Title of the clinical research project :
Prediction of Response related to IMmune age T cell fitness in Elderly patients with relapsed and refractory multiple myeloma

Sponsor of the clinical research project: Hematology Department – University Hospital of Brussels (HUB)

Principal Investigator (PI): Marie Vercruyssen, MD

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Declaration of the Principal Investigator

I agree to conduct this clinical research project in accordance with the design and specific provisions of this protocol and will only modify the protocol after informing the sponsor.

I understand that I may terminate or suspend the research project at any time if it becomes necessary to protect the well-being of the participants in this research. The project may also be terminated by the CUB – Hôpital Erasme, with or without cause.

I agree to conduct or personally supervise this research project and to ensure that all associates, colleagues, and employees participating in this study are informed of their obligations to comply with these commitments.

I will conduct the research project in accordance with Good Clinical Practice, the Declaration of Helsinki, and the moral, ethical, and scientific principles that justify medical research. The research project will be conducted in accordance with all relevant laws and regulations relating to clinical studies and patient protection.

I will ensure that the requirements relating to review and approval by the Ethics Committee are met. I will provide the CUB – Hôpital Erasme with all material submitted to the Ethics Committee for ethical approval.

I agree to keep adequate and accurate records and to make them available for audit and inspection in accordance with relevant regulatory requirements.

I agree to promptly report to the Ethics Committee any changes in research activity and any unforeseen problems that may pose risks to human subjects or others. Furthermore, I will not make any changes to the research without Ethics Committee approval, except when necessary to ensure the safety of the research participants.

VERCRUYSSEN Marie 27/05/2026

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1 List of abbreviations

Abbreviation	Description
AE	Adverse Event
AL	Amyloid Light-chain
ANC	Absolute Neutrophil Count
ASTCT	American Society for Transplantation and Cellular Therapy
sBCMA	soluble Serum B-cell maturation antigen
BH	Benjamini-Hochberg
CAR-T	Chimeric Antigen Receptor T cell
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CNS	Central Nervous System
CRS	Cytokine Release Syndrome
CTCAE	Common Terminology Criteria for Adverse Events
DXA	Dual-energy X-ray Absorptiometry
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EOS	End of Study
EOT	End of Treatment
FDR	False Discovery Rate
FLC	Free Light Chain
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HUB	Hôpitaux Universitaires de Bruxelles
ICF	Informed Consent Form
ICANS	Immune Effector Cell-Associated Neurotoxicity Syndrome
Ig	Immunoglobulins
IHC	Immunohistochemistry

IMWG	International Myeloma Working Group
IMWG-FI	International Myeloma Working Group Frailty Index
IP	Immunophenotyping
LDH	Lactate Dehydrogenase
MM	Multiple Myeloma
PET-CT	Positron Emission Tomography – Computed Tomography
PI	Principal Investigator
PS	Performance Status
RRMM	Relapsed/Refractory Multiple Myeloma
RBC	Red Blood Cell
sBCMA	soluble B-cell Maturation Antigen
SOP	Standard Operating Procedure
SPEP	Serum Protein Electrophoresis
Th	T helper
TNF-α	Tumor Necrosis Factor alpha
UPEP	Urine Protein Electrophoresis
VGPR	Very Good Partial Response
WBC	White Blood Cell

2 Protocol - summary

Study Title	Prediction of Response related to IMmune age T cell fitness in Elderly patients with relapsed and refractory multiple myeloma
Study Name	PRIME
Sponsor	Hôpitaux Universitaires de Bruxelles Rue Meylemeersch 90 1070 Anderlecht, Belgium
Indication(s)	T cell fitness and exhaustion assessment by flow cytometry and to assess their relation with the treatment response
Target Study Population	Patients ≥ 65 years old with relapsed/refractory multiple myeloma eligible for a Chimeric Antigen Receptor (CAR) T cell or bispecific antibody therapies
Phase	NA
Study Design	Prospective, multicenter exploratory discovery study Multiparametric flow cytometry will be used to characterize peripheral T-cell compartment, including differentiation status, senescence-associated phenotypes and exhaustion markers, as well as Th1/Th17 and Treg. The following markers will be used : CD57, CD25, CD3, CD45RO, CD38, CD27, CD8, CD4, CD45, KLRG1, CD127, CD28, TIGIT, CCR4, LAG-3, CD3, CCR6, CD8, CD4, CD45, PD-1, CXCR3, TIM-3. A serum sample will be collected to measure soluble B-cell Maturation Antigen (sBCMA) and senescence-associated soluble markers. Patients will be followed longitudinally according to routine clinical schedules during treatment with CAR-T/bispecifics ; baseline characteristics (oncogeriatric assessment, sarcopenia evaluation, disease characteristics) clinical outcomes and adverse events will be collected
Study Rationale	Multiple myeloma (MM) is a plasma cell malignancy that predominantly affects older adults and remains incurable despite major therapeutic advances. The introduction of T-cell–based immunotherapies, including bispecific antibodies and chimeric antigen receptor (CAR) T-cell therapy, has significantly improved response rates in relapsed and refractory (RRMM); however, clinical outcomes remain highly heterogeneous, particularly among elderly patients (1-2). The International Myeloma Working Group Frailty Index (IMWG-FI) is widely used to stratify elderly patients as fit, intermediate-fit, or frail, and has consistently demonstrated strong prognostic value for progression-free and overall survival (3). Furthermore, sarcopenia is a central component of frailty in elderly patients and has been associated with increased toxicity, poorer functional status, and reduced survival. It correlates with established frailty scores and

	may serve as an objective marker of physiological reserve (4). Nevertheless, while frailty scores capture functional status and comorbidity burden, they provide limited insight into immune competence and fail to explain the pronounced inter-individual variability in response to modern T-cell–dependent immunotherapies observed among patients with similar frailty profiles (5). Immunosenescence and T-cell exhaustion represent two distinct but frequently overlapping mechanisms of immune impairment. These changes are associated with impaired proliferative capacity, reduced cytotoxic function. While exhaustion may be at least partially reversible (6), immunosenescence is considered irreversible, making their distinction biologically and clinically relevant in the context of T-cell–based therapies (7-10). Recent studies suggest that immune aging and T-cell fitness defined by T-cell differentiation, senescence, and exhaustion profiles, may predict treatment efficacy and toxicity more accurately than chronological age or clinical frailty alone. Moreover, the Th17/Treg imbalance and baseline immune profile influence multiple myeloma progression (11). Peripheral blood immunophenotyping and evaluation of soluble markers offer a minimally invasive and reproducible approach to assess systemic immune fitness and may serve as a complementary prognostic tool alongside established frailty scores (12-14). By integrating detailed immunological profiling with established clinical assessments such as the IMWG Frailty Index, this project aims to develop a biologically informed framework for risk stratification in elderly patients with RRMM treated with T-cell–based immunotherapies.
Objectives	• Primary - to identify immune profile associated with Very Good Partial Response (VGPR) rate at 3 months • Secondary - to assess the association between Adverse events (AEs) and baseline T cell phenotypes - Association between geriatric assessment and baseline T cell phenotypes - Association between sarcopenia and baseline T cell phenotypes
Endpoints	• Primary - Rate of ≥VGPR or better according to IMWG criteria at 3 months. The association of VGPR and immune profile will be assessed. • Secondary - the rate and severity of specific adverse events Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) / Cytokine Release Syndrome (CRS) and non-ICANS

	neurotoxicities according to ASTCT criteria and non specific AEs according to CTCAE v5.0 will be evaluated regarding the baseline immune profile of the patients - baseline oncogeriatric assessment will be evaluated regarding the baseline T cell profile - baseline sarcopenia assessed by dual-energy X-ray absorptiometry (DXA) will be evaluated regarding the baseline T cell profile
Number of subjects	• Estimated number of subjects to enrol /randomise: 31 • Number of evaluable subjects required: 28 • Sample size was calculated based on the primary objective of the study
Inclusion Criteria	• ≥ 65 years old • Relapsed/refractory multiple myeloma • Eligible for a CAR-T cell or bispecific antibody therapies
Exclusion Criteria	• <65 years old • Active cancer other than myeloma • Active amyloid light-chain (AL) amyloidosis • Central nervous system (CNS) involvement
Non-Investigational Medicinal Product(s) Dose/Route/Regimen	NA
Concomitant Medications	NA
Assessment of Efficacy	NA
Assessment of Safety	NA
Assessment of Quality of life	NA
Statistical Analyses	This is an exploratory discovery study. The investigators will assess about 20-25 differentiation status/senescence-associated phenotypes/exhaustion markers and also a measurement of sBCMA and senescenece-associated soluble markers. The expected VGPR rate for these population is about 60%-70%. The comparison of senescence-associated phenotypes/exhaustion markers between patients with VGPR vs non-VGPR will be performed Wilcoxon test. Benjamini-Hochberg (BH) procedure will be used to control the False Discovery Rate (FDR) set at 0.05. With 28 evaluable patients, it is expected to discover features/variables that are strongly different between the two groups

	(VGPR vs non-VGPR). 31 patients will be recruited accounting for possible drop outs/non-evaluable rate. Based on this results/insight and clinical considerations, certain cutoffs will be derived to classify the younger and older immune profile. The baseline characteristics and outcome variables of the two groups (higher vs younger immune-response) will be assessed. Comparisons in binary variables (e.g. VGPR rate, AE rate, rate of sarcopenia) will be assessed using the Fisher Exact test. Comparisons in continuous variables will be assessed using the t-test and Wilcoxon test. A full statistical analysis plan will be written prior to the statistical analyses.
Stratification factors	NA
Translational research(es)	NA
Length of the study	Planned recruitment period (in months): 9-12 months
End of study	3 months after the last patient inclusion

3 Background and scientific rationale

Multiple myeloma (MM) is a plasma cell malignancy that predominantly affects older adults and remains incurable despite major therapeutic advances. The introduction of T-cell–based immunotherapies, including bispecific antibodies and chimeric antigen receptor (CAR) T-cell therapy, has significantly improved response rates in relapsed and refractory (RRMM); however, clinical outcomes remain highly heterogeneous, particularly among elderly patients (1-2).

The International Myeloma Working Group Frailty Index (IMWG-FI) is widely used to stratify elderly patients as fit, intermediate-fit, or frail, and has consistently demonstrated strong prognostic value for progression-free and overall survival (3). Furthermore, sarcopenia is a central component of frailty in elderly patients and has been associated with increased toxicity, poorer functional status, and reduced survival. It correlates with established frailty scores and may serve as an objective marker of physiological reserve (4). Nevertheless, while frailty scores capture functional status and comorbidity burden, they provide limited insight into immune competence and fail to explain the pronounced inter-individual variability in response to modern T-cell–dependent immunotherapies observed among patients with similar frailty profiles (5).

Immunosenescence and T-cell exhaustion represent two distinct but frequently overlapping mechanisms of immune impairment. These changes are associated with impaired proliferative capacity, reduced cytotoxic function. While exhaustion may be at least partially reversible (6), immunosenescence is considered irreversible, making their distinction biologically and clinically relevant in the context of T-cell–based therapies (7-10). Recent studies suggest that immune aging and T-cell fitness defined by T-cell differentiation, senescence, and exhaustion profiles, may predict treatment efficacy and toxicity more accurately than chronological age or clinical frailty alone. Moreover, the Th17/Treg imbalance and baseline immune profile influence multiple myeloma progression (11). Peripheral blood immunophenotyping and evaluation of soluble markers offer a minimally invasive and reproducible approach to assess systemic

immune fitness and may serve as a complementary prognostic tool alongside established frailty scores (12-14).

By integrating detailed immunological profiling with established clinical assessments such as the IMWG Frailty Index, this project aims to develop a biologically informed framework for risk stratification in elderly patients with RRMM treated with T-cell–based immunotherapies.

4 Objectives and endpoints of the study

4.1 Primary objective and endpoint

4.1.1 Primary objective

To identify immune profile associated with VGPR rate at 3 months

4.1.2 Primary endpoint

- Rate of ≥VGPR or less according to IMWG criteria at 3 months. The association of VGPR and immune profile will be assessed.

4.2 Secondary objectives and endpoints

4.2.1 Secondary objectives

- to assess the difference in VGPR rate at 3 months between patients with a young immune profile (baseline T cell phenotype) and patients with an older immune-profile

- to assess the association between AEs and baseline T cell phenotypes

- Association between geriatric assessment and baseline T cell phenotypes

- Association between sarcopenia and baseline T cell phenotypes

4.2.2 Secondary endpoints

- Rate of ≥VGPR or less according to IMWG criteria at 3 months. The association between young/older immune profile classification and VGPR will be assessed.

- the rate and severity of specific adverse events (ICANS/CRS – Non ICANS neurotoxicities) according to ASTCT criteria and non specific AEs according to CTCAE v5.0

- baseline oncogeriatric assessment will be evaluated regarding the baseline T cell profile

- baseline sarcopenia assessed by dual-energy X-ray absorptiometry (DXA) will be evaluated regarding the baseline T cell profile

5 Methods and study design

This is a prospective, non-interventional, exploratory discovery, multicenter study including HUB, Cliniques universitaires Saint-Luc and Centre Hospitalier Universitaire (CHU) Saint-Pierre .

Patients will be enrolled prior to treatment initiation. Baseline assessments will include clinical data, disease characteristics, frailty evaluation, oncogeriatric assesment, sarcopenia assessed by dual-energy X-ray absorptiometry (DXA). A peripheral blood sampling for immune profiling. Multiparametric flow cytometry will be used to characterize peripheral T-cell compartment, including differentiation status, senescence-associated phenotypes and exhaustion markers. The following markers will be used : CD57, CD25, CD3, CD45RO, CD38, CD27, CD8, CD4, CD45, KLRG1, CD127, CD28, TIGIT, CCR4, LAG-3, CD3, CCR6, CD8, CD4, CD45, PD-1, CXCR3, TIM-3. A serum sample will be collected to measure sBCMA and senescence-associated soluble markers.

Patients will be followed longitudinally according to routine clinical schedules during treatment with CAR-T/bispecifics ; baseline characteristics (oncogeriatric assessment, sarcopenia evaluation, disease characteristics) clinical outcomes and adverse events will be collected. Statistical and exploratory analyses, will be used to identify predictors of response, survival, and toxicity. The planned recruitment period is 9–12 months, and the study will end 3 months after the inclusion of the last patient.

6 Selection of subjects

6.1 Inclusion criteria

Subjects must meet all of the following criteria in order to be eligible for this study:

- Male or female of at least 65 years at the time of signing the informed consent.
- Confirmed diagnosis of MM according to the IMWG revised criteria.
- Must have a biologically active (relapsing or refractory) and measurable disease as defined by at least one of the following:
    - Serum M-protein ≥ 0.5 g/dL by serum protein electrophoresis (SPEP) or quantitative immunoglobulins (Ig);
    - Urinary M-protein of at least 200 mg/24 hours by urine protein electrophoresis (UPEP);
    - Serum free light chain (FLC) ≥ 100 mg/L, provided that FLC ratio is abnormal;
    - If SPEP is felt to be unreliable for routine M-protein measurement (example, for IgA or IgD MM), then quantitative Ig levels by nephelometry or turbidimetry are acceptable.
- Eligible for a CAR-T cell or bispecific antibody therapies
- Written informed consent obtained prior to any study procedure.

6.2 Exclusion criteria

Subjects meeting one of the following criteria are not eligible for this study:

- Male or female less than 65 years old
- Patient with an active cancer other than myeloma
- Patient with AL amyloidosis
- Central nervous system (CNS) involvement

7 Clinical and biological data

During the screening period, eligible patients are identified after informed consent through collection of demographic data, medical and cancer history, prior treatments, concomitant medications, and a full clinical assessment including physical examination, vital signs, ECOG performance status, and geriatric assesment.

Laboratory tests include :

- Hematology : complete white blood cell (WBC) count with differential (neutrophils with Absolute Neutrophil Count (ANC), lymphocytes, monocytes, eosinophils and basophils), hemoglobin, hematocrit, RBC count, platelet count;
- Biochemistry : total protein, glucose, albumin, indirect and total bilirubin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (ALP), Lactate Dehydrogenase (LDH), electrolytes (sodium, potassium, and calcium), serum creatinine (glomerular filtration rate estimation by CKD-EPI equation), urea, uric acid; M-Protein by SPEP or quantitative immunoglobulins (Ig), Serum Free Light Chain
- Urinalysis : total protein and M-protein by urine protein electrophoresis (UPEP).

Imaging :

- Osteoporosis and sarcopenia assessed by dual-energy X-ray absorptiometry (DXA)

Additional procedures on a blood sample :

- T cell markers by flow cytometry immunophenotyping (IP) : CD57, CD25, CD3, CD45RO, CD38, CD27, CD8, CD4, CD45, KLRG1, CD127, CD28, TIGIT, CCR4, LAG-3, CD3, CCR6, CD8, CD4, CD45, PD-1, CXCR3, TIM-3
- SBCMA
- Serum cytokines : IL-1b, IL-2, Il-6, IL-8, IL10, IL-17, TNF-a, IFN-g, TGF-b

8 Statistical design

This is an exploratory discovery study. The investigators will assess about 20-25 differentiation status/senescence-associated phenotypes/exhaustion markers and also a measurement of sBCMA and senescenece-associated soluble markers.

The expected VGPR rate for these population is about 60%-70%. The comparison of senescence-associated phenotypes/exhaustion markers between patients with VGPR vs non-VGPR will be performed Wilcoxon test. Benjamini-Hochberg (BH) procedure will be used to control the False Discovery Rate (FDR) set at 0.05.

With 28 evaluable patients, it is expected to discover features/variables that are strongly different between the two groups (VGPR vs non-VGPR). 31 patients will be recruited accounting for possible drop outs/non-evaluable rate.

Based on this results/insight and clinical considerations, certain cutoffs will be derived to classify the younger and older immune profile.

The baseline characteristics and outcome variables of the two groups (higher vs younger immune-response) will be assessed. Comparisons in binary variables (e.g. VGPR rate, AE rate, rate of sarcopenia)

will be assessed using the Fisher Exact test. Comparisons in continuous variables will be assessed using the t-test and Wilcoxon test.

A full statistical analysis plan will be written prior to the statistical analyses.

9 Risk and Benefits of the Clinical Research Project

This is a non-interventional study with minimal risk, as all treatments and most assessments are part of standard clinical care including a DXA. Additional procedures are limited to peripheral blood sampling, which may cause minor discomfort or local complications.

No direct clinical benefit is expected for participants. However, the study may improve understanding of immune fitness and its impact on treatment outcomes, potentially contributing to better patient selection and personalized therapeutic strategies in the future.

The expected scientific benefits outweigh the minimal risks associated with participation.

10 Study Location and list of sites

Coordinating institution: Hôpitaux Universitaires de Bruxelles, Jules Bordet Institute
Address: Rue Meylemeersch 90, 1070 Anderlecht, Belgium
Department: Department of Hematology
Site Principal Investigator: Dr. Marie Vercruyssen

Participating institution: Cliniques Universitaires Saint-Luc, King Albert II Institute
Address: Avenue E. Mounier 34, 1200 Woluwe-Saint-Lambert, Belgium
Department: Department of Hematology
Site Principal Investigator: Prof. Marie-Christiane Vekemans

Participating institution: CHU Saint-Pierre
Address: Rue aux Laines 105, 1000 Brussels, Belgium
Department: Department of Hematology
Site Principal Investigator: Dr. Nicolas Cilla

11 Treatment and Data Protection

☒ **Coded data**

Personal data will be processed using pseudonymization. Each participant will be assigned a unique study identification code that allows linkage to the participant's medical record while preventing direct identification within the study database.

The study identification code will be generated according to the following format:

[Center]-[Patient Number]

Examples:

- HUB-01: first participant enrolled at HUB
- UCL-02: second participant enrolled at UCL

Only the Principal Investigator, **Dr. Marie Vercruyssen**, will maintain the identification key linking study codes to participant identities. This file will be stored as a password-protected electronic Excel file on the study site only. The research fellow, **Ahmet-Fatih Karadag**, will also have authorized access to this identification file for study purposes.

The identification key will be stored separately from the clinical study database.

At HUB, study data will preferably be collected using the secure internal **REDCap** electronic Case Report Form (eCRF). The participant identification code will be entered in the first CRF (CRF1). Variables containing directly identifiable personal information will be designated as identifiers and will therefore not be included in exported datasets used for statistical analyses.

Only data strictly necessary for the objectives of the study will be collected. Data collection procedures will minimize the risk of participant re-identification. The study database will not contain participants' initials or complete dates of birth; only the year of birth or age at inclusion will be recorded.

No copies of medical records or source documents containing directly identifiable information may leave the participating institution unless all personal identifiers have been replaced by the study identification code.

12 References

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13 Appendix

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