Protocol

A prospective international observational study on Epithelioid Haemangioendothelioma 1/describing the clinical presentation, natural history, and treatment outcomes, 2/evaluating cytokines and hormones as biomarkers and 3/generating patient-derived preclinical models as a tool to assess the activity of anticancer agents and validate novel therapeutic targets

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A PROSPECTIVE INTERNATIONAL OBSERVATIONAL STUDY ON EPITHELIOID HAEMANGIOENDOTHELIOMA: 1/DESCRIBING THE CLINICAL PRESENTATION, NATURAL HISTORY, AND TREATMENT OUTCOMES; 2/EVALUATING CYTOKINES AND HORMONES AS BIOMARKERS; 3/GENERATING PATIENT-DERIVED PRECLINICAL MODELS AS A TOOL TO ASSESS THE ACTIVITY OF ANTICANCER AGENTS AND VALIDATE NOVEL THERAPEUTIC TARGETS.

1. RATIONALE AND BACKGROUND

Epithelioid hemangioendothelioma (EHE) is an ultra-rare (incidence rate < 1/1,000,000 of population), translocated, vascular soft tissue sarcoma. It shows a peak of incidence in the 4th decade of life, and it is more commonly diagnosed in females, with reported disease onset during pregnancy[1,2].

Two specific translocations have been identified in EHE, representing an hallmark in diagnosis today: the fusion of Transcriptional Co-activator with a PDZ-motif (TAZ) with Calmodulin Binding Transcription Activator 1 (CAMTA1) which is present in almost 90 % of cases, and the fusion of Yes-associated Protein (YAP) and Transcription Factor E3 (TFE3) genes (YAP-TFE3), which can be found in around 10% of the patients[3,4]. YAP and TAZ are well-defined downstream effectors in the Hippo pathway. Forced activation of YAP/TAZ is thought to drive EHE and contribute to key aspects of the cancer phenotype, including metastasis and fibrosis[5].

EHE most often presents as multifocal or metastatic at diagnosis, with lung, liver and bone being the most commonly involved sites. The clinical course ranges from cases naturally stable over time to those highly aggressive and rapidly fatal. Pleural effusion, lymph node metastases and pathologic features (nuclear pleomorphism, mitotic figures and presence of necrosis) have been reported to be associated with a worse outcome, but biological and molecular predictors are still lacking[6]. In particular, there is a subgroup of EHE presenting with serosal involvement, typically associated with chronic mild fever, weight loss, asthenia, anorexia, severe disease- related pain, (more responsive to anti- inflammatory pain killers than morphine), and dyspnoea which seems to perform very poorly. The biological basis sustaining this presentation is completely unknown.

As of today, there are no reports available in the literature providing a comprehensive

description of the specific radiological features of EHE, both for primary and metastatic disease at different sites, and their potential prognostic role has not been explored. In addition, there are no published data to indicate the optimal routine follow-up policy of surgically treated EHE patients with localised disease and the routine follow-up schedules differ across institutions. The appropriate frequency of imaging in cases suffering from distant metastases is also left to be determined.

Also, the definition of radiological progression and the assessment of treatment response in EHE remain a major challenge. The appearance or worsening of serosal effusion, the changes in serosal involvement and the limited increase in size over a short-time interval in slow-growing variants are not properly captured by Response Evaluation Criteria for Solid Tumor (RECIST) definitions for disease progression. This makes the use of such criteria unsatisfactory in this complex disease and could potentially lead to a delay in progression recognition and treatment start. Similarly, being frequently observed in EHE under treatment, improvement of serosal effusion, reduction in size <30%, and correlation between radiology and symptoms should be considered when assessing treatment response.

Surgery is the mainstay of care in the local setting. Active surveillance can be a reasonable strategy for patients with naturally stable or asymptomatic, slowly progressive disease, reserving medical treatment to symptomatic or progressive cases[7].

Data on conventional chemotherapy in advanced EHE are limited to case reports and single-institution experiences and suggest a limited role for the drugs commonly used in adult-type soft tissue sarcomas[8,10]. Signs of activity have been reported with the use of anti-angiogenics, including pazopanib, sorafenib, bevacizumab, alone or in combination with chemotherapy, and apatinib[11-14]. Due to the unique natural history of the disease, the value of antiangiogenics and/or immunomodulatory agents has also been explored, with responses described with sirolimus, thalidomide, interferon, and celecoxib[15-18].

In absence of any available active treatment, EHE is a neglected disease, and the identification of new potentially active compounds, especially for patients affected by the more aggressive EHE variant is critically important. To this end, it is of major importance to identify the mechanism of disease progression, the “inflammatory-like” disease presentation, and the prevalence of the disease in young females.

Several lines of evidence have highlighted the significance of inflammation at the local and/or systemic level in human tumor pathobiology. Indeed, inflammation can influence

tumor progression, metastasis and therapeutic outcome by establishing a tumor supportive immune microenvironment. These processes are mediated through a variety of cytokines and hormones that exert their biological actions either locally or distantly via systemic circulation.

Estrogen signaling is mediated *via* several receptor proteins. In addition to the classical ERα and ERβ[19], the membrane-bound G-protein coupled estrogen receptor (GPER) mediates both the genomic and non-genomic effects of estrogen and has been implicated in the development of other tumors such as breast cancer[20]. Interestingly, GPER stimulation activates YAP and TAZ as key effectors of the Hippo pathway[21]. Insulin-like growth factor-1(IGF-1) has also been shown to regulate GPER expression and function, suggesting a crosstalk between growth factors and ERs[22].

The availability of translatable preclinical models of human EHE, able to properly recapitulate tumor biology and response to treatment of the clinical tumors, appears instrumental for the development of innovative and effective treatments. Patient-derived xenograft (PDX) models preserve the original histomorphological and molecular characteristics of the originating clinical tumors. We previously demonstrated the consistency between preclinical data obtained on PDXs of different soft-tissue sarcoma histotypes (solitary fibrous tumor, epithelioid sarcoma and dedifferentiated liposarcoma) and clinical results concerning the activity of several cytotoxic and molecularly targeted drugs, providing novel insight into the antitumor effect of different combinations that was instrumental to design novel clinical trials[23-27].

MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. Their proven deregulation in several types of human cancer, and the possibility to be reliably detected in both tissue and blood specimens, have prompted the assessment of miRNAs as novel cancer biomarkers[21]. No information is currently available on miRNA expression and function in EHE.

2. RESEARCH OBJECTIVES

2.1 Clinical research objectives

1. To provide a demographic description of the population affected by advanced EHE
2. To provide a description of clinical presentation, natural history, and treatment pattern in patients with advanced EHE. In particular, a specific objective will be to prospectively assess antitumor activity of sirolimus in patients affected by progressing EHE (group B, D)
3. To provide a description of tumour-related symptoms and their changes over time
4. To provide a tumor-related pain assessment
5. To prospectively identify clinical prognostic factors
6. To describe the radiological features of the disease (group A, B, C, D, E)
7. To correlate radiologic features with the outcome and the tested plasma levels of the cytokines and hormones (group A, B, C, D, E)
8. To assess radiologic response to systemic treatments by comparing different assessment criteria (group B, D)

2.2 Translational research objectives

1. To assess *i)* the longitudinal profiles of circulating cytokines, hormones, and miRNAs, and *ii)* the ERα, Erβ and GPER expression and the YAP/TAZ activation in tumor tissues, according to the clinical course of the disease. To this end the analysis will be conducted onthe whole study patient population compared to healthy controls, and by stratifying EHE patients who will enter the study in 3 subgroups according to disease behaviour (non- growing disease, slow- growing disease, highly- aggressive disease)
2. To generate preclinical tumor models (PDXs and cell lines) for comparatively assessing the activity of anticancer agents and inform the design of new clinical trials. EHE preclinical models will be also used for validating novel therapeutic targets.
3. To identify and validate novel biomarkers to inform patient management (prognosticators and predictors of response to medical agents) as well as potential therapeutic targets.

3. STUDY DESIGN

In this observational, prospective study all consecutive patients diagnosed with EHE treated

at participating centers will be included. We will enroll a minimum number of 100 EHE patients, including:

- Group A: newly diagnosed, advanced, EHE, naturally stable disease on follow-up
- Group B: newly diagnosed, advanced, EHE with progressive disease requiring treatment
- Group C: previously diagnosed, advanced, EHE already on active surveillance
- Group D: previously diagnosed, advanced, EHE already on treatment
- Group E: localized EHE will also be included.

As detailed in the Appendix, to allow prospective evaluation of the activity of sirolimus in the disease, at least 15 evaluable patients with evidence of disease progression prior starting sirolimus and evaluable for the response who started sirolimus after signing for the study (Group B, patients progressing on group A, C and E while on study) will be prospectively collected. With the same intent of prospectively evaluating sirolimus activity in EHE patients, data from patients undergoing treatment with sirolimus in group B and D and who discontinue the drug for any reason (e.g., toxicity, patient's preference, clinical indication, etc) will be thoroughly collected in order to evaluate time to subsequent progression and sirolimus activity at time of treatment restart whenever indicated (at least 10 patients).

The stratification according to disease behavior (non-growing disease, slow-growing disease, highly- aggressive disease) foreseen by the translational study will be done according to the following criteria:

a. "Non-growing disease": absence of progressive disease over 12 months.
b. "Slow-growing disease": evidence of progressive disease between 6 and 12 months and patient belonging to group D (provided that treatment was started in evidence on progressive disease).
c. "Highly-aggressive disease": evidence of progressive disease within 6 months.

Progressive disease is defined as:

- Evidence of RECIST 1.1 progression
- any increase in tumor size of the known lesions (even not meeting RECIST 1.1 definition of progression) in association with worsening of at least two tumour related symptoms (tumour-related pain*, fever, weight loss, asthenia)
- new appearance / any worsening of serosal effusion / involvement in association with worsening of at least two tumour-related symptoms (tumour- related pain*, fever, weight loss, asthenia)

* Worsening of pain is defined as an increase in tumour-related pain of 2 points on NRS from 0 to 10 of at least 2-week duration or new onset of tumour-related pain of at least 3/10 of at least 1 week duration

3.1 Pathological review

A centralized review of the pathological diagnosis will be required for all patients (group A-E) entering the study.

The following data will be recorded:

- Evidence of nuclear pleomorphism, mitotic figures and presence of necrosis
- Immunohistochemical staining for CAMTA1
- Molecular testing for WWTR1-CAMTA1 and / or YAP-TFE3

3.2 Clinical assessments and data collection

3.2.1 Baseline

The following information will be recorded for all patients (group A-E) entering the study at the baseline:

- Demographics – patient ID, DOB, gender, data of diagnosis; childbearing potential, use of oral contraception or post-menopausal hormonal therapies for female; date of last menstrual cycle; history of autoimmune disease, malignancies, allergy, prolonged immunosuppressive treatment
- Data on disease extent – single lesion, metastatic multifocal; metastatic multicentric

- Data on staging – evidence of primary disease, lymph nodal metastases, liver involvement, lung involvement, bone involvement, other sites of metastatic disease, evidence of serosal involvement, evidence of serosal effusion
- Data on treatment – surgery, active surveillance, radiation therapy (site, dose), medical treatment (type), other
- Data on symptoms – pain (NRS), weight loss in the last 3 months (%), episodes of temperature (>37.5°) in the last 4 weeks, presence of asthenia in the last 4 weeks, anorexia in the last 4 weeks, night sweat in the last 4 weeks, and dyspnoea in the last 4 weeks.
- Concomitant medications
- Physical examination (including BMI)
- Quality of life assessment by the ESAS-r questionnaire
- Pain assessment if pain NRS ≥4 with visit and completion of the pain assessment form

The following tests will be performed for all patients (group A-E) at baseline according to standard of care:

- Blood tests (complete blood count, AST, ALT, GGT, ALP, total bilirubin, creatinine, BUN, sodium, potassium, calcium, PT, PTT, fibrinogen, glycaemia, total cholesterol, LDL, HDL, triglycerides, screening for HBV and HCV); plasmatic B- HCG (for female);
- Echocardiogram and ECG
- Radiological assessment: CT scan, MRI, bone scan (as clinically indicated)
- Gynaecological assessment: blood tests (FSH, LH, progesterone, estradiol, prolactin), clinical assessment, US

3.2.2 Clinical monitoring

Patient on active surveillance (group A and C) will be monitored every 3-4 months for the first 2 years and every 6 months thereafter as follows:

- Data on symptoms – pain (NRS), weigh loss in the last 3 months (%), episodes of temperature (>37.5°) in the last 4 weeks, presence of asthenia in the last 4 weeks, anorexia in the last 4 weeks, night sweat in the last 4 weeks, dyspnoea in the last 4 weeks.
- Pain medications
- Physical examination – BMI
- Quality of life assessment by the ESAS-r questionnaire
- Blood tests (complete blood count, AST, ALT, GGT, ALP, total bilirubin, creatinine, BUN, sodium, potassium, calcium, PT, PTT, fibrinogen, glycaemia, total cholesterol, LDL, HDL, triglyceride); plasmatic B-HCG (for female)
- Radiological assessment: CT scan, MRI, bone scan (as clinically indicated)

Patient on active treatment (group B and D) will be monitored clinically after 2 weeks from treatment start, then every 4 weeks (for the first 3 months) and every 8 weeks thereafter as follows:

- Data on symptoms – pain (NRS), weight loss in the last 3 months (%), episodes of temperature (>37.5°) in the last 4 weeks, presence of asthenia in the last 4 weeks, anorexia in the last 4 weeks, night sweat in the last 4 weeks, dyspnoea in the last 4 weeks.
- Pain medications
- Childbearing potential (females) and length of menstrual cycle (fertile females)
- Physical examination – BMI
- Quality of life assessment by the ESAS-r questionnaire
- Blood tests (complete blood count, AST, ALT, GGT, ALP, total bilirubin, creatinine, BUN, sodium, potassium, calcium, PT, PTT, fibrinogen, glycaemia, total cholesterol, LDL, HDL, triglycerides); plasmatic B-HCG (for female)
- Radiological assessment (CT scan, MRI, bone scan as clinically indicated) will be

performed every 3-4 months. Gynecological assessment (FSH, LH, progesterone, estradiol, prolactin, clinical assessment, US) will be performed every 6-8 months. For patients on sirolimus, sirolimus plasma levels will be monitored after 2 weeks and 4 weeks from treatment start and every 4 weeks thereafter. For patients in group B receiving sirolimus and for patients discontinuing sirolimus treatment in group D, clinical assessments will follow the same timepoints detailed above. In these patients, radiological assessments will be performed every 12 weeks (+/- 1 week). Sirolimus plasma level target will be ≥ 15 ng/ml. Treatment with sirolimus in group B will be continued until disease progression, unacceptable toxicity, or a patient's refusal to continue treatment.

Patients with localised disease (group E) will be monitored every 3-4 months for the first 2 years, every 6 months up to year 5 and yearly thereafter, as follows:

- Data on symptoms — pain (NRS), weight loss in the last 3 months (%), episodes of temperature (>37.5°) in the last 4 weeks, presence of asthenia in the last 4 weeks, anorexia in the last 4 weeks, night sweat in the last 4 weeks, dyspnoea in the last 4 weeks
- Pain medications
- Physical examination – BMI
- Quality of life assessment by the ESAS-r questionnaire
- Blood tests (complete blood count, AST, ALT, GGT, ALP, total bilirubin, creatinine, BUN, sodium, potassium, calcium, PT, PTT, fibrinogen, glycaemia, total cholesterol, LDL, HDL, triglycerides); plasmatic B-HCG (for female)
- Radiological assessment: CT scan, MRI, bone scan (as clinically indicated)

3.2.3 Radiologic assessment

Radiologic images (CT and/or MRI scans) will be uploaded on to the centralized XNAT

platform and reviewed by a sarcoma dedicated radiologist. The XNAT Platform is a cross-platform, open-source tool designed to support imaging research with its core function to manage the import, archiving, processing, annotation and secure distribution of imaging and related study data. The radiological review will 1) describe the radiological features of the disease (group A, B, C, D, E), 2) assess response to systemic treatments by RECIST, by CHOI criteria and by RESCORe (Response Evaluation by Symptomatic Change and Outcomes Reporting) criteria (group B, D).

3.2.4 Progression

At the time of progression, the following data will be recorded, and the following tests performed for all patients (group A-D):

- Data on treatment choice – surgery, active surveillance, radiation therapy (site, dose), medical treatment (type), other
- Data on symptoms – pain (NRS), weight loss in the last 3 months (%), episodes of temperature (>37.5°) in the last 4 weeks, presence of asthenia in the last 4 weeks, anorexia in the last 4 weeks, night sweat in the last 4 weeks, dyspnoea in the last 4weeks.
- Pain medications
- Physical examination – BMI
- Quality of life assessment by the ESAS-r questionnaire
- Blood tests (complete blood count, AST, ALT, GGT, ALP, total bilirubin, creatinine, BUN, sodium, potassium, calcium, PT, PTT, fibrinogen, glycaemia, total cholesterol, LDL, HDL, triglycerides); plasmatic B-HCG (for female)
- Radiological assessment: CT scan, MRI, bone scan (as clinically indicated)
- Gynaecological assessment: blood tests (FSH, LH, progesterone, estradiol, prolactin), clinical assessment, US

4. STUDY POPULATION

We plan to include at least 100 patients (range: 100-150) in 72 months, followed by a follow-up time of 3 years.

4.1 Inclusion criteria

- Histological diagnosis of EHE according to 2020 WHO classification, performed on biopsy or surgical specimen
- Signed informed consent
- Adequate patient compliance to treatment or follow up
- No age limits

4.2 Exclusion criteria

- Impossibility to ensure adequate compliance

5. TRANSLATIONAL STUDY

The translational part of the study will be carried out on two independent case series:

INT-Milano series: overall, we plan to collect blood (plasma and serum) samples from ≥ 50 molecularly confirmed EHE patients and FFPE tissues from at least 20 molecularly confirmed EHE patients among those in which the blood sample is collected. We will collect blood samples from all consecutive molecularly confirmed EHE patients entering INT irrespective of the disease phase (group A-E). In addition to the baseline sample, for each patient we will collect longitudinal samples after 1 month from baseline, at 6 months and in case of evidence of progression. For patients starting a medical treatment, samples will be collected at baseline then after 2 weeks, 1 and 6 months of treatment and in case of

disease progression.

Only patients with a pathologic diagnosis of EHE confirmed by the presence of *WWTR1-CAMTA1* or *YAP-TAZ* fusion gene will be considered eligible.

Blood samples from healthy individuals, will be also collected for comparative purpose with EHE cases; gender and age will also be registered in order to perform adjusted comparative analyses.

For EHE patients undergoing surgical procedures, a tumor sample will be transplanted into immune-compromised mice to generate PDXs (Section 5.6). Corresponding cell lines will be then established following PDX disaggregation.

Clinical data of all EHE patients who will enter this study will be collected prospectively in a dedicated database, also recording samples for study-related translational research analysis, and progressively updated with patient outcome information.

Institute of Cancer Research (ICR)/Royal Marsden (RM) - London series:

Similar to the INT series, we will be collecting blood and tissue samples from all patients who are currently being treated at or are referred to the Royal Marsden Hospital (RMH).

We expect the number of patients diagnosed with EHE at RMH to be approximately 5-6 patients per year (approx. 18 over the 3-year period).

Mirroring the blood collection at INT, we will be collecting at the following time points:

Patients who undergo observation

- Diagnosis (Baseline Pre-treatment)
- Baseline +1month
- Baseline +6months
- Treatment Baseline

Patients who undergo a systemic therapy

Treatment Baseline + 2 weeks

- Treatment Baseline +1 month

- Treatment Baseline +6 months
- Progression

RMH will also collect tissue alongside these patient groups including diagnostic FFPE and any excess surgical resection tissue (fresh frozen and FFPE). RMH will also collect samples from patients who are already being treated at RMH for EHE, to maximize sample numbers. All blood and tissue will be stored in the UK's National EHE Biobank, the set up of which was funded by the EHE Rare Cancer Charity, and is situated at The Royal Marsden Hospital NHS Foundation Trust in line with HTA regulations and released for analysis when required.

ICR will also carry out CRISPR whole genome screen on PDX-derived cell lines generated at INT to identify new therapeutic targets for the disease.

The Royal Marsden Hospital (RMH) will participate in the outlined research project as a hosted non-commercial study and will submit the protocol proposal to the UK regulatory authorities before commencing consent and tissue collection. Once the appropriate regulatory approvals are in place, the Human Tissue Manager based at RMH, who is part funded by the EHE Rare Cancer Charity, will consent any patients to this study and funding for this work is already covered within the percentage of funded whole time equivalent. In addition, samples collection can be undertaken by the unit's Biological Specimen Coordinator.

The study will be started on the INT case series (Training set) and the circulating and/or tissue factors emerging as candidate biomarkers will be assessed on the ICR case series (Testing set) by using the same experimental approaches and biostatistics pipeline. If results generated in the Training set will identify specific biomarkers able to provide useful information for a specific subgroup of patients, the number of patients in that group will be enriched.

Based on an already established collaboration, the top candidates selected in the previous

steps will be validated on an independent case series (Validation set) collected at MSKCC-New York.

5.1 Circulating cytokines

Cytokines will be initially analysed in plasma samples from EHE patients by using a protein array able to simultaneously detect the expression of 105 different cytokines.
Successively, cytokines differentially expressed between patients and healthy donors, or between different groups of patients, will be assessed in the same plasma samples using specific ELISA assays.

5.2 Circulating hormone profiles

Serum concentrations of estradiol, estrone, estriol, progesterone, DHEAS, androstenedione, testosterone, dihydrotestosterone, luteinizing hormone, follicle-stimulating hormone, prolactin, sex hormone-binding globulin and IGF-1 will be determined by different kinds of immunoassays using commercial kits.

5.3 Circulating miRNA profiles

The expression profiling of plasmatic miRNAs will be carried out by the qRT-PCR-based OpenArray Technology (which simultaneously evaluate the expression of 754 different miRNAs and 4 control RNAs in replicates).

5.4 Immunohistochemistry

Immunostaining of ERα, phosphoERα, ERβ, GPER, YAP, AR, PR, phosphoYAP, TAZ, phosphoTAZ and aromatase will be performed on 5-µm thick FFPE sections using specific moAbs and standard immunohistochemical techniques. (This analysis will be carried out, in part, at the Policlinico Gemelli, Rome)

Whenever possible, the expression of ERα, ERβ, AR and PR on PBMCs will be also detected by flow-cytometric analysis.

5.5 Mass spectrometry

To complement the immunohistochemistry analysis, comprehensive proteomic profiling of FFPE specimens by mass spectrometry will be undertaken (30 samples combined from INT and RM) at the ICR using established protocols developed in the team.

5.6 PDX model generation

Patients who will have surgery at INT will be considered for generation of a patient-derived xenograft. PDXs will be obtained by directly implanting freshly resected tumor pieces subcutaneously/orthotopically into immune-compromised (nude or SCID) mice and characterized for consistence with the originating clinical tumors in terms of histo-morphology, presence of specific translocations (WWTR1-CAMTA1 or YAP-TFE3), genomic and transcriptomic profiles.

PDX will be then mechanically or enzymatically disaggregated into single cells to establish cell lines.

5.7 CRISPR whole genome screen

A human genome-wide knockout CRIPSR library consisting of 90,709 single guide RNA (sgRNA) sequences to target 18,010 human genes, will be used to stably express a single sgRNA per cell in a cas9 expressing PDX-derived cell line.

6 STATISTICAL ANALYSIS

Descriptive statistics will be used to summarize patient and tumour characteristics. Contingency tables will be used to describe the associations between pairs of categorical variables. Multivariate association between clinical characteristics, such as symptoms at baseline and new symptoms during follow-up, baseline metastatic sites and sites of progressive disease, and treatments will be studied by applying cluster analysis, the results of which will be represented using heat map plots. The identified patient clusters will be compared with the 5 groups A-B-C-D-E defining the EHE diagnosis to better characterize disease heterogeneity.

Overall survival (OS) and progression-free survival (PFS) curves will be estimated with the Kaplan-Meier method in the 5 groups along with survival rate estimations at predefined endpoints (i.e., 6-, 12-, 18-, 24-month). We will also estimate the post- progression OS (ppOS) curves for patients who will develop progressive disease. Multivariable prognostic analyses will be performed using Cox models; due to the low number of cases and events the analyses will be performed by applying penalized likelihood methods. Variable selection will be performed beforehand by applying random forest procedures for survival data for inclusion in subsequent multivariable Cox models for OS and PFS; selection criteria will be minimal depth (the lowest the best) and variable importance (the highest the best).

Particularly, for patients treated with sirolimus, overall response rate (ORR = complete response, CR + partial response, PR) will be computed according to RECIST v1.1 and to Choi criteria. Clinical benefit rate (CBR) will consider patients achieving a CR, PR or stable disease lasting ≥12 weeks.

Duration of response will be computed with the Kaplan-Meier method as the difference between first assessment of response and disease progression or death whichever occurs first.

In the absence of the event of interest, all survival endpoints will be censored at the date of last follow up where the patients were free from the event.

In the translational study, to adjust for the gender and age heterogeneity between EHE cases and controls we will estimate a propensity score (PS) (REF: Rosenbaum PR, Rubin DB. Reducing bias in observational studies using subclassification on the propensity score. J Am Stat Assoc 1984; 79: 516 – 24) as a balancing score and use a PS function as weight (REF: Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. Stat Med 2015; 34: 3661-79) in the comparative analyses.

Unsupervised analysis of blood tests, cytokines, hormones, miRNA and immunohistochemistry data will be performed by applying cluster analysis, the results of which will be represented using heat map plots. We will also try to use appropriate deep learning algorithms to integrate multi-omics data into the aim of identifying/deriving prognostic signatures. The analyses will be carried out using the SAS® and R software. We will consider a statistical test as significant when achieving a p value <0.05.

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