STUDY PROTOCOL
RET-HE

A) TRIAL IDENTIFICATION CLINICAL	
SPONSOR CODE NUMBER: ICO-2017-01	
TRIAL TITLE: Study Evaluating Reticulocyte Hemoglobin Content (RET-He) for the Management of Functional Iron Deficiency Anemia in Patients with Solid Tumors	
ABBREVIATED TITLE: RET-HE	
COORDINATOR: Dr. Mathilde DUPE	
ESTIMATED NUMBER OF CENTERS: 1	**NUMBER OF PATIENTS**: 80

B) IDENTIFICATION OF THE PROMOTER
NAME OF ORGANIZATION: ICO, DRCI-Promotion Unit
CONTACT PERSON: **Ms. Nadia ALLAM** Tel: 02 40 67 98 26 Nadia.allam@ico.unicancer.fr

C) GENERAL INFORMATION ABOUT THE TRIAL

INDICATION:

Patients with Solid Tumors experiencing Functional Iron Deficiency Anemia

METHODOLOGY:

The study has the following characteristics:

- single-center,
- prospective,
- open-label,
- non-randomized

PRIMARY OBJECTIVE AND ENDPOINT:

Primary objective:

To determine the value of baseline measurement of reticulocyte hemoglobin content (RET-He) in predicting response to iron therapy in patients with solid tumors presenting with functional iron deficiency anemia, as defined by NCCN 2016 (with or without inflammation). The aim is to refine the current definition of functional iron deficiency in order to optimize iron prescribing in oncology, administering iron only when necessary, i.e., in patients with low RET-He levels.

Primary Endpoint:

Assessment of the correlation between baseline RET-He values and response to intravenous (IV) iron therapy at 1 month. Responders are defined as patients with baseline functional iron deficiency (hemoglobin < 11 g/dL, transferrin saturation [TSAT] < 20%, and serum ferritin 30–800 ng/mL) who achieve an increase of at least 1 g/dL in hemoglobin (compared with baseline) following IV iron administration (with or without erythropoiesis-stimulating agents [ESAs]).

The mean baseline RET-He level will be compared between responders and non-responders.

SECONDARY OBJECTIVES AND ENDPOINTS

Secondary Objectives:

a. To assess the effectiveness of therapeutic management of iron deficiency anemia using intravenous (IV) iron (with or without erythropoiesis-stimulating agents [ESAs]) at 1 month (excluding transfusion) at the site.
b. To determine whether there are factors limiting the use or interpretation of the RET-He parameter (e.g., baseline reticulocyte count, etc.).

Secondary Endpoints:

a. Calculation of the percentage of patients who respond to IV iron therapy (with or without erythropoiesis-stimulating agents [ESAs]) in the setting of functional iron deficiency.
b. Construction of the receiver operating characteristic (ROC) curve for baseline RET-He and determination of the optimal cut-off value.
c. Calculation of descriptive diagnostic performance parameters for prediction, including sensitivity, specificity, and positive and negative predictive values.

INCLUSION CRITERIA:

1. Age ≥ 18 years.
2. Solid tumor (metastatic or non-metastatic).
3. Functional iron deficiency anemia (according to NCCN 2016 guidelines), defined as:
   - Hemoglobin (Hb) < 11 g/dL,
   - Transferrin saturation (TSAT) < 20%, and
   - Serum ferritin between 30 and 800 ng/mL.
4. Baseline anemia work-up performed at the site.

EXCLUSION CRITERIA:

1. Patients with hematologic malignancies.
2. Patients with chronic kidney disease, defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m² using the CKD-EPI formula.
3. Patients with vitamin B12 deficiency, folate deficiency, hemolysis, and/or hemoglobinopathy.
4. Patients with active infection.
5. Patients with bone marrow involvement by tumor. Confirmation by bone marrow biopsy or aspiration is not required to exclude the patient.
6. Patients who received erythropoiesis-stimulating agents (ESAs) within the 2 months prior to baseline assessment.
7. Patients who received iron therapy (oral or intravenous) within the 2 months prior to baseline assessment.
8. Patients who received a red blood cell transfusion within 1 month prior to baseline assessment.

D) TREATMENT, PROCEDURE UNDER STUDY

- **Study Intervention/Procedure:**

Measurement of reticulocyte hemoglobin content (RET-He) performed prior to initiation of treatment aimed at correcting anemia, consisting of intravenous (IV) iron administration (with or without erythropoiesis-stimulating agents [ESAs]).

- **Treatment Duration / Follow-up:**

RET-He is measured at baseline before initiation of anemia treatment. Patient follow-up to assess response to iron therapy is conducted between week 5 (Day 36) and Week 12 (Day 84) after treatment initiation.

D) STUDY IMPLEMENTATION AND CONDUCT

Patients will be enrolled consecutively as they are managed at the study site. An approximate delay of 3 months is required for data completion and validation. Patient records will be reviewed at regular intervals to document follow-up data.
The initiation of therapy to correct anemia—consisting of intravenous (IV) iron administration (with or without erythropoiesis-stimulating agents [ESAs])—will be at the discretion of the physician.

Follow-up assessments for each patient will be based on standard evaluations conducted in accordance with NCCN recommendations:

- **ASSESSMENT 1:** Between Day 21 (Week 3) and Day 35 (Week 5) after treatment initiation (Day 0). This assessment must include at minimum a complete blood count (CBC) and reticulocyte count with RET-He (performed at the ICO laboratory). This evaluation will allow an interim assessment of treatment efficacy by the physician and consideration of potential ESA initiation.
- **ASSESSMENT 2:** Between Day 36 (Week 6) and Day 84 (Week 12) after treatment initiation (Day 0). This assessment must include at minimum a CBC (performed at the site laboratory).

Patients will be secondarily excluded from the protocol if they receive a red blood cell transfusion between baseline and ASSESSMENT 1. Results from ASSESSMENT 2 will not be considered if a transfusion occurs between ASSESSMENT 1 and ASSESSMENT 2.

E) STATISTICAL CONSIDERATIONS

CALCULATION OF THE NUMBER OF SUBJECTS REQUIRED:

Based on the study by Mauro Buttarello et al., a difference (delta) in RET-He of approximately 2.3 between the two groups is expected. The standard deviation (SD) of RET-He in that study was estimated at around 3.5, and similar values are anticipated in the present study.
The response rate at 1 month is expected to be approximately 50%. To detect a difference of at least 2.3 between the two groups, with a type I error (α) of 5% and a type II error (β) of 20% (i.e., 80% power), a total of 74 evaluable patients is required.
To account for potential loss to follow-up, a total of 80 patients will be included.

STATISTICAL ANALYSIS PLAN:

Baseline and 1-month variables, whether continuous or categorical— including complete blood count (CBC) parameters, reticulocyte count, RET-He, iron studies (serum iron, transferrin, ferritin), C-reactive protein (CRP), vitamins B12 and B9, creatinine, haptoglobin, and thyroid-stimulating hormone (TSH)— will be described using appropriate summary statistics: mean, standard deviation (SD), median,

interquartile range (IQR), and range for continuous variables; and frequency distributions for categorical variables.

Responders and non-responders will be compared for quantitative variables (including baseline RET-He, the primary endpoint) using Student's t-test (or the Wilcoxon rank-sum test if assumptions are not met), and for qualitative variables using the Pearson chi-square test (or Fisher's exact test when appropriate).

The receiver operating characteristic (ROC) curve for baseline RET-He (predicting treatment response) will be constructed, and the area under the curve (AUC) will be calculated. The optimal RET-He cut-off (maximizing correct classification) will be determined, along with its diagnostic performance characteristics (sensitivity, specificity, positive predictive value, and negative predictive value).

A multivariate analysis using logistic regression may be performed to assess the independent prognostic value of RET-He if several relevant factors are identified in the initial univariate analysis. The presence of inflammation will be systematically included in the analyses, as well as any use of erythropoiesis-stimulating agents (ESAs).

F) EXPECTED DURATION OF THE TRIAL	
INCLUSION PERIOD:	**36 MONTHS**
FOLLOW-UP PERIOD:	**12 WEEKS**
EXPECTED DURATION UNTIL ANALYSIS OF THE PRIMARY ENDPOINT:	**48 MONTHS**