Clinical Study Protocol

Study Title:

A Real-World Observational Study on Patient-Reported Outcomes in Allogeneic Stem Cell Transplantation (Allo-SCT) and CAR-T Therapy (QOL-ONE PRO-CT)

Protocol ID: [To be assigned]

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Sponsor/Collaborator: QOL-ONE Research Association

Study Sites:

• Policlinico Gemelli, Rome, Italy

• La Sapienza University, Rome, Italy

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Sponsor: Associazione QOL-ONE

Steering committee members: Di Rocco Alice, Ionova Tatyana, Iori Anna Paola, Oliva Esther Natalie, Salek Sam, Sica Simona, Tripepi Rocco

This protocol has been written and will be conducted according to the Declaration of Helsinki, and with the principles established according to Good Clinical Practice guidelines

Approval of the protocol

Sponsor/Collaborator: QOL-ONE Research Association

Legal Representative

Rocco Tripepi

Signature _____ **Date** __/__/_____

Principal investigator approval of the protocol

I undersigned_____

(name and surname)

Principal Investigator of the CENTER_____

 Declare to conduct the study **QOL-ONE PRO-CT** in compliance with the protocol.

 Acknowledge that I am responsible for overall study conduct. I agree to personally conduct or supervise the described clinical study.

 Agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receive the appropriate information throughout the study.

Investigator name: _____

Investigator signature: _____

Date: ___/___/__

(dd / mm / yy)

Participating sites

1.	**Prof. Simona Sica** (coordinating site)	Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Ematologia e Trapianto di cellule staminali emopoietiche, Rome, Italy
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Summary

1. Background and Rationale

Advancements in cellular therapies for hematologic malignancies (HM)—notably allogeneic stem cell transplantation (allo-SCT) and chimeric antigen receptor T-cell (CAR-T) therapies—have significantly improved clinical outcomes and survival for patients with otherwise limited treatment options [1–3]. Allo-SCT remains a curative approach for many malignant hematologic conditions through graft-versus-leukemia effects but is associated with considerable short- and long-term morbidity, including graft-versus-host disease (GVHD), infectious complications, and delayed quality of life recovery [4–6]. CAR-T therapy, by contrast, has revolutionized the management of relapsed or refractory B-cell malignancies, offering durable responses in heavily pretreated patients. However, CAR-T is characterized by unique acute toxicities such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which often occur during the index hospitalization and substantially affect symptom burden [7–9].

Despite the transformative impact of these therapies, there remains a paucity of real-world data describing short-term patient-reported outcomes (PROs) during the acute inpatient phase of treatment. While long-term quality of life trajectories have been studied in both allo-SCT and CAR-T cohorts [10–12], less is known about patients' immediate experiences throughout hospitalization, when symptom burden is highest and when clinical and supportive care decisions are most critical. Importantly, a recent prospective longitudinal study directly compared PROs across CAR-T, autologous SCT, and allo-SCT recipients. This study demonstrated that although all groups experienced a decline in quality of life with a nadir around week 2, CAR-T recipients had a significantly less severe decline and a faster recovery compared to allo-SCT patients, who experienced the greatest symptom burden and slowest recovery [13]. These findings underscore the need for further prospective, real-world research to better characterize short-term PRO trajectories in these populations and to confirm these observations in diverse clinical settings. Understanding these short-term experiences has implications for optimizing symptom management, resource utilization, discharge readiness, and early survivorship care [14,15].

The Hematological Malignancy Patient-Reported Outcome (HM-PRO) is a validated instrument specifically developed to assess health-related quality of life (HRQoL) and symptom burden in patients with HM [16–20]. It consists of two parts: Part A evaluates the impact of disease and treatment on HRQoL, and Part B assesses the severity of symptoms. HM-PRO has undergone rigorous psychometric validation, demonstrating strong content validity, construct validity, reliability, and sensitivity to change across a broad spectrum of hematologic malignancies [16–18]. Its disease-specific design makes it particularly suitable for capturing the dynamic and treatment-specific symptom profiles observed in allo-SCT and CAR-T recipients.

Given the distinct toxicity and recovery profiles of allo-SCT and CAR-T therapy, a prospective observational study is warranted to compare short-term PRO trajectories between these two patient groups during the inpatient treatment course. Observational methodology is appropriate, as treatment allocation is determined by clinical indication rather than

randomization, and such a design enables the capture of real-world patient experiences in a non-interventional setting [21,22]. By employing the HM-PRO to collect PRO data at baseline, throughout hospitalization, and at discharge, this study will generate high-resolution, real-world evidence on patient experiences that are currently underrepresented in the literature. These findings will help inform supportive care strategies, improve patient-centered decision-making, and contribute to comparative effectiveness research in advanced cellular therapies.

2. Study Objectives

Primary Objective:
- To assess and compare short-term patient-reported outcomes [PROs (symptom burden and QoL impact)] between patients receiving allo-SCT and CAR-T therapy using the HM PRO from admission to hospital discharge.

Secondary Objectives:
- To describe PRO trajectories during hospitalization.
- To assess differences in PROs based on:
- Treatment modality (allo-SCT vs CAR-T)
- Disease type (e.g., AML, lymphoma, myeloma)
- Disease status (remission vs progression)
- Treatment line (first-line vs multiple lines)
- To validate the use of the HM-PRO in the context of inpatient care for advanced therapies.

3. Study Design

3.1. Overview

Prospective, multicenter, observational cohort study comparing short-term PROs measured with the HM-PRO between two exposure groups: patients undergoing allogeneic stem cell transplantation (allo-SCT) and patients receiving CAR-T cell therapy. Patients will be enrolled at hospital admission for the index inpatient procedure and followed through the inpatient stay (admission → discharge). The study is non-randomized and designed to describe trajectories of symptoms and HRQoL and to estimate the between-group difference in deterioration of HM-PRO scores (primary estimand: mean difference in change score, CAR-T vs allo-SCT).

3.2 Setting:

Two tertiary care hospitals in Rome, Italy, with active allo-SCT and CAR-T in patient programs; multicenter to improve generalizability.

3.2 Duration:

1 year enrollment, participation from hospital admission to discharge (maximum 3 months).

4. Population

One hundred and sixty-two adults (≥18 years) with a hematologic malignancy admitted for either (a) allo-SCT (N=81) or (b) CAR-T cell therapy (N=81).

4.1 Inclusion Criteria:

- Age ≥ 18 years
- Diagnosed with a hematologic malignancy (e.g., leukemia, lymphoma, multiple myeloma)
- Undergoing either allogeneic SCT or CAR-T therapy
- Able and willing to provide written informed consent
- Sufficient Italian proficiency to complete the HM-PRO
- Signed informed consent

4.2 Exclusion Criteria:

- Cognitive or physical impairments that preclude the ability to complete questionnaires
- Estimated life expectancy < 7 days at the time of admission

5. Assessment Schedule

Patient-reported outcomes will be collected using the HM-PRO instrument at the following time points:
- T1: Hospital admission
- T2: Day of infusion (CAR-T or stem cell infusion)
- T3: Day 3 post-infusion
- T4: Day 10 post-infusion
- T5: Day of hospital discharge

6. Collected Variables

Demographics:
- Age, Gender, Education level, occupation, civil status

Clinical Variables:
- Comorbidities, Disease type, Disease status (remission/progression), Line of treatment

Laboratory:
- Full blood counts

Treatment:
- CAR-T or allo-SCT treatment details, including conditioning regimen and product specifics

Adverse Events:
- Documented using CTCAE criteria, including:
  - Mucositis, Infections, GvHD, CRS, ICANS

Patient-Reported Outcomes:
- HM-PRO (Impact Scale, Symptoms Scale)

7. Endpoints

Primary Endpoint:
- Difference in HM-PRO total and domain scores between allo-SCT and CAR-T groups at each time point (T1–T5)

Secondary Endpoints:
- Change in HM-PRO scores over time within each group
- Comparison of PROs by disease status and treatment line
- Frequency and severity of adverse events and correlation with PRO data

8. Statistical Analysis

- All analyses will be performed using two-sided tests with a nominal significance level of $\alpha = 0.05$ unless otherwise stated. Estimates will be reported with 95% confidence intervals.

Primary analysis population include all enrolled participants with at least one post-baseline HM-PRO assessment (intention-to-observe principle).

-Baseline demographic and clinical variables will be summarized by treatment group (allo-SCT vs CAR-T).

- **Continuous variables:** mean (SD) and median (IQR) as appropriate.
- **Categorical variables:** counts and percentages.

- Between-group baseline comparisons will be descriptive (t-tests/Wilcoxon tests or chi-square/Fisher exact tests) to display balance; inferential testing of baseline covariates is not the primary focus. A baseline table will be produced.

8.1 Primary longitudinal analysis (HM-PRO)

- **Primary modelling approach:** linear mixed-effects models (LMM) for repeated measures of HM-PRO scores to evaluate within-hospital trajectories and between-group differences. The base model will include:
    - Fixed effects for **timepoint (categorical: T1, T2, T3, T4, T5)**, **treatment group**, and the **group × time** interaction (to test for differential trajectories).
    - A random intercept for participant to account for within-subject correlation; random slope for time will be considered if supported by data and model fit.
    - Prespecified covariates: baseline HM-PRO score (if modelling discharge score via ANCOVA alternatively), age, sex, disease type, disease status, line of therapy, major comorbidity index, and site (as fixed effect or as random effect in a hierarchical model).

- Parameter of interest: the group × time interaction contrasts (e.g., difference in mean change from baseline to discharge between groups). Report estimated marginal means (least-squares means), differences, 95% CIs and p-values.
- **Model diagnostics:** check residuals for normality and heteroscedasticity, influence diagnostics, and perform sensitivity analyses using robust standard errors if necessary. If severe non-normality persists, consider transformation or a generalized linear mixed model (GLMM) or rank-based mixed model.

Repeated measures ANOVA

- As a supportive analysis (or when assumptions are met and complete data are available), repeated-measures ANOVA (with Greenhouse-Geisser correction if sphericity violated) may be used to summarize overall time and group effects. Primary inference will rely on mixed models which better accommodate missingness and unequal intervals.

Between-group comparisons & ANCOVA

- For the primary endpoint specified as change from baseline to discharge, an **ANCOVA** model will be used (discharge HM-PRO as outcome, baseline HM-PRO as covariate, plus the same prespecified covariates). This produces an adjusted estimate of the between-group difference in mean change.
- Unadjusted two-sample t-tests (or nonparametric Wilcoxon tests) will be presented for transparency.

Subgroup analyses

- Exploratory subgroup analyses will be performed for: disease type (AML vs lymphoma vs myeloma), disease status (remission vs progression), age strata, and treatment line. Each subgroup analysis will include a formal **interaction test** (group × subgroup) within the primary model rather than separate stratified unadjusted tests. Subgroup results are exploratory and interpreted cautiously.

Correlation / association between adverse events and PROs

- **Correlation analyses:** relationship between AE burden (grade or counts) and HM-PRO scores will be examined using Spearman's rank correlation (for ordinal AE grades) or Pearson correlation if assumptions hold.
- **Regression analyses:** to examine whether specific AEs (e.g., CRS, ICANS, mucositis, infections, GvHD) are associated with concurrent or subsequent HM-PRO scores, include AE indicators or graded severity as fixed effects in the mixed model (time-varying covariates). Logistic or Poisson regression (or negative binomial) may be used where appropriate (e.g., modelling probability of clinically meaningful deterioration, or AE counts). Report adjusted effect estimates (e.g., mean differences, odds ratios, incidence rate ratios) with 95% CIs.

- Pre-specify temporal directionality in the SAP (e.g., whether AEs occurring before an HM-PRO assessment are considered predictors).

Handling of missing data

- **Item-level HM-PRO missingness:** per the HM-PRO scoring manual, **missing item responses will not be substituted**; scores will be calculated according to the instrument's guidance.
- **Missing assessments / visit-level missingness:** for missing entire HM-PRO assessments (e.g., missed T2, T3, T4 or T5), the primary approach will be **multiple imputation by chained equations (MICE)** under a missing at random (MAR) assumption. Imputation models will include baseline and post-baseline HM-PRO items/scores, demographic and clinical covariates, laboratory markers, site, and indicators predictive of missingness. We will perform at least m = 20 imputations (or more if fraction of missing information is high) and combine estimates by Rubin's rules.
- **Sensitivity analyses:** compare results from: complete-case analysis, multiple imputation, and worst-case / tipping-point analyses to assess robustness to missing not at random (MNAR) mechanisms. The SAP will detail variables included in imputation models and diagnostics for imputation quality.

Multiplicity and inference

- Multiple secondary endpoints and subgroup tests are planned; these will be described as exploratory. Where formal control of family-wise error is desired for predefined secondary endpoints, procedures (e.g., Holm or Benjamini-Hochberg false discovery rate) will be specified in the SAP.

Additional analyses

- **Proportion with clinically meaningful deterioration:** compute proportion of participants with ≥6-point worsening from baseline to discharge and compare groups using chi-square tests and logistic regression adjusted for covariates; report risk differences and adjusted odds ratios with 95% CIs.
- **Sensitivity for clustering:** if substantial between-site heterogeneity is present, use mixed models with random site intercepts or a generalized estimating equations (GEE) approach with robust SEs clustered by site.
- **Exploratory trajectories:** use latent class growth modelling or clustering of longitudinal profiles if warranted to identify common PRO trajectory phenotypes (reported descriptively).

Reporting

- All results will be reported according to STROBE guidance for observational studies and following best practices for PRO reporting (including instrument scoring details, number of missing items, and sensitivity analyses). Statistical code and de-identified

analytic datasets will be archived as per institutional policies and will be made available on reasonable request, consistent with GDPR and ethics approvals.

8.2. Sample Size:

The primary objective was to detect a between-group difference in HM-PRO between groups based on the difference in HM-PRO total score of Δ = 6 points (the established Minimal Important Difference). Assuming a two-sided α = 0.05 and 80% power, the required sample size per group was calculated using the two-sample t-test formula:
n=(2(z_(1-α/2)+z_(1-β))^2 σ^2)/Δ^2.
Because the SD of HM-PRO change in our population is uncertain, we present results for a range of plausible SDs: for SD = 12 the required sample size is 63 per group (80% power); for SD = 15 it is 98 per group. If the primary analysis adjusts for baseline HM-PRO using ANCOVA and the correlation between baseline and outcome is r, the residual variance is approximately σ^2 (1-r^2) and the required sample size is reduced accordingly (for example, with SD = 15 and r=0.5, n ≈ 74 per group). We inflated the calculated sample size by 10% to allow for anticipated loss to follow-up (final target n per group = 81). All sample-size calculations were performed in R using power.t.test.

9. Methods

9.1 Ethical considerations

The study will be performed in accordance with the principles of the **Declaration of Helsinki**, **Good Clinical Practice (GCP)** guidelines, and all applicable national regulations. Approval will be obtained from the local **Institutional Review Boards (IRB) / Research Ethics Committees (REC)** of all participating centers prior to study initiation.

All participants (or their legally authorized representatives) will provide **written informed consent** before enrollment. Patients will be informed about study objectives, procedures, potential risks, and their right to withdraw at any time without any impact on their standard clinical care.

9.1.1 Data protection

Data protection: All study data will be **de-identified** prior to analysis and processed in accordance with the requirements of the **European Union General Data Protection Regulation (EU GDPR)**. Pseudonymized identifiers will be used for data entry into the REDCap system, and re-identification keys will be stored separately under secure, access-controlled conditions. Only study personnel will have access to identifiable information.

9.1.2 Risk assessment:

There is **no anticipated physical risk** to participants beyond standard clinical care, as this is a non-interventional, observational study limited to collection of PROs and routinely available clinical data.

9.2 Data collection and management

Data will be collected prospectively and entered into secure **electronic case report forms (eCRFs)** using the **REDCap platform** hosted on institutional servers. The Contract Research Organization (CRO) DIELNET SRL will be responsible for the creation, setup, and maintenance of the eCRFs. REDCap access will be role-restricted, password-protected, and audited to ensure data integrity.

9.3 Patient-reported outcomes

The **HM-PRO instrument**, a validated PRO tool for patients with hematological malignancies, will be used to assess symptom burden and health-related quality of life (HRQoL). HM-PRO will be self-administered in paper format by patients at standardized time points during hospitalization:

- **T1:** Hospital admission (baseline)
- **T2:** Day of infusion (CAR-T or stem cell infusion)
- **T3:** Day 3 post-infusion
- **T4:** Day 10 post-infusion
- **T5:** Day of hospital discharge

Study staff will facilitate administration of the instrument and verify completeness of responses. Where patients are unable to self-complete due to temporary health-related limitations, assistance will be provided in accordance with HM-PRO guidance to preserve data validity. The completed questionnaires will be scanned and uploaded into the REDCap system to ensure secure storage and source documentation.

9.4 Collected variables

Demographics

- Age
- Gender
- Education level
- Occupation
- Civil status

Clinical variables

- Comorbidities (documented at admission)
- Disease type (e.g., AML, lymphoma, myeloma)
- Disease status (remission vs progression)
- Line of treatment (first-line vs subsequent lines)

Laboratory

- Full blood counts at baseline and as per standard of care during hospitalization

Treatment-related data

- Treatment modality (CAR-T or allo-SCT)
- Conditioning regimen details
- Product specifics (CAR-T product characteristics or stem cell source/donor type)

Adverse events

Adverse events will be systematically captured and graded according to **Common Terminology Criteria for Adverse Events (CTCAE, version 5.0)**. The following specific toxicities of interest will be documented:

- Mucositis
- Infections
- Graft-versus-host disease (GvHD; allo-SCT group only)
- Cytokine release syndrome (CRS; CAR-T group only)
- Immune effector cell-associated neurotoxicity syndrome (ICANS; CAR-T group only)

10. Schedule of Procedures

Procedure / Assessment	T1: Admission	T2: Infusion	T3: Day 3 Post-Infusion	T4: Day 10 Post-Infusion	T5: Discharge
Informed consent	✓	–	–	–	–
Eligibility check	✓	–	–	–	–
Demographics	✓	–	–	–	–
Clinical variables (comorbidities, disease type, status, line of treatment)	✓	–	–	–	–
Laboratory tests (full blood counts)	✓ (baseline)	As per SOC	As per SOC	As per SOC	As per SOC
Treatment details (conditioning regimen, product specifics)	–	✓	–	–	–

Procedure / Assessment	T1: Admission	T2: Infusion	T3: Day 3 Post-Infusion	T4: Day 10 Post-Infusion	T5: Discharge
Adverse events (CTCAE vX.X)	–	✓ (from infusion)	✓	✓	✓
HM-PRO questionnaire	✓	✓	✓	✓	✓
Questionnaire scan & upload to REDCap	✓	✓	✓	✓	✓

✓ = performed
SOC = standard of care

11. Dissemination of Results

Study results will be submitted for presentation at scientific conferences and for publication in peer-reviewed journals. All publications will maintain patient confidentiality and comply with authorship standards.

12. Funding and Conflicts of Interest

This study is sponsored by QOL-ONE Research Association. No conflicts of interest are declared by the principal investigators or collaborators.

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