 CD44v6

Despite the remarkable results obtained by targeting CD19, widespread application of CAR strategy is however limited by the current lack of a CAR specific for antigens different from CD19. Furthermore, CD19 is not exactly a “leukemia specific” antigen. Normal B cells express CD19 and CD19 CAR-T cell therapy is therefore associated with the loss of CD19-positive normal B cells from blood and bone marrow resulting in profound hypogammaglobulinemia (*Park et al., 2016*). The impressive response rates observed in clinical trials of CD19 CAR T-cells have in fact led to the rapid proliferation of preclinical studies testing new targets and methods to make CAR T-cell therapy safer and more broadly applicable to various tumor types. Strategies to genetically modify T cells to improve their targeting, enhance their tissue penetration, and control their expansion and persistence are all ways to engineer better CAR T-cells (*Maus et al., 2016*).

The hyaluronate receptor CD44 is a class I membrane glycoprotein overexpressed in hematologic and epithelial tumors. It plays a crucial role in the bone marrow homing of initiating leukemia cells and in interactions with the microenvironment. It has been shown that CD44 inhibition drives leukemia cells into differentiation and apoptosis by dislodging them from osteogenic niches (*Singh et al., 2013*). The isoform variant 6 (CD44v6) has recently emerged as one of the most promising tumor-associated antigens (TAA) for AML. CD44v6 is expressed at variable levels in 16 of 25 (64%) AML cases belonging to different French-

American-British subtypes and World Health Organization categories and in 13 of 15 (87%) MM cases at different stages according to the Durie-Salmon classification or the International Staging System (*Casucci et al., 2013)*. Preclinical studies have shown that CD44v6-silenced THP-1 (AML) and MM.1S (MM) cells are severely impaired in their capacity to engraft in immune-compromised mice and, similarly, CD44v6-silenced primary leukemic blasts fail to initiate leukemia in vivo, providing further rationale for targeting the CD44v6 antigen in AML and MM since the potential generation of antigen lost variants is circumvented by the reduced growth of CD44v6 negative tumor cells.

1.1.5 EURE-CART Project

This clinical study protocol is Working Package 4 of grant N. 733297 awarded by the European Commission for the action entitled '***EURopean Endeavour for Chimeric Antigen Receptor Therapies — EURE-CART***.

The nine Parties described in Annex 1 formed a consortium and signed a grant agreement with the European Commission, under the coordination of MolMed, which acts also as a Sponsor of this clinical study.

1.2 Drug description

Generation of the medicinal product (MLM-CAR44.1 T cells)

The medicinal product (MLM-CAR44.1 T cells) is defined as frozen T lymphocytes genetically modified to express CAR CD44v6 by the retroviral vector CAR CD44v6ΔNL encoding the CAR CD44v6 and HSV-TK Mut2 genes in the final formulation and container closure system, ready for intended medical use. It is a patient-specific product prepared starting from a lymphocyte apheresis of the patient.

The retroviral vector CAR CD44v6ΔNL is generated from the SFCMM3 Mut2 vector currently used for manufacturing Zalmoxis (i.e. encoding the HSV-TK Mut2 suicide gene and the ΔLNGFR marker gene) by substituting the marker gene with CAR CD44v6.

In its final version, the CAR CD44v6ΔNL retroviral vector carries the HSV-TK Mut2 gene under the transcriptional control of the viral 5'LTR promoter and an internal expression cassette with the SV40 promoter driving the CAR CD44v6.

The CAR CD44v6 includes:

- the single-chain variable fragment (scFv) of the humanized mAb BIWA-8 (*Verel et al., 2002*) specific for the CD44v6 antigen;
- a spacer formed by the extracellular domain of the human low-affinity nerve growth factor receptor (ΔLNGFr) that allows the selection of cells to be infused in patients and their monitoring using specific antibodies (*Casucci et al., 2018*);
- the transmembrane and intracellular domain of the human costimulatory molecule CD28;
- the intracellular signaling domain of the human CD3ζ chain.

The HSV-TK Mut2 suicide gene encodes a mutated form of the thymidine kinase enzyme of Herpes Simplex Virus I and provides a safety valve in case of toxicity. Indeed, the derived enzyme is functional and used both *in vitro* and *in vivo* to selectively eliminate the transduced

cells in presence of Ganciclovir (GCV). The HSV-TK Mut2 variant was generated by site-directed mutagenesis with the introduction of a silent T to C transition at a splicing donor site of the original HSV-TK. The mutation has been proven to avoid the generation of GCV-resistant HSV-TK spliced forms.

To generate the stable packaging cell line producing the CAR CD44v6ΔNL retroviral vector, the LTK-S44v6ΔNL plasmid was used to transfect the ecotropic GP+E86 packaging cells line (ATCC n° CRL-9642). The supernatant from the transient transfection was harvested and used to infect the PG13 packaging cell line (ATCC n° CRL-10686) expressing the Gibbon Ape Leukemia virus envelope protein (Galv). The integration of the construct in the packaging cell line DNA allows the production of retroviral vectors able to infect a broad range of mammalian cells, including human cells. The PG13 bulk cell population was selected, following transduction, based on expression of CD44v6 CAR using antibodies specific for the LNGFR spacer conjugated to magnetic beads. The resulting transduced cells were then plated in limited dilution conditions to obtain producer cell clones. The clones were tested for expression of CD44v6 CAR, good growth capacity in culture, efficiency of transduction of T lymphocytes, stability, absence of contaminating adventitious viruses and of replication-competent forms. The producer clone PG13-187#68 was chosen for further development.

The MLM-CAR44.1 T-cell manufacturing process essentially consists in: i) stimulation of peripheral blood mononuclear cells isolated from patients with TransAct, a polymeric nanomatrix conjugated to anti-CD3 and anti-CD28 mAbs, IL15 and IL7; ii) transduction of the stimulated cells with the CAR CD44v6ΔNL retroviral vector using RetroNectin®; iii) immunoselection of the transduced cells using CD271 Microbeads. Selected cells are then further expanded before the final formulation in frozen medium.

Of note, the overall yield of the MLM-CAR44.1 T-cells manufacturing process carried out with AML starting material is quite variable and lower to what observed with peripheral blood mononuclear cells (PBMC) from healthy donors due to the lower $CD3^+$ percentages and the presence of variable degrees of leukemic cells. However, the expansion of $CD3^+$ cells during the culture period is good, indicating that if a proper number of $CD3^+$ cells is present in the patient apheresis/starting material, it should be feasible to reach the desired final dose of MLM-CAR44.1 T-cells.

The entire manufacturing process lasts 10 days and is performed in an open system, except for cell washing and CD271 selection at day 8, which employ the CliniMACS Prodigy instrument.

Overall, the transduced cells are positive for the CD45 leukocyte marker, and for $CD3^+$. No relevant proportions of monocytes ($CD14^+$), B cells ($CD19^+$) and NK cells ($CD3^-$ $CD16^+$ and $CD56^+$) are found. T-lymphocyte subpopulations ($CD4^+$ and $CD8^+$) are represented in variable degrees among different patients.

1.3 Drug activity

The expression of CAR CD44v6 allows the transduced T-cells to acquire a potent and specific reactivity against tumor cells expressing the CD44v6 antigen, including primary AML and MM cells.

Moreover, the expression of the CAR CD44v6 on the cell membrane allows for the immunoselection of genetically modified cells using mAb conjugated to magnetic beads that recognize the LNGFR spacer. The expression of the LNGFR protein can be used in vivo as a marker of genetically modified cells once infused into the patients and allows the assessment of drug product persistence in the human body following treatment, possible cell population expansion or reduction and characterization in terms of lymphocyte subtype and state of activation.

HSV-TK Mut2 makes the transduced cell sensitive to GCV. Following its administration, GCV is first monophosphorylated by the HSV thymidine kinase enzyme expressed in the genetically modified cells and then transformed to the triphosphate form by other cellular kinases. This active, triphosphate form of GCV induces the blockage of the synthesis of new DNA strands during mitosis, and hence provokes the death of proliferating cells.

1.3.1 Non-clinical studies

Pre-clinical studies: efficacy

The original CD44v6 CAR construct (CH2CH3-spaced CAR) was developed at the research laboratories of the San Raffaele Institute. When co-cultured with an excess of target cells, CD44v6.CAR28z T cells efficiently eliminated CD44v6$^+$, but not CD44v6$^-$, primary leukemic blasts and malignant plasma cells. The antitumor activity of these CAR T cells was then assessed *in vivo* in NOD/SCID/IL2Rnull (NSG) mice previously infused with primary leukemic blasts and then treated with a single infusion of CD44v6.CAR28z or control CTR.CAR28z T cells from the same mouse. Although control mice had increasing percentages of circulating leukemic blasts over time, mice receiving CD44v6.CAR28z T cells had progressive disappearance of leukemia and, at sacrifice, were completely cleared of tumor cells in the bone marrow.

The original CD44v6 CH2CH3-spaced CAR however mediated suboptimal long-term antitumor activity in vivo when challenged against high tumor burdens due to undesired interaction with Fc receptor (FcR)-bearing myeloid cells and premature clearance. To develop a more effective CAR construct that could enable enrichment and tracking of CD44v6 CAR T cells, the CH2CH3 spacer was replaced with the extracellular domain from the human low affinity nerve growth factor receptor (ΔNL) currently used as cell surface marker in Zalmoxis cell therapy. In co-culture experiments with tumor cells expressing CD44v6 and/or FcR, the new construct killed CD44v6$^+$ THP-1 myeloid leukemia cells and MM.1S myeloma cells but spared CD44v6$^-$ BV173 lymphoid leukemia cells. They also failed to kill CD44v6$^-$/FcR$^+$ HL60 myeloid leukemia cells, indicating efficient abrogation of FcR recognition. Importantly, maintenance of potent antitumor reactivity in these CD44v6ΔNL CAR T cells was confirmed by efficient and specific killing of both primary AML blasts and malignant plasma cells expressing CD44v6. Finally, antitumor activity was tested in vivo in the THP-1 leukemia and MM.1S myeloma models. Treated mice benefited from substantial antitumor effects, as indicated by normalization of THP-1 cell infiltrated liver weight. Moreover, similar results were obtained when testing against myeloma.

A GLP-like study was then conducted to determine the antitumor efficacy of CAR T cells transduced with CD44v6ΔNL CAR and HSV-TK-encoding retrovirus according to the final drug product manufacturing process (44v6.28z cells). 44v6.28z cells were compared to CD19ΔNL CAR T cells identically cultured and manipulated (19.28z cells). In co-culture

experiments with tumor cells, 44v6.28z cells were able to efficiently kill MM.1S cells, proliferate and produce inflammatory cytokines. THP-1 cells were also targeted, although with inferior intensity due to lower CD44v6 expression levels. Moreover, CD44v6^{neg} BV-173 cells were completely spared.

The in vivo functionality of 44v6.28z cells was tested in NSG mice engrafted with tumor cells. MM.1S myeloma cells and THP-1 leukaemia cells were modified to express a secreted luciferase, which has the great advantage of being released by the cells and is therefore detectable in blood allowing monitoring of tumor progression in mice. In the myeloma model, treatment did not cause toxicity *per se*, as indicated by the general well-being of mice and the lack of consistent weight loss. 44v6.28z cells significantly inhibited tumor progression in mice, as indicated by bioluminescence signals measured in blood. The antitumor effect of 44v6.28z cells was confirmed at sacrifice, when no or very low percentages of tumor cells were found both in the bone marrow and in the spleen. Notably, antitumor activity was paralleled by CAR T cell expansion and contraction. 44v6.28z cells were predominantly CD4$^+$ and, in accordance with antigen-specific recognition, were strongly activated, as indicated by transient increases of HLA-DR. Importantly, the expression of PD1 was only transiently up-regulated at the peak of T cell activation and then returned to basal levels, indicating no CAR T cell exhaustion and thus long-term antitumor potential.

In the leukemia model, treatment with 44v6.28z cells was well tolerated and able to significantly inhibit tumor progression, as indicated by both luciferase levels and liver weight at sacrifice. CAR-T cells expanded in peripheral blood, were predominantly CD4$^+$ and were activated upon antigen encounter. Also in this setting, 44v6.28z cells were detectable at sacrifice both in the bone marrow and spleen, still included a proportion of early differentiated cells and did not express exhaustion and senescence markers.

Full details of preclinical studies are available in the Investigator's brochure.

Pre-clinical studies: safety

In clinical trials, tumor responses by CAR-redirected T cells were often associated with toxicities deriving from off-tumor expression of the target. Cases of fatal lung toxicity and the frequent observation of prolonged B-cell depletion were observed using HER2 and CD19 CAR T-cells, respectively. Despite promising activity against epithelial tumors, the administration of the CD44v6-specific mAb (bivatuzumab) used for deriving our CAR scFv showed reversible myelosuppression and mucositis when conjugated with radioisotopes (*Borjesson et al., 2003*), and showed skin toxicity, including a fatal case, when conjugated with the potent cytotoxic drug mertansine (*Tijink et al., 2006*).

In accordance with CD44v6 expression on monocytes and keratinocytes, but not on HSCs and progenitor cells, the infusion of CD44v6 CAR T cells in human hematochimeric mice caused selective and reversible monocytopenia, indicating preservation of the HSC pool. Indeed, after in vivo exhaustion of CD44v6-targeted T cells, NSG-3G mice reconstituted monocytes de novo (*Casucci et al., 2013*).

Keratinocytes appear to be resistant to CD44v6 CAR T activity in vitro, whereas they are killed by EGFR CAR T-cells. To verify in vivo the absence of activity against skin-resident keratinocytes, we developed a human-skin xenograft mouse model. Full-thickness human skin fragments were implanted subcutaneously into immunodeficient NSG mice. After healing of

the skin graft, mice were treated with either vehicle, unrelated CD19 CAR T-cells (negative control), EGFR CAR T-cells (positive control) or CD44v6 CAR T-cells (test item). Three days after CAR T-cell infusion, no differences were found in the number of circulating T cells between the different groups, while we observed an increased percentage of cells expressing HLA-DR in EGFR CAR T-cells as compared to CD44v6 CAR T-cells, indicating a more activated state. Two weeks after CAR T-cell infusion, skin xenografts were recovered and analyzed by immunohistochemistry for the presence of infiltrating human T cells. Mice that were infused with EGFR CAR T-cells showed the highest CD3+ cell infiltration in the derma portion of the skin xenograft, while mice infused with CD44v6 CAR T-cells showed only low CD3+ cell infiltration, similarly to control mice receiving CD19 CAR T-cells. At the dermal-epidermal junction of the skin, we observed a similar trend without reaching statistical significance. These results were confirmed by using a skin explant model in which human skin biopsies were directly co-cultured with CAR T cells for 3 days before being analyzed by immunohistochemistry. Also in this model, minimal skin infiltration by CD44v6ΔNL CAR T cells was reported.

The TK/ganciclovir system incorporated into the CAR construct was designed to address potential CAR T-cell toxicities, and its efficacy was confirmed in animal models. 44v6.28z cells generated from two different healthy donors expanded in the peripheral blood of mice receiving saline, while they remained nearly undetectable in mice treated with ganciclovir (GCV). These results were confirmed at sacrifice as no 44v6.28z cells were identified in the bone marrow of mice treated with GCV, while they were present in control mice. Besides being effective, the suicide system proved to be specific since no significant differences were observed in mice infused with untransduced T cells and treated with either saline or GCV.

All together, these results and the presence of a suicide gene in the transduced cells, strongly suggest that therapeutic doses of CD44v6 CAR T-cells are likely associated with acceptable and/or reversible toxicities.

Full details of preclinical safety studies are available in the Investigator's brochure.

1.3.2 Clinical studies

Not Applicable

1.4 Rationale – risks and benefits

1.4.1 Possible benefits of CAR-T cell therapy

Even in this epoch of great drug development, there is urgent need to investigate how new therapies and interventions may further benefit patients with MM and AML. In this context, CAR T-cells represent an exciting frontier in cancer therapy: antitumor T cells with high-avidity recognition of tumor antigens can be expanded in vitro in large numbers, genetically engineered, and/or activated ex vivo to acquire antitumor functions. In addition, the host can be manipulated before cell transfer to eliminate suppressor cells and promote in vivo expansion of transferred lymphocytes, which can then function as "living drugs" that induce long-term protection (*Houot et al., 2015*).

CAR T-cell therapy is entering a new era, transitioning from an experimental approach being tested in a handful of centers to a more mainstream and broadly investigated therapeutic

platform with significant efforts directed towards commercial translation. Ongoing innovations into the design and application of CAR T-cells are aimed at improving antitumor potency and, at the same time, ensuring safety. The remarkable clinical efficacy demonstrated with CD19 CARs has been achieved at multiple institutions, each evaluating their own CAR T-cell platforms and trial designs. The principles that have arisen from this wealth of clinical experience has helped shape the parameters key to achieving therapeutic success, as well as the management of potential toxicity (*Brown et al., 2016*).

The results of preclinical studies have demonstrated that CD44v6ΔNL CAR T cells efficiently and consistently inhibit tumor growth in both MM and AML models. The correlation between antitumor activity and level of antigen expression suggests that CD44v6ΔNL CAR T cells have highly specific targeting properties that should favor low toxicity in vivo. Moreover, their phenotypes (i.e. significant proportion of early differentiated T cells and few exhausted cells) may potentially provide long-term antitumor effects in patients.

Given the refractory and relapsing nature of AML and MM, the high levels of CD44v6 surface expression on tumor cells and the results of preclinical studies, the aim of the present study is to take CAR T-cell therapy one step further by investigating the treatment of these diseases with CD44v6 CAR T-cells (MLM-CAR44.1 T-cells). The purpose of this first-in-man, Phase I-IIa study is to evaluate the safety and antitumor activity of MLM-CAR44.1 T-cells in patients with AML and MM.

Considering the "*first in human"* nature of this clinical study, the Bayesian Optimal Interval Design (BOIN) (*Liu and Yuan, 2015*) has been chosen to minimize any risks of exposure to the novel MLM-CAR44.1 T-cells during dose escalation. The BOIN design is a novel Bayesian dose-finding method that minimizes the decision errors of dose assignment (for example the chance of exposing patients to overly toxic doses) and thus ensures patient ethics. This design has performance superior to the traditional 3+3 design (ref Liu and Yuan, 2015), but shares with it the fact that it can be easily implemented, differently from the well-known continual reassessment method (CRM), the gold standard of Phase 1 dose finding. The BOIN design yields an average comparable performance compared to CRM in selecting the MTD but has a lower risk of assigning patients to sub-therapeutic or overly toxic doses.

1.4.2 Possible risks of CAR T-cell Therapy

The adverse effects of CAR T-cell therapy can vary from mild constitutional syndrome (flu-like syndrome, malaise, fever, etc.) to life threatening situations requiring intensive care. Toxicities can be summarized as follows (*Hartman et al., 2017*):

Cytokine-release syndrome (*CRS*): A systemic inflammatory response resulting in non-infectious fever with elevated levels of inflammatory cytokines such as interleukin-6 and interferon-γ.

On-target/off-tumor toxicity: Adverse effects caused by the killing of healthy tissue by CAR T-cells due to target antigen expression outside tumor tissue.

Off-target toxicity: Side effects in CAR T-cell-treated patients due to cross-reactivity of the engineered antigen binding domain with a non-related surface protein.

Neurotoxicity: Presence of neurocognitive deficits.

Allergic reactions and Tumor Lysis Syndrome (TLS), the latter a group of metabolic complications that can occur due to the breakdown of dying cells.

Infectious diseases caused by opportunistic agents in immune-compromised patients after administration of chemotherapy followed by CAR T-cells.

Insertional oncogenesis, transformation of a T-cell clone and clonal expansion.

1.4.2.1 Cytokine release syndrome

Cytokine release syndrome (CRS) is a novel critical illness syndrome characterized by a series of inflammatory symptoms resulting from cytokine elevations associated with T-cell engagement and proliferation. In most patients, CRS symptoms are mild and flulike, with fever and myalgia. However, some patients have experienced a severe inflammatory syndrome that includes vascular leakage, hypotension, pulmonary edema, and coagulopathy, resulting in multiorgan system failure and in death, the latter related to treatment with blinatumomab. In the study by Maude et al., severe cytokine-release started a median of one day after infusion, whereas non-severe CRS started 4 days later (Maude et al., 2014).

A widely referenced mechanism for grading CRS due to T-cell therapies is the one proposed by Neelapu et al. Grade 1 symptoms include fever and Grade 1 organ toxicity and require only symptomatic management. Grade 2 symptoms respond to moderate intervention and include oxygen requirement <40%, grade 2 organ toxicity, or hypotension responding to IV fluids or low doses of one vasopressor (e.g., <20 μg/min of norepinephrine). Grade 3 CRS includes an oxygen requirement ≥40%, hypotension requiring high-dose or multiple vasopressors, grade 3 or 4 transaminitis, and grade 3 organ toxicity at other sites. Grade 4 CRS is defined as the presence of life-threatening symptoms requiring ventilator support or grade 4 organ toxicity other than transaminitis (*Neelapu et al., 2017*). Grade 4 CRS will be considered a Dose Limiting Toxicity (DLT) for the definition of Maximum Tolerated Dose (MTD) in Phase I of the present study. The same applies to Grade 3 or higher CRS not responsive to therapy with steroids and or tocilizumab/siltuximab within 24 hours.

A strategy for managing CRS is to target cytokines directly. The cytokines most significantly elevated in CRS associated with CD19 CAR T-cells and blinatumomab were IL-10, IL-6, and IFN-γ. IL-10, primarily produced by monocytes/macrophages, regulates both innate and cell-mediated immunity by inhibiting activated macrophages. IL-10 can also be produced by mast cells, B cells, regulatory T cells, and helper T cells (T_H2), but not commonly by cytotoxic T lymphocytes. As a negative regulator, IL-10 may not be an ideal cytokine to target in CRS. Conversely, IL-6 is an inflammatory cytokine involved in many processes within the immune system, such as neutrophil trafficking, acute phase response, angiogenesis, B-cell differentiation, and autoantibody production. IL-6 is produced by monocytes/macrophages, dendritic cells, T cells, fibroblasts, keratinocytes, endothelial cells, adipocytes, myocytes, mesangial cells, and osteoblasts. Interferon-γ is also an inflammatory cytokine and is involved in macrophage activation, T_H1 differentiation, major histocompatibility complex class 1 induction, and B-cell isotype switching. Interferon-γ is produced by cytotoxic T cells, helper T cells (T_H1), and natural killer cells. As an effector cytokine released by activated cytotoxic T cells after engagement, IFN-γ elevations are both anticipated and likely required for efficacy, making it a potentially undesirable target for toxicity management (*Maude et al., 2014*).

Based on these considerations, targeting IL-6 represents an effective strategy for reducing CRS toxicity without compromising the clinical efficacy of T-cell therapies. IL-6 blockade by tocilizumab determined rapid, dramatic reversal of life-threatening CRS in patients treated with CD19 CAR T-cells or blinatumomab (*Grupp et al., 2013; Maude et al., 2014*). In patients who responded to tocilizumab, fever and hypotension often resolved within a few hours, and vasopressors and other supportive care measures were weaned quickly thereafter. In some cases, symptoms may however not completely resolve, and continued aggressive support may be necessary for several days, along with the administration of a second dose of tocilizumab and/or a second immunosuppressive agent such as corticosteroids (*Lee et al., 2014*).

Tocilizumab is a recombinant humanized monoclonal antibody that blocks IL-6 from binding to its receptor and is approved in the EU for the treatment of moderate to severe active rheumatoid arthritis in adult patients and for the treatment of juvenile idiopathic arthritis in children as young as 2 years. On 30 August 2017, the FDA granted Priority Review and Orphan Drug Designation to tocilizumab intravenous injection for the treatment of CAR T cell-induced severe or life-threatening CRS in patients two years of age and older. The approval was based on a retrospective analysis of pooled outcome data from clinical trials of CAR T-cell therapies for blood cancers (*Grupp et al., 2016*). The study population included 45 pediatric and adult patients treated with tocilizumab, with or without additional high-dose corticosteroids, for severe or life-threatening CRS. Thirty-one patients (69%; 95% CI: 53%–82%) achieved a response, defined as resolution of CRS within 14 days of the first dose of tocilizumab, no more than two doses of tocilizumab were needed, and no drugs other than tocilizumab and corticosteroids were used for treatment. No adverse reactions related to tocilizumab were reported. A second study confirmed resolution of CRS within 14 days using an independent cohort that included 15 patients with CAR T cell-induced CRS.

Grade 4 CRS or Grade 3 CRS not responsive to steroids and/or tocilizumab within 24 hours may set off suicide gene activation.

1.4.2.2 On-target/off tumor recognition

The ideal target antigen is restricted to the tumor cell. Unfortunately, most targets of CAR T-cells have shared expression on normal tissues and some degree of "on-target/off-tumor" toxicity occurs through engagement of target antigen on nonpathogenic tissues. One of the earliest trials utilizing a carboxyanhydrase-IX-specific CAR T-cell for renal cell carcinoma resulted in the development of cholestasis due to expression of carboxyanhydrase-IX on bile duct epithelium (*Lamers et al., 2013*). In the setting of CD19-specific CAR T-cells, the targeting of normal B cells results in B-cell aplasia, which may require intermittent infusion of pooled immunoglobulin as prophylaxis from infectious complications (*Bonifant et al., 2016*). A fatal example of "on-target/off-tumor" recognition is that of a patient treated with CAR T-cells specific for the cancer-associated antigen HER-2/neu. The patient developed rapid respiratory failure, multi-organ dysfunction, and subsequent death attributed to reactivity against pulmonary tissue expression of HER-2/neu (*Morgan et al., 2010*). However, this unforeseen toxicity was potentially provoked by the substantial dose of infused CAR T cells (1×10^{10} CAR T cells) as subsequent studies utilizing a different HER2/neu-specific CAR (without prior conditioning chemotherapy) have proven safe at significantly lower CAR T-cell doses (*Ahmed et al., 2015*).

CD44v6 has a low level of expression on normal cells, including activated T cells, monocytes, and keratinocytes. Preclinical studies have shown that on-target off-tumor toxicities associated with CD44v6 CAR T-cells are acceptable and/or reversible (see Section 1.3.1) Nevertheless, given the potential for off-tumor toxicity of CD44v6 CAR T-cells, patients will be closely monitored for skin toxicities and monocyte levels. Moreover, patients with multiple antibiotic resistant diseases will not be eligible for this study. A DLT for Phase I of this study is the presence of NCI-CTC grade 3 or higher erythroderma (generalized exfoliative dermatitis), Stevens-Johnson Syndrome, Toxic Epidermal Necrolysis (TEN), and any other grade 3 or higher skin toxicity suspected of being related to CAR T-cell treatment.

Based on the clinical judgment of the Investigator, and in the presence of the following off-tumor skin toxicities, ganciclovir may be administered to set off suicide gene activation:

- Grade 3 erythroderma not responsive to steroids (methylprednisolone 2mg/kg for 72 hours or rapidly evolving)
- Grade 2 or higher bullous dermatitis
- Any grade Stevens Johnson Syndrome
- Any evidence for Toxic Epidermal Necrolysis (TEN)

1.4.2.3 Off-target antigen recognition

Most CAR T-cells recognize antigen through scFv derived from monoclonal antibodies, some of which may have a proven safety record in clinical use while others may not. Organ damage could hypothetically occur when CAR T-cells cross-react with antigens expressed on normal tissue that are similar to the target antigen expressed by the malignancy. This toxicity has not been documented in clinical trials of CARs but has been observed in clinical trials of T cells genetically modified to express T-cell receptors (*Brudno et al., 2016*).

Predicting off-target antigen recognition and subsequent toxicities is difficult in first-in-man studies. To this end, patients will be diligently monitored to recognize any potential toxicity promptly.

1.4.2.4 Neurotoxicity

Neurologic toxicities including confusion, delirium, expressive aphasia, obtundation, myoclonus, and seizure have been reported in patients receiving CD19-specific CAR T-cells (*Maude et al., 2014, Davila et al., 2014, Lee et al., 2015*).

The pathophysiology of these neurologic side effects is unknown although it is plausible that elevated cytokine levels are partly responsible. Conversely, direct CAR T-cell toxicity on the central nervous system is possible but has not been demonstrated. Neurological events may occur at different times than CRS or in the absence of CRS toxicities (*Maude et al., 2014*), suggesting that neurologic toxicity might have a different mechanism than other toxicities such as fever and hypotension.

In most instances, neurologic events are self-limiting, and there are no definitive guidelines regarding best management of these events. It is unclear if tocilizumab has any beneficial effect. Because tocilizumab is a monoclonal antibody, its size makes efficient Blood-Brain Barrier (BBB) penetration unlikely. The smaller IL-6 molecule is known to cross the BBB and has been shown to cause neurologic defects. Saturation of IL-6 receptors following systemic tocilizumab

administration may increase serum IL-6 levels, theoretically leading to an increase in CSF IL-6 levels that might worsen neurologic toxicity. As for other groups *(Lee et al., 2014)*, the Transplantation and Immunology Branch of the US National Cancer Institute treats severe neurologic toxicities with systemic corticosteroids rather than tocilizumab as the first-line agent. (*Brudno et al., 2016*).

Grade 3 or higher neurotoxicity will be considered a DLT in this study and may trigger suicide gene activation.

1.4.2.5 Allergic reactions and Tumor Lysis Syndrome (TLS)

Allergic reactions to CAR T-cells have been reported. A patient with pleural mesothelioma received multiple infusions of autologous T cells transduced with an antimesothelin CAR. Although the patient tolerated the first two cell infusions well, he experienced anaphylaxis and cardiac arrest following completion of the third infusion, with dramatically elevated serum tryptase levels. The patient received cardiopulmonary resuscitation and recovered *(Maus et al., 2013)*.

Although chemotherapy may have caused TLS in some cases, the infusion of CAR T cells in the absence of prior conditioning chemotherapy has led to TLS *(Kochenderfer et al., 2013; Grupp et al., 2013)*.

Given the growing interest in CAR T-cell therapies, the CAR-T-cell therapy-associated Toxicity (CARTOX) Working Group (comprising investigators from multiple institutions and medical disciplines who have experience in treating patients with various CAR-T-cell therapy products) has published a series of recommendations for monitoring, grading, and managing the acute toxicities that can occur in patients treated with CAR T-cell therapy (*Neelapu et al, 2017*). These recommendations are aimed at reducing CAR T-cell associated mortality and morbidity by optimizing intensive monitoring, accurate grading, and prompt management of these toxicities with aggressive supportive care, anti-IL-6 therapy, and/or corticosteroids for severe cases.

1.4.2.6 Infectious diseases

Patients on CAR T-cell clinical trials frequently become neutropenic and lymphopenic after administration of chemotherapy followed by CAR T-cells, predisposing them to opportunistic infection. In this setting, the fevers, tachycardia, and hypotension associated with CRS can be difficult to differentiate from sepsis. In an early report, a patient with chronic lymphocytic leukemia who received chemotherapy and anti-CD19 CAR T-cells died with fever, hypotension, and renal failure. It was later found that this patient had elevated serum levels of inflammatory cytokines before CAR T-cell infusion, suggesting that the patient had a prior infection (*Brentjens et al., 2010*). Bacteremia, *Salmonella*, urinary tract infections, and viral infections such as influenza, respiratory syncytial virus, and herpes zoster virus, have also occurred following CAR T-cell infusion.

Given their predisposition to infections, patients with multiple antibiotic resistant diseases will not be eligible for this study.

Suicide gene activation may be considered in case of grade 3-4 potentially life-threatening infections.

1.4.2.7 Insertional oncogenesis

Insertion of a transgene into differentiated T cells carries the risk of induced malignant transformation. However, no adverse or toxic events related to the gene transfer procedure have been reported to date. Accordingly, no genotoxic effect of integrating vectors, nor clonal dominance of gene modified T-cells has been observed (*Recchia et al., 2006; Lupo Stanghellini et al., 2014*).

1.4.3 Suicide gene

As described above, CAR T-cell therapy may lead to several types of toxicity due to the inability to control T-cell activity and some tumor-associated antigens that are presented by both tumor and healthy tissue. A safe and efficient means of resolving these adverse effects is the incorporation of suicide genes into the CAR T-cells. With this approach, T cells are engineered to express signaling pathways that cause the T cell to destroy itself after a defined number of cell divisions.

Several "suicide-gene" strategies that allow selective destruction of administered T-cells on demand have been developed. A suicide gene codes for a protein able to convert, at a cellular level, a non-toxic prodrug into a toxic product. Expression of the herpes simplex virus thymidine kinase (HSV-TK) renders modified cells susceptible to treatment with the acyclic nucleoside analog ganciclovir (GCV). Once expressed, HSV-TK catalyzes phosphorylation of GCV resulting in competitive inhibition of guanosine incorporation with subsequent disruption of DNA polymerization and synthesis (*Greco et al., 2015*). The killing mechanism of the TK/GCV suicide gene is unique in that GCV kills only proliferating engineered cells, allowing for resting lymphocytes to be spared for subsequent therapy and immune reconstitution (*Oliveira et al., 2012*). GCV administration in most of the cases eliminates more than 90% of circulating TK cells (*Ciceri et al., 2009*). Of note, transduced T-cells can be easily tracked in patients thanks to the presence of the LNGFR spacer in the MLM-CAR44.1 T-cell, thus providing a unique tool to study the fate of the CAR T-cells *in vivo* (*Bonini et al., 2003*).

Since suicide gene therapy for allogenic HSCT has demonstrated the potential to safely balance graft versus tumor (GvT) and graft versus host disease (GvHD) (*Greco et al., 2015*), the implementation of a suicide gene in MLM-CAR44.1 T-cells should help to mitigate their risks while preserving their therapeutic effects (*Casucci et al., 2013*). Although CAR T-cells are in general well tolerated, their broader use requires having solid strategies to treat on-target, off-tumor effects and cytokine storms (*Hoyos et al., 2010*). Applying suicide gene modification to MLM-CAR44.1 T-cells may greatly increase their safety profile and facilitate their clinical development.

In conclusion, the risk to subjects in this trial should be minimized by eligibility criteria, pre-infusion criteria, a CRS treatment algorithm, study procedures and close clinical monitoring. Based on the high response rates and lasting remissions of CTL019 therapy, the potential benefit of treatment with MLM-CAR44.1 T-cells in the target patient population outweighs its potential risks.

2 OBJECTIVES

2.1 Phase I objectives

2.1.1 Primary objectives

1. To determine the maximum tolerated dose (MTD) and recommended Phase II dose of MLM-CAR44.1 T-cells in patients with relapsed/refractory acute myeloid leukemia (AML) or multiple myeloma (MM) expressing CD44v6.

   The MTD will be established through a BOIN study design and the following dose-limiting toxicities (DLT) occurring within 30 days following CAR T-cell infusion:

   a. Grade 4 CRS
   b. Grade 3 or higher CRS not responsive to therapy with steroids and/or tocilizumab/siltuximab within 24 hours
   c. NCI-CTC grade 3 or higher toxicity at least possibly related to treatment with MLM-CAR44.1 T-cells excluding hematological toxicities
   d. Skin toxicities:
      - NCI-CTC grade 3 or higher erythroderma (generalized exfoliative dermatitis)
      - Stevens-Johnson Syndrome
      - Toxic Epidermal Necrolysis (TEN)
      - Any other grade 3 or higher skin toxicity histologically confirmed as being related to CAR T-cell treatment
   e. Grade 3 or higher neurotoxicity

2. To evaluate the overall safety of treatment with MLM-CAR44.1 T-cells.

   Overall safety will be evaluated by analyzing the type, frequency and severity of adverse events (AE) and by monitoring for systemic reactions (fever, tachycardia, nausea and vomiting, joint pain, skin rash) for 30 days following CAR T-cell infusion.

3. The absence of replication-competent retrovirus (RCR) will be monitored by DNA PCR 3, 6, 12 and 24 months after infusion and then yearly as defined in the long-term follow-up.

2.1.2 Secondary objectives

1. To evaluate hematologic response to MLM-CAR44.1 T-cells in AML and MM.

   The endpoints for assessing hematologic response in Phase I are defined as follows:

   a. AML: complete remission (CR), incomplete response (CRi) and partial response (PR) according to European LeukemiaNet (ELN) criteria, 1 and 2 months following infusion.
   b. MM: overall response rate (ORR): stringent complete response (sCR), CR, very good partial response (VGPR) and partial response (PR) according to International Myeloma Working Group (IMWG) criteria, 1 and 3 months following infusion.

2. To characterize the in vivo pharmacokinetic profile (engraftment, persistence, trafficking) of MLM-CAR44.1 T-cells by evaluating levels of circulating MLM-CAR44.1 T-cells by flow cytometry.

3. To assess suicide gene activation and elimination of transduced cells through administration of ganciclovir in case of CRS and other MLM-CAR44.1 T-cell related toxicities, by evaluating levels of circulating MLM-CAR44.1 T-cells by flow cytometry.
4. To conduct an ancillary study including but not limited to the characterization of the cellular composition of circulating LNGFR+ (CAR T-cells) and LNGFR- cells, and the monitoring of the patient's immune status through analyses of peripheral blood mononuclear cells (PBMC).
5. To set-up a validation process among the participating centers for the CD44v6 marking of tumor cells (AML and MM).

2.2 Phase IIa objectives

2.2.1 Primary objective

To evaluate hematologic response to MLM-CAR44.1 T-cells at 2 and 3 months after infusion in AML and MM, respectively

The primary endpoint is disease response, defined as:

AML: CR, CRi and PR rates as per ELN criteria, 2 months after MLM-CAR44.1 T-cell infusion.

MM: ORR: (sCR, CR, VGPR and PR) as per IMWG criteria, 3 months after T-cell infusion.

2.2.2 Secondary objectives

1. To evaluate the antitumor activity of MLM-CAR44.1 T-cells

   The secondary endpoints for assessing antitumor activity are as follows:

   - Disease response.

     AML: CR, CRi and PR rates as per ELN criteria, 1 month and 6 months after MLM-CAR44.1 T-cell infusion. Morphologic leukemia-free state (MLFS) 1, 2 and 6 months after infusion.

     MM: ORR (sCR, CR, VGPR and PR) as per IMWG criteria, 1, 2 and 6 months after T-cell infusion.
   - Overall survival (OS) at 2 years: time from MLM-CAR44.1 T-cell infusion to death due to any cause.
   - Disease-free survival (DFR) in AML patients: time from achievement of response to relapse or death due to any cause during response.
   - Event Free Survival (EFS) in AML patients: time from the date of MLM-CAR44.1 T-cell infusion to the date of the earliest of the following: last follow-up, resistance, relapse or death due to any cause.
   - Progression-free survival (PFS) in MM patients at 2 years: time from MLM-CAR44.1 T-cell infusion to progression/relapse or death due to any cause.
   - Duration of response (DOR) in MM patients: time from achievement of response to relapse or death from any cause.

2. To characterize the in vivo pharmacokinetic profile (engraftment, persistence, trafficking) of MLM-CAR44.1 T-cells.
    - Endpoint: Levels of circulating MLM-CAR44.1 T-cells by flow cytometry.
3. To evaluate the feasibility of treatment with MLM-CAR44.1 T-cells

   The endpoints for assessing feasibility are as follows:
    - Percentage of eligible patients for each indication after screening who undergo both lymphocyte apheresis and CAR T-cell infusion.
    - Percentage of patients who are eligible for lymphocyte apheresis and are then effectively infused with CAR T-cells.
4. To evaluate the safety of MLM-CAR44.1 T-cells.

   The endpoints for assessing safety are as follow:
    - Adverse events (any grade) and monitoring for systemic reactions (fever, tachycardia, nausea and vomiting, joint pain, skin rash).
    - Absence of RCR monitored by DNA PCR for the Galv gene 3, 6, 12 and 24 months after infusion and then yearly as defined in the long-term follow-up.
    - Absence of abnormal MLM-CAR44.1 T-cell clonal expansion monitored by clinical and laboratory surveillance, as well as by TCR-Vbeta repertoire study 6 months after infusion.
    - Suicide gene activation and elimination of transduced cells through administration of ganciclovir in case of CRS and other MLM-CAR44.1 T-cell related toxicities.
5. To conduct an ancillary study including but not limited to the characterization of the cellular composition of circulating LNGFR+ (CAR T-cells) and LNGFR- cells, and the monitoring of the patient's immune status through analyses of peripheral blood mononuclear cells (PBMC).

2.2.3 Exploratory objectives

To evaluate minimal residual disease (MRD):

- AML: proportion of patients with a molecular CR, 2 months after infusion.
- MM: proportion of patients with a molecular CR, 3 months after infusion.

3 TRIAL DESIGN

This is a seamless Phase I/IIa, open-label, multicenter clinical trial that combines Phase I dose escalation based on toxicity with Phase IIa dose expansion based on antitumor activity.

3.1 Phase I dose escalation

A Bayesian Optimal Interval (BOIN) design with cohorts of 3 patients for each indication (AML and MM) will be used to single out the MTD based on a target toxicity rate of 30%.

The BOIN design is a novel Bayesian dose-finding method that minimizes the decision errors of dose assignment (for example the chance of exposing patients to overly toxic doses) and thus safeguards patient ethics and safety by allowing decisions to be made at any time during the study, even when one of the patients in the cohort is not evaluable. This design has performance superior to the traditional 3 + 3 design, but shares with it the fact that it can be easily implemented, differently from the well-known continual reassessment method (CRM), the gold standard design of phase I dose finding. The BOIN design yields an average performance comparable with CRM in selecting the MTD but has a lower risk of assigning patients to sub therapeutic or overly toxic doses.

According to this design, the dose assignment for patients who will sequentially enter the study is pre-specified and guided by the observed probability of dose-limiting toxicity (i.e. the number of patients experiencing DLT at the current dose divided by the total number of patients treated at the current dose). The decision boundaries identified by the method, which are optimal in the sense that they minimize the chance of incorrect decisions in dose assignments, are 24.7% and 34.8% (given a tolerance toxicity interval of 20-40%) and the trial will use them as follows:

- dose escalation, if the observed toxicity rate is smaller than the lower boundary of 24.7% (E in Table 3-1),
- dose de-escalation, if the observed toxicity rate is greater than the upper boundary of 34.8% (D in Table 3-1)),
- stay at the current dose, if the observed toxicity rate is between 24.7 and 34.8% (S in Table 3-1)).

A Bayesian safety rule will also be applied to eliminate from the trial, and to consider not admissible for MTD selection, the doses that are overly toxic (DU in Table 3.1)). This might happen when the risk that the toxicity exceeds the target 30% is very high (>95%), given the current data (i.e. Pr(toxicity rate>30%|current data)>95%), based on a beta posterior distribution.

The first AML cohort and first MM cohort will be made up of adult patients and will be treated with the lowest dose of autologous MLM-CAR44.1 T-cells (0.5 x 10E6/Kg).

The first three enrolled patients will be treated **regardless of indication** at lowest dose of 0.5 x 10E6/Kg one at a time at intervals of at least 36 days to evaluate the occurrence of any toxicity, to save the rights, safety, and well-being of the trial patients in this fist-in-human trial setting. The second patient will undergo lymphodepleting chemotherapy (from day -5 to day -3 prior to CAR T-cell infusion), only once the first patient has completed the 30-day follow-up period after CAR T-cell infusion (30 + 5 days). The third patient will undergo lymphodepleting

chemotherapy only once the second one has completed the 30-day follow-up after CAR T-cell infusion.

The remaining patients to complete the first cohort for each indication will be treated without staggering once the first three enrolled patients have completed follow-up and the dose has proven to present a manageable safety profile.

After all patients of the first AML cohort or first MM cohort (according to BOIN Design) have completed the 30-day safety monitoring period, the decision on whether to escalate to the next dose will be made together with a Data and Safety Monitoring Board (DSMB) based on the rate of dose-limiting toxicities (DLT) and the decision rules presented in Table 3-1.

The first three patients of the second dose level (1 x 10E6/kg) will be treated with staggering regardless of indication only if both indications (AML and MM) have completed the first cohort dose, otherwise only the indication that has completed the first cohort dose can be treated. The remaining patients to complete the second cohort for each indication will be treated without staggering once the first three enrolled patients have completed follow-up and the dose has proven to present a manageable safety profile.

Then, as outlined for the previous cohort level, the first three patients of the third dose level (2 x 10E6/kg) will be treated with staggering regardless of indication only if both indications (AML and MM) have completed the second cohort dose, otherwise only the indication that has completed the second cohort dose can be treated. The remaining patients to complete the third cohort for each indication will be treated without staggering once the first three enrolled patients have completed follow-up and the dose has proven to present a manageable safety profile.

The second and then third dose will be administered to cohorts of new patients with an escalation process that continues until the MTD or the maximum sample size of 15 patients for each indication has been reached. The duration of the evaluation period for each patient (30 days) is based on previous studies with similar cellular products, in which severe CRS (main DLT for the study) was observed for up to 7-15 days after infusion.

0.5 x 10E6/Kg, 1 x 10E6/Kg and 2 x 10E6/Kg are the three doses of autologous MLM-CAR44.1 T-cells for this study. The recommended Phase IIa dose corresponds to the MTD. If no DLT is reported, the dose will be the maximum dose investigated (2 x 10E6/kg).

TABLE 3-1 TRANSITION RULES FOR EACH DOSE

Number of patients with at least one DLT	Cumulative Number of Patients: 3	6	9	12	15
0	E	E	E	E	E
1	S	E	E	E	E
2	D	S	E	E	E
3	DU	D	S	S	E
4		DU	D	S	S
5		DU	DU	D	S
6		DU	DU	D	D
7			DU	DU	D
8			DU	DU	DU
9			DU	DU	DU
10				DU	DU
11				DU	DU
12				DU	DU
13					DU
14					DU
15					DU

E: Escalate to the next higher dose; **S**: Stay at the same dose; **D**: De-escalate to the previous lower dose;
DU: De-escalate to the previous lower dose and the current dose will never be used again in the trial (safety rule).

3.2 Phase IIa dose expansion

The activity of the MTD will be assessed on 14 patients of each indication (AML and MM separately) using Simon's two-stage design. These cohorts will include the patients enrolled in Phase I treated with the MTD, and in the case of AML, also children.

In the first stage, the hematologic response to treatment of the first 6 patients with each indication will be evaluated, and if no response is observed the study will be terminated. Otherwise, the study will proceed to stage two and the remaining 8 subjects will be enrolled.

Transition from Phase I to Phase II will be managed through the submission of a substantial amendment to the protocol, which will be evaluated in consultancy with the Data Safety Monitoring Board (DSMB).

3.3 Follow-up

After being infused with MLM-CAR44.1 T-cells, patients will be monitored closely in the hematology or pediatric ward for at least the first 15 days. Follow up will be twice weekly through Month 1, weekly for the second month, every two weeks for the third month, monthly until Month 6 and then every 3 months until Month 24 to evaluate the safety, antitumor activity and feasibility of treatment with MLM-CAR44.1 T-cells.

3.4 End of study

The end of study is defined as the last patient's last visit (LPLV) planned at Month 24 (end of primary follow-up), or the time of premature withdrawal. Patients who discontinue primary follow-up before month 24 will nevertheless continue to be monitored through a secondary follow-up for up to 2 years after MLM-CAR44.1 T-cell infusion to collect data required by health authorities (e.g. delayed adverse events). Discontinuation of primary follow-up may be

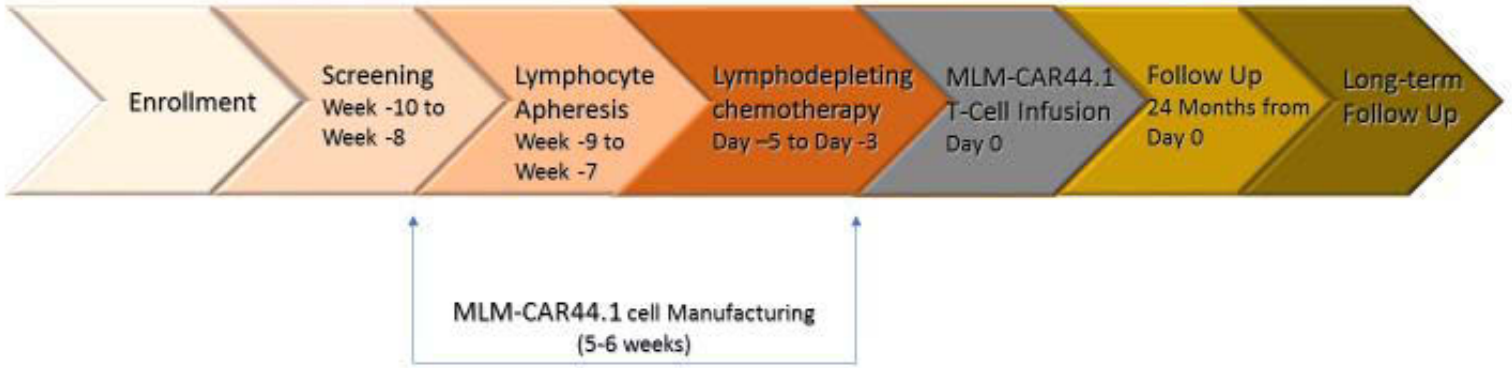
due to progression of disease, treatment failure, relapse after remission, pursuing HSCT while in remission, or withdrawal from follow-up.

Furthermore, evaluations will be performed for up to 15 years (Long-term follow-up) on all patients as recommended by health authority guidance for patients treated with gene therapies. Separate informed consent/assent forms will be provided for all patients who either complete or prematurely discontinue from the study and choose to enroll into the long-term follow-up protocol.

The study can be terminated at any time for any reason by the sponsor or if any of the stopping criteria described in Section 5.12 are met. Additional procedures may need to be followed to safeguard patient safety.

The overall study design is shown in Figure 1

FIGURE 1 EURE-CART-1 STUDY DESIGN



4 PATIENT SELECTION CRITERIA

4.1 Target population

The study population is made up of patients with relapsed/refractory AML or MM expressing CD44v6.

The trial will include pediatric patients with AML (<18 years old) only in Phase IIa, with the exception of the center in the Czech Republic, and if all the following criteria have been met by adult patients in Phase I:

1. No SAEs related to lymphocyte apheresis.
2. No immediate SAEs related to MLM-CAR44.1 T-cell infusion (within 48 hours of infusion).
3. No early SAEs related to infusion (within 30 days).
4. Evidence of MLM-CAR44.1 T-cell engraftment.

Should one or more of these criteria not be met, the DSMB (including a pediatrician) will evaluate the risk/possible benefit profile of study treatment and provide suggestions on whether to include pediatric patients.

The protocol will be amended to classify pediatric patients according to age groups

4.2 Inclusion criteria

Patients must meet all the following inclusion criteria to be eligible for the study.

1. Written informed consent before any study-related procedure.
2. Adults and children:
   a) Adults 18 to 75 years old with AML or MM or 18 to 63 years old for CZ site.
   b) Children 1 to 17 years old with AML, only in Phase IIa, except for CZ site.
3. Confirmed diagnosis of AML or MM as follows:
   a) **AML**: Primary or secondary AML (any subtype except acute promyelocytic leukemia) according to World Health Organization (WHO) classification.
   b) **MM** with measurable disease defined at least one of the following:
      - Serum M protein ≥ 5g/dL (≥ 5 g/L)
      - Urine M protein ≥ 100 mh/24 hours
      - abnormal serum free light chain (sFLC) assay: FLC ratio <0.26 or >1.65 and/or involved Serum free light chain >=100 mg/L
4. Patients with relapse or refractory disease:
   a) **AML** patients must be unlikely to benefit from cytotoxic chemotherapy as follows:
      - Leukemia refractory to at least 2 induction attempts (use of a hypomethylating agent for at least 4 cycles can be considered a line of treatment).

- Leukemia in relapse within 1 year following CR after at least 2 induction attempts (use of a hypomethylating agent for at least 4 cycles can be considered a first line of treatment).
- High-risk leukemia in adults according to 2017 ELN (Appendix 9) in first relapse after a hypomethylating agent or a cycle containing cytarabine at a dose ≥1g/sqm a day (e.g. FLAG-IDA), except for FLT3-mutated AML.
- High-risk leukemia in children as defined by the Italian Association of Pediatric Hematology and Oncology (AIEOP).

b) Patients with **MM** must have a relapse or refractory disease after at least 4 different prior treatments in 3 treatment lines, or 4 treatments in 2 treatment lines in case of early relapsing patients (relapse in less than 1.5 years). Treatments include:

- Proteasome inhibitor
- High-dose alkylating agent if patients less than 70 years old
- Immunomodulatory drug (IMID)
- A monoclonal antibody (i.e. anti CD38 monoclonal antibody)

5. Positive CD44v6 expression on tumor cells by flow cytometry ≥ 20%.
6. Eastern Cooperative Oncology Group (ECOG) performance status 0-2.
7. Life expectancy of at least 12 weeks.
8. Adequate organ function:
   a) Alanine aminotransferase (ALT) level within 2.5 times the institutional upper limit of normal (ULN).
   b) aspartate aminotransferase (AST) level ≤2.5 x ULN.
   c) Total bilirubin level ≤1.5 x ULN, or ≤2.5 x ULN in case of history of Gilbert's disease.
   d) Serum creatinine ≤ 2.0 mg/dL or a calculated or measured creatinine clearance ≥ 45mL/min.
   e) Corrected Diffusing Capacity of Carbon Monoxide (DLCO) (via Dinakara Equation) or forced expiratory volume in one second (FEV1) of ≥ 66% without dyspnea on slight activity after hemoglobin correction.
   f) Left ventricular ejection fraction >45%.
9. Recovery from toxicities of clinical consequence attributed to previous chemotherapy to CTCAE v5.0 Grade 1 (i.e., certain toxicities such as alopecia will not be considered in this category).
10. Ability to comply with study procedures, including hospitalization and protocol-specified acquisition of blood and/or bone marrow specimens.
11. Willing to be followed up long term, i.e. a 15-year follow up as required by health authorities for cell and gene therapy products.
12. Women of childbearing potential must test negative for pregnancy at enrolment and during the study and agree to use an effective method of contraception until 1 year after CAR T-

cell infusion. Male patients with partners of childbearing potential must be either vasectomized or agree to use a condom in addition to having their partners use another method of contraception resulting in a highly effective method of birth control. See Section 6. 7 for details.

4.3 Exclusion criteria

4.3.1 At screening

Patients must meet none of the following exclusion criteria to be eligible for the study:

1. History of or candidate for allogeneic stem cell transplantation.
2. Cardiovascular, pulmonary, renal, and hepatic functions that in the judgment of the investigator are insufficient for the patient to undergo investigational CAR T-cell therapy.
3. Any history of or suspected current autoimmune disorders (apart from vitiligo, resolved childhood atopic dermatitis, Graves' disease clinically controlled).
4. History of rheumatologic disorders requiring specific treatment at any time in the patient's medical history.
5. Second primary malignancy that requires active therapy. Adjuvant hormonal therapy is allowed.
6. Known or suspected central nervous system (CNS) leukemia.
7. Presence or history of myeloid sarcoma or any extramedullary mass.
8. Any medical or psychiatric condition that may limit compliance or increase safety risks, such as:
   a) Active uncontrolled infection (including, but not limited to viral, bacterial, fungal, or mycobacterial infection).
   b) Patients with known multiple antibiotic resistant infections in their clinical history.
   c) Known human immunodeficiency virus infection, active or chronic hepatitis B or C infection.
   d) Grade 3 or 4 bleeding.
   e) Uncontrolled hypertension (systolic pressure >180 mm Hg or diastolic pressure >100 mm Hg).
   f) Clinically significant arrhythmia, clinically significant baseline QTcF, or QTcF > 480 msec.
   g) Unstable angina.
   h) Myocardial infarction within 6 months prior to study entry.
   i) Clinically significant heart disease (e.g. CHF NYHA III or IV, unstable coronary artery disease, myocardial infarction < 6 months prior to study entry).
   j) Pregnancy or breastfeeding.

k) Major surgery or trauma within 4 weeks before enrollment.

l) Dementia or altered mental status that would preclude sufficient understanding to provide informed consent.

Once all other eligibility criteria are confirmed, a lymphocyte apheresis product of non-mobilized cells must be received and accepted by the manufacturing site. Note: the lymphocyte apheresis product will not be shipped or assessed for acceptance by the manufacturing site until documented confirmation of all other eligibility criteria has been received

4.4 Prior to lymphocyte apheresis (week -9 to -7).

The following criteria must be met:

1. Peripheral blast count ≤ 20,000/mm^3 (AML).
2. No treatment with any other investigational agent in the previous 4 weeks.
3. No treatment with an immunostimulatory agent (IMIDs are allowed) or any cell therapy in the previous 30 days.
4. Negative to the following tests: HCV (Antibody, NAT), HIV 1-2 (p24, AB, Ag and NAT), total Ig Treponema Pallidum (if positive perform specific test), Australia HBsAg, total anti HB core Ab (if positive perform HBV DNA NAT), mycoplasma (PCR or IgM) and HTLV I-II.
5. No treatment with steroids at least 3 days before lymphocyte apheresis

4.5 Prior to lymphodepleting chemotherapy/ MLM-CAR44.1 T-cell infusion (day -5 to day -3)

The following criteria must be met:

1. Evidence of active disease at the beginning of lympho-depleting chemotherapy
2. The following medications are excluded and should not be administered:
   a. Monoclonal antibodies in the 8 weeks prior to MLM-CAR44.1 T-cell infusion are prohibited.
   b. Salvage chemotherapy is allowed (e.g. clofarabine, cytosine arabinoside >100 mg/m^2, anthracyclines, cyclophosphamide, proteasome inhibitors, IMIDs) but must be stopped >2 weeks prior to lymphodepleting chemotherapy
   c. Granulocyte colony stimulating or granulocyte-macrophage colony stimulating factor in the 2 weeks prior to study CAR T-cell administration.
   d. Immunosuppressant medications in the 2 weeks prior to CAR T-cell administration.
   e. Cytosine arabinoside < 100 mg/m^2/day must be stopped > 1 week prior to MLM-CAR44.1 T-cell infusion.
   f. Therapeutic systemic doses of steroids must be stopped >72 hours prior to product infusion. However, < 12 mg/m^2/day hydrocortisone or equivalent are allowed as physiological replacement doses of steroids.
   g. Hydroxyurea must be stopped >72 hours prior to MLM-CAR44.1 T-cell infusion.

3. No cardiovascular, pulmonary, renal, and hepatic functions that in the judgment of the investigator are insufficient for the patient to undergo investigational CAR T-cell therapy.
4. Female patients of childbearing potential must have a negative pregnancy test within 24 hours prior to starting lymphodepleting therapy.

5 EXPERIMENTAL PRODUCT

5.1 Study drug information

The medicinal product consists of autologous T-cells (MLM-CAR44.1 T-cells) genetically modified by retroviral vector LTK-S44v6ΔNL to express the CD44v6 CAR and HSV-TK Mut2 genes. The drug product is defined as the frozen MLM-CAR44.1 T-cell suspension formulated in frozen medium at the concentration of 1.0-10 x 10E6 cells/mL and ready for intended medical use. The product is patient specific as it is prepared starting from lymphocytes of the patient collected through lymphocyte apheresis. See Section 1.2 for details about drug description.

5.2 Study drug supply

The cryopreserved MLM-CAR44.1 T-cell product will be shipped to the trial center from the manufacturing site. Upon receipt, the T-cell product must be received by designated personnel at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designated site personnel have access. Any damaged or unusable cell product must be documented and notified to the study Sponsor.

5.3 Study drug accountability, disposal and destruction

Traceability between the patient's autologous apheresis and the MLM-CAR44.1 T-cell product will be compliant with Regulation (EC) 1394/2007, Directive 2004/23/EC (including all related directives) as well as EU "Detailed guidelines on good clinical practice specific to advanced therapy medicinal products." The data contributing to the full traceability of the cells are stored for a minimum of 30 years. A unique patient identifier will maintain a chain of identity between the apheresis product and the MLM-CAR44.1 T-cell batch, and the link between patient identity and unique patient identifier will be confirmed prior to infusion.

The investigator or designee must maintain an accurate record of the shipment and dispensing of the MLM-CAR44.1 T-cell product in a drug accountability log. Drug accountability will be reviewed by the monitor during site visits and at the completion of the study.

The investigator will dispose of used and unused MLM-CAR44.1 T-cell product, packaging, product labels as per local institutional standard operating procedures, and return a copy of the completed drug accountability log to the study monitor.

See the *MLM-CAR44.1 Study Manual* for details.

5.4 Formulation, packaging, labelling and storage

Each infusion bag will contain 10-50 mL of cells containing a cell dose of 0.5 or 1 or 2 x 10E6/Kg of autologous CD44v6 CAR T-cells. Each infusion bag will be labelled according to GMP for advanced therapy and GCP guidelines. Labels will include at least the following information: protocol name, the Principal Investigator's name, batch number, patient code, storage conditions, manufacturing date, total number of cells, total volume, concentration, route of administration, Sponsor's name, "For clinical trial use only" and the expiry date. Text might be in different order on the labels and will be adapted to the country specific local requirements. Additional label elements required by local regulations will also be included. Prior to the

infusion, two individuals will confirm identity according to local institutional guidelines to ensure that the patient receives only their autologous product.

The investigational product must be stored safely and properly. The cryopreserved cell product should be kept in the vapor phase of liquid nitrogen until infusion. The product should be thawed near the patient's bedside so the time between thawing and completion of the infusion does not exceed 60 minutes, thus maintaining maximum product viability. After thawing, the MLM-CAR44.1 T-cell product should NOT be washed prior to infusion. If the product bag appears to be damaged or is leaking, the product should not be infused and should be disposed of according to local institutional procedures.

See the *MLM-CAR44.1 Study Manual* for details.

5.5 Dosage schedule

Patients will receive a single dose of MLM-CAR44.1 T-cells: 0.5 x 10E6/Kg or 1 x 10E6/Kg or 2 x 10E6/Kg according to the BOIN design in Phase I, and a dose corresponding to the MTD in Phase IIa.

5.5.1 Dose modification

Dose escalation or de-escalation will take place during Phase I following the BOIN design outlined in Section 3 and considering the following dose limiting toxicities.

- Grade 4 CRS
- Grade 3 or higher CRS not responsive to therapy with steroids and/or tocilizumab/siltuximab within 24 hours
- NCI-CTC grade 3 or higher toxicity at least possibly related to treatment with MLM-CAR44.1 T-cells excluding hematological toxicities
- Skin toxicities:
  - NCI-CTC grade 3 or higher erythroderma (generalized exfoliative dermatitis)
  - Stevens-Johnson syndrome
  - Toxic Epidermal Necrolysis (TEN)
  - Any other grade 3 or higher skin toxicity histologically confirmed as being related to CAR T-cell treatment
- Grade 3 or higher neurotoxicity

Dose modifications are not applicable in Phase IIa.

5.5.2 Treatment duration

The MLM-CAR44.1 T-cell product will be administered as a single intravenous infusion.

5.6 Lymphocyte apheresis

The first step in manufacturing CAR T-cells is to collect autologous lymphocytes by apheresis. Patients will undergo non-stimulated lymphocyte apheresis at least 7 weeks prior to MLM-CAR44.1 T-cell infusion to allow cell production, quality and release controls, and completion of lymphodepleting chemotherapy. All efforts will be made to perform CAR T-cell manufacturing within 5-6 weeks after lymphocyte apheresis.

Apheresis should be scheduled prior to the administration of chemotherapy, immunomodulators, or steroids to ensure an adequate absolute lymphocyte count (ALC). Prior treatment with an immunostimulatory agent (IMIDs are allowed) or any cell therapy in the 30 days prior to lymphocyte apheresis is prohibited. Patients may be required to undergo multiple apheresis procedures to collect a target of 7 × 10E8 CD3+ cells or a minimum of 5 x 10E8, or, when it is not possible, approximately 10 x 10E9 WBC, for the preparation of the investigational product. AML patients should however present a peripheral blast count ≤20,000/mm^3 at time of lymphocyte apheresis.

The lymphocyte apheresis product will not be shipped or assessed for acceptance by the manufacturing site until documented confirmation of eligibility criteria has been received. See section 4.4. Full details of apheresis requirements, as well as of the procedures for procurement, handling and shipment of the apheresis product are present in the *MLM-CAR44.1 Study Manual*.

In most patients undergoing CAR T-cell therapy, lymphocyte apheresis is well tolerated, and adequate numbers of lymphocytes are collected (*Allen et al., 2017*). Some patients may however experience adverse effects including fatigue, nausea, dizziness, feeling cold, and tingling in the fingers and around the mouth. More serious complications, including abnormal heart rate and seizures, can occur but are extremely rare. A retrospective analysis of 15,763 apheresis procedures found only 59 moderate-to-severe adverse events (AEs) (0.37%), including dizziness or fainting episodes (0.12%), citrate toxicity (0.02%), a combination of dizziness/fainting and citrate toxicity (0.11%), vascular injuries (0.07%), and miscellaneous events (0.04%) (*Yuan et al., 2010*).

5.7 Salvage chemotherapy

Patient can undergo a salvage chemotherapy (e.g. clofarabine, cytosine arabinoside > 100 mg/m^2, anthracyclines, cyclophosphamide, proteasome inhibitors, IMIDs) after lymphocyte apheresis and prior to lymphodepleting chemotherapy.

Salvage chemotherapy must be stopped > 2 weeks prior to lymphodepleting chemotherapy, so it should last at most 4 weeks.

It is recommended to communicate to Sponsor the salvage chemotherapy intended to be administered.

5.8 Lymphodepleting chemotherapy

Lymphodepleting chemotherapy prior to T-cell infusion has been shown to favor T-cell engraftment and proliferation. This preconditioning creates space for the expansion of infused cells, limits the competition for homeostatic gamma chain cytokines IL-7 and IL-15, depletes regulatory T cells, and activates the innate immune system.

Patients must meet the eligibility criteria for lymphodepleting chemotherapy stated in Section 4.5. Should this not occur, the Investigator is to delay lymphodepleting chemotherapy for up to 60 days until the patient's conditions are deemed suitable for treatment. Any delay of more than 1 week must be discussed with the Sponsor in order to evaluate how to proceed with the treatment of the next patient, who has to be infused at interval of 36 days from the previous one.

The Investigator must inform the Sponsor of the start date of lymphodepleting chemotherapy to confirm the delivery date of the MLM-CAR44.1 T-cells.

The following criteria must be met:

a. Evidence of active disease at the beginning of lympho-depleting chemotherapy.
b. Salvage chemotherapy (e.g. clofarabine, cytosine arabinoside > 100 mg/m^2, anthracyclines, cyclophosphamide, proteasome inhibitors, IMIDs) must have been stopped > 2 weeks prior to lymphodepleting chemotherapy.
c. Female patients of childbearing potential must have a negative pregnancy test within 24 hours prior to starting lymphodepleting therapy.
d. From October through May, patients should undergo a rapid influenza diagnostic test within 10 days prior to lymphodepleting chemotherapy. If the patient is positive, oseltamivir phosphate or zanamivir should be administered for 10 days as preventative treatment and the test repeated prior to lymphodepleting chemotherapy. If flu-like symptoms are present, lymphodepleting chemotherapy should be delayed until the patient is asymptomatic.

Patients will undergo planned lymphodepleting chemotherapy with: cyclophosphamide i.v. (500 mg/m^2) and fludarabine i.v. (30 mg/m^2) daily from day -5 to day -3.

NOTE: Patients should be closely monitored for tumor lysis syndrome before and after lymphodepleting chemotherapy through blood tests for potassium and uric acid (see section 11.6.1.5). Patients with elevated uric acid or high tumor burden should receive prophylactic allopurinol, or a non-allopurinol alternative

5.9 Treatment with MLM-CAR44.1 T-cells

The MLM-CAR44.1 T-cell product will be released by the manufacturing facility and shipped to the study site approximately 4 weeks after the start of manufacturing. Details of the storage and handling requirements for the cryopreserved MLM-CAR44.1 T-cell product are available in the *MLM-CAR44.1 Study Manual*.

The following should be considered prior to MLM-CAR44.1 T-cell infusion:

1. Patient should not present a significant worsening of clinical status or laboratory abnormalities that would, in the opinion of the Investigator, increase the risk of adverse events associated with T-cell treatment or compromise the likelihood of completing the study protocol. MLM-CAR44.1 T-cell infusion should be delayed until clinically appropriate for up to 10 days.

   Patients with significant progression of disease following lymphodepleting chemotherapy are at high risk of severe CRS and must not receive T-cell infusion.
2. T-cell infusion should be delayed for up to 10 days in case of significant unresolved toxicities from preceding lymphodepleting chemotherapy, especially toxicities requiring supplemental oxygen, uncontrolled cardiac arrhythmia, hypotension requiring vasopressor support and uncontrolled active infection.

3. The following medications must be stopped prior to MLM-CAR44 T-cell infusion at the following times:
    a. Monoclonal antibodies: 8 weeks before.
    b. Granulocyte colony stimulating or granulocyte-macrophage colony stimulating factor and immunosuppressant medications: 2 weeks before.
    c. Cytosine arabinoside < 100 mg/m^2/day: 1 week before.
    d. Immunosuppressant medications in the 2 weeks prior to CAR T-cell administration
    e. Therapeutic systemic doses of steroids must be stopped > 72 hours prior to product infusion. However, < 12 mg/m^2/day hydrocortisone or equivalent are allowed as physiological replacement doses
    f. Hydroxyurea should be stopped > 72 hours prior to MLM-CAR44.1 T-cell infusion
    g. Ganciclovir/valganciclovir should be stopped at least 48 hours prior to MLM-CAR44.1 T-cell infusion

5.9.1 Premedication

Systemic corticosteroids other than physiologic replacement of hydrocortisone should not be administered except in the case of life-threatening emergency as this may negatively affect MLM-CAR44.1 T-cell expansion and function. Patients should be pre-medicated with paracetamol and an H1 antihistamine for infusion side effects such as fever, chills and nausea.

5.9.2 MLM-CAR44.1 T-cell infusion

Detailed instructions for the preparation and administration of the MLM-CAR44.1 T-cell product are available in the *MLM-CAR44.1 Study Manual*.

The CAR T-cell product should not be washed after cell thawing, and the infusion should be completed within 60 minutes of thawing to maintain cell viability.

Patients will receive one dose of MLM-CAR44.1 T-cells during a single intravenous infusion, preferably through a central line and latex free i.v. tubing without a leukocyte filter (approximately 10 to 20 mL per minute). The tubing setup should be fitted with a connector and an attached supplemental saline bag to be used after the initial infusion is completed allowing any remaining product remaining in the bag and tubing to be recovered and infused.

Vital signs (temperature, heart rate, diastolic and systolic blood pressure) are to be taken:

- prior to MLM-CAR44.1 T-cells infusion
- during infusion
- at the end of infusion
- after the infusion, approximately every 15 minutes for one hour and then every hour for the next two hours, or until these signs are satisfactory and stable.

Emergency medical equipment should be available during the infusion in case the patient has a significant infusion reaction such as CRS or anaphylaxis. **Tocilizumab/siltuximab must be available on site prior to CAR T-cell infusion to manage CRS** and supportive therapy such

as oxygen, bronchodilators, epinephrine, antihistamines, and corticosteroids. These medications should be given according to the safety monitoring guidelines in Section 11.6.1.

5.10 Rescue medication

Rescue medications are those given for toxicity due to MLM-CAR44.1 T-cell therapy. Tocilizumab (recommended dose 8 mg/kg i.v.), siltuximab (11 mg/kg IV over 1 hour) and steroids may be used for the treatment of suspected severe CRS as described in Section 11.6.1. Prior to MLM-CAR44.1 T-cell infusion, the site must confirm that two doses of tocilizumab are on site and available for administration.

Significant off-tumor toxicity can be controlled by setting off suicide gene activation through administration of ganciclovir 10 mg/kg/day divided into 2 administrations, or valganciclovir 900 mg twice per day orally for 14 days. Ganciclovir should be avoided right before and right after the infusion: a wash out of 48 hours is recommended. There is no issue in using others drugs such as Foscarnet if needed in a timeframe closer to the infusion period. Ganciclovir is commonly used in the field of congenital and acquired pathologies causing immunodepression for the prevention and therapy of cytomegalovirus reactivation and for the treatment of GvHD in patients infused with lymphocytes genetically modified with HSV-tk. The dose in both cases is equal to 10 mg/kg/day, with reduced dosage in case of impaired renal function. Other rescue medications such as the ones indicated previously can continue to be administered until the effects of suicide gene activation are clinically relevant. The use of ganciclovir can be accompanied by side effects such as marrow depression, gastrointestinal toxicity and impaired renal function. However, these side effects occur especially after long-term administration.

Based on the clinical judgment of the Investigator, and in the presence of the following toxicities at least possibly related to MLM CAR44.1 T-cells, ganciclovir may be administered to set off suicide gene activation:

- Grade 3 or higher neurotoxicity
- Grade 4 CRS
- Grade 3 CRS not responsive to steroids after tocilizumab failure within 24 hours (see details in Table 11-3).
- Grade 3 erythroderma not responsive to steroids (methylprednisolone 2mg/kg for 72 hours or rapidly evolving)
- Grade 2 or higher bullous dermatitis
- Any grade Stevens Johnson Syndrome
- Any evidence for Toxic Epidermal Necrolysis (TEN)
- Any other grade 3 or higher toxicity suspected of being related to CAR T-cell treatment not responsive to appropriate first-line treatment or judged to be potentially life threatening by the investigator. Patients who remain monocytopenic after MLM-CAR44.1 T-cell therapy should be considered immune-depressed. Suicide gene activation should thus also be considered in case of grade 3-4 potentially life-threatening infections.

In case of administration of ganciclovir/valganciclovir for activation of suicide gene, an immune phenotype analysis of MLM-CAR44.1 T-cells by flow cytometry (peripheral blood) must be performed at the following time-points:

- before ganciclovir/valganciclovir administration
- 4 days after ganciclovir/valganciclovir administration
- one day after the discontinuation of ganciclovir/valganciclovir administration

It is strongly recommended to share with Sponsor the decision to activate suicide gene.

All rescue medications must be listed on the concomitant medication CRF. Suicide gene activation shall be reported immediately to the Sponsor and DSMB.

5.11 Concomitant therapy

Clinically significant prescription and nonprescription medications will be recorded in the patient's medical record and in the CRF at every visit following the screening visit. Concomitant therapies to be avoided during or shortly prior to MLM-CAR44.1 T-cell therapy are outlined in Section 5.9.

5.12 Stopping rules

Early discontinuation of the study will be based on review of SAEs. Only unexpected SAEs related to treatment with MLM-CAR44.1 T-cells should be considered. The review of these adverse events, and any decision to prematurely stop patient enrollment, will be discussed with the DMSB and reviewed by the IRB/IEC.

Premature termination may occur because of a regulatory authority decision, change in opinion of the IRB/IEC or DMSB, or may be related to issues concerning cell product generation or safety at the discretion of the study investigators.

The study will be terminated prematurely also if no disease response to treatment is detected in the first 6 patients with each indication evaluated in Phase IIa (first stage of Simon's two-stage design).

5.12.1 Criteria for interrupting or discontinuing the study

5.12.1.1Phase I

- Grade 4 or higher toxicity that is unmanageable, unexpected and unrelated to chemotherapy and attributable to MLM-CAR44.1 T-cell therapy will be promptly assessed with the DSMB (see section 10). Decision to interrupt or discontinue the study will be taken case by case, based on risk/benefit assessment. CRS, neurotoxicity, ICU admission, dialysis and mechanical ventilation may be required and are not considered unexpected.

5.12.1.2Phase IIa

- Uncontrolled T-cell proliferation in any patient beyond 8 weeks from CAR T-cell infusion that does not respond to management.
- Detectable replication competent retrovirus (RCR) during the study in any patient.

- The Investigator, Sponsor, DMSB, or any independent review board or regulatory body decides for any reason that patient safety may be compromised by continuing the study.
- Lack of disease response.

The study will be interrupted pending investigation and possible protocol amendment if any patient experiences any of the following events within three weeks of CAR T-cell infusion:

- Grade 4 toxicity that is unmanageable, unexpected and unrelated to chemotherapy and attributable to MLM-CAR44.1 T-cell therapy. CRS, neurotoxicity, ICU admission, dialysis and mechanical ventilation are not considered unexpected. The same may apply to grade 4 liver toxicity, nephrotoxicity and other organ involvement.

Death suspected of being related to MLM-CAR44.1 T-cell therapy.

6 STUDY ASSESSMENTS AND PROCEDURES

The Tables provided in Section 7 present all the planned assessments and procedures. Patients who are prematurely discontinued from the study will continue to be monitored through a secondary follow-up to collect data required by health authorities (e.g. delayed adverse events, etc.).

6.1 Acute Myeloid Leukemia

Table 7-1 presents all the planned assessments and procedures for patients with AML.

6.1.1. ***Screening (Week -10 to Week -8) and enrollment***In Phase I, the first three enrolled patients (regardless of indication) for each dose will be treated one at a time at intervals of at least 36 days. Each of these three patients will undergo lymphodepleting chemotherapy only once the previous patient has completed the 30-day follow-up or until the previous patient experiences a DLT within the completion of 30-day follow-up period (see section 9.1 for the definition of "evaluable" patient). The remaining patients for each dose will be enrolled once the dose has proven to be safe in the first three enrolled patients.

Children with AML will be enrolled in Phase IIa only, except for CZ site.

Any screening assessment that is done outside the screening window must be repeated prior to apheresis.Each patient will be identified by a unique patient number, assigned centrally through a web interface. This patient number will be used for the entire study. See the *MLM-CAR44.1 Study Manual* for details on enrollment procedures.

The screening and leukemia workup include the following assessments:

- Evaluation of CD44v6 expression by tumor cells (see section below)
- Demography (year of birth, age, sex, childbearing status of females, race)
- Inclusion/exclusion criteria
- Medical history, AML diagnosis/history
- Prior/concomitant medications and antineoplastic therapies
- Bone marrow aspirate/biopsy and peripheral blood draw for morphology, immunophenotyping, cytogenetics, molecular cytogenetics by means of fluorescence in situ hybridization (FISH) and MRD
- Brain MRI scan
- Extramedullary disease (physical exam and CNS system assessment)
- Complete physical examination including height and weight
- Vital signs (temperature, heart rate, diastolic and systolic blood pressure)
- ECOG performance status
- DLCO or FEV1
- Pulse oximetry
- MUGA or ECHO
- ECG
- Serum pregnancy test for women of childbearing potential

- Local laboratory evaluations (hematology, clinical chemistry, urine analysis, creatinine clearance)
- CD3/mcl count in peripheral blood
- Coagulation panel
- Serum β2-microglobulin and LDH
- Viral serology (HAV, HBV, HCV, HIV, VZV, HSV 1-2, HSV-6, CMV, EBV) mycoplasma and toxoplasma. Not required if performed as part of clinical routine within 4 weeks prior to informed consent.
- Adverse events
- Absence of active infections and/or viral infections requiring the use of ganciclovir

6.1.1.1 Re-screening

In case of screening failure, for example for an adverse event, that has delayed the execution of screening assessments, the investigator can re-include the patient in the clinical trial only if the following conditions are satisfied:

1. Positivity to CD44v6 marker at first screening
2. Absence of active infections and/or viral infections requiring the use of ganciclovir

Re-screened patient will be identified with another screening code, but he/she will be linked with the previous screening code. The outcome of the re-screening phase will be considered for the purposes of the study.

6.1.1.2 Evaluation of CD44v6 expression on tumor cells

In study Phase I, CD44v6 expression on tumor cells in peripheral blood and bone marrow will be evaluated by a centralized laboratory at the San Raphael Hospital. The same assessment will also be performed at each local laboratory to set-up a validation process for CD44v6 marking.

AML patient's enrolment: CD44v6 positivity is required for bone marrow and peripheral blood sample, a positivity at least for the peripheral blood sample is required for some AML with extra-medullar involvement such as cutaneous localization (see M4 AML).

The threshold value of CD44v6 positivity on tumor cells should be ≥ 20%.

In case of the result of central laboratory is different from that of local laboratory, the central assessment will be considered. Details on sample collection are provided in the *MLM-CAR44.1 Study Manual.*

6.1.2 Lymphocyte apheresis (Week -9 to Week -7)

Lymphocyte apheresis is described in Section 5.6, and full details are provided in the *MLM-CAR44.1 Study Manual*. The procedure should take place no longer than 7 weeks prior to MLM-CAR44.1 T-cell infusion and prior to the administration of chemotherapy, immunomodulators, or steroids to ensure an adequate ALC.

Patients may be required to undergo multiple apheresis procedures to collect a target of 7 × 10E8 CD3+ cells or a minimum of 5 x 10E8, or when is not possible approximately 10 x 10E9 WBC, for the preparation of the investigational product. Patients should however present a peripheral blast count ≤ 20,000/mm^3 at time of lymphocyte apheresis.

Prior to lymphocyte apheresis, patients should undergo a check of inclusion/exclusion criteria and concomitant therapies. Only after informed consent and confirmation of all eligibility criteria will the patient's apheresis product be shipped to the manufacturing facility.

6.1.3 Pre-chemotherapy evaluation visit (Week -2)

Before lymphodepleting chemotherapy begins, the patient will undergo blood collection for safety assessments and other assessments as listed below:

- Check of inclusion/exclusion criteria
- Physical examination and vital signs
- Concomitant medications
- Hematology, clinical chemistry
- Disease evaluation in bone marrow and peripheral blood (morphology and flow cytometry)
- Adverse events
- Rapid influenza test (within 10 days of infusion) If the patient is positive, oseltamivir phosphate or zanamivir should be administered for 10 days as preventative treatment prior to CAR T-cell infusion

At the discretion of the Investigator, assessments may be repeated for up to 60 days until the patient is considered suitable for lymphocyte depleting chemotherapy.

6.1.4 Lymphodepleting chemotherapy (Day -5 to Day -3)

Patients will undergo lymphodepleting chemotherapy with cyclophosphamide i.v. (500 mg/m^2) and fludarabine i.v. (30 mg/m^2) daily from day -5 to day -3.

The following assessments should be performed within 24 hours prior to starting lymphodepleting chemotherapy.

- Physical examination and vital signs
- Weight
- Urine pregnancy test for women of childbearing potential
- Concomitant medications
- Adverse events
- Verification of criteria detailed in Section 5.8

6.1.5 Pre-infusion visit (Day -1)

The following exams must be performed one day prior to MLM-CAR44.1 T-cell infusion.

- Physical exam, weight and vital signs
- ECOG performance status assessment
- Hematology, clinical chemistry and coagulation panel
- MLM-CAR44.1 T-cell infusion prerequisites (see Section 5.9)
- Concomitant medications
- Adverse events

6.1.6 MLM-CAR44.1 T-cell infusion (Day 0)

See Section 5.9 for details of MLM-CAR44.1 T-cell infusion. Patients will undergo the following assessments the day of infusion (prior to infusion):

- Physical examination, weight, vital signs pre-, during and post-infusion as indicated in Section 5.9.2
- Pulse oximetry
- ECOG performance status
- Hematology and clinical chemistry
- ECG
- Concomitant medications
- MLM-CAR44.1 PK (pre-infusion) by flow cytometry in peripheral blood
- MLM-CAR44.PK (pre-infusion) by PCR in peripheral blood
- Blood sample collection (pre-infusion) for ancillary studies
- Blood sample collection for RCR monitoring (pre-infusion) by DNA PCR
- Plasma sampling (pre-infusion) for cytokine monitoring
- Adverse events
- See Section 11.6.1 for safety monitoring guidelines following infusion

6.1.7 Post-infusion visits: D4±1d, D7±1d, D11±1d, D14±1d, D18±1d, D21±1d, D±25±1d and D28±1d (Month 1)

Patient hospitalization with close monitoring is recommended for at least 15 days after CAR-T-cell infusion. See Section 11.6.1 for details about safety monitoring.

The following assessments will be performed at the post-infusion visits:

- Physical examination and vital signs: all visits
- Pulse oximetry: D4, D7, D11, D14
- ECOG performance status: D7, D14, D21, D28
- Hematology and clinical chemistry: all visits
- Coagulation panel: D7, D14, D28
- Urine analysis: D7 and D21
- Concomitant medications: all visits
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry: D7, D14, D21, D28
- MLM-CAR44.1 PK (peripheral blood) by PCR: D7, D14, D21, D28
- MLM-CAR44.1 PK (bone marrow) by flow cytometry: Day 28
- Blood sample collection for ancillary studies: D7, D14, D21, D28
- Plasma sampling for cytokine monitoring: D7, D14, D21, D28
- Adverse events: all visits
- Weight: D28
- See Section 11.6.1 for safety monitoring guidelines following infusion

Assessments will include an evaluation of disease response as follows:

- Tumor cell assessment by flow cytometry in peripheral blood on D7, D14, D21 and D28

- Peripheral blood for morphologic blasts on D28
- Bone marrow aspirate/biopsy – morphology and flow cytometry on D28
- MRD in bone marrow aspirate if CR/CRi is confirmed
- Extramedullary disease on D28
- Response assessment by Investigator: D28

Frequent monitoring of serum CRP, ferritin and cytokines should be considered during CRS of any severity, especially in case of persisting fever, hemodynamic instability, respiratory distress and rapid clinical deterioration. See Section 11.6.1 for CRS management.

6.1.8 Post-infusion visits: Weekly from W5±3d to W8±3d (Month 2)

From Week 5 to Week 8 patients will undergo one or more of the following assessments during weekly visits:

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry (Day 60 only)
- MLM-CAR44.1 PK (peripheral blood) by PCR (Day 60 only)
- Sample collection (peripheral blood) for ancillary studies (Day 60only)
- Plasma sampling for cytokine monitoring (Day 60 only)
- MLM-CAR44.1 PK (bone marrow) by flow cytometry (Day 60 only)
- Concomitant medications
- Adverse events
- Disease assessments at Week 8 only:
  - Tumor cell assessment by flow cytometry in peripheral blood
  - Peripheral blood for morphologic blasts
  - Bone marrow aspirate/biopsy for morphology and flow cytometry
  - MRD in bone marrow if CR or CRi is confirmed
  - Extramedullary disease
  - Disease response by Investigator

6.1.9 Post-infusion visit: W10±3d and W12±3d (Month 3)

The following assessments will be performed on Week 10 and Week 12:

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- Urine analysis at Week 10
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry (Day 90 only)
- MLM-CAR44.1 PK (peripheral blood) by PCR (Day 90 only)
- Blood sample collection for ancillary studies (Day 90 only)
- Plasma sampling for cytokine monitoring (Day 90 only)
- RCR monitored by DNA PCR (Day 90)
- Concomitant medications

- Adverse events
- Disease assessments at Week 12 only:
  - Tumor cell assessment by flow cytometry in peripheral blood
  - Peripheral blood for morphologic blasts
  - Extramedullary disease
  - Disease response by Investigator

6.1.10 Post-infusion visits: Monthly from M4±14d to M6±14d

The following exams will be performed monthly:

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry (Day 180 only)
- MLM-CAR44.1 PK (peripheral blood) by PCR (Day 180 only)
- Blood sample collection for ancillary studies (Day 180 only)
- Plasma sampling for cytokine monitoring (Day 180 only)
- MLM-CAR44.1 PK (bone marrow) by flow cytometry (Day 180 only)
- RCR monitored by DNA PCR (Day 180 only)
- Concomitant medications
- Adverse events

One or more of the following disease assessments will be performed monthly:

- Tumor cell assessment by flow cytometry in peripheral blood at Month 6
- Peripheral blood for morphologic blast (M4-M6)
- Bone marrow aspirate/biopsy at Month 6 for morphology and flow cytometry
- MRD in bone marrow at Month 6 if CR or CRi is confirmed
- Extramedullary disease (M4-M6)
- Response assessment by Investigator

6.1.11 Post-infusion visits: M9±14d, M12±14d, M15±14d, M18±14d, M21±14d, M24 ±14d (end of primary follow-up)

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- RCR monitored by DNA PCR (M12 and M24)
- Concomitant medications
- Adverse events
- Disease assessments
  - Tumor cell assessment by flow cytometry in peripheral blood
  - Peripheral blood for morphologic blasts
  - Extramedullary disease
  - Response assessment by Investigator

6.2 Multiple Myeloma

Table 7-2 presents all the planned assessments and procedures for patients with MM

6.2.1 Screening (Week -10 to Week -8) and enrollment

In Phase I, the first three enrolled patients (regardless of indication) for each dose will be treated one at a time at intervals of at least 36 days. Each of these three patients will undergo lymphodepleting chemotherapy only once the previous patient has completed the 30-day follow-up or until the previous patient experiences a DLT within the completion of 30-day follow-up period (see section 9.1 for the definition of "evaluable" patient). The remaining patients for each dose will be enrolled once the dose has proven to be safe in the first three enrolled patients

Any screening assessment that is done outside the screening window must be repeated prior to apheresis.

Each patient will be identified by a unique patient number, assigned centrally through a web interface. This patient number will be used for the entire study. See the *MLM-CAR44.1 Study Manual* for details on enrollment procedures.

The screening and multiple myeloma workup include the following assessments:

- Evaluation of CD44v6 expression by tumor cells (see section below).
- Demography (year of birth, age, sex, childbearing status of females, race).
- Inclusion/exclusion criteria.
- Medical history, MM diagnosis/history.
- Prior/concomitant medications and antineoplastic therapies including surgery and chemo-, biologic-, immunologic- and radiation therapies.
- International Staging System (ISS) and R-ISS (Revised-ISS): The ISS is based on the assessment of two blood test results, beta 2-microglobulin (ß2-M) and albumin, and has shown significant prognostic power for multiple myeloma. R-ISS evaluates the prognostic effect of adding to ISS the LDH value and the presence of high risk FISH results (see Appendix 2)
- Bone marrow aspirate/biopsy for cytogenetics by means of fluorescence in situ hybridization (FISH).
- Brain MRI (not required if patient has negative brain MRI within 5 weeks prior to screening).
- Complete physical examination including height and weight.
- Vital signs (temperature, heart rate, diastolic and systolic blood pressure)
- ECOG performance status
- DLCO or FEV1
- Pulse oximetry
- MUGA or ECHO
- ECG
- Serum pregnancy test for women of childbearing potential

- Local laboratory evaluations (hematology, clinical chemistry, urine analysis, creatinine clearance)
- CD3/mcl count in peripheral blood
- Coagulation panel
- Serum β2-microglobulin and LDH
- Viral serology (HAV, HBV, HCV, HIV, VZV, HSV 1-2, HSV-6, CMV, EBV) mycoplasma and toxoplasma. Not required if performed as part of clinical routine within 4 weeks informed consent. The details of tests requested for lymphocyte apheresis are specified in section 4.4
- Absence of active infections and/or viral infections requiring the use of ganciclovir
- Adverse events
- Disease assessments
  - In serum:
    - M-protein by protein electrophoresis (PEP) and immunofixation electrophoresis (IFE)
    - Free light chain (FLC) protein assessment
    - Corrected calcium
  - In urine (24-h urine collection required):
    - M-protein by PEP and IFE;
  - Bone marrow aspirate/biopsy: plasma cell count, CD44v6 expression on tumor cells, MRD
  - Assessment of soft tissue plasmacytomas (STP)
  - Local full body skeletal survey by X-ray and/or CT/MRI

6.2.1.1 Re-screening

In case of screening failure, for example for an adverse event, that has delayed the execution of screening assessments, the investigator can re-include the patient in the clinical trial only if the following conditions are satisfied:

1. Positivity to CD44v6 marker at first screening
2. Absence of active infections and/or viral infections requiring the use of ganciclovir

Re-screened patient will be identified with another screening code, but he/she will be linked with the previous screening code. The outcome of the re-screening phase will be considered for the purposes of the study.

6.2.1.2 Evaluation of CD44v6 expression on tumor cells

During study Phase I, CD44v6 expression on tumor cells in peripheral blood and bone marrow will be evaluated by a centralized laboratory at the San Raphael Hospital. The same assessment will also be performed at each local laboratory to set-up a validation process for CD44v6 marking

Patients with CD44v6 expression positive in bone marrow can be enrolled also if they have CD44v6 negative peripheral blood samples.

The threshold value of CD44v6 positivity on tumor cells should be ≥ 20%.

In case of the result of central laboratory is different from that of local laboratory, the central assessment will be considered.

Details on sample collection are provided in the *MLM-CAR44.1 Study Manual.*

6.2.2 Lymphocyte apheresis (Week -9 to Week -7)

Lymphocyte apheresis is described in Section 5.6, and full details are provided in the *MLM-CAR44.1 Study Manual*. The procedure should take place no longer than 7 weeks prior to MLM-CAR44.1 T- cell infusion and prior to the administration of chemotherapy, immunomodulators, or steroids to ensure an adequate ALC.

Patients may be required to undergo multiple apheresis procedures to collect a target of 7 × 10E8 CD3+ cells or a minimum of 5 x 10E8, or when is not possible approximately 10 x 10E9 WBC, for the preparation of the investigational product.

Prior to apheresis, patients should undergo a check of inclusion/exclusion criteria and concomitant therapies. Only after informed consent and confirmation of all eligibility criteria will the patient's apheresis product be shipped to the manufacturing facility.

6.2.3 Pre-chemotherapy evaluation visit (Week -2)

Before lymphodepleting chemotherapy begins, the patient will undergo blood collection for assessments as listed below:

- Inclusion/exclusion criteria
- Physical examination and vital signs
- Concomitant medications
- Hematology, clinical chemistry
- Bone marrow aspirate/biopsy for plasma cell count
- Adverse events
- Rapid influenza test (within 10 days of infusion) If the patient is positive, oseltamivir phosphate or zanamivir should be administered for 10 days as preventative treatment prior to CAR T-cell infusion

At the discretion of the Investigator, assessments may be repeated for up to 60 days until the patient is considered suitable for lymphocyte depleting chemotherapy.

6.2.4 Lymphodepleting chemotherapy (Day -5 to Day -3)

Patients will undergo lymphodepleting chemotherapy with cyclophosphamide i.v. (500 g/m^2) and fludarabine i.v. (30 mg/m^2) daily from day -5 to day -3.

The following assessments should be performed within 24 hours prior to starting lymphodepleting chemotherapy.

- Physical examination and vital signs
- Weight
- Urine pregnancy test for women of childbearing potential
- Concomitant medications

- Adverse events
- Verification of criteria detailed in Section 5.8

6.2.5 Pre-infusion visit (Day -1)

The following exams are to be performed one day prior to MLM-CAR44.1 T-cell infusion.

- Physical exam, vital signs and weight
- ECOG performance status assessment
- Hematology, clinical chemistry and coagulation panel
- MLM-CAR44.1 T-cell infusion prerequisites (see Section 5.9)
- Concomitant medications
- Adverse events
- Disease assessments
  - In serum:
    - M-protein by PEP and IFE
    - FLC protein assessment
    - Corrected calcium
  - In urine (24-h urine collection required):
    - M-protein by PEP and IFE;

6.2.6 MLM-CAR44.1 T-cell infusion (Day 0)

See Section 5.9 for details of MLM-CAR44.1 T-cell infusion. Patients will undergo the following assessments the day of infusion (but prior to infusion):

- Physical examination, weight and vital signs pre-, during and post-infusion as indicated in Section 5.9.2.
- Pulse oximetry
- ECOG performance status
- Hematology and clinical chemistry
- ECG
- MLM-CAR44.1 PK (pre-infusion) by flow cytometry in peripheral blood
- MLM-CAR44.PK (pre-infusion) sample collection for PCR
- Blood sample collection (pre-infusion) for ancillary studies
- Plasma sampling (pre-infusion) for cytokine monitoring
- Blood sample collection for RCR monitoring (pre-infusion) by DNA PCR
- Concomitant medications
- Adverse events
- See Section 11.6.1 for safety monitoring guidelines following infusion

6.2.7 Post-infusion visits: D4±1d, D7±1d, D11±1d, D14±1d, D18±1d, D21±1d, D±25±1d, D28±1d (Month 1)

Patient hospitalization with close monitoring is recommended for at least 15 days after CAR-T-cell infusion. See Section 11.6.1 for details.

The following assessments will be performed at the post-infusion visits:

- Physical examination and vital signs: all visits
- Weight: D28
- Pulse oximetry: D4, D7, D11, D14
- ECOG performance status: D7, D14, D21, D28
- Hematology and clinical chemistry: all visits
- Urine analysis: D7 and D21
- Coagulation panel: D7, D14, D28
- CD44v6 expression on tumor cells in peripheral blood: D7, D14, D21 and D28
- CD44v6 expression on tumor cells in bone marrow: D28
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry: D7, D14, D21 and D28
- MLM-CAR44.1 PK (peripheral blood) by PCR: D7, D14, D21 and D28
- MLM-CAR44.1 PK (bone marrow) by flow cytometry on D28
- Blood sample collection for ancillary studies: D7, D14, D21 and D28
- Plasma sampling for cytokine monitoring: D7, D14, D21, D28
- Concomitant medications: all visits
- Adverse events: all visits
- See Section 11.6.1 for safety monitoring guidelines following infusion

Frequent monitoring of serum CRP, ferritin and cytokines should be considered during CRS of any severity, especially in case of persisting fever, hemodynamic instability, respiratory distress and rapid clinical deterioration. See Section 11.6.1 for CRS management.

On Day 28, assessments will include an evaluation of disease response as follows:

- In serum:
  - M-protein by PEP and IFE
  - FLC protein assessment (if serum PEP or urine PEP or both is/are non-measurable and to identify sCR)
  - Corrected calcium
- In urine (24-h urine collection required):
  - M-protein by PEP and IFE
- Bone marrow aspirate/biopsy for plasma cell count
- MRD in bone marrow in case of CR.
- Response assessment by Investigator
- Assessment of soft tissue plasmacytomas

6.2.8 Post-infusion visits: Weekly from W5±3d to W8±3d (Month 2)

From Week 5 to Week 8 patients will undergo the following assessments during weekly visits:

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- CD44v6 expression on tumor cells in peripheral blood (Day 60 only)
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry (Day 60 only)
- MLM-CAR44.1 PK (peripheral blood) by PCR (Day 60 only)
- Blood sample collection for ancillary studies (Day 60 only)
- Plasma sampling for cytokine monitoring (Day 60 only)Concomitant medications
- Adverse events

On Week 8, assessments will include an evaluation of disease response as follows:

- In serum:
  - M-protein by PEP and IFE
  - FLC protein assessment (if serum PEP or urine PEP or both is/are non-measurable and to identify sCR)
  - Corrected calcium
- In urine (24-h urine collection required):
  - M-protein by PEP and IFE
- Assessment of soft tissue plasmacytomas
- Disease response by Investigator

6.2.9 Post-infusion visit: W10±3d and W12±3d (Month 3)

The following assessments will be performed on Week 10 and on Week 12:

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- Urine analysis (Week 10)
- CD44v6 expression on tumor cells in peripheral blood (Day 90 only)
- CD44v6 expression on tumor cells in bone marrow (Day 90 only)
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry (Day 90 only)
- MLM-CAR44.1 PK (peripheral blood) by PCR (Day 90 only)
- MLM-CAR44.1 PK (bone marrow) by flow cytometry (Day 90 only)
- Blood sample collection for ancillary studies (Day 90 only)
- Plasma sampling for cytokine monitoring (Day 90 only)
- RCR monitored by DNA PCR (Day 90 only)
- Concomitant medications
- Adverse events
- Disease assessments (Week 12 only)

- In serum:
  - M-protein by PEP and IFE
  - FLC protein assessment (if serum PEP or urine PEP or both is/are non-measurable and to identify sCR)
  - Corrected calcium
- In urine (24-h urine collection required):
  - M-protein by PEP and IFE
- Bone marrow aspirate/biopsy for plasma cell count
- MRD in bone marrow in case of CR
- Assessment of soft tissue plasmacytomas
- Disease response by Investigator

6.2.10 Post-infusion visits: Monthly from M4±14d to M6±14d

One or more of the following assessments will be performed monthly from Month 4 to Month 6:

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- CD44v6 expression on tumor cells in peripheral blood (Day 180 only)
- CD44v6 expression on tumor cells in bone marrow (Day 180 only)
- MLM-CAR44.1 PK (peripheral blood) by flow cytometry (Day 180 only)
- MLM-CAR44.1 PK (peripheral blood) by PCR (Day 180 only)
- Blood sample collection for ancillary studies (Day 180 only)
- Plasma sampling for cytokine monitoring (Day 180 only)
- MLM-CAR44.1 PK (bone marrow) by flow cytometry (Day 180 only)
- RCR monitored by DNA PCR (Day 180 only)
- Concomitant medications
- Adverse events
- Disease assessments
  - In serum:
    - M-protein by PEP and IFE
    - FLC protein assessment (if serum PEP or urine PEP or both is/are non-measurable and to identify sCR)
    - Corrected calcium
  - In urine (24-h urine collection required):
    - M-protein by PEP and IFE
  - Bone marrow aspirate/biopsy for plasma cell count – Month 6 only
  - MRD in bone marrow in case of CR at Month 6

- Assessment of soft tissue plasmacytomas
- Response assessment by Investigator

6.2.11 Post-infusion visits: M9±14d, M12±14d, M15±14d, M18±14d, M21±14d, M24 ±14d (end of primary follow-up)

One or more of the following assessments will be performed during visits at Months 9, 12, 15, 18, 21 and 24:

- Physical examination, vital signs and weight
- ECOG performance status
- Hematology and clinical chemistry
- CD44v6 expression on tumor cells in peripheral blood
- RCR monitored by DNA PCR (M12 and M24)
- Concomitant medications
- Adverse events
- Disease assessments
  - In serum:
    - M-protein by PEP and IFE
    - FLC protein assessment (if serum PEP or urine PEP or both is/are non-measurable and to identify sCR)
    - Corrected calcium
  - In urine (24-h urine collection required):
    - M-protein by PEP and IFE
  - Assessment of soft tissue plasmacytomas
  - Response assessment by Investigator

6.3 End of primary follow-up and premature withdrawal

Patients will be followed up for 24 months from the date of infusion. If a patient discontinues prematurely from the primary follow-up, a visit should be scheduled as soon as possible, at which time all the assessments listed for the Month 24 visit will be performed, except for RCR evaluation.

Patients may voluntarily withdraw from treatment and primary follow-up at any time or be discontinued from the study due to reasons including:

- Disease progression/Relapse after remission
- Pursuing HSCT while in remission
- Voluntary withdrawal

Patients who discontinue or withdraw from primary follow-up early will be asked to continue the study in a secondary follow-up phase through Month 24. For patients who are lost to follow-up, the investigator should make every effort to contact the patient.

Following completion of primary follow-up, patients will be followed for survival every 3 months until the end of the study or will be enrolled in a long-term follow-up study, whichever occurs first.

6.4 Secondary follow-up

Patients who discontinue primary follow-up will continue to be followed in the secondary follow-up phase to collect health authority requested data (e.g. protocol defined adverse events) up to 2 years after MLM-CAR44.1 T-cell infusion. Depending on when discontinuation occurs, patients will have visits at M3, M6, M9, M12, M18 and M24 as outlined in Table 7-3 and undergo the following assessments:

- RCR by PCR: M3, M6, M12, M24
- Adverse events and adverse events of special interest (including new malignancies), pregnancies as detailed in Section 11.4.
- Antineoplastic therapies

If the patient cannot attend a visit, he or she should be contacted by phone so at least survival status can be determined.

6.5 Long-term follow-up

Patients will be followed for 15 years after MLM-CAR44.1 infusion as per health authority guidelines.

Annual evaluations will be performed on all patients who have received an MLM-CAR44.1 T-cell product infusion as recommended by the FDA and EMA and will consist in assessing overall health including new/recurrent malignancies, neurologic disorders, hematologic disorders, autoimmune disorders, unexpected medical problems and hospitalizations through a specific checklist.

For the first 5 years, a blood sample for RCR testing will be collected from treated patients. In case the samples collected until M24 were always negative, the subsequent yearly samples will be taken but not analysed; in case of one or more positive samples, the culture test will be performed for confirmation.

The purpose of long-term follow-up is to detect adverse reactions, manage their clinical consequences, and thereby collect information on the long-term safety of the treatment. For these reasons, the long-term follow-up should be maintained also for patients who have interrupted treatment and who have withdrawn from the study. A separate informed consent/assent form will be provided for this protocol.

Patient alert cards that inform treating physicians about the study treatment will be provided to patients. These alert cards will be approved by the Ethics Committee and contain as minimum the name of the patient, the investigator's contact number and information regarding the medical treatment received

6.6 Quality of Life assessment

Not Applicable

6.7 Contraception

A woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

For the purpose of this document, a man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

Women of childbearing potential and all male participants must use highly effective methods of contraception for at least 12 months following MLM-CAR44.1 CAR T-cell infusion and until CAR T-cells are no longer present by flow cytometry on two consecutive tests. Highly effective contraception methods include:

a) Total abstinence. Periodic abstinence and withdrawal are not acceptable.

b) Female sterilization (hysterectomy, bilateral salpingectomy and bilateral oophorectomy)

c) Male sterilization (at least 6 months prior to screening).

d) BOTH of the following forms of contraception must be utilized:

- Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormonal vaginal ring or transdermal hormonal contraception
- Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository.

Sexually active males must accept to use a condom during intercourse for 12 months after treatment. A condom is required to be used also by vasectomized men (as well as during intercourse with a male partner or sterile female partner) as WBCs are a normal part of semen and transmission of CAR T transduced cells may occur.

7 SCHEDULE OF ASSESSMENTS

TABLE 7-1 SCHEDULE OF ASSESSMENTS - ACUTE MYELOID LEUKEMIA

	Screening	Pre-Treatment				Treatment and Primary Follow-Up														Survival follow-up every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion													
							Month 1								Month 2	Month 3				
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 18**	**Day 21**	**Day 25**	**Day 28**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 ± 14 days**	
Informed consent	X																			
Demography	X																			
Inc./Exc. criteria	X	X	X																	
Medical history	X																			
Prior antineoplastic therapy	X																			
AML diagnosis/history	X																			
Bone marrow / blood: Morphology Immunophenotyping and if applicable Cytogenetics Fluorescence in situ hybridization (FISH)	X																			
Brain MRI scan	X																			
CD44v6 expression in bone marrow and	X																			

	Screening	Pre-Treatment				Treatment and Primary Follow-Up														Survival follow-up every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion													
							Month 1								Month 2	Month 3				
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 18**	**Day 21**	**Day 25**	**Day 28**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 ± 14 days**	
peripheral blood (central assessment)																				
Safety Assessments																				
Physical examination	X	Symptom-directed physical examination																		
Vital signs	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Height	X																			
Weight	X			X	X	X								X	X	X	X	X	X	
Post-infusion safety monitoring						See Section 11.6.1														
Pulse oximetry	X					X	X	X	X	X										
Performance status score (ECOG)	X				X	X		X		X		X		X	X	X	X	X	X	
Adverse events	Continuously																			X
Prior/concomitant medication, transfusions	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
DLCO/FEV1	X																			
Serology (HAV, HBV, HCV, HIV, VZV, HSV 1-2, HSV-6, CMV, EBV, toxoplasma)	X																			
Serum pregnancy test	X																			
Urine pregnancy test				X																
Rapid influenza A and B testing			Within 10 days of infusion																	
MUGA/ECHO	X																			

	Screening	Pre-Treatment				Treatment and Primary Follow-Up														Survival follow-up every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion													
							Month 1								Month 2	Month 3				
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 18**	**Day 21**	**Day 25**	**Day 28**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 ± 14 days**	
ECG	X					X	As clinically indicated													
Lab Assessments																				
Hematology	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CD3/mcl count in peripheral blood	X																			
Clinical chemistry	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine analysis	X							X				X				X				
Coagulation (PT, aPTT, INR, fibrinogen, D-dimer)	X				X			X		X				X						
Mycoplasma test	X																			
Disease assessments																				
Peripheral blood for morphologic blasts	X		X											X	Week 8		X	X	X	
Tumor cell assessment by flow cytometry in peripheral blood (tumor cells and CD44v6 assessment)	X							X		X		X		X	Week 8		X	Month 6 only	X	
Bone marrow aspirate/biopsy morphology and flow cytometry	X		X											X	Week 8			Month 6		
MRD in bone marrow aspirate/biopsy	X													X[1]	Week 8[1]			Month 6[1]		

	Screening	Pre-Treatment				Treatment and Primary Follow-Up													Survival follow-up every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion												
							Month 1								Month 2	Month 3			
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 18**	**Day 21**	**Day 25**	**Day 28**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 ± 14 days**	
Extramedullary disease	X													X	Week 8		X	X	X	
Response assessment by Investigator														X	Week 8		X	X	X	
CD44v6 Assessment, Pharmacokinetics, safety and ancillary studies																				
CRS assessments in peripheral blood (cytokines, markers)						As clinically indicated														
CD44v6 assessment in bone marrow	X													X	Week 8			Day 180 only		
Plasma sample for cytokine monitoring						X (pre-)		X		X		X		X	Day 60		Day 90	Day 180 only		
MLM-CAR44.1 PK by flow cytometry and PCR (peripheral blood)[2]						X (pre-)		X		X		X		X	Day 60		Day 90	Day 180 only		
MLM-CAR44.1 PK bone marrow by flow cytometry														X	Day 60			Day 180 only		
RCR monitored by DNA PCR						X (pre-)											Day 90	Day 180	Month 12 and Month 24	
Sample collection for ancillary studies[3]		X				X (pre-)		X		X		X		X	Day 60		Day 90	Day 180		
Intervention																				
MLM-CAR44.1 T-cell infusion						X														
Lymphocyte apheresis		X																		

	Screening	Pre-Treatment				Treatment and Primary Follow-Up														Survival follow-up every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion													
							Month 1								Month 2	Month 3				
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4**	**Day 7**	**Day 11**	**Day 14**	**Day 18**	**Day 21**	**Day 25**	**Day 28**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 ± 14 days**	
Lymphodepleting chemotherapy				X																
CD44v6 infusion prerequisite					X															
Survival follow-up																				X

[1] If CR/CRi is confirmed

[2] Flow cytometry to be performed both locally and at a central laboratory, PCR at a central laboratory

[3] Ancillary studies: Characterization of T-cells on Days 0, 7, 14, 21, 28, Week 8, Week 12 and Month 6 (Bambino Gesù Pediatric Hospital laboratory)
CAR T immunogenicity on lymphocyte apheresis visit (Week -9 to Week -7), day 28 and Month 6 (MolMed laboratory)

TABLE 7-2 SCHEDULE OF ASSESSMENTS - MULTIPLE MYELOMA

	Screening	Pre-Treatment				Treatment and Primary Follow-Up													Survival every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion												
							Month 1								Month 2	Month 3			
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4 ± 1d**	**Day 7 ± 1d**	**Day 11 ± 1d**	**Day 14 ± 1d**	**Day 18 ± 1d**	**Day 21 ± 1d**	**Day 25 ± 1d**	**Day 28 ± 1d**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 (end of follow-up) ± 14 days**	
Informed consent	X																			
Demography	X																			
Inc./Exc. Criteria	X	X	X																	
Medical history	X																			
Prior antineoplastic therapy	X																			
MM diagnosis/history	X																			
Internal Staging System (ISS and R-ISS)	X																			
Brain MRI[1]	X																			
Cytogenetics (FISH)	X																			
CD44v6 expression in bone marrow and peripheral blood (central assessment)	X																			
Safety Assessments																				
Physical examination	X	Symptom-directed physical examination																		
Vital signs	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Height	X																			
Weight	X			X	X	X								X	X	X	X	X	X	
Post-infusion safety monitoring						See Section 11.6.1														

	Screening	Pre-Treatment				Treatment and Primary Follow-Up														Survival every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion													
							Month 1								Month 2	Month 3				
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4 ± 1d**	**Day 7 ± 1d**	**Day 11 ± 1d**	**Day 14 ± 1d**	**Day 18 ± 1d**	**Day 21 ± 1d**	**Day 25 ± 1d**	**Day 28 ± 1d**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 (end of follow-up) ± 14 days**	
Pulse oximetry	X					X	X	X	X	X										
Performance status score (ECOG)	X				X	X		X		X		X		X	X	X	X	X	X	
Adverse events	Continuously																			X
Prior/concomitant medication, transfusions	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
DLCO or FEV1	X																			
Serology (HAV, HBV, HCV, HIV, VZV, HSV 1-2, HSV-6, CMV, EBV, toxoplasma)	X																			
Serum pregnancy test	X																			
Urine pregnancy test				X																
Rapid influenza A and B testing			Within 10 days of infusion																	
MUGA/ECHO	X																			
ECG	X					X	As clinically indicated													
Lab Assessments																				
Hematology	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CD3/mcl count in peripheral blood	X																			
Clinical chemistry	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine analysis	X							X				X				X				
Coagulation (PT, aPTT, INR, fibrinogen, D-dimer)	X				X			X		X				X						

	Screening	Pre-Treatment				Treatment and Primary Follow-Up														Survival every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion													
							Month 1								Month 2	Month 3				
Study day/week/month	**W -10 to W - 8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4 ± 1d**	**Day 7 ± 1d**	**Day 11 ± 1d**	**Day 14 ± 1d**	**Day 18 ± 1d**	**Day 21 ± 1d**	**Day 25 ± 1d**	**Day 28 ± 1d**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 (end of follow-up) ± 14 days**	
Serum β2-microglobulin, LDH	X																			
Mycoplasma test	X																			
Efficacy Assessments																				
Efficacy / Disease Assessment required to confirm response or disease progression																				
SPEP, UPEP	X				X									X	Week 8		X	X	X	
SIFE, UIFE	X				X		.							X	Week 8		X	X	X	
						To confirm CR regardless of whether measurable M-protein was present at baseline.														
SFLC and UFLC assay	X				X									X	Week 8		X	X	X	
						Analyzed only when serum M-protein or urine M-protein or both assessed by PEP is/are non-measurable and to identify sCR in case CR criteria are met.															
Plasma cell count in bone marrow	X		X											X			X	Month 6 only		
MRD in bone marrow	X													X[2]			X[2]	Month 6 only[2]		
Corrected calcium	X				X									X	Week 8		X	X	X	
Skeletal survey	X	.				As clinically indicated; CT/MRI in case of newly symptomatic areas with no X-ray finding.														
Assessment of extramedullary soft tissue plasmacytoma	X													X	Week 8		X	X	X	
Response assessment by Investigator														X	Week 8		X	X	X	
CD44v6 Assessment, Pharmacokinetics, safety and ancillary studies																				
CRS assessments in peripheral blood (cytokines, markers)						As clinically indicated														

	Screening	Pre-Treatment				Treatment and Primary Follow-Up													Survival every 3 months
		Lymphocyte apheresis	Pre-chemotherapy	Lymphodepleting chemotherapy	Pre-infusion	Infusion	Post-infusion												
							Month 1								Month 2	Month 3			
Study day/week/month	**W -10 to W -8**	**W -9 to W -7**	**Week -2**	**Day -5 to day -3**	**Day -1**	**Day 0**	**Day 4 ± 1d**	**Day 7 ± 1d**	**Day 11 ± 1d**	**Day 14 ± 1d**	**Day 18 ± 1d**	**Day 21 ± 1d**	**Day 25 ± 1d**	**Day 28 ± 1d**	**Week 5 to Week 8**	**Week 10**	**Week 12**	**Month 4 to Month 6 ± 14 days**	**Month 9, 12, 15, 18, 21, 24 (end of follow-up) ± 14 days**	
CD44v6 assessment in peripheral blood	X							X		X		X		X	Week 8		X	Day 180	X	
CD44v6 assessment in bone marrow	X													X			X	Day 180		
Plasma sample for cytokine monitoring						X (pre)		X		X		X		X	Day 60		Day 90	Day 180		
MLM-CAR44.1 PK by flow cytometry and PCR (peripheral blood)[3]						X (pre)		X		X		X		X	Day 60		Day 90	Day 180		
MLM-CAR44.1 PK – bone marrow by flow cytometry														X			Day 90	Day 180		
RCR monitored by DNA PCR						X (pre)											Day 90	Day 180	Month 12 and Month 24	
Sample collection for ancillary studies[4]		X				X (pre)		X		X		X		X	Day 60		Day 90	Day 180		
Intervention																				
MLM-CAR44.1 T-cell infusion						X														
Lymphocyte apheresis		X																		
Lymphodepleting chemotherapy				X																
CD44v6 infusion prerequisite					X															
Survival follow-up																				X

M protein by electrophoresis in serum (SPEP) and urine (UPEP). M protein by immunofixation in serum (SIFE) and urine (UIFE). Free light chain protein assessment (SFLC).

Minimal residual disease (MRD)
[1] Not required if negative MRI available within 5 weeks prior to screening.
[2] In case of CR.
[3] Flow cytometry to be performed both locally and at a central laboratory, PCR at a central laboratory
[4] Ancillary studies: Characterization of T-cells on Days 0, 7, 14, 21, 28, Week 8, Week 12 and Month 6 (Bambino Gesù Pediatric Hospital laboratory)
CAR T immunogenicity on lymphocyte apheresis visit (Week -9 to Week -7), day 28 and Month 6 (MolMed laboratory)

TABLE 7-3 SCHEDULE OF ASSESSMENTS – SECONDARY FOLLOW-UP (AML AND MM)

	Secondary follow-up							
	Post-infusion							
Study month		**M3 ±14d**	**M6±30d**	**M9±30d**	**M12±30d**	**M18±60d**	**M24±60d**	**Survival every 3 months after study completion**
Antineoplastic medications		X	X	X	X	X	X	
Adverse events		X	X	X	X	X	X	
Pregnancies		X	X	X	X	X	X	
RCR by DNA PCR		X	X		X		X	
Survival follow-up								X

8 EVALUATION CRITERIA

8.1 Expression of CD44v6 on tumor cells – AML and MM patients' study inclusion

In study phase I, positivity of tumor cells in peripheral blood and bone marrow to CD44v6 will be evaluated by a centralized laboratory at the San Raphael Hospital during the screening phase.

AML patients: CD44v6 positivity is required for bone marrow and peripheral blood samples; a positivity at least for the peripheral blood sample is required for some AML with extra-medullar involvement such as cutaneous localization (see M4 AML).

MM patients: CD44v6 positivity is required for bone marrow; patients with CD44v6 expression positive in bone marrow can be enrolled also if they have CD44v6 negative peripheral blood sample.

The threshold value of CD44v6 positivity of tumor cells should be ≥ 20%.

This is a mandatory requirement, as CD44v6 is the target antigen of transduced MLM-CAR44.1 T-cells.

The same evaluation will be performed at each local laboratory.

In case of the result of central laboratory is different from that of local laboratory, the central assessment will be considered.

8.2 Maximum tolerated dose (Phase I) - AML and MM

The maximum tolerated dose (MTD) and recommended Phase IIa dose of MLM-CAR44.1 T-cells will be established through a BOIN study design (see Section 3).

8.3 Disease response (Phase I and Phase IIa) – AML

Response or disease progression will be assessed by the investigator based on ELN guidelines as indicated in Appendix 3. Response is based on bone marrow and blood morphologic criteria, physical examination findings, along with laboratory assessments of bone marrow minimal MRD. Disease assessments should be performed as reported in Table 8-1.

TABLE 8-1 DISEASE ASSESSMENTS AML

Peripheral blood for morphologic blasts	Screening, Week -2 (pre-chemotherapy), monthly until Month 6 and every 3 months thereafter
Flow cytometry of peripheral blood (tumor cell, CD44v6 assessment)	Screening, Days 7, 14, 21, 28 and Months 2, 3, 6, 9, 12 15, 18, 21 and 24.
Flow cytometry of bone marrow (CD44v6 assessment)	Screening, Day 28 (Month1), Week 8 (Month 2) and Month 6
Bone marrow aspirate/biopsy for morphologic blast cell count/flow cytometry	Screening, Week -2 (pre-chemotherapy), Day 28 (Month1), Week 8 (Month 2) and Month 6
MRD assessments in bone marrow by flow cytometry	Screening, Day 28 (Month1), Week 8 (Month2) and Month 6 in case of CR/CRi

8.3.1 Morphology

Cell morphology will be performed according to standard procedure. Myeloblasts, monoblasts, and megakaryoblasts are included in the blast count. In AML with monocytic or myelomonocytic differentiation, monoblasts and promonocytes, but not abnormal monocytes, are counted as blast equivalents. Erythroblasts are not counted as blasts except in the rare instance of pure erythroid leukemia.

8.3.2 Tumor cell assessment by flow cytometry

Flow cytometry of peripheral blood (tumor cells and CD44v6 assessment) will be performed at screening, , Days 7, 14, 21, 28 and Months 2, 3, 6, 9, 12, 15, 18, 21 and 24. Bone marrow exams will be performed at screening, Day 28, Week 8 and Month 6.

Flow cytometry provides an insight into differentiation pathways, maturation stages and abnormal features of the cell populations which are clinically relevant for the diagnosis of hematological malignancies. The presence and absence of antigens on or in the cell populations are recognized by various Monoclonal Antibodies (MAb) which gives characteristic immunostaining defining the cell lineages thus helping in making the diagnosis of acute leukemias. Flow cytometric immunophenotyping is a rapid reliable method not only to diagnose but also to assess prognosis, decision making for targeted therapy and follow up (in minimal residual disease evaluation) in acute leukemia (*Gajendra 2016*).

Flow cytometric immunophenotyping of peripheral blood and bone marrow samples will be initially performed locally.

8.3.3 Minimal residual disease (MRD) in bone marrow

MRD in AML refers to the presence of leukemic cells below the threshold of detection using conventional morphologic methods. Patients with a CR according to morphologic assessment alone can potentially harbor a large number of leukemic cells in the bone marrow. The most frequently use methods for MRD assessment include multicolor flow cytometry to detect abnormal immunophenotypes and qPCR assays to detect NPM mutation if positive at baseline. For MRD evaluation with qPCR, sampling of bone marrow mononuclear cells (MNC) is preferred.

8.3.4 Extramedullary disease

Extramedullary disease is to be assessed via physical examination, CSF assessment, and if required, by imaging techniques. Patients with suspected CNS leukemia must be evaluated by lumbar puncture and be free of CNS disease prior to study entry. Previously treated CNS leukemia is allowed provided adequate treatment has been given and the patient is free of CNS disease.

8.3.5 Molecular cytogenetics by means of fluorescence in situ hybridization (FISH)

Fluorescent in situ hybridization (FISH) is an option to detect gene rearrangements, such as RUNX1-RUNX1T1, CBFB-MYH11, MLL and EVI1 gene fusions, or loss of chromosome 5q and 7q material. FISH is frequently necessary to identify MLL fusion partners in 11q23 translocations. A cytogenetic analysis of bone marrow cells will be performed at screening.

8.4 Disease response (Phase I and Phase IIa) MM

Response or disease progression will be assessed by the investigator based on the analysis of serum and urine protein electrophoresis (SPEP and UPEP), serum and urine immunofixation (SIFE and UIFE), serum free light chain protein (SFLC), imaging and bone marrow assessments as per modified IMWG guidelines (Appendix 4).

SPEP, UPEP, and serum calcium corrected for albumin should be collected as shown in Table 8-2 until disease progression. After screening, IFE should be done only when endogenous M-protein is 0 or nonquantifiable.

TABLE 8-2 DISEASE ASSESSMENTS MM

M-protein by electrophoresis Serum (SPEP), Urine (UPEP)	Screening Pre-infusion Monthly from Month 1 to Month 6, then every 3 months from Month 9 to Month 24. As needed to confirm disease progression.
M-protein by immunofixation Serum[1] (SIFE), Urine (UIFE)	Screening Pre-infusion Monthly from Month 1 to Month 6, then every 3 months from Month 9 to Month 24 in subjects with disappearance of M-protein by PEP. As needed to confirm disease progression
Free light chain Serum (SFLC), Urine (UFLC)	Screening Pre-infusion Monthly from Month 1 to Month 6, then every 3 months from Month 9 to Month 24 if serum PEP or urine PEP or both is/are non-measurable. As needed to confirm sCR and confirm disease progression for subjects with serum FLC only disease.
Clinical assessment of soft tissue plasmacytoma (STP)	Screening Monthly from Month 1 to Month 6, then every 3 months from Month 9 to Month 24. As needed to confirm disease progression
CT/MRI assessment of STP	At screening and as clinically indicated.

Plasma cell count in bone marrow	At screening, Week -2 (pre-chemotherapy), Day 28 (Month 1), Week 12 (Month 3) and Month 6
Skeletal survey by X-ray and/or CT/MRI	At screening and as clinically indicated, CT/MRI in case of newly symptomatic areas with no X-ray finding.
Corrected calcium	Screening Pre-infusion Monthly from Month 1 to Month 6, then every 3 months from Month 9 to Month 24. As needed to confirm disease progression
MRD by means of RT-qPCR	At screening, Day 28 (Month 1), Week 12 (month 3) and Month 6 in case of CR.
Flow cytometry of peripheral blood (CD44v6 assessment)	Screening Days 7, 14, 21, 28 and Months 2, 3, 6, 9, 12 15, 18, 21 and 24 if positive at screening
Flow cytometry of bone marrow aspirate (CD44v6 assessment)	Screening, Day 28, Week 12 (Month 3) and Month 6.
[1] IFE should only be done when endogenous M-protein is 0 or non-quantifiable.	

Confirmation of response

Response must be confirmed for all categories other than stable disease (SD) to rule out errors. A consecutive assessment can be performed at any time and confirmation should be obtained by M-protein assessments.

- Bone marrow assessments do not need to be repeated, but at least 1 bone marrow assessment is required to substantiate a CR.
- If imaging studies were done, they need to rule out new lytic bone lesions.
- Should confirmation assessments reveal a better category (e.g., VGPR after PR), the response category of the previous assessment will be considered as confirmed (PR).
- Should confirmation assessments reveal a worse category, (e.g., VGPR after CR), the response category of the subsequent assessment will be considered as confirmed (VGPR).
- Should repeated measurements of a variable result in more values, the worst assessment is to be considered.

Confirmation of progressive disease

Confirmation is based on M-protein measurement or serum FLC levels. Confirmation of an increase in M-protein or FLC should be obtained as soon as possible.

8.4.1 M-protein Assessment by PEP and IFE

Response to treatment is based on M-protein levels in serum and urine. Two methods are required:

- Protein electrophoresis provides quantitative measurements.
- Immunofixation provides qualitative measurements (present/absent). It is a more sensitive method than PEP and is used to confirm the absence of M-protein by PEP (CR).

- Serum and urine IFE is required at baseline and to confirm CR regardless of whether measurable M-protein was present at baseline.
- Subjects with measurable disease with SPEP will be assessed for response based on SPEP and not by the serum FLC assay.
- Subjects with measurable disease in both SPEP and UPEP will be assessed for response based on these two tests and not by the serum FLC assay.

Analysis by IFE will be done for all subjects at screening and baseline, and thereafter (see Table 7-2) only in subjects with disappearance of M-protein by PEP.

8.4.2 Free Light Chain (FLC) Protein Assessment

The serum FLC assay measures free kappa light chain (0.33-1.94 mg/dL) and free lambda light chain (0.57-2.63 mg/dL). The FLC ratio is defined as the kappa serum level divided by the lambda serum level. Lambda is the involved light chain if FLC ratio < 0.26. Kappa is the involved light chain if FLC ratio is > 1.65. The FLC ratio is considered normal if FLC ratio is within 0.26-1.65, and abnormal if FLC ratio is <0.26 or >1.65.

The kappa/lambda ratios by urine FLC immunoassays and then the cut-offs for the kappa/lambda ratio are defined as 5.5 for BJP K and 0.1 for BJP lambda.

FLC will be analyzed only when serum M-protein or urine M-protein or both assessed by PEP is/are non-measurable (i.e., serum M-protein < 0.5 g/dL or urine M-protein < 200 mg per 24 hours) and to identify sCR if CR criteria are met.

8.4.3 Plasma cell count in bone marrow

A bone marrow aspirate and biopsy for plasma cell quantification will be performed and the percentage of plasma cells determined through cytological/histological examination. Clonal plasma cell will be determined by either flow cytometry or immunohistochemistry or immunofluorescence, considering the following MM markers: CD45, CD14, CD2, CD19, CD27, CD56, CD38, CD138, CD117 and CD81.

Either bone marrow aspirate or biopsy can be used for this assessment, but the same method should be used throughout the study. Bone marrow aspirate is required for MRD and flow cytometry assessment.

A bone marrow aspirate and biopsy should be performed for every subject at screening, Week -2 (pre-chemotherapy, Day 28 (Month 1), Week 12 (month 3) and Month 6.

8.4.4 Assessment of soft tissue plasmacytoma (STP)

Clinical assessment

The investigator should perform a clinical exam to assess the presence of STP at screening and as indicated in Table 7-2. If the presence of a STP is suspected during the study, a CT or MRI must be performed immediately to confirm and document the lesions' location.

CT/MRI

- Screening: A CT/MRI will be performed at screening. Any imaging assessments already completed during the regular work-up of the subject within 40 days prior to lymphocyte apheresis can be considered as the baseline assessment.

- Post-baseline: Assessments should be performed using the same imaging technique used at baseline. In case of suspicion of STP and/or disease progression (based on clinical exam or symptoms), a CT/MRI should be performed promptly to confirm suspicion

8.4.5 Skeletal survey

A skeletal survey is to be performed by conventional radiography for osteolytic disease at screening. The survey will be performed during the study if clinically indicated. Use of conventional or low -dose CT scan or MRI is acceptable. If imaging is performed on treatment for assessment of progression, the same imaging technique as the one used at screening must be used. The number and location of skeletal lesions and whether they are lytic should be recorded on the eCRF. An on-treatment survey should record any increase or improvement in the number or size of lytic lesions.

8.4.6 Minimal residual disease (MRD)

MRD assessment by RT-qPCR or NGS (Next-Generation-Sequencing) is an effective tool in the assessment of patients with MM. MRD will be analyzed when bone marrow sample is obtained at screening to establish the baseline clone, and at Day 28 (Month 1), Week 12 (Month 3) and Month 6 in case of CR. Additional assessments are to be performed 12, 18 and 24 months post CR/sCR in subjects who maintain CR or sCR, until PD is observed.

8.4.7 Corrected calcium

Corrected calcium in serum for determination of hypercalcemia as part of response assessment will be evaluated at screening and as indicated in Table 7-2 until disease progression using the following formula:

Corrected Calcium, mg/dL = (0.8 x [normal albumin, g/dL - subject's albumin, g/dL] + serum Ca, mg/dL).

8.4.8 Cytogenetics by means of fluorescence in situ hybridization (FISH)

A cytogenetic analysis of bone marrow cells will be performed at screening through FISH; should be considered at least the following abnormalities del17p, t(4;14), t(14,16). The pathological bone marrow cells from patients with MM are characterized by genetic instability resulting in numerical and structural chromosomal abnormalities that may serve as prognostic factors.

8.4.9 CD44v6 evaluation by flow cytometry

CD44v6 evaluation by flow cytometry of peripheral blood will be performed at screening and on Days 7, 14, 21, 28 and Months 2, 3, 6, 9, 12, 15, 18, 21 and 24 if positive at screening.

Bone marrow exams will be performed at screening, Day 28, Week 12 (Month 3) and Month 6.

8.5 Safety assessments (AML and MM)

See Section 11.6.1 for safety monitoring guidelines following MLM-CAR44.1 T-cell infusion.

8.5.1 Adverse Events and safety monitoring

The overall safety of treatment with MLM-CAR44.1 T-cells will be evaluated by analyzing the type, frequency and severity of adverse events (AE) and by monitoring for systemic reactions (fever, tachycardia, nausea and vomiting, joint pain, skin rash) for 30 days following CAR T-cell infusion. See Section 11.4 for details about adverse events and Section 11.6 for guidelines about safety monitoring.

8.5.2 Physical examination

Physical examinations will be performed daily until Day 15 and then at every scheduled visit. A full physical examination will be performed at screening, whereas targeted exams will occur during the treatment and follow-up periods according to the investigator's observations and complaints from the patient.

Significant findings that were present prior to the signing of informed consent must be included in the Medical History eCRF page. Significant new findings that begin or worsen after informed consent must be recorded on the Adverse Event page of the eCRF.

8.5.3 Eastern Cooperative Oncology Group (ECOG) Performance Status

ECOG Performance Status score will be evaluated using the criteria described in Appendix 6. The assessment should be completed prior to any study-related procedures or assessments.

8.5.4 Vital signs

Vital signs include temperature, blood pressure, pulse and respiratory rate.

8.5.5 Height and weight

Height in centimeters (cm) and body weight (to the nearest 0.1 kilogram in indoor clothing, but without shoes).

8.5.6 Cardiac assessments

An ECG will be performed at screening, day 0 (prior to infusion) and throughout the study as clinically indicated. An ECHO/MUGA test is required to be completed at screening. Clinically significant abnormalities present when the patient signed the informed consent should be reported on the Medical History eCRF page. New or worsened clinically significant findings occurring after informed consent must be recorded on the Adverse Events CRF page. Patients must have a left ventricular ejection fraction (LVEF) >45% to be included into the study.

8.5.7 DLCO or FEV1

Carbon monoxide diffusing capacity (DLCO) or forced expiratory volume in one second (FEV1) will be assessed at screening.

8.5.8 Pregnancy test

Women of childbearing potential will be required to undergo a serum pregnancy test at screening and a urine pregnancy test prior to lymphodepleting chemotherapy.

8.5.9 Replication-competent retrovirus (RCR) testing

Although extremely unlikely, an RCR may be generated during CAR-T-cell manufacturing or following infusion. The development of RCR could represent a risk to both the patient and their

close contacts, and therefore monitoring for RCR will take place during the trial by means of DNA PCR. If a positive RCR assay is obtained from a blood specimen, the patient will be rescheduled for a retest. Should the test be confirmed, public health officials are to be informed and the patient should be followed up intensively together with gene therapy experts, study investigators and Health Authorities.

8.6 Laboratory tests

Laboratory assessments will be performed according to Table 8.3. Additional assessments should be performed between visits as clinically indicated and to follow adverse events or events related to treatment with MLM-CAR44.1 T-cells. See Section 11.6.1 for safety monitoring guidelines following MLM-CAR44.1 T-cell infusion.

TABLE 8-3 LOCAL CLINICAL LABORATORY ASSESSMENTS

Hematology	Hematocrit, hemoglobin, MCHC, MCV, platelets, red blood cells, white blood cells with complete differential, flow cytometry panel
CD44v6 expression	
Chemistry	Glucose, blood urea nitrogen (BUN), creatinine, creatinine clearance (at screening only), sodium, potassium, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, ALT (SGPT), AST (SGOT), magnesium, phosphorus, LDH, ferritin, uric acid and C-reactive protein (CRP), Serum β2-microglobulin, IgA, IgG, IgM
Urinalysis	Macroscopic panel (dipstick) (bilirubin, blood, glucose, ketones, leukocytes esterase, nitrite, Ph, protein, specific gravity). If macroscopic panel is abnormal then perform microscopic panel (red blood cells, white blood cells, casts, crystals, bacteria, epithelial cells)
Coagulation panel	Prothrombin time (PT) or International normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer
Pregnancy screen	Serum or urine tests
Influenza	Rapid Influenza A & B Test
Serology	HAV, HBV, HCV, HIV, VZV, HSV 1-2, HSV-6, CMV, EBV, toxoplasma, treponema pallidum and mycoplasma
MLM-CAR44.1 T-cell PK	Flow cytometry for CAR detection in peripheral blood
MLM-CAR44.1 T-cell PK	Flow cytometry for CAR detection in bone marrow at disease evaluation

TABLE 8-4 CENTRAL LABORATORY ASSESSMENTS

CD44 v6 expression	
MLM-CAR44.1 T-cell PK	Flow cytometry and PCR of peripheral blood**
Cytokine monitoring	Multiplex technology of cytokine plasma levels**
Assessments for ancillary studies	Functional and phenotypic characterization of T cells** CAR T immunogenicity*
Replication competent retrovirus (RCR)	PCR in peripheral blood*

**Studies performed at MolMed laboratory*
***Studies performed at Ospedale Pediatrico Bambino Gesù laboratory*

8.7 Pharmacokinetics (AML and MM)

Blood sampling for local PK evaluations by flow cytometry will be performed during the infusion visit (Day 0) prior to infusion, on Days 7, 14, 21, 28, 60, 90 and 180. Additional unscheduled samples may be collected as needed depending on the clinical course of CRS. An unscheduled PK sample should be collected in case of relapse.

Bone marrow sampling for PK will take place on Day 28, 60, and 180 in AML patients and on Day 28, 90 and 180 in MM patients.

Samples will also be properly stored and sent to a central laboratory, where peripheral blood, and plasma samples will be analyzed through flow cytometry, qPCR and multiplex cytokine technology.

8.8 Ancillary studies

In addition to assessing the efficacy and safety of MLM-CAR44.1 T-cells, the study aims to perform exploratory assessments that address specific features and concerns related to CAR T-cell therapy.

We aim to characterize the cellular composition of circulating LNGFR+ (i.e. CAR-T cells) and LNGFR- T cells in patients on Days 0, 7, 14, 21, 28, 60, 90 and 180. Characterization will include, but is not limited to, the presence of naïve (CD45RA+/CD95-/CD62L+), stem memory (CD45RA+/CD95+/CD62L+), central memory (CD45RA-/CD62L+), effector memory (CD45RA-/CD62L-), terminally differentiated (CD45RA+/CD62L) as well as T regulatory cells. Moreover, the expression of activation and exhaustion markers will be assessed.

If sufficient biological material is available on the sampling days mentioned above, and if more than 10% of LNGFR+ T cells are present in the patient's biological material, functional analyses will be performed including:

1) Activation by challenging with CD44v6+ and CD44v6- AML and MM tumor cell lines. In this case the readout will be specific T cell proliferation and/or cytokine production.
2) By-standard activation of tumor specific T cells recognizing tumor-specific peptides (i.e. WT1, PR1 and NYESO1 pepmix). In this case the readout will be specific T cell cytokine production.

To this aim, frozen PBMC collected at the time points defined above will be shipped to a central laboratory. A priority level has been set in case the recovered PBMC are not sufficient to perform all the proposed analysis.

The immunogenicity of the CAR polypeptide as well as against suicide genes has been described in several studies. If an immune response to MLM-CAR44.1 T-cells occurs, the cells might be rejected. To monitor T cell-mediated immune responses against CAR CD44v6 or TK products, PBMC will be collected at lymphocytes apheresis, d28, d180, or after CAR T-cell disappearance, and shipped to a central laboratory. Samples will be analyzed in case of CAR T-cells disappearance: they will be stimulated in vitro in the presence of irradiated autologous CAR T cells and tested against target cells expressing TKmut2 and /or CAR CD44v6.

Additional samples from peripheral blood and/or bone marrow may be collected after MLM-CAR44.1 T cell infusion according to medical and scientific judgement (i.e. during CRS or other clinically relevant event).

If the patient agrees, the remaining biomarker and/or PK samples collected as well as any patient's baseline lymphocyte apheresis product that is not used for manufacturing and any MLM-CAR44.1 manufactured product that is not infused will be stored for up to 15 years and further analyzed to address scientific questions related to MLM-CAR44.1 or cancer, including research related to improvements in the manufacturing process. The assessments will include but are not limited to immune-related DNA, RNA, or protein markers, qPCR, and/or gene expression profiling. A decision to perform such additional research studies would be based on outcome data from this study or from new scientific findings related to the drug class or disease, as well as on reagent and assay availability.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical design

This is a seamless Phase I/IIa, open label, multicenter clinical trial that combines a Phase I dose-escalation component based on toxicity with a Phase IIa dose expansion based on anti-tumor activity for AML and MM populations separately.

Phase I: To select the Maximum Tolerated Dose (MTD) from the three specified doses, we assumed a maximum sample size of 15 patients (in AML and MM separately) treated in cohorts of three and a target toxicity rate of 30%.

A patient is defined "evaluable" if, after CAR-T cell infusion, can be included in one of the following cases:

- The patient completes the 30 days of safety follow-up period with no occurrence of DLTs;
- The patient experiences a DLT within the completion of 30-day follow-up period;
- The patient dies before the completion of the 30-day follow-up period with no previous occurrence of DLTs. In this case, the patient is considered as having experienced a DLT.

A patient is defined "not evaluable" and should be replaced during the study if one of the following conditions occurs before the completion of the 30-day follow-up period:

- The patient receives a rescue medication (i.e.: Ganciclovir or Valganciclovir administered for Grade 2 or higher bullous dermatitis; Tocilizumab/Siltuximab administered for Grade 3 or higher CRS responsive to therapy within 24 hours OR for Grade 2 or lower CRS OR for Grade 2 or lower CRES) without previous occurrence of DLTs;
- The patient prematurely discontinues the study (alive) without the occurrence of DLTs.

Phase IIa: A total of 14 patients with each indication will be treated at the MTD (AML and MM separately) according to a Simon's two-stage design, considering alpha (type 1 error) = 0.01, power = 0.90 and assuming p_0=0.10 and p_1=0.50, being p_0 the probability of hematological disease response in case of a poorly effective drug and p_1 the desired probability required to proceed to further development.

9.1.1 Primary study variable

Phase I

- DLT

The proportion of patients with DLT occurring within one month from CAR T infusion will be calculated for each dose level along with the 95% exact Clopper-Pearson confidence intervals.

- Overall safety: type and frequency of SAEs, of treatment-emergent AEs and of systemic reactions

SAEs, treatment-emergent AEs and systemic reactions (i.e. fever, tachycardia, nausea and vomiting, joint pain, skin rash) will be summarized for each dose level by system organ class and/or preferred term and severity by means of frequency tables. The description will be done for

1) Pre-infusion period (from the day of apheresis to the day before CAR T-cell infusion

2) Post-infusion period (30 days following CAR T-cell infusion).

- Absence of replication competent retrovirus

The proportion of patients with absence of replication competent retrovirus monitored by DNA PCR at 3, 6, 12 and 24 months after infusion of MLM-CAR44.1 T-cells will be calculated for each dose level. The corresponding 95% exact Clopper-Pearson confidence intervals will also be calculated.

Phase IIa

- Hematologic disease response for AML at 2 months

The percentages of patients either in CR, Cri or PR (ELN criteria) at 2 months following MLM-CAR44.1 T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

- Hematologic disease response for MM at 3 months

The overall response rate (ORR) is defined as the proportion of patients in: stringent complete response (sCR), CR, very good partial response (VGPR) and partial response (PR) (IMWG criteria). The percentages of overall response at 3 months following MLM-CAR44.1 T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

9.1.2 Secondary/exploratory study variables

Phase I

- Hematologic disease response for AML

The percentages of patients either in CR, CRi or PR (ELN criteria) at 1 and 2 months following MLM-CAR44.1 T-cell infusion will be estimated at each dose level and the 95% exact Clopper-Pearson confidence intervals calculated.

- Hematologic disease response for MM

The overall response rate (ORR) is defined as the proportion of patients in: stringent complete response (sCR), CR, very good partial response (VGPR) and partial response (PR) (IMWG criteria). The percentages of overall response at 1 and 3 months following MLM-CAR44.1 T-cell infusion will be estimated at each dose level and the 95% exact Clopper-Pearson confidence intervals calculated.

- Suicide gene activation

The percentage of patients for whom the activation of the suicide gene was needed will be calculated for each dose level along with the corresponding 95% exact Clopper-Pearson confidence intervals.

The levels of transduced cells in patients presenting CRS or other significant MLM-CAR44.1 T-cell related toxicities and treated with ganciclovir will be listed and described at each dose level with measures of location (i.e., mean, median) and variability (i.e., standard deviation, interquartile range, range), as appropriate.

- In vivo pharmacokinetic profile

Details of the pharmacokinetic analysis will be provided in the statistical analysis plan.

- To set-up a validation process among the participating centers for the CD44v6 marking of tumor cells (AML and MM).

Details of the analysis will be provided in the statistical analysis plan.

- Ancillary study

Details of the ancillary study will be provided in the statistical analysis plan

Phase IIa

Efficacy

- Hematologic disease response for AML

The percentages of patients either in CR, CRi or PR (ELN criteria) at 1 and 6 months after MLM-CAR44.1 T-cell infusion and those of patients in morphologic leukemia-free state (MLFS) 1, 2 and 6 months after infusion will be estimated and the 95% exact Clopper-Pearson confidence intervals calculated.

- Hematologic disease response for MM

ORR at 1, 2 and 6 months following MLM-CAR44.1 T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

- Overall Survival (OS)

Overall Survival (OS) is defined as the time from the date of MLM-CAR44.1 T-cell infusion to the date of last follow-up or death due to any cause, whichever occurs first.

Censoring may be due to:

- study closure
- lost to follow-up
- withdrawn consent

A description of the overall survival time will be performed using median and range. The Overall Survival distribution function will be estimated at 2 years using the Kaplan-Meier method on the Full Analysis Set and 95% CI will also be calculated. An additional analysis of OS will be performed not censoring at the new anticancer therapy (including >HSCT), so that these patients should be followed-up for survival.

- Disease-Free Survival (DFS) in AML patients

Disease Free Survival (DFS) is the time from achievement of response to the earliest of the following: lost follow-up, relapse or death due to any cause.

Censoring may be due to:

- study closure
- lost to follow-up
- withdrawn consent
- new anticancer therapy (including HSCT)

A description of the DFS will be performed using median and range. The DFS distribution function will be estimated at 2 years using the Kaplan-Meier method on the Full Analysis Set

and 95% CI will also be calculated. Additionally, a sensitivity analysis of DFS will be performed without censoring at SCT.

- Progression Free Survival (PFS) in MM patients

Progression Free Survival (PFS) is the time from the date of MLM-CAR44.1 T-cell infusion to the earliest of the following: last follow-up, progression/relapse or death due to any cause.

Censoring may be due to:

- study closure
- lost to follow-up
- withdrawn consent.
- new anticancer therapy (including HSCT)

A description of the PFS will be performed using median and range. The PFS distribution function will be estimated at 2 years using the Kaplan-Meier method and 95% CI will also be calculated. Additionally, a sensitivity analysis of PFS will be performed without censoring at SCT.

- Duration of response (DOR) in MM patients

Duration of response (DOR) is defined as the time from achievement of response to the date of last follow-up, relapse or death due to any cause, whichever occurs first.

Censoring may be due to:

- study closure
- lost to follow-up
- withdrawn consent.
- new anticancer therapy (including HSCT)

A description of the DOR will be performed using median and range. The DOR distribution function will be estimated using the Kaplan-Meier method and 95% CI will also be calculated. Additionally, a sensitivity analysis of DOR will be performed without censoring at SCT.

- Event-Free Survival (EFS) in AML patients

Event Free Survival (EFS) is the time from the date of MLM-CAR44.1 T-cell infusion to the date of the earliest of the following: last follow-up, resistance, relapse or death due to any cause.

Censoring may be due to:

- study closure
- lost to follow-up
- withdrawn consent
- new anticancer therapy (including HSCT)

A description of the EFS will be performed using median and range. The EFS distribution function will be estimated at 2 years using the Kaplan-Meier method on the Full Analysis Set and 95% CI will also be calculated. Additionally, a sensitivity analysis of EFS will be performed without censoring at SCT.

- Minimal Residual Disease for AML

The percentages of patients with a molecular CR at 2 months following MLM-CAR44.1 T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

- Minimal Residual Disease for MM

The percentages of patients with a molecular CR at 3 months following MLM-CAR44.1 T-cell infusion will be estimated, and the 95% exact Clopper-Pearson confidence intervals calculated.

Feasibility

- Percentage of eligible patients who undergo both lymphocyte apheresis and CAR T-cell infusion

The percentages of eligible patients who undergo both lymphocyte apheresis and CAR T-cell infusion will be estimated and the 95% exact Clopper-Pearson confidence intervals calculated

- Percentage of eligible patients effectively infused with CAR T-cells after undergoing lymphocyte apheresis.

The percentages of eligible patients effectively infused with CAR T-cells after undergoing lymphocyte apheresis will be estimated and the 95% exact Clopper-Pearson confidence intervals calculated.

Safety

- Overall safety: type and frequency of SAEs, treatment-emergent AEs and systemic reactions

SAEs, treatment-emergent AEs and systemic reactions (i.e. fever, tachycardia, nausea and vomiting, joint pain, skin rash) will be summarized for each dose level by system organ class and/or preferred term and severity by means of frequency tables. The description will be done for 1) the pre-infusion period (from the day of apheresis to the day before CAR T-cell infusion) and 2) for the post-infusion period (30 days following CAR T-cell infusion).

- Absence of replication competent retrovirus

The proportion of patients with absence of replication competent retrovirus monitored by DNA PCR at 3, 6 and 12 and 24 months after infusion of MLM-CAR44.1 T-cells will be calculated. The corresponding 95% exact Clopper-Pearson confidence intervals calculated.

- Absence of abnormal MLM-CAR44.1 T-cell clonal expansion

The proportion of patients with absence of abnormal MLM-CAR44.1 T-cell clonal expansion monitored by clinical and laboratory surveillance, as well as by TCR-Vbeta repertoire study will be calculated 6 months after infusion. The corresponding 95% exact Clopper-Pearson confidence intervals will also be calculated.

- Suicide gene activation

The percentage of patients for whom activation of suicide gene was needed will be calculated along with the corresponding 95% exact Clopper-Pearson confidence intervals.

The levels of transduced cells in patients presenting CRS or other significant MLM-CAR44.1 T-cell related toxicities and treated with ganciclovir will be listed and described with measures of location (i.e., mean, median) and variability (i.e., standard deviation, interquartile range, range), as appropriate.

Pharmacokinetic profile

Details of the pharmacokinetic analysis will be provided in the statistical analysis plan.

Ancillary study

Details of the ancillary study will be provided in the statistical analysis plan

9.1.3 Analysis populations

The analysis sets are shown in the Table below. The analyses will be generally performed separately on AML and MM, unless otherwise specified. The safety and the efficacy analyses will be conducted on the Full Analysis Set.

Analysis set	Description
Full Analysis Set (Safety Set)	All screened patients who have received infusion of MLM-CAR44.1 T-cells
Per-Protocol Set	Subset of the patients in the Full Analysis Set who are compliant with major requirements of the clinical study protocol. Major protocol deviations leading to exclusion from the Full Analysis Set are: - patients who receive a dose which differs for more than 10% of the target dose (±10%).

9.1.4 Data Quality Assurance

Data collection

Designated investigator staff will enter the data required by the protocol into the eCRF using fully validated software that conforms to 21 CFR Part 11 requirements. Designated investigator site staff will not be given access to the Electronic Data Capture system until they are trained.

Web-based software will be used, and no installation procedure is needed. Each site will be authorized by the administrator to access the eCRF. Each site-qualified personnel will be allowed to access the eCRF by means of a 'login mask' requiring user ID and password and may read, modify, and update only the information entered at his or her site and according to their profile. Each page reports site code and subject code.

On-line validation programs will check for data discrepancies and, by generating appropriate error messages, allow the data to be confirmed or corrected before transfer to the CRO working on behalf of the Sponsor. The investigator will certify that the data entered in the eCRF are complete and accurate.

After database lock, the investigator will receive a CD-ROM of subject data for archiving at the investigational site.

Database management and quality control

The CRO working on behalf of the Sponsor will review the data entered in the eCRF by investigational staff for completeness and accuracy and instruct site personnel to make any necessary corrections or additions. The Data Manager will perform the cleaning session by reviewing the warning messages raised by on-line checks and by running post-entry checks by means of validation programs and data listings specific for the study. The occurrence of any protocol deviations will also be checked.

If clarifications are needed, the Data Manager will raise queries through the web application. Designated investigator site staff will be required to respond to queries and the Data Manager will make the correction to the database according to the responses.

Data collection and query flows, as well as the on-line and off-line checks, are detailed in the Data Management Plan and Data Validation documents.

Concomitant medications and prior medications entered into the database will be coded using the WHO Drug Reference List, which employs the ATC classification system. Medical history/current medical conditions and AEs will be coded using MedDRA.

The database will be locked after all the above actions have been completed and the database has been declared complete and accurate.

9.2 Sample size

Phase I

No formal sample size calculation was performed for this study as no inference is foreseen. However, simulation study for the dose escalation process was done to assess the performances of the BOIN design for different sample sizes.

Phase IIa

A total of 14 patients will be treated at the MTD for each indication (AML and MM separately) according to a Simon's two-stage design, considering alpha (type 1 error) = 0.01, power = 0.90 and assuming p_0=0.10 and p_1=0.50, being p_0 the probability of hematological disease response in case of a poorly effective drug and p_1 the desired probability required to proceed to further development.

10 DATA AND SAFETY MONITORING BOARD (DSMB)

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB).

10.1 DSMB roles and responsibilities

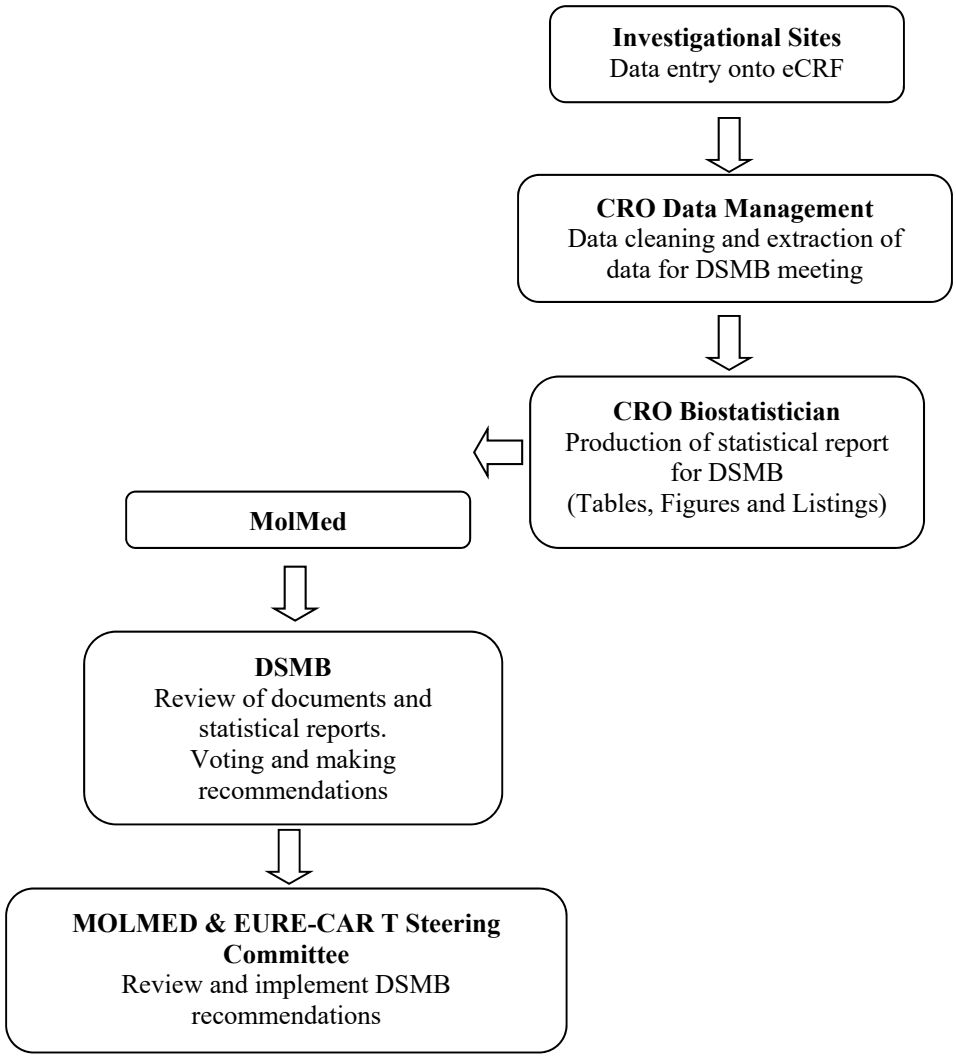
This DSMB will

- Review the Statistical Analysis Plan for DSMB
- Meet periodically to review aggregate and individual subject data related to safety, data integrity and overall conduct of the trial.
- Provide recommendations to continue or terminate the trial depending upon these analyses.
- Communicate other recommendations or concerns as appropriate.
- Suggest/request additional analyses (if necessary)
- Operate according to the procedures described in the DSMB Charter and all regulatory guidelines in place.
- Follow conflict of interest guidelines as detailed in the DSMB Charter.
- Comply with confidentiality procedures as described in the DSMB Charter.
- Maintain documentation and records of all activities as in the DSMB Charter.

10.2 Composition of the DSMB

During Phase I, the DSMB will be made up of a Hematologist with expertise in CAR T-cell therapy, a Biostatistician and a Dermatopathologist collectively have expertise in the management of patients with the study indication, experience in Phase I clinical trials, statistical methods and safety monitoring. A Pediatrician will join the DSMB during phase IIa

10.3 Organizational diagram and data flow



10.4 DSMB governance

The DSMB will operate under the rules of an approved charter and its members will be independent from study conduct and free of conflict of interest.

The initial meeting of the DSMB will be organizational and will be held after the DSMB group has been established to let the members review and approve the DSMB charter, to establish a meeting schedule, review the study modifications and/or termination guidelines and discuss the format of the open and closed reports that will be used to present trial results at future DSMB meetings

11 SAFETY

11.1 Definitions

An **Adverse Event** (**AE**) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a casual relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An **Adverse Drug Reaction** (**ADR**) is any untoward and unintended response to an investigational medicinal product related to any dose administered.

An **Unexpected Adverse Drug Reaction** is any adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure).

A **Serious Adverse Event** (**SAE**) or a **Serious Adverse Drug Reaction** (**SADR**) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is medically significant
- requires inpatient hospitalization or prolongation of existing hospitalization
- hospitalizations for the following reasons should not be reported as SAEs:
  - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
  - treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above

Medical and scientific judgment should be exercised in deciding whether expedited reporting 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. These situations should also usually be considered Serious Adverse Events.

Any clinically significant laboratory test value that meets the definition of a SAE must be reported as a SAE.

11.2 Criteria of evaluation

All adverse events will be recorded on the case report forms (CRFs) according to CTC version 5.0 except for CRS and CRES, which will follow table 11-2. The investigator will decide if the events are drug related and the decision will be recorded for all adverse events.

The adverse events not listed on the NCI CTC grading system will be graded on a five points scale and reported in detail on the CRF, as indicated:

Mild	Discomfort noticed but no disruption of normal daily activity
Moderate	Discomfort sufficient to reduce or affect daily activity
Severe	Inability to work or perform normal daily activity
Life-threatening	Represents an immediate threat of life
Death	Death related to the adverse event

Relationship of the adverse event to the treatment should also be assessed using the following categories for determining relationship:

Not related	The adverse event is clearly not related to the investigational product
Related to MLM-CAR44.1 T-cells	The adverse event is considered related to the investigational product.
Related to lymphocyte apheresis	The adverse event is considered related to the lymphocyte apheresis.
Related to lymphodepleting chemotherapy	The adverse event is considered related to the chemotherapy.
Unknown	This category should be used when it is difficult to determine if the adverse event is drug related or not, or information about it is unavailable, missing or incomplete.

ALL SAEs and AEs are collected regardless of their relationship with the experimental product.

Laboratory abnormalities that constitute an AE (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded on the Adverse Events CRF. Whenever possible, a diagnosis, rather than a symptom should be provided (e.g. anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found.

11.3 Adverse events of special interest (AESI)

The following adverse events are defined as being of special interest: all AE related to study procedures, cytokine release syndrome, any cutaneous toxicity, tumor lysis syndrome, neutropenic fever, cytopenia >28 days, neurotoxicity, any fever/infection with monocytopenia and hepatic events.

11.4 Reporting procedures

11.4.1 Adverse Events

Adverse events that begin or worsen after informed consent/assent will be recorded in the patient's source documents. New or worsening adverse events prior to starting study treatment (lymphocyte apheresis) are to be recorded in the Adverse Events CRF if they meet one of the following criteria:

- All infections
- All clinical AEs grade ≥ 3
- All clinically significant laboratory abnormalities
- All AEs related to a study procedure
- All AEs leading to study discontinuation
- All SAEs.

Once the patient begins lymphocyte apheresis, all new or worsening adverse events regardless of causality will be recorded in the Adverse Events CRF up to the Month 12 visit. Events occurring during salvage chemotherapy but prior to CAR T-cell infusion will however be excluded from the adverse event reporting period, except for SAE.

Following the Month 12 visit, and through the Month 24 visit, adverse events should only be reported and recorded in the Adverse Events CRF if they meet one of the following criteria:

- SAEs
- Events leading to death
- Events related to a study procedure
- Infections: serious or opportunistic infections
- New incidence or exacerbation of a pre-existing neurologic disorder
- New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
- New incidence of other hematologic disorder
- Any condition suspected of being related to MLM-CAR44.1 T-cells.
- Positive RCR test result
- Vector insertion site sequencing result with a mono-or oligoclonality pattern or in a location near a known human oncogene
- New malignancy other than the primary malignancy
- Progressive multifocal leukoencephalopathy (PML)
- Hepatitis B reactivation

11.4.2 Serious Adverse Events

Every SAE, regardless of suspected causality, occurring after signature of informed consent to Month 24 must be reported to **within 24 hours** of learning of its occurrence and be inserted in CRF.

The follow-up information of SAE should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.

Information about all SAEs will be recorded using the safety tool integrated into the eCRF.

In case of technical difficulties, SAE notification can be carried out by contacting the Contract Research Organization (CRO) in charge of Pharmacovigilance via email at all_phv@opis.it or by fax using the following number: Fax: +39 0362 633622; Tel: +39 0362 311, +39 349 3925306

The event is nevertheless to be reported on the eCRF once technical difficulties have been resolved.

As this is a "first in man" study, Serious Adverse Drug Reactions (SADR) are not present in the reference safety information of the Investigator's brochure. All events are therefore to be considered "unexpected" for reporting purposes.

11.4.3 DLTs

All DLTs must be reported **within 24 hours** of learning of their occurrence and be inserted in the specific page of the eCRF

11.5 Procedures to be followed in the event of pregnancy

The investigator shall report all pregnancy exposure occurring in a female patient or in a male patient's partner within 24 hours to the Sponsor using the safety tool integrated into the eCRF.

In case of technical difficulties, pregnancy notification can be carried out by contacting the Contract Research Organization (CRO) OPIS in charge of Pharmacovigilance via email at all_phv@opis.it or by fax using the following number: Fax: +39 0362 633622; Tel: +39 0362 311, +39 349 3925306

The investigator should counsel the subject; discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the subject should continue until conclusion of the pregnancy

11.6 Risk management plan

11.6.1 Safety monitoring

The two most commonly observed toxicities with CAR T-cell therapies are:

- cytokine-release syndrome (CRS), characterized by high fever, hypotension, hypoxia, and/or multiorgan toxicity;
- CAR T-cell related encephalopathy syndrome (CRES), characterized by a toxic encephalopathic state with symptoms of confusion and delirium, and occasionally seizures and cerebral edema (*Neelapu et al., 2017; Lee et al., 2014; Brudno et al., 2016; Maude et al., 2014*).

Rare cases of fulminant macrophage-activation syndrome (MAS) characterized by severe immune activation, lymphohistiocytic tissue infiltration, and immune-mediated multiorgan failure, have also been reported.

11.6.1.1Patient monitoring after CAR-T-cell infusion

Patient hospitalization with close monitoring is recommended for at least 15 days after CAR-T-cell infusion. Monitoring should include assessment of vital signs at least every 4 h, and daily review of organ systems, physical exam, complete blood count with differential, complete metabolic profile, coagulation profiles, and measurement of serum CRP and ferritin levels (see Tables below). Laboratory tests, including blood counts and chemistry panel, might need to be performed more than once daily, especially for patients at high risk of severe CRS and/or CRES, or those with a high tumor burden, who are at risk of tumor lysis; for the latter group,

precautions to avoid tumor lysis should be used, as per standard institutional guidelines. Owing to a high risk of arrhythmias, cardiac monitoring by telemetry is advised from the time of CAR-T-cell infusion until resolution of any emergent CRS symptoms. Additional investigations, such as chest radiography, electrocardiography, echocardiography, electroencephalography (EEG), and imaging studies, can be performed as needed, depending on the toxicities that arise. Daily fluid balance and bodyweights should be strictly monitored, and maintenance of intravenous hydration is recommended for all patients with, or at risk of developing CRS. Central venous access is recommended, preferably with a double or triple lumen catheter, before CAR-T-cell infusion, to facilitate the timely delivery of any medications needed to manage toxicities. Packed red blood cells and platelets can be transfused according to standard institutional guidelines. Corticosteroids should be avoided for the management of fever or for premedication before blood transfusions to avoid limiting the effectiveness of CAR-T-cell therapy, but growth-factor support with filgrastim can be provided for neutropenia. Patients who develop fever should be assessed for infection using blood and urine cultures, chest radiography, and additional tests, such as cytomegalovirus PCR, respiratory viral screening, and CT of the chest, as indicated. conditional orders for fever and hypotension should be put in place for all patients receiving CAR-T-cell infusion, in order that appropriately trained nursing staff can act quickly in the event of toxicities, thereby minimizing delays in intervention (Neelapu et al., 2017).

TABLE 11-1 SUPPORTIVE-CARE CONSIDERATIONS FOR CAR-T-CELL THERAPY

Before and during CAR-T-cell infusion

• Baseline brain MRI to rule out any central nervous system (CNS) disease.
• Central venous access, preferably with double or triple lumen catheter, for intravenous fluid and other infusions in case of toxicities.
• Cardiac monitoring by telemetry starting on the day of CAR-T-cell infusion and continued until cytokine-release syndrome (CRS) resolves to detect arrhythmias.
• Tumor lysis precautions for patients with bulky tumors, as per standard institutional guidelines.
• Hospitalization recommended for at least 15 days after CAR-T-cell therapy

Patient monitoring after CAR-T-cell infusion

- Day 1-15

• Assess vital signs every 4 h, close monitoring of oral and intravenous fluid input and urine output, and daily measurement of bodyweight.
• Daily review of patient history and physical examination.
• Daily blood counts, complete metabolic profiling, and coagulation profiling.
• C-reactive protein and ferritin levels measured daily, starting on the day of infusion.
• Assessment and grading of CRS should be done at least twice daily, and whenever the patient's status changes
• Assessment and grading of CRES using the CAR-T-cell-therapy-associated toxicity 10-point neurological assessment (CARTOX-10; Appendix 7) should be done at least every 8 h.
• Maintenance intravenous fluids with normal saline to ensure adequate hydration.

- Day 15-30

• Assess vital signs every 4 h, close monitoring of oral and intravenous fluid input and urine output, and daily measurement of bodyweight.
• Daily review of patient history and physical examination.
• Daily blood counts,
• Complete metabolic profiling, coagulation profiling, C-reactive protein and ferritin levels measured three times a week.

• Assessment and grading of CRS should be done at least daily, and whenever the patient's status changes
• Assessment and grading of CRES using the CAR-T-cell-therapy-associated toxicity 10-point neurological assessment (CARTOX-10; Appendix 7) should be done daily.

Notifications and contingency orders

• The physician should be notified on detection of any of the following: systolic blood pressure (SBP) >140 mmHg or <90 mmHg; heart rate >120 bpm or <60 bpm, or arrhythmia; respiratory rate >25 breaths per min or <12 breaths per min; arterial oxygen saturation <92% on room air; urine output <1,500 ml per day; upward trend in blood creatinine levels or the results of liver function tests; tremors or jerky movements in extremities; change in mental status (alertness, orientation, speech, ability to write a sentence, or CARTOX-10 score).
• For patients with a temperature ≥38.3 °C, order blood cultures (central and peripheral), urinalysis and urine cultures, portable chest radiography, and notify physician.
• For patients with neutropenia and fever, start empiric broad-spectrum antibiotics.
• Corticosteroids should not be administered unless approved by physician.
• If patient develops CRES, withhold oral intake of food, fluids, and medicine, and notify physician.

11.6.1.2Cytokine release syndrome (CRS)

CRS is the most common toxicity of cellular immunotherapy and is triggered by the activation of T cells on engagement with antigens expressed by tumor cells. The activated T cells release cytokines and chemokines, as do bystander immune cells such as monocytes and/or macrophages. CRS typically presents with constitutional symptoms, such as fever, malaise, anorexia, and myalgias, but can affect any organ system in the body, including cardiovascular, respiratory, integumentary, gastrointestinal, hepatic, renal, hematological, and nervous systems. Patients at high risk of severe CRS include those with bulky disease, comorbidities, and those who develop early onset CRS within 3 days of cell infusion. The onset of CRS toxicity usually occurs within the first week after CART-cell therapy, and typically peaks within 1–2 weeks of cell administration. See Section 1.4 for more details.

CRS should be suspected if at least one of the following four symptoms or signs is present within the first 3 weeks of cellular immunotherapy: fever ≥38° C; hypotension with systolic blood pressure <90 mmHg; hypoxia with an arterial oxygen saturation of <90% on room air; and/or evidence of organ toxicity.

This study will adopt the CRS grading system and treatment recommendations proposed by Neelapu et al. and the CAR T-cell therapy associated TOXicity (CARTOX) Working Group (*Neelapu et al., 2017*), which incorporate and expand on the criteria proposed previously by Lee et al. (*Lee et al., 2014*). The grading system is shown in the Table below:

TABLE 11-2 GRADING OF CYTOKINE-RELEASE SYNDROME (CRS)

Symptom or sign of CRS	CRS grade 1*	CRS grade 2‡	CRS grade 3‡	CRS grade 4‡
Vital signs				
Temperature ≥38 °C (fever)	Yes	Any	Any	Any
Systolic blood pressure <90 mmHg (hypotension)	No	Responds to IV fluids or low-dose vasopressors	Needs high-dose or multiple vasopressors§	Life-threatening
Needing oxygen for SaO_2 >90% (hypoxia)	No	FiO_2 <40%	FiO_2 ≥40%	Needing ventilator support
Organ toxicities‖				
Cardiac: tachycardia, arrhythmias, heart block, low ejection fraction • Respiratory: tachypnea, pleural effusion, pulmonary edema • GI: nausea, vomiting, diarrhea • Hepatic: increased serum ALT, AST, or bilirubin levels • Renal: acute kidney injury (increased serum creatinine levels), decreased urine output • Dermatological: rash (less common) • Coagulopathy: disseminated intravascular coagulation (less common)	Grade 1	Grade 2	Grade 3 or grade 4 transaminitis	Grade 4 except grade 4 transaminitis

1. Adapted from Lee *et al*. The CRS grade should be determined at least twice a day, and whenever a change in the patient's status is observed. ALT, alanine aminotransferase; AST, aspartate aminotransferase; FiO_2, fraction of inspired oxygen; GI, gastrointestinal; IV, intravenous; SaO_2, arterial oxygen saturation.
2. *Grade 1 CRS can manifest as fever and/or grade 1 organ toxicity.
3. ‡For grades 2, 3, or 4 CRS, any one of the criteria other than fever is sufficient.
4. §High-dose vasopressors are defined as any of the following (as reported by Lee *et al*.): noradrenaline ≥20 µg/min; dopamine ≥10 µg/kg/min; phenylephrine ≥200 µg/min; adrenaline ≥10 µg/min; if on vasopressin, vasopressin plus noradrenaline equivalent of ≥10 µg/min; and if on combination vasopressors (not including vasopressin), noradrenaline equivalent of ≥20 µg/min. The noradrenaline equivalent dose is calculated using the VASST trial vasopressor equivalent equation: [noradrenaline (µg/minute)] + [dopamine (µg/kg/minute)/2] + [adrenaline (µg/minute)] + [phenylephrine (µg/minute)/10].
5. ‖Grading of organ toxicities is performed according to Common Terminology Criteria for Adverse Events.

Supportive care and anti-cytokine therapy have been used for effective management of CRS. Prompt responses to tocilizumab have been seen in most patients. Those with a suboptimal response to the first dose of tocilizumab may receive a second or third dose. If the patient experiences ongoing CRS despite administration of repeated doses of tocilizumab, activation of the MLM-CAR44.1 T-cell suicide gene through administration of ganciclovir (GCV) may be considered.

A treatment algorithm for the management of CRS is presented in Table 11-3 and should be followed by investigators. The algorithm is designed to avoid life-threatening toxicities, while allowing the CAR T-cells to proliferate.

TABLE 11-3 CRS MANAGEMENT ALGORITHM

CRS grade	Symptom or sign	Management
Grade 1	Fever or organ toxicity	Acetaminophen and hypothermia blanket for the treatment of fever Ibuprofen can be used as second treatment option for fever, if not contraindicated Assess for infection using blood and urine cultures, and chest radiography Empiric broad-spectrum antibiotics and filgrastim if neutropenic Maintenance intravenous (IV) fluids for hydration Symptomatic management of constitutional symptoms and organ toxicities. Consider tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV for persistent (lasting >3 days) and refractory fever.
Grade 2	Hypotension	IV fluid bolus of 500–1,000 ml of normal saline. Can give a second IV fluid bolus if systolic blood pressure (SBP) remains <90 mmHg. Tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV for the treatment of hypotension that is refractory to fluid boluses; tocilizumab can be repeated after 6 h if needed. If hypotension persists after two fluid boluses and anti-IL-6 therapy, start vasopressors, consider transfer to intensive-care unit (ICU), obtain echocardiogram, and initiate other methods of hemodynamic monitoring In patients at high-risk‡ or if hypotension persists after 1–2 doses of anti-IL-6 therapy, dexamethasone can be used at 10 mg IV every 6 h. Manage fever and constitutional symptoms as in grade 1.
	Hypoxia	Supplemental oxygen. Tocilizumab or siltuximab ± corticosteroids and supportive care, as recommended for the management of hypotension.
	Organ toxicity	Symptomatic management of organ toxicities, as per standard guidelines. Tocilizumab or siltuximab ± corticosteroids and supportive care, as indicated for hypotension.
Grade 3	Hypotension	IV fluid boluses as needed, as recommended for the treatment of grade 2 CRS. Tocilizumab and siltuximab as recommended for grade 2 CRS, if not administered previously. Vasopressors as needed. Transfer to ICU, obtain echocardiogram, and perform hemodynamic monitoring as in the management of grade 2 CRS. Dexamethasone 10 mg IV every 6 h; if refractory, increase to 20 mg IV every 6 h. Manage fever and constitutional symptoms as indicated for grade 1 CRS. Activation of suicide gene with ganciclovir should be considered in case of Grade 3 CRS that does not respond to steroids after tocilizumab administration within 24 hours.
	Hypoxia	Supplemental oxygen including high-flow oxygen delivery and non-invasive positive pressure ventilation. Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above. Activation of suicide gene with ganciclovir as described above.

CRS grade	Symptom or sign	Management
	Organ toxicity	Symptomatic management of organ toxicities as per standard guidelines. Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above. Activation of suicide gene with ganciclovir as described above.
Grade 4	Hypotension	IV fluids, anti-IL-6 therapy, vasopressors, and hemodynamic monitoring as defined for the management of grade 3 CRS. Methylprednisolone 1 g/day IV. Manage fever and constitutional symptoms as in grade 1 CRS. Activate suicide gene with ganciclovir.
	Hypoxia	Mechanical ventilation. Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above. Activate suicide gene with ganciclovir.
	Organ toxicity	Symptomatic management of organ toxicities as per standard guidelines. Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above. Activate suicide gene with ganciclovir.

Neelapu et al. 2017.

All medication doses indicated are for adults. *Maximum amount of tocilizumab per dose is 800 mg. ‡High-risk patients include those with bulky disease, those with comorbidities, and those who develop early onset CRS within 3 days of CAR-T-cell infusion.

Patients with grade 3 or 4 CRS should be treated in the ICU to enable continuous monitoring, management of life-threatening arrhythmias, hemodynamic shock, non-invasive positive pressure ventilation, mechanical ventilation, and/or dialysis. Both anti-IL-6 therapy and corticosteroids should be used for the management of grades 3 and 4 CRS, and the associated organ toxicities. Suicide gene activation should be considered in case of Grade 4 CRS and Grade 3 CRS that does not respond to steroids after tocilizumab administration within 24 hours.

Serum CRP levels are a useful marker to monitor in patients undergoing cellular immunotherapy because IL-6 induces the production of CRP by hepatocytes. Thus, an increase in serum CRP level is typically detected after the onset of CRS and correlates with increased levels of IL-6. Moreover, the return of CRP levels to baseline indicates that the CRS phase of CAR-T-cell therapy has ended, and the patient can be considered for discharge from the hospital, assuming other toxicities that require monitoring and/or intervention have resolved. Of note, the correlation between CRP levels and CRS is variable, and is not observed in all patients.

11.6.1.3CAR T-cell related encephalopathy syndrome (CRES)

The earliest signs of CRES are diminished attention, language disturbance, and impaired handwriting; other symptoms and signs include confusion, disorientation, agitation, aphasia, somnolence, and tremors. In severe cases of CRES (grade >2), seizures, motor weakness, incontinence, mental obtundation, increased intracranial pressure, papilloedema, and cerebral oedema can also occur. The manifestation of CRES can be biphasic, with the first phase

occurring concurrently with high fever and other CRS symptoms within the first 5 days after CAR T-cell therapy, and the second after the fever and other CRS symptoms subside, often beyond 5 days after cell infusion. See Section 1.4 for more details. CRES typically lasts for 2–4 days but can vary in duration from a few hours to weeks.

Like CRS, the management of CRES is based on the toxicity grade (CARTOX-10 – see Appendix 7). Grade 1 CRES are managed primarily through supportive care. The head of the patient's bed should be elevated by at least 30 degrees to minimize aspiration risks and to improve cerebral venous flow. A neurology consultation should be requested for thorough neurological evaluation, including EEG and fundoscopic examination to rule out papilloedema. Neuroimaging and CSF opening pressure, if available, are much better surrogates of increased intracranial pressure and possible cerebral edema than papilloedema; however, lumbar puncture might also be infeasible when patients are restless or have coagulopathy. In patients with an ommaya reservoir, opening pressure can be measured in the supine position with the base of the manometer placed at heart level. Combinations of these techniques should be considered to diagnose increased intracranial pressure and cerebral edema. In particular, repeated neuro imaging, preferably with review of the results by a neuro radiologist, is recommended to detect early signs of cerebral edema in patients with grade 3 or 4 CRES, and in patients with rapid changes in the CRES grade (increase in grade by two levels, for example, grade 1 CRES worsening to grade 3). The clinical status of the patient often dictates the choice of neuroimaging modality: MRI of the brain is preferred, but cannot be performed for unstable or agitated patients, whereas CT can be. The development of cerebral edema in patients treated with CAR T cells is associated with other acute and clinically significant neurological changes, such as a low CARTOX-10 score and/or seizures.

Anti-IL-6 therapy is recommended for patients with CRES and concurrent CRS. If not associated with CRS, corticosteroids are the preferred treatment and can be tapered after improvement. The optimal duration of corticosteroid therapy remains unknown, although short courses of steroids have been associated with resolution of neurological toxicities without impaired antitumor responses. Patients should be monitored closely for recurrence of neurotoxicity symptoms during corticosteroid tapering. Monitoring in the ICU is recommended for patients with grade 3 CRES and is required for all patients with grade 4 CRES because they might need mechanical ventilation for airway protection. Activation of suicide gene with ganciclovir should be considered in grade 3 or higher neurotoxicity. Non-convulsive and convulsive status epilepticus should be managed with benzodiazepines and additional antiepileptics (preferably with levetiracetam), as needed. The response of some patients to benzodiazepine is rapid, with improvements in both EEG findings and mental status. After levetiracetam, phenobarbital is the preferred second antiepileptic for the management of CRES-related seizures: phenytoin and lacosamide are associated with higher risks of cardiovascular adverse effects, therefore, their use in patients with concurrent CRS should be excluded to avoid arrhythmias and hypotension. CRES with raised intracranial pressure should be managed promptly with corticosteroids and acetazolamide; patients who develop grade 4 CRES with cerebral oedema should receive high-dose corticosteroids, hyperventilation, and hyperosmolar therapy (*Neelapu et al., 2017*).

TABLE 11-4 RECOMMENDATIONS FOR MANAGEMENT OF CAR-T-CELL CRES

Grade 1
• Vigilant supportive care; aspiration precautions; intravenous (IV) hydration
• Withhold oral intake of food, medicines, and fluids, and assess swallowing
• Convert all oral medications and/or nutrition to IV if swallowing is impaired
• Avoid medications that cause central nervous system depression
• Low doses of lorazepam (0.25–0.5 mg IV every 8 h) or haloperidol (0.5 mg IV every 6 h) can be used, with careful monitoring, for agitated patients
• Neurology consultation
• Fundoscopic exam to assess for papilloedema
• MRI of the brain with and without contrast; diagnostic lumbar puncture with measurement of opening pressure; MRI spine if the patient has focal peripheral neurological deficits; CT scan of the brain can be performed if MRI of the brain is not feasible
• Daily 30 min electroencephalogram (EEG) until toxicity symptoms resolve; if no seizures are detected on EEG, continue levetiracetam 750 mg every 12 h
• If EEG shows non-convulsive status epilepticus, treat as per algorithm in BOX 3
• Consider anti-IL-6 therapy with tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV, if CRES is associated with concurrent cytokine-release syndrome (CRS)

Grade 2
• Supportive care and neurological work-up as described for grade 1 CRES
• Tocilizumab 8 mg/kg* IV or siltuximab 11 mg/kg IV if associated with concurrent CRS
• Dexamethasone 10 mg IV every 6 h or methylprednisolone 1 mg/kg IV every 12 h if refractory to anti-IL-6 therapy, or for CRES without concurrent CRS
• Consider transferring patient to intensive-care unit (ICU) if CRES associated with grade ≥2 CRS

Grade 3
• Supportive care and neurological work-up as indicated for grade 1 CRES
• ICU transfer is recommended
• Anti-IL-6 therapy if associated with concurrent CRS, as described for grade 2 CRES and if not administered previously
• Corticosteroids as outlined for grade 2 CRES if symptoms worsen despite anti-IL-6 therapy, or for CRES without concurrent CRS; continue corticosteroids until improvement to grade 1 CRES and then taper
• Activation of suicide gene with ganciclovir may be considered
• Consider repeat neuroimaging (CT or MRI) every 2–3 days if patient has persistent grade ≥3 CRES

Grade 4
• Supportive care and neurological work-up as outlined for grade 1 CRES
• ICU monitoring; consider mechanical ventilation for airway protection
• Anti-IL-6 therapy and repeat neuroimaging as described for grade 3 CRES
• High-dose corticosteroids continued until improvement to grade 1 CRES and then taper; for example, methylprednisolone IV 1 g/day for 3 days, followed by rapid taper at 250 mg every 12 h for 2 days, 125 mg every 12 h for 2 days, and 60 mg every 12 h for 2 days
• Activation of suicide gene with ganciclovir

All medication doses indicated are for adults. CAR, chimeric antigen receptor. *Maximum amount of tocilizumab per dose is 800 mg

11.6.1.4On-target/off tumor recognition

CD44v6 has a low level of expression on normal cells, including activated T cells, monocytes, and keratinocytes (see Section 1.4.2.2). Nevertheless, given the potential for off-tumor toxicity of CD44v6 CAR T-cells, patients will be closely monitored for skin toxicities and monocyte levels.

In case of skin rash, it is strongly recommended to perform:

- a dermatological visit to evaluate the possibility of drug reaction
- a dermatological diagnosis by a specialist to identified:
    - specific skin reaction compatible with drug reaction (erythema multiforme, DRESS syndrome, acute generalized exanthematous pustulosis, Stevens-Johnson Syndrome, …) → optional but recommended biopsy
    - nonspecific skin reaction (e.g. esantematic reaction), but compatible with drug reaction → recommended biopsy
    - skin reaction not compatible with drug reaction → if specific diagnosis, optional biopsy; if uncertain diagnosis, recommended biopsy.

Details on biopsy are provided in the *MLM-CAR44.1 Study Manual.*

Antihistamines and topical or systemic cortisone-based drugs should be used for treatments. In case of specific or controversial diagnosis, therapy should be decided by a specialist dermatologist.

A DLT for Phase I of this study is the presence of: NCI-CTC grade 3 or higher erythroderma (generalized exfoliative dermatitis), Stevens-Johnson Syndrome (SJS), Toxic epidermal Necrolysis (TEN), and any other grade 3 or higher skin toxicity suspected of being related to CAR T-cell treatment.

The following off-tumor skin toxicities may set off suicide gene activation through administration of ganciclovir 10 mg/kg/day divided into 2 administrations, or valganciclovir 900 mg twice per day orally for 14 days:

- Grade 3 erythroderma not responsive to steroids (methylprednisolone 2mg/kg for 72 hours or rapidly evolving)
- Grade 2 or higher bullous dermatitis
- Any grade Stevens Johnson SyndromeAny evidence for Toxic Epidermal Necrolysis (TEN)

Patients have to be monitored for SJS and TEN before and after chemotherapy and CAR T cell infusion.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous hypersensitivity reactions. The exact mechanism of Stevens-Johnson syndrome and toxic epidermal necrolysis is unknown; however, one theory holds that altered drug metabolism (eg, failure to clear reactive metabolites) in some patients triggers a T-cell-mediated cytotoxic reaction to drug antigens in keratinocytes. CD8+ T cells have been identified as important mediators of blister formation: SJS/TEN, from an immunologic standpoint, appears to behave most like a delayed-type hypersensitivity reaction (DTH) [9].

The most common drug causes include:

- Sulfa drugs (eg, cotrimoxazole, sulfasalazine)
- Other antibiotics (eg, aminopenicillins [usually ampicillin or amoxicillin], fluoroquinolones, cephalosporins)
- Antiepileptics (eg, phenytoin, carbamazepine, phenobarbital, valproate, lamotrigine)
- Miscellaneous individual drugs (eg, piroxicam, allopurinol, chlormezanone)
- Cases that are not caused by drugs are attributed to:
- Infection (mostly with Mycoplasma pneumoniae)
- Vaccination
- Graft-vs-host disease

Within 1 to 3 week after the start of the offending drug, patients develop a prodrome of malaise, fever, headache, cough, and keratoconjunctivitis. Macules, often in a target configuration, then appear suddenly, usually on the face, neck, and upper trunk. These macules simultaneously appear elsewhere on the body, coalesce into large flaccid bullae, and slough over a period of 1 to 3 days. Nails and eyebrows may be lost along with epithelium. The palms and soles may be involved. Skin, mucosal, and eye pain are common. In some cases, diffuse erythema is the first skin abnormality of toxic epidermal necrolysis.

In severe cases of toxic epidermal necrolysis, large sheets of epithelium slide off the entire body at pressure points (Nikolsky sign), exposing weepy, painful, and erythematous skin. Painful oral crusts and erosions, keratoconjunctivitis, and genital problems (eg, urethritis, phimosis, vaginal synechiae) accompany skin sloughing in up to 90% of cases. Bronchial epithelium may also slough, causing cough, dyspnea, pneumonia, pulmonary edema, and hypoxemia. Glomerulonephritis and hepatitis may develop.

Mortality can be as high as 7.5% in children and 20 to 25% in adults but tends to be lower with early treatment.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are clinically similar except for their distribution. By one commonly accepted definition, changes affect < 10% of body surface area in SJS and > 30% of body surface area in TEN; involvement of 15 to 30% of body surface area is considered SJS/TEN overlap.

Diagnosis is often obvious from appearance of lesions and rapid progression of symptoms. Histologic examination of sloughed skin shows necrotic epithelium, a distinguishing feature.

The severity-of-illness score for TEN (see Table: Severity-of-Illness Score for Toxic Epidermal Necrolysis (SCORTEN)) systematically scores 7 independent risk factors within the first 24 h of presentation to the hospital to determine the mortality rate for a particular patient.

TABLE 11-5 SEVERITY-OF-ILLNESS SCORE FOR TOXIC EPIDERMAL NECROLYSIS (SCORTEN)

Risk* Factor	Score 0	Score 1
Age	< 40 yr	≥ 40 yr
Associated Cancer	No	Yes
Heart rate (beats/min)	<120	≥ 120
Serum BUN, mg/dL (mmol/L)	≤ 28 (10)	> 28 (10)
Serum bicarbonate, mEq/L (mmol/L)	≥ 20 (20)	<20 (20)
Serum glucose, mg/dL (mmol/L)	≤ 252 (14)	>252 (14)

*More risk factors indicate a higher score and a higher mortality rate (%) as follows:

0–1 = 3.2% (CI: 0.1 to 16.7)

2 = 12.1% (CI: 5.4 to 22.5)

3 = 35.3% (CI: 19.8 to 53.5)

4 = 58.3% (CI: 36.6 to 77.9)

≥ 5 => 90% (CI: 55.5 to 99.8)

CI = confidence interval.

Data from Bastuji-Garin S, Fouchard N, Bertocchi M, et al: SCORTEN: A severity-of-illness score for toxic epidermal necrolysis. Journal of Investigative Dermatology 115:149–153, 2000.

Cyclosporine, plasma exchange or IVIG (Intravenous immunoglobulin) and early pulse corticosteroid therapy have been used for treatments.

Potentially causative drugs should be stopped immediately. Patients are isolated to minimize exposure to infection and are given fluids, electrolytes, blood products, and nutritional supplements as needed. Skin care includes prompt treatment of secondary bacterial infections and daily wound care as for severe burns. Prophylactic systemic antibiotics are controversial and often avoided.

Ganciclovir may be administered to set off suicide gene activation in the presence of the following off-tumor skin toxicities:

- Grade 3 erythroderma not responsive to steroids (methylprednisolone 2mg/kg for 72 hours or rapidly evolving)
- Grade 2 or higher bullous dermatitis
- Any grade Stevens-Johnson Syndrome
- Any evidence for Toxic Epidermal Necrolysis

Concerning the potential **on-target/off-tumor risk of monocytopenia**, the following measures of close patient monitoring have been implemented:

- Close monitoring of the haematological parameters, including the measurement of complete blood count with differentia including monocytes, at each clinical study visit
- Precautionary hospitalization in a hematology ward, during the first 15 days after CAR-T dosing

- Twice-weekly measurement of monocytes during the first month after CAR-T dosing, followed by weekly measurement for the second month, every two weeks for the third month, monthly until Month 6 and then every 3 months until Month 24

Patients who remain monocytopenic after MLM-CAR44.1 T cell therapy should be considered immune-depressed. Suicide gene activation may thus also be considered in case of grade 3-4 potentially life-threatening infections.

11.6.1.5Tumor lysis syndrome (TLS)

Patients are to be monitored for TLS before and after chemotherapy and CAR T-cell infusion.

Prophylactic allopurinol, or a non-allopurinol alternative (e.g. febuxostat), and increased oral/IV hydration should be given prior to lymphodepleting chemotherapy and CAR T-cell infusion in patients with elevated uric acid or high tumor burden. Supportive care (i.v. hydration and rasburicase) should be implemented promptly if uric acid continues to rise despite allopurinol/febuxostat and fluids.

Patients should be monitored frequently from the start of lymphodepleting chemotherapy for potassium, phosphorus, calcium, creatinine and uric acid.

Laboratory TLS is defined as two or more of the following values within three days before or

in the days following MLM-CAR44.1 T-cell infusion:

- Uric acid ≥8 mg/dL or 25% increase from baseline
- Potassium ≥6 mEq/L or 25% increase from baseline
- Phosphorus ≥ 4.5 mg/dL or 25% increase from baseline
- Calcium ≤7 mg/dL or 25% decrease from baseline

In case of laboratory TLS, patients should be managed with i.v. fluids, laboratory blood tests every 6 to 8 hours and inpatient care. Cardiac monitoring should be considered, and rasburicase should be considered if uric acid levels remain elevated. If zero or one of the laboratory values are abnormal, continue to manage with allopurinol or a non-allopurinol alternative (e.g. febuxostat) and oral hydration. IV fluids and rasburicase should be considered if uric acid levels remain elevated.

Clinical TLS is defined as the presence of laboratory TLS plus ≥1 of these criteria in the absence of other causes.

- Serum creatinine ≥ 1.5 times the upper limit of normal range
- Symptomatic hypocalcemia
- Cardiac arrhythmia

If Clinical TLS exists, manage with IV fluids, laboratory blood tests every 6 to 8 hours, cardiac monitoring, rasburicase/allopurinol/febuxostat and inpatient care (consider ICU). (*Cairo et al, 2004*).

11.6.1.6Infections

Patients who develop fever should be assessed for infection using blood and urine cultures, chest radiography, and additional tests, such as cytomegalovirus PCR, respiratory viral screening, and CT of the chest, as indicated. Such tests should also be performed before

initiation of CAR-T-cell therapy when infection is suspected. Therapy with CAR T-cells should be delayed until infection has been controlled or ruled out as undiagnosed infections can have catastrophic consequences in patients with CRS, probably owing to exacerbation of systemic inflammation associated with the underlying infection. Empiric broad-spectrum antibiotic therapy, including Gram-negative bacterial coverage, should be implemented because sepsis and CRS have overlapping symptoms and the absence of positive cultures cannot rule out pathogenic infection in immunocompromised patients with cancer (*Neelapu et al., 2017*). Patients who remain monocytopenic after MLM-CAR44.1 T-cell therapy should be considered immune-depressed. Suicide gene activation should thus also be considered in case of grade 3-4 potentially life-threatening infections.

11.6.1.7Uncontrolled T-cell proliferation

MLM-CAR44.1 T-cells could theoretically proliferate without control of normal homeostatic mechanisms. In pre-clinical studies and clinical experience (*Grupp et al 2013, Maude et al 2014*), CTL019 transduced cells have only proliferated in response to physiologic signals or upon exposure to CD19 antigen. In the context of MLM-CAR44.1 T-cell therapy, it is expected that the T cells will proliferate in response to signals from the CD44v6 expressing malignant tumor and normal monocytes. If uncontrolled T-cell proliferation occurs (e.g. expansion in the absence of CD44v6), patients may be treated with corticosteroids such as methylprednisolone (2 mg/kg/d i.v.), chemotherapy, such as high dose cyclophosphamide or ganciclovir to activate the suicide gene.

11.6.1.8Replication-competent retrovirus (RCR) testing.

A review of 29 clinical trials using gene-modified T cell products in HIV and cancer patients that were manufactured with gamma-retroviral vectors reported no RCR in T cell products tested. In addition, in the 629 follow-up patient samples from these trials, ranging from 1 month to 8 years, no RCR were detected (*Bear et al 2012*). The absence of replication-competent RCR will be monitored in this study by DNA PCR for the Galv and gag-pol genes.

11.6.2 Urgent Safety Measures (USM)

To reduce any extra risks that may arise from multicenter clinical trial, appropriate measures have been taken to ensure the prompt communication of serious adverse events and suspected unexpected serious adverse reactions (SUSARs) or serious safety-related protocol deviations between the sponsor, all study sites and investigators, patients and Competent Authorities and IECs according to the regulatory requirements.

In the case of emerging safety issues, the Sponsor via Urgent Safety Measure (USM) dissemination shall inform Competent Authorities and IECs using the fastest means of communication followed by a written report as well as Investigators and participants (at any site) as soon as possible, and at least prior to any planned next dosing.

USMs are a group of expedited actions that can be made in the exceptional circumstances of events occurred that can affect the participating subject's safety and without prior approval of the relevant authorities, which aim is to protect the subjects against any immediate hazard.

Details and procedures on USM management and dissemination are specified in the study Safety Management Plan.

The Investigator must immediately inform the Sponsor in case of any relevant safety issue in the clinical trial which has the potential to expose subjects to an immediate hazard or any unexpected event that is likely to affect the benefit-risk ratio. Sponsor contacts DSMB for making a decision whether USM is necessary to protect participants against any immediate

hazard. If trial participants are at risk, the Sponsor redacts a written document for the urgent safety measure with the proposed corrective/preventative action to be disseminated to the trial Investigators immediately (i.e. within 24 hours of the decision) with acknowledgment of its receipt and implementation received from each Principal Investigator.

12 ETHICS AND GENERAL STUDY ADMINISTRATION

12.1 Ethical aspects

The investigator will ensure that this study is conducted in full conformity with either the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice, Hong Kong, South Africa and Edinburgh) or the laws and regulations of the country in which the study was conducted, whichever affords the greater protection to the individual.

The protocol has been written and the study will be conducted in conformity with the "Guideline for Good Clinical Practice" and local regulations.

12.2 Independent Ethics Committees/Institutional Review Board

This protocol and any accompanying material provided to the subject, such as subject information sheets or descriptions of the study used to obtain informed consent, will be submitted to an Institutional Review Board (IRB)/Independent Ethics Committee (IEC).

Approval from the committee must be obtained before starting the study and should be documented in a letter specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted to the committee in accordance with local procedures and regulatory requirements.

12.3 Informed consent

All patients will be informed of the aims of the study, the possible adverse events and the procedures and possible hazards to which they will be exposed. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that participation is voluntary and that the patient can refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient’s subsequent care.

It is the responsibility of the investigator or a person designated by the investigator (if acceptable by local regulations) to obtain written informed consent from each subject participating in this study before his/her registration in the trial.

For patients unable to read, an impartial witness should be present during the entire informed consent discussion. After the subject and representative have orally consented to participation in the trial, the witness’ signature on the form will attest that the information in the consent form was accurately explained and understood.

For minors (<18 years), parents or legal representatives must give permission by signing a specific informed consent form according to local laws.

If new safety information results in significant changes in the risk/benefit assessment, the consent form is reviewed and approved, if necessary. All patients, including those already being treated, should be informed of the new information and give their consent to continue the study

after receiving a copy of the revised informed consent form. The Eligibility Screening Form ESF and CRF for this study contain a section for documenting informed patient consent and these must be completed appropriately.

12.4 Conditions for modifying the protocol

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator. Protocol modifications must be discussed by a representative of the sponsor and the principal investigator. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Sponsor.

All protocol modifications must be submitted to the appropriate regulatory authorities, Independent Ethics Committee and/or Ministry of Health for information and approval in accordance with local requirements. Approval must be obtained before any changes can be implemented except for changes necessary to eliminate an immediate hazard to trial subjects or when the change(s) involve only logistical or administrative aspect of the trial.

12.5 Conditions for terminating the study

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, MolMed and the investigators will assure that adequate consideration is given to the protection of the patient's interest.

12.6 Study documentation: CRF and record keeping

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into 2 different categories:

- Investigator's study file
- Subject clinical source documents

The investigators' study file will contain the protocol/amendments, CRF (Case Report Form) and query forms, IRB/IEC approvals with correspondence, sample of informed consent, drug/product records, staff CVs and authorization forms and other appropriate documents/correspondence.

Subject clinical source documents include patient hospital/clinic records, physician's and nurse notes, appointment book, original laboratory reports, ECG, EEG, x-ray, pathology and special assessment reports, signed informed consent forms, consultant letters and subject screening and enrolments logs. The investigator must keep these two categories of documents on file until at least 2 years after the last approval of a marketing application or at least 15 years after completion or discontinuation of the study. After that period, the documents may be destroyed, subject to local regulations.

All traceability records should be kept for a minimum of 30 years after the end of study by each party: lymphocyte apheresis establishments/procurement, manufacturer, Sponsor and

investigator/clinical site, as outlined in the annex of "Detailed guidelines on good clinical practice specific to advanced therapy medicinal products" and in Regulation 1394/2007.

Should the investigator not be able to guarantee this archiving requirement at the investigational site for any or all the documents special arrangements must be made between the investigator and MolMed to store these in a sealed container outside of the site so that they can be returned sealed to the investigator in case of regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storing outside of the site.

12.7 Source documents and background data

The investigator shall supply the sponsor with any required background data from the study documentation or clinical records. In case of special problems and/or governmental queries or request for audit inspections, it is also necessary to have access to the complete study records provided that patient confidentiality is protected.

12.8 Audits and inspections

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from the MolMed Quality Assurance Unit or its designees or to health authorities after appropriate notification.

12.9 Case Report Forms

For each patient enrolled, an electronic case report form (eCRF) must be completed by the principal investigator or one of his/her authorized staff members within 5 days from the clinical visit.

If a patient withdraws from the study the reason must be noted on the eCRF.

If a patient is withdrawn from the study because of a treatment limiting adverse event, thorough efforts should be made to clearly document the outcome.

The investigator should ensure the accuracy, completeness and timelines of the data reported to the sponsor in the eCRF and in all required reports.

eCRF instructions will be provided in a detailed manual.

12.10 Monitoring the study

It is understood that the MolMed monitor or designee will contact and visit the investigator regularly and will be allowed on request to inspect the various records of the trial provided that patient confidentiality is maintained in accord with local requirements.

It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to laboratory test reports and other patient records needed to verify the entries on the CRF.

The investigator agrees to cooperate with the monitor to ensure that any problems detected during these monitoring visits are resolved.

The end of study in a center corresponds to the last patient's last visit.

The end of the completion of the trial in all countries corresponds to the date of the end of study at the last center.

12.11 Confidentiality of trial documents and subject records

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor, subjects should not be identified by their names but by an identification code. The investigator should keep a subject enrolment log showing code names. The investigator should maintain documents not for submission to MolMed (e.g. written informed consent) in strict confidence.

12.12 Publication of data and protection of trade secrets

The results of this study may be published or presented at scientific meetings. In this case the investigator agrees to discuss all manuscripts or abstracts with MolMed prior to submission. This allows the sponsor to protect proprietary information and to provide comments based on information from other studies that may not be yet available to the investigator. EURE CAR T requirements for publication and dissemination will also be considered.

In accord with standard editorial and ethical practice, MolMed will generally support publication of multicentric trials only in their entirety and not as individual centre data. Authorship will be determined by agreement.

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Appendix 1: BOIN design

We performed a simulation study in the specific context we are evaluating to

1) define the most suitable sample size for the trial.
2) assess the operating characteristics at the chosen sample size.

We considered a BOIN design with the same parameters of the current study: 3 doses, cohorts of 3 patients, a target toxicity at 30%, a tolerance toxicity interval of 20%-40%, identifying an optimal interval for decision of 24.7%-34.8% and a value for overdose control of 95%.

We simulated 2000 potential trial configurations, using seven different scenarios of toxicity assigned to the doses as follows:

	Scenario	D_1	D_2	D_3
1	**ALL TOX**	0.45	0.55	0.60
2	**D_1**	0.30	0.45	0.55
3	**D_1/D_2**	0.20	0.40	0.50
4	**D_2**	0.15	0.30	0.50
5	**D_2/D_3**	0.15	0.25	0.35
6	**D_3**	0.07	0.15	0.30
7	**NO TOX**	0.01	0.10	0.15

Specifically, i) the first and last scenarios reflect the extreme cases where all or no doses are toxic, ii) scenarios 2, 4 and 6 correspond to MTD exactly at D_1, D_2 and D_3, and iii) in scenarios 3 and 5 the MTD is included between two consecutive doses (i.e. D_1-D_2 and D_2-D_3) and thus not directly assigned to a specific dose.

Evaluation of sample size

To compare the performances of the BOIN design at different sample sizes (n=12, 15, 18) we considered the accuracy in MTD selection. The results in terms of percentage of MTD selection at each dose level are shown in Figure 1.

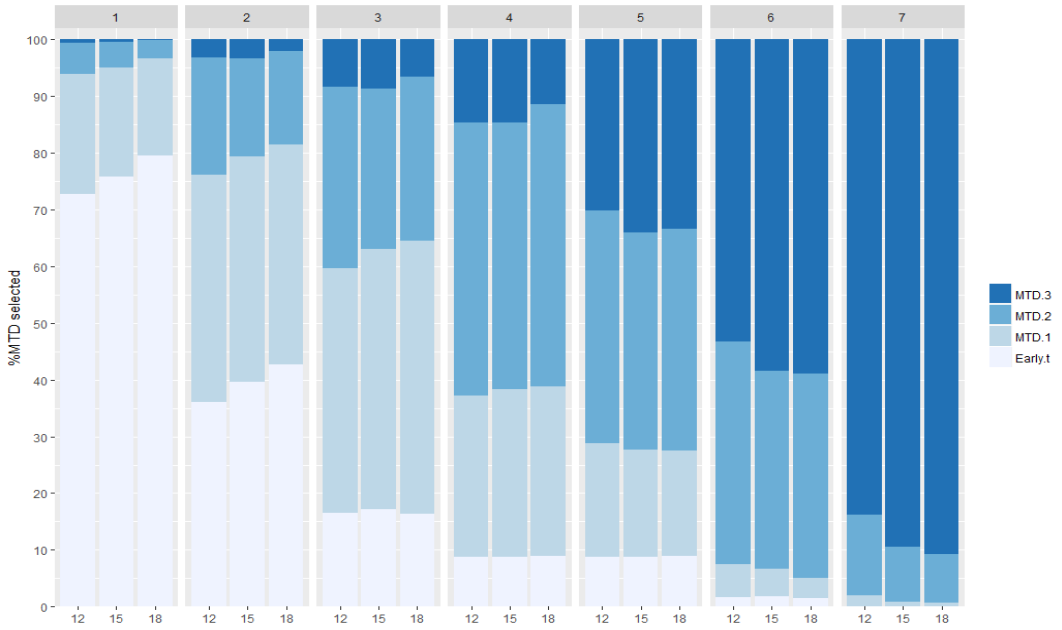

FIGURE 1: PERCENTAGE OF MTD SELECTION AT EACH DOSE LEVEL AND EARLY TERMINATION (WITHOUT SELECTING MTD) IN 7 SCENARIOS OF TOXICITY WITH N=12,15,18

As regards safety, trials are terminated when high levels of toxicity are seen (scenario 1) and the percentage of early termination increase, increasing sample size. Conversely, in the absence of toxicity (scenario 7), larger samples increase the chance of identifying the last dose as MTD. In general, results are similar for n=15 and 18, while the worst performances are consistently seen with n=12. In light of these results, a sample size of 15 patients was defined for the EURE-CART-1 study.

Operating characteristics

The operating characteristics of the BOIN design for the EURE CAR T-1 trial with n=15 are shown in the table below.

		Dose level			% early stopping	Number of pts
		1	**2**	**3**		
1	**True DLT rate**	0.5	0.6	0.6		
	Selection %	19.3	4.5	0.5	75.8	7.7
	# pts treated	6.0	1.5	0.2		
2	**True DLT rate**	**0.3**	0.5	0.6		
	Selection %	39.7	17.4	3.3	39.7	11.4
	# pts treated	7.1	3.6	0.7		
3	**True DLT rate**	***0.2***	***0.4***	0.5		
	Selection %	45.9	28.3	8.7	17.2	13.3

		Dose level			% early stopping	Number of pts
		1	2	3		
	# pts treated	6.7	5.2	1.3		
4	**True DLT rate**	0.2	**0.3**	0.5		
	Selection %	29.4	47.1	14.7	8.9	14.1
	# pts treated	5.7	6.0	2.4		
5	**True DLT rate**	0.15	***0.25***	***0.5***		
	Selection %	18.9	38.3	34.0	8.9	14.1
	# pts treated	5.3	5.5	3.3		
6	**True DLT rate**	0.1	0.2	**0.3**		
	Selection %	4.9	34.9	58.4	1.9	14.8
	# pts treated	3.9	5.2	5.7		
7	**True DLT rate**	0.0	0.1	0.2		
	Selection %	0.9	9.7	89.4	0	15.0
	# pts treated	3.2	4.3	7.5		

The operating characteristics show that the BOIN design selects the true MTD with high probabilities and allocates more patients to the dose levels with the DLT rate closest to the target of 0.3.

In Figure 2A we have also reported the most common potential trial configurations (≥1%) in scenarios 4, from the most to the less probable, with the occurrence (n, %) reported in each pattern. In each scatter-plot the dose escalation process and the MTD selection following the BOIN design are shown. The x and y axes represent patients and doses, respectively, while the individual DLT response to treatment are reported as: no toxicity=filled circle and toxicity=cross. The horizontal red line corresponds to the selected MTD.

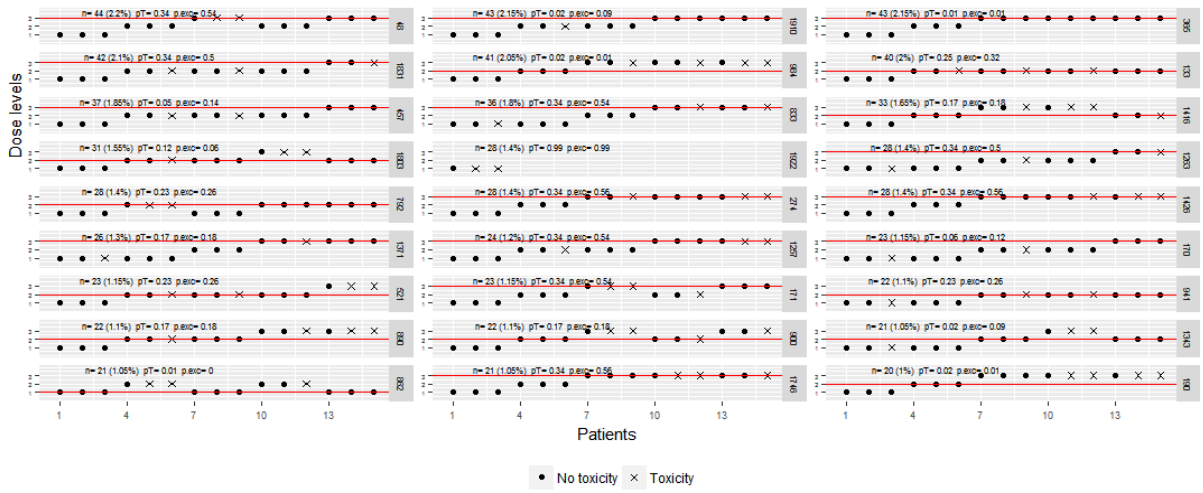

FIGURE 2: MOST COMMON TOXICITY PATTERNS SEEN IN THE SIMULATION OF SCENARIO 4. NUMBERS OUTSIDE THE PLOTS INDICATE THE SIMULATION ID

Appendix 2: International Staging System (ISS) and revised-ISS (R-ISS) for multiple myeloma

Table 1. Standard Risk Factors for MM and the R-ISS

Prognostic Factor	Criteria
ISS stage	
I	Serum β_2-microglobulin $<$ 3.5 mg/L, serum albumin $\geq$ 3.5 g/dL
II	Not ISS stage I or III
III	Serum β_2-microglobulin $\geq$ 5.5 mg/L
CA by iFISH	
High risk	Presence of del(17p) and/or translocation t(4;14) and/or translocation t(14;16)
Standard risk	No high-risk CA
LDH	
Normal	Serum LDH $<$ the upper limit of normal
High	Serum LDH $>$ the upper limit of normal
A new model for risk stratification for MM	
R-ISS stage	
I	ISS stage I and standard-risk CA by iFISH and normal LDH
II	Not R-ISS stage I or III
III	ISS stage III and either high-risk CA by iFISH or high LDH

Abbreviations: CA, chromosomal abnormalities; iFISH, interphase fluorescent in situ hybridization; ISS, International Staging System; LDH, lactate dehydrogenase; MM, multiple myeloma; R-ISS, revised International Staging System.

Palumbo et al. 2015

Appendix 3: European LeukemiaNet (ELN) Response Criteria for Acute Myeloid Leukemia

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested and by method used.
Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100 000/μL)	MRD^+ or unknown
CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100 000/μL])	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1-2 clinical trials
Treatment failure		
Primary refractory disease	No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	
Death in aplasia	Deaths occurring ≥7 d following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 d of death, without evidence of persistent leukemia	
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 d following its completion; or deaths occurring ≥7 d following completion of initial therapy with no blasts in the blood, but no bone marrow examination available	
Response criteria for clinical trials only		
Stable disease	Absence of CR_{MRD-}, CR, CR_i, PR, MLFS; and criteria for PD not met	Period of stable disease should last at least 3 months
Progressive disease (PD)[*],[†]	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood:	Category mainly applies for older patient given low-intensity or single-agent "targeted therapies"

Category	Definition	Comment
	• >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 mo.; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/μL], and/or platelet count to >50 × 10^9/L [50 000/μL] nontransfused); or	In general, at least 2 cycles of a novel agent should be administered
	• >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome)[†]; or	Some protocols may require blast increase in 2 consecutive marrow assessments at least 4 wk apart; the date of progression should then be defined as of the first observation date
	• New extramedullary disease	Some protocols may allow transient addition of hydroxyurea to lower blast counts
		"Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms
Relapse		
Hematologic relapse (after CR_{MRD-}, CR, CR_i)	Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
	If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported.

From Dohner et al., Blood 2017; 129(4): 424-447
ANC, absolute neutrophil count; IDH, isocitrate dehydrogenase; MLFS, morphologic leukemia-free state; WBC, white blood cell.
*The authors acknowledge that this new provisional category is arbitrarily defined; the category aims at harmonizing the various definitions used in different clinical trials.
†Certain targeted therapies, for example those inhibiting mutant IDH proteins, may cause a differentiation syndrome, that is, a transient increase in the percentage of bone marrow blasts and an absolute increase in blood blasts; in the setting of therapy with such compounds, an increase in blasts may not necessarily indicate PD.

Appendix 4: Modified International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma

Response	*IMWG criteria*
Stringent complete response (sCR)	CR as defined below plus normal FLC ratio and absence of clonal cells in bone marrow[1] by immunohistochemistry or immunofluorescence.[2]
Complete response (CR)	Negative immunofixation on serum and urine and disappearance of any soft tissue plasmacytomas and < 5% plasma cells in bone marrow.[1]
Very good partial response (VGPR)	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥ 90% reduction in serum M-protein plus urine M-protein level < 100 mg/24 h
Partial response (PR)	≥ 50% reduction of serum M-protein and reduction in 24 hours urinary M-protein by ≥90% or to < 200 mg/24 h. If serum and urine M-protein are unmeasurable, [3] a ≥ 50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria. If serum and urine M-protein are not measurable, and serum free light assay is also not measurable, ≥ 50% reduction in plasma cells is required in place of M protein, provided baseline bone marrow plasma cell percentage was ≥ 30%. In addition to the above listed criteria, if present at baseline, a ≥ 50% reduction in the size of soft tissue plasmacytomas is also required.
Minor (Minimal) Response (MR)	25-49% reduction of serum M-protein and reduction in 24-hour urine M-protein by 50-89%, which still exceeds 200 mg per 24 hours. In addition, if present at baseline, 25-49% reduction in the size of soft tissue plasmacytomas is also required. No increase in the size or number of lytic bone lesions (development of compression fracture does not exclude response).
No change/Stable disease (SD)	Not meeting criteria for CR, VGPR, PR, or progressive disease.
Progressive disease (PD)[3]	Any of the following: • Increase of ≥ 25% from lowest response value in any one or more of the following: o Serum M-component and/or (the absolute increase must be ≥ 0.5 g/dL)[4] o Urine M-component and/or (the absolute increase must be ≥ 200 mg/24 h) o Only in patients without measurable serum and urine M-protein levels; the difference between involved and uninvolved FLC levels. The absolute increase must be > 10 mg/dL o Bone marrow plasma cell percentage; the absolute percentage must be ≥ 10%[5] • Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas (≥50% increase from nadir in SPD[7] of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in the short axis).

Response	*IMWG criteria*
	• Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.87 mmol/L) that can be attributed solely to the plasma cell proliferative disorder
Relapse	Clinical relapse requires one or more of: Direct indicators of increasing disease and/or end organ dysfunction (CRAB features).[4] It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice • Development of new soft tissue plasmacytomas or bone lesions • Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of the measurable lesion • Hypercalcemia (> 11.5 mg/dL) [2.87 mmol/L] • Decrease in hemoglobin of > 2 g/dL [1.24 mmol/L] • Rise in serum creatinine by 2 mg/dL or more [177 μmol/L or more]
Relapse from CR[3] (To be used only if the endpoint studied is DFS)[6]	Any one or more of the following: • Reappearance of serum or urine M-protein by immunofixation or electrophoresis • Development of ≥ 5% plasma cells in the bone marrow[5] • Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion, or hypercalcemia)

Note: A clarification to IMWG criteria for coding CR and VGPR in patients in whom the only measurable disease is by serum FLC levels: CR in such patients is defined as a normal FLC ratio of 0.26–1.65 in addition to CR criteria listed above. VGPR in such patients is defined as a >90% decrease in the difference between involved and uninvolved free light chain (FLC) levels.

[1] Confirmation with repeat bone marrow biopsy not needed.

[2] Presence/absence of clonal cells is based upon the kappa/lambda ratio. An abnormal kappa/lambda ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is kappa/lambda of > 4:1 or < 1:2.

[3] All relapse categories require two consecutive assessments made at any time before classification as relapse or disease progression and/or the institution of any new therapy. In the IMWG criteria, CR patients must also meet the criteria for progressive disease shown here to be classified as progressive disease for the purposes of calculating time to progression and progression-free survival. The definitions of relapse, clinical relapse and relapse from CR are not to be used in calculation of time to progression or progression-free survival.

[4] For progressive disease, serum M-component increases of >1 gm/dL are sufficient to define relapse if starting M-component is >5 g/dL.

[5] Relapse from CR has the 5% cut-off versus 10% for other categories of relapse.

[6] For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease.

[7] SPD = sum of the products of the maximal perpendicular diameters of measured lesions

Appendix 5: WHO classification of myeloid leukemia

AML with certain genetic abnormalities

- AML with a translocation between chromosomes 8 and 21
- AML with a translocation or inversion in chromosome 16
- APL with the PML-RAPA fusion gene
- AML with a translocation between chromosomes 9 and 11
- AML with a translocation between chromosomes 6 and 9
- AML with a translocation or inversion in chromosome 3
- AML (megakaryoblastic) with a translocation between chromosomes 1 and 22
- AML with the BCR-ABL1 (BCR-ABL) fusion gene*
- AML with mutated NPM1 gene
- AML with biallelic mutations of the CEBPA gene (that is, mutations in both copies of the gene)
- AML with mutated RUNX1 gene

*This is still a "provisional entity," meaning it's not yet clear if there's enough evidence that it's a unique group

AML with myelodysplasia-related changes

AML related to previous chemotherapy or radiation

AML not otherwise specified (This includes cases of AML that don’t fall into one of the above groups and is similar to the FAB classification.)

- AML with minimal differentiation (M0)
- AML without maturation (M1)
- AML with maturation (M2)
- Acute myelomonocytic leukemia (M4)
- Acute monocytic leukemia (M5)
- Acute erythroid leukemia (M6)
- Acute megakaryoblastic leukemia (M7)
- Acute basophilic leukemia
- Acute panmyelosis with fibrosis

Myeloid sarcoma (also known as granulocytic sarcoma or chloroma)

Myeloid proliferations related to Down syndrome

Undifferentiated and biphenotypic acute leukemias (leukemias that have both lymphocytic and myeloid features). Sometimes called ALL with myeloid markers, AML with lymphoid markers, or mixed phenotype acute leukemias.

Appendix 6: ECOG performance status scale

Grade	Performance scale
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix 7: Grading of CAR-T-cell-related encephalopathy syndrome (CRES)

Symptom or sign	Grade 1	Grade 2	Grade 3	Grade 4
Neurological assessment score (CARTOX-10*)	7-9 (mild impairment)	3-6 (moderate impairment)	0-2 (severe impairment	Patient in critical condition, and/or obtunded and cannot perform assessment of tasks
Raised intracranial pressure	NA	NA	Stage 1–2 papilloedema‡, or CSF opening pressure <20 mmHg	Stage 3–5 papilloedema‡, or CSF opening pressure ≥20 mmHg, or cerebral oedema
Seizures or motor weakness	NA	NA	Partial seizure, or non-convulsive seizures on EEG with response to benzodiazepine	Generalized seizures, or convulsive or non-convulsive status epilepticus, or new motor weakness

CAR, chimeric antigen receptor; CARTOX-10, CAR-T-cell-therapy associated toxicity 10-point neurological assessment CSF, cerebrospinal fluid; EEG, electroencephalogram; NA, not applicable.
*In the CARTOX-10, one point is assigned for each of the following tasks that is performed correctly (normal cognitive function is defined by an overall score of 10): orientation to year, month, city, hospital, and President/Prime Minister of country of residence (total of 5 points); name three objects — for example, point to clock, pen, button (maximum of 3 points); write a standard sentence, for example, 'our national bird is the bald eagle' (1 point); count backwards from 100 in tens (1 point).
‡Papilloedema grading is performed according to the modified Frisén scale[98].

Appendix 8: Common terminology criteria for Adverse Events

In the present study adverse events and/or adverse drug reactions will be recorded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.
At the time this protocol was issued, the full CTC document was available on the NCI web site, at the following address:
http://ctep.cancer.gov/reporting/ctc.html

Appendix 9: 2017 European LeukemiaNet (ELN) recommendations for diagnosis and management of AML

AML and related precursor neoplasms, and acute leukemias of ambiguous lineage (WHO 2016)

AML and related neoplasms	AML and related neoplasms (cont'd)
AML with recurrent genetic abnormalities	Acute myelomonocytic leukemia
AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1	Acute monoblastic/monocytic leukemia
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11	Pure erythroid leukemia#
Acute promyelocytic leukemia with PML-RARA*	Acute megakaryoblastic leukemia
AML with t(9;11)(p21.3;q23.3); MLLT3-KMT2A†	Acute basophilic leukemia
AML with t(6;9)(p23;q34.1); DEK-NUP214	Acute panmyelosis with myelofibrosis
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1)	Myeloid sarcoma
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); RBM15-MKL1‡	Myeloid proliferations related to Down syndrome
Provisional entity: AML with BCR-ABL1	Transient abnormal myelopoiesis
AML with mutated NPM1§	Myeloid leukemia associated with Down syndrome
AML with biallelic mutations of CEBPA§	Blastic plasmacytoid dendritic cell neoplasm
Provisional entity: AML with mutated RUNX1	Acute leukemias of ambiguous lineage
AML with myelodysplasia-related changes‖	Acute undifferentiated leukemia
Therapy-related myeloid neoplasms{	MPAL with t(9;22)(q34.1;q11.2); BCR-ABL1**
AML, NOS	MPAL with t(v;11q23.3); KMT2A rearranged
AML with minimal differentiation	MPAL, B/myeloid, NOS
AML without maturation	MPAL, T/myeloid, NOS
AML with maturation	

For a diagnosis of AML, a marrow blast count of $20% is required, except for AML with the recurrent genetic abnormalities t(15;17), t(8;21), inv(16), or t(16;16). Adapted from Arber et al.[3]

MPAL, mixed phenotype acute leukemia; NK, natural killer.

*Other recurring translocations involving RARA should be reported accordingly: for example, AML with t(11;17)(q23;q12); ZBTB16-RARA; AML with t(11;17)(q13;q12); NUMA1-RARA; AML with t(5;17)(q35;q12); NPM1-RARA; or AML with STAT5B-RARA (the latter having a normal chromosome 17 on conventional cytogenetic analysis).

†Other translocations involving KMT2A (MLL) should be reported accordingly: for example, AML with t(6;11)(q27;q23.3); MLLT4-KMT2A; AML with t(11;19)(q23.3;p13.3); KMT2A-MLLT1; AML with t(11;19)(q23.3;p13.1); KMT2A-ELL; AML with t(10;11)(p12;q23.3); MLLT10-KMT2A.

‡Rare leukemia most commonly occurring in infants.

§Diagnosis is made irrespective of the presence or absence of multilineage dysplasia.

‖At least 20% ($20%) blood or marrow blasts AND any of the following: previous history of MDS or MDS/MPN; myelodysplasia-related cytogenetic abnormality (see list below); multilineage dysplasia; AND absence of both prior cytotoxic therapy for unrelated disease and aforementioned recurring genetic abnormalities. Cytogenetic abnormalities sufficient to diagnose AML with myelodysplasia-related changes are: Complex karyotype (defined as 3 or more chromosomal abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with BCR- ABL1); Unbalanced abnormalities: 27 or del(7q); 25 or del(5q); i(17q) or t(17p); 213 or del(13q); del(11q); del(12p) or t(12p); idic(X)(q13); Balanced abnormalities: t(11; 16)(q23.3;p13.3); t(3;21)(q26.2;q22.1); t(1;3)(p36.3;q21.2); t(2;11)(p21;q23.3); t(5;12)(q32;p13.2); t(5;7)(q32;q11.2); t(5;17)(q32;p13.2); t(5;10)(q32;q21.2); t(3;5) (q25.3;q35.1).

{Cases should be classified with the related genetic abnormality given in the diagnosis.

#The former subgroup of acute erythroid leukemia, erythroid/myeloid type ($50% bone marrow erythroid precursors and $20% myeloblasts among nonerythroid cells) was removed; myeloblasts are now always counted as percentage of total marrow cells. The remaining subcategory AML, NOS, pure erythroid leukemia requires the presence of .80% immature erythroid precursors with $30% proerythroblasts.

**BCR-ABL1[1] leukemia may present as MPAL; treatment should include a tyrosine kinase inhibitor.

2017 ELN risk stratification by genetics

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); RUNX1-RUNX1T1
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
	Mutated NPM1 without FLT3-ITD or with FLT3-ITD^{low}†
	Biallelic mutated CEBPA
Intermediate	Mutated NPM1 and FLT3-ITD^{high}†
	Wild-type NPM1 without FLT3-ITD or with FLT3-ITD^{low}† (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); MLLT3-KMT2A‡
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); DEK-NUP214
	t(v;11q23.3); KMT2A rearranged
	t(9;22)(q34.1;q11.2); BCR-ABL1
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1)
	25 or del(5q); 27; 217/abn(17p)
	Complex karyotype,§ monosomal karyotype‖
	Wild-type NPM1 and FLT3-ITD^{high}†
	Mutated RUNX1{
	Mutated ASXL1{
	Mutated TP53#

Frequencies, response rates, and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.

*Prognostic impact of a marker is treatment-dependent and may change with new therapies.

†Low, low allelic ratio (,0.5); high, high allelic ratio ($0.5); semiquantitative assessment of FLT3-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve "FLT3-ITD" divided by area under the curve "FLT3- wild type"; recent studies indicate that AML with NPM1 mutation and FLT3-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic HCT.[57-59,77]

‡The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

§Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with BCR-ABL1.

‖Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).[116]

{These markers should not be used as an adverse prognostic marker if they co- occur with favorable-risk AML subtypes.

#TP53 mutations are significantly associated with AML with complex and monosomal karyotype.[37,66-69]

Response criteria in AML

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD2})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	Bone marrow blasts ,5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC $1.0 3 10^9/L (1000/mL); platelet count $100 3 10^9/L (100 000/mL)	MRD[1] or unknown
CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia (,1.0 3 10^9/L [1000/mL]) or thrombocytopenia (,100 3 10^9/L [100 000/mL])	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts ,5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1-2 clinical trials
Treatment failure		
Primary refractory disease	No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	Regimens containing higher doses of cytarabine (see Table 8) are generally considered as the best option for patients not responding to a first cycle of 7 + 3; the likelihood of responding to such regimens is lower after failure of a first
Death in aplasia	Deaths occurring $7 d following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 d of death, without evidence of persistent leukemia	
Death from indeterminate cause	Deaths occurring before completion of therapy, or ,7 d following its completion; or deaths occurring $7 d following completion of initial therapy with no blasts in the blood, but no bone marrow examination available	
Response criteria for clinical trials only		
Stable disease	Absence of CR_{MRD2}, CR, CR_i, PR, MLFS; and criteria for PD not met	Period of stable disease should last at least 3 mo.
Progressive disease (PD)*,†	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood: - .50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with ,30% blasts at baseline; or persistent marrow blast percentage of .70% over at least 3 mo; without at least a 100% improvement in ANC to an absolute level (.0.5 3 10^9/L [500/mL], and/or platelet count to .50 3 10^9/L [50 000/mL] nontransfused); or - .50% increase in peripheral blasts (WBC 3 % blasts) to .25 3 10^9/L (.25 000/mL) (in the absence of differentiation syndrome)†; or - New extramedullary disease	Category mainly applies for older patient given low-intensity or single-agent "targeted therapies" in clinical trials In general, at least 2 cycles of a novel agent should be administered Some protocols may require blast increase in 2 consecutive marrow assessments at least 4 wk apart; the date of progression should then be defined as of the first observation date Some protocols may allow transient addition of hydroxyurea to lower blast counts *Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms
Relapse		
Hematologic relapse (after CR_{MRD2}, CR, CR_i)	Bone marrow blasts $5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD2})	If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

ANC, absolute neutrophil count; IDH, isocitrate dehydrogenase; MLFS, morphologic leukemia-free state; WBC, white blood cell.

*The authors acknowledge that this new provisional category is arbitrarily defined; the category aims at harmonizing the various definitions used in different clinical trials.

†Certain targeted therapies, for example, those inhibiting mutant IDH proteins, may cause a differentiation syndrome, that is, a transient increase in the percentage of bone marrow blasts and an absolute increase in blood blasts; in the setting of therapy with such compounds, an increase in blasts may not necessarily indicate PD.

Selected conventional care regimens for patient with AML

Selected conventional care regimens

Patients eligible for intensive chemotherapy	
Induction therapy (all ages) ("713")*,†,‡	• 3 d of an IV anthracycline; daunorubicin at least 60 mg/m^2; idarubicin 12 mg/m^2; or mitoxantrone 12 mg/m^2, and 7 d of continuous infusion cytarabine (100-200 mg/m^2)
Consolidation therapy‡,§	
Younger patients (18-60/65 y)	
• Favorable-risk genetics	• 2-4 cycles of IDAC (1000-1500 mg/m^2 IV over 3 h q12h, d1-3; or 1000-1500 mg/m^2 IV over 3 h d1-5 or 6)
• Intermediate-risk genetics	• Allogeneic HCT from matched-related or unrelated donor
	• 2-4 cycles of IDAC (1000-1500 mg/m^2 IV over 3 h q12h, d1-3; or 1000-1500 mg/m^2 IV over 3 h d1-5 or 6), or
	• High-dose therapy and autologous HCT
• Adverse-risk genetics	• Allogeneic HCT from matched-related or unrelated donor
Older patients (.60/65 y)	
• Favorable-risk genetics	• 2-3 cycles of IDAC (500-1000 mg/m^2 IV over 3 h q12h, d1-3; or 500-1000 mg/m^2 IV over 3 h d1-5 or 6)
• Intermediate/adverse-risk genetics	• No established value of intensive consolidation therapy; consider allogeneic HCT in patients with low HCT-Comorbidity Index, or investigational therapy
Patients considered not candidates for intensive chemotherapy‖	
Azacitidine{	75 mg/m^2, SC, d1-7, q4 wk, until progression
Decitabine#	20 mg/m^2, IV, d1-5, q4 wk, until progression
Low-dose cytarabine**	Low-dose cytarabine (20 mg q12h, SC, d1-10, q4 wk; until progression); not recommended in patients with adverse-risk genetics
Best supportive care	Including hydroxyurea; for patients who cannot tolerate any antileukemic therapy, or who do not wish any therapy
Common salvage regimens in patients not responding to a first induction cycle or with relapsed disease who are candidates for intensive therapy	
IDAC†† (with or without anthracycline)	IDAC (1000-1500 mg/m^2 IV over 3 h q12 h, d1-3 [500-1000 mg/m^2 in patients .60 y]; or 1000-1500 mg/m^2 IV over 3 h d1-5 or 6 [500-1000 mg/m^2 in patients .60 y]); with or without daunorubicin 45-60 mg/m^2, IV, d1-3; idarubicin 8-10 mg/m^2, IV, d3-5, or mitoxantrone 8-10 mg/m^2, IV, d1-3
FLAG-IDA‡‡	Fludarabine 30 mg/m^2 IV, d2-6; cytarabine 1500-2000 mg/m^2 IV over 3 h, starting 4 h after fludarabine infusion, d2-6; idarubicin 10 mg/m^2 IV, d2-4; G-CSF 5 mg/kg, SC, d1-5; additional G-CSF may be administered starting 7 d after end of chemotherapy until WBC count .500/uL Consider dose reduction in patients .60 y: fludarabine 20 mg/m^2; cytarabine 500-1000 mg/m^2; idarubicin 8 mg/m^2
MEC	Mitoxantrone 8 mg/m^2, d1-5; etoposide 100 mg/m^2, d1-5; cytarabine 1000 mg/m^2, d1-5
Allogeneic HCT	Consider transplantation for patients with primary refractory disease, for patients in second CR or with major cytoreduction but still active disease following salvage therapy Consider second transplantation under certain conditions (see "Salvage treatment") Perform early HLA typing

Patients should go on clinical trials if possible.

EMA, European Medicines Agency; FLAG-AMSA, FLAG 1 amsacrine; FLAG-MITO, FLAG 1 mitoxantrone; q, every; SC, subcutaneously.

*Regimens containing higher doses of cytarabine are generally considered as the best option for patients not responding to a first cycle of "713" (see common salvage regimens).

†Older patients (in general .65 y) and patients with adverse genetics are less likely to respond to conventional induction therapy and may receive hypomethylating agents, or, preferably, investigational therapy.

‡Patients, at least those aged 18 to 60 y, with newly diagnosed AML and activating FLT3 mutations may be considered to receive additional therapy with midostaurin (administered after the chemotherapy).[61]

§Results from assessment of MRD should be taken into account for selecting the appropriate consolidation therapy.

‖For discussion of patients not considered candidates for intensive chemotherapy see first 2 paragraphs of "Current therapy."

{Approved by FDA and EMA for adult patients who are not eligible for HCT with AML with 20% to 30% blasts and multilineage dysplasia; in addition, approved by EMA for patients who are not eligible for allogeneic HCT with AML with .30% marrow blasts.

#Approved by EMA (not by FDA) for patients with newly diagnosed de novo or secondary AML, who are not candidates for standard induction chemotherapy.

**In some countries used in a dosage of 20 mg/m2 SC once daily.

††Evidence from pharmacologic studies and clinical trials in first-line treatment indicate that doses higher than 1500 mg/m2 are above the plateau of the maximal therapeutic effect;147 single-agent IDAC should not be used in patients relapsing within 6 mo following consolidation with higher doses of cytarabine.

‡‡Idarubicin may be replaced by mitoxantrone 10 mg/m2, IV, days 2 to 4 (FLAG-MITO); or by amsacrine 100 mg/m2, days 2 to 4 (FLAG-AMSA).

Appendix 10: Grant members

This **Agreement** ('the Agreement') is **between** the following parties:

on the one part, *the **European Union** ('the EU'), represented by the European Commission ('the Commission')*1*,* represented for the purposes of signature of this Agreement by the Head of Unit, DIRECTORATE GENERAL FOR RESEARCH & INNOVATION, Health, Administration and finance, Mila BAS SANCHEZ, **and on the other part,**

1. 'the coordinator':
MOLECULAR MEDICINE SPA (MLM) SPA, 1506630, established in VIA OLGETTINA 58, MILANO 20132, Italy, VAT number IT11887610159, represented for the purposes of signing the Agreement by CEO, by Riccardo PALMISANO and the following other beneficiaries, if they sign their 'Accession Form' (see Annex 3 and Article 56):

2. **OSPEDALE SAN RAFFAELE SRL (OSR)** SRL, 1972938, established in VIA OLGETTINA 60, MILANO 20132, Italy, VAT number IT07636600962,

3. **UNIVERSITAETSKLINIKUM WUERZBURG - KLINIKUM DER BAYERISCHEN JULIUS-MAXIMILIANS-UNIVERSITAT (UKW)**, not applicable, established in JOSEFSCHNEIDER- STRASSE 2, WURZBURG 97080, Germany, VAT number DE250013752,

4. **OSPEDALE PEDIATRICO BAMBINO GESU (OPBG)**, -, established in PIAZZA SANT ONOFRIO 4, ROMA 00165, Italy, VAT number VAT exemption,

5. **FUNDACIO PRIVADA INSTITUT DE RECERCA DE L'HOSPITAL DE LA SANTA CREU I SANT PAU (PAU)** ES3, 708, established in CALLE SANT ANTONI M CLARET 167, BARCELONA 08025, Spain, VAT number ESG60136934,

6. **FAKULTNI NEMOCNICE S POLIKLINIKOU OSTRAVA FOUNDATION (UHO)**, 00843989, established in 17 LISTOPADU 1790, OSTRAVA PORUBA 708 52, Czech Republic, VAT number CZ00843989,

7. **ISTITUTO SUPERIORE DI SANITA (ISS)**, 80211730587, established in Viale Regina Elena 299, ROMA 00161, Italy, VAT number IT03657731000,

8. **ACROMION GMBH (ACRO)** GMBH, HRB45379, established in EUROPAALLEE 27-29, FRECHEN 50226, Germany, VAT number DE224978890,

9. **ARTTIC (ART)** SAS, 344112396, established in RUE DU DESSOUS DES BERGES 58A, PARIS 75013, France, VAT number FR53344112396,

Appendix 11: Amendment history

Protocol Version	Protocol Approval Date	Rationale and Substantial Changes
A	27 August 2019	First release
B	22 July 2019	Version released for the German clinical center, but not implemented as not approved by the Competent Authority of Germany (PEI).
C	8 January 2020	Version released to implement mainly more details/clarifications on study procedures, as follows: • Section 3.1: it has better clarified that the first 3 patients of all cohorts will be treated according to the staggered approach • Section 4.2 inclusion criteria 3: measurable criteria for MM disease have been defined • Section 4.2 inclusion criteria 5: it has been defined the threshold value of ≥ 20% for CD44v6 positivity on tumor cells • Section 4.4: no treatment with steroids at least 3 days before lymphocyte apheresis has been added • Section 5.5.1: Toxic Epidermal Necrolysis (TEN) has been included as DLT. All sections on DLTs have been updated. • Section 5.6: it has been defined the amount of CD3+ cells required for the lymphocyte apheresis collection • Section 5.7: it has been better defined the possibility to administer salvage chemotherapy • Section 5.8: it has been included the communication to Sponsor of any delay ≥ 1 week for the lymphodepleting chemotherapy. Timeline for execution of rapid influenza diagnostic test has been update. • Section 5.9: it has been specified the delay for up 10 days for the MLM-CAR44.1 T-cell infusion and the recommendation of GCV/valGCV wash-out at least 48 h prior to infusion • Section 5.10: it has been included the immune phenotype analysis of MLM-CAR44.1 T-cells in case of GCV/valGCV administration as rescue medication (day 0, 4 and at the end of admin.) • Section 5.12.1.1: it has been better defined the criteria for interrupting/discontinuing the study • Section 6.1.1.1/6.1.1.1: it has been included the possibility of patient re-screening in exceptional cases • Section 6.1.1.2/6.2.1.2 and 8: it has been better detailed the CD44v6 assessment for AML patients and MM patients • Section 6.2: assessment of CD44v6 expression on tumor cells in PB and BM has been included also for MM patients

		• Section 6.2: R-ISS assessment has been also included. (appendix 2 has been updated) • Section 6.5: it has been better clarified the RCR collection/analysis during the Long-Term Follow-up • Section 8.4.2: it has been added the evaluation of urine FLC • Section 8.4.3: MM markers have been added • Section 8.4.8: more details on FISH assessments have been added • Section 8.6 Chemistry: IgA, IgG and IgM have been included • Section 9.1: it has been included the definition of "evaluable" and "not evaluable" patient • Chapter 10: DSMB information has been updated according to the Data Safety Monitoring Board Charter • Section 11.6.1.4: recommendations in case of skin rash have been added. A clear and a comprehensive description of symptoms/diagnostic markers for TEN and SJS, including appropriate treatment management, has been detailed. • Section 11.6.2: procedures for ensuring the prompt communication of significant safety matters (Urgent Safety Measures) between the sponsor, all study sites and investigators and trial subjects have been added. • Section 12.9: timelines for eCRF completion have been added. • Appendix 9: guidance for definition of high risk leukemia in adults has been added In addition, this version includes also the changes required by the State Institute for Drug Control (Státní ústav pro kontrolu léčiv) of Czech Republic (CZ), previously implemented in a local protocol version (IPR/33.A-LA-001). The protocol and corresponding sections of the synopsis have been modified accordingly as follows: • Pediatric patients with AML expected in phase II, will not be enrolled in CZ • patients enrolled in Phase I must be enrolled between the ages of 18 and 63 years. Following a substantial amendment and transition to Phase IIa, all adult patients may be enrolled without further age restriction.