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# Bando 2019 - Programma 5 per mille anno 2019
# Investigator Grant (IG)

## TRANSLATIONAL RESEARCH

LILT will support research projects in the field of cancer aimed at improving cancer diagnosis and treatment. Particularly considered will be those translational research projects that promise short-medium term effects in clinical practice, concerning new diagnostic methodologies and new therapies. Multicentric studies with national coordination, aimed at validating new diagnostic methods, diagnostic, prognostic and predictive tumor markers, able to improve the clinical management of cancer patients are potentially eligible for funding. Specific research projects on new oncological therapeutic approaches are also eligible for LILT funding as IG. For this type of grants it is necessary to demonstrate solid preliminary experimental data supported by a rigorous biological rationale.

## 1. Principal investigator's full name and qualification:

P.I.'s CV is attached as Supplementary Document 1

2. Proposal title: Minimally and non-invasive methods for early detection and/or progression of endometrial cancer

3. Primary area of Relevance: Gynecology Oncology

4. Relevance for the National Health System: Investigation of serum metabolomic biomarkers for detecting endometrial cancer and predicting recurrence following initial surgery or medical treatment in order to ultimately improve patient survival based on better stratification and informed treatment decisions.

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6. Authorized Administrative Official: Cristina Grandi; address: Largo Rosanna Benzi 10, 16132, Genova. Phone 010/5558222 e-mail cristina.grandi@hsanmartino.it

7. Proponent's signature.....

8. Authorized Administrative Official's signature.Cristina Grandi

9. Place and date: Genoa, 10-02-2020

## SELF EVALUATION FORM

1. Investigator's full name: (PI) **Simone Ferrero**
2. Total papers: **274** IF: **910,288**
3. Total papers (last 10 years): **193** IF: **541,953**
4. Total Papers as first/last author or corresponding author: **227**
5. Total H-index **43**

## PROPOSAL MAIN BODY

1. **Proposal title**

Minimally and non-invasive methods for early detection and/or progression of endometrial cancer

2. **Abstract**

**Rationale:** Endometrial cancer (EC) is the most frequent gynecological malignancy in the developed world. Optimal treatment of EC depends on early diagnostics and pre-operative stratification to appropriately select the extent of surgery and to plan further therapeutic approach. Currently, invasive endometrial histology is the gold standard for diagnosis, as there are no valid non-invasive methods available, and patient stratification is based on histopathology and surgical findings. There is a great need for efficient and reliable screening test for asymptomatic women with high risk of EC (such as patients with Lynch syndrome and those treated with tamoxifen). In addition, a prognostic test is needed to stratify pre-operatively EC patients with high risk of progression who need radical surgery together with adjuvant chemo/ratio therapy from EC patients with good prognosis. In our project we are addressing this lack of non-invasive diagnostic and prognostic biomarkers of EC.
**Objective**: We aim to identify diagnostic serum metabolite and protein biomarker signatures for early detection of cancer in asymptomatic high-risk population and (secondary objective) prognostic biomarkers for selection of patients with poor prognosis.
**Study design:** Prospective observational case / control study. Serum from women diagnosed with EC (150) and controls (150) will be analyzed using non-targeted and targeted metabolomics and semi-targeted proteomics approaches. Subjects will also fill a life-style questionnaire.
**Study population:** Subject older than 18 years will be included. Inclusion criteria for the case group: endometrioid, serous, clear cell or mucinous EC; dedifferentiated EC; any FIGO (International Federation of Gynecology and Obstetrics) stage; high grade or low grade. Exclusion criteria: atypical hyperplasia, other types of cancer, sarcoma uteri or uterine metastasis, previous EC. Inclusion criteria for control group: benign uterine diseases, e.g. myoma uteri, prolapsed uterus, prophylactic hysterectomy for Lynch syndrome. Exclusion criteria: cancer, ovarian unknown masses, previous EC, pregnancy at the time of enrolment.
**Intervention (if applicable)**: Blood sampling (6 mL) prior to standard care (e.g. surgery, medical treatment).
**Main study parameters/endpoints:** Clinical data (via a case report form) and epidemiological data (via a questionnaire) will be collected prospectively. Blood samples will be taken before treatment. In case of hysterectomy, an endometrial biopsy will be collected for immunohistochemical marker analyses. Blood metabolome comprising over 850 metabolites will be analyzed by ultra-performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS) and electrospray ionization tandem mass spectrometry (ESI-MS/MS). Blood proteome including 900 different cancer-related proteins will be analyzed in parallel using high

content antibody microarrays. Bioinformatics/biostatistical analysis will be used to derive diagnostic and prognostic algorithms based on blood metabolites, proteins and clinical data. Algorithms in the biomarker discovery study will be developed by comparing EC and patients with benign uterine pathologies and by comparing EC patients with low risk and high risk for cancer progression and recurrence.

**Nature and extent of the burden and risks associated with participation, benefit and group relatedness:** The burden for subjects enrolled is minimal, being the only intervention a blood sampling (6 mL) and giving information about lifestyle via a questionnaire. The expected benefits are the discovery of diagnostic biomarker signatures for early diagnosis and for development of screening test, and the discovery of prognostic biomarkers for pre-surgical selection of EC patients with poor prognosis. Thus, this would lead to early diagnosis of EC non-invasively and improved treatment outcomes for the high-risk patients, likely decreasing the cases of under and over treatment.

## 3. Introduction

Endometrial cancer (EC) is the most common gynecological malignancy in the developed world with 319,605 new cases and 76,160 deaths in 2012 only (1). The incidence and mortality for EC are still increasing (2). The majority of EC patients are postmenopausal, but 14% of patients are premenopausal and 4% of these women are in the reproductive age (3, 4). Although most patients are diagnosed and treated at an early stage, the cancer recurs in 15% to 20% of patients with no signs of advanced disease at the time of primary intervention (5). For uterine cancer, the staging system developed by the International Federation of Obstetrics and Gynecology (FIGO) is largely employed.

There is no reliable screening test for asymptomatic women with high risk for EC, although there is a great need, as this population includes patients treated with tamoxifen, a standard therapy for the majority of 1.6 million breast cancer patients identified yearly worldwide (1) and patients with Lynch syndrome, with over one million cases in Europe alone (6).

## 4. Background and rational

The endometrial biopsy has been recommended for the evaluation of women at high risk for EC, but this invasive approach is not used as general screening test (6). The only non-invasive diagnostics for EC relies on the combination of ultrasound, optional MRI and tumor marker CA-125, where none of these is completely satisfactory (4). CA-125 is most frequently used as a biomarker of ovarian cancer, although it has some diagnostic/prognostic value also in EC (7). However, CA-125 lacks specificity, with increased levels seen in other cancers, other gynecological and non-gynecological diseases, and several normal physiological conditions (8). Reliable non-invasive diagnostics would be important as a screening test for early diagnosis of cancer followed by tailored treatment. Biomarkers are also needed for prognosis, for pre-operative stratification of EC patients with high risk of progression and recurrence, who need radical surgery and adjuvant chemo/radio therapy from those with low risk, who have low chances to develop metastases and do not need radical surgery with lymphadenectomy (9). In several European countries lymphadenectomy is still routinely performed, although it is associated with severe complications, including lymphedema, deep vein thrombosis, neurological and vascular injuries and does not bring benefits to disease recurrence and survival (10, 11). Additional biomarkers are thus needed to provide pre-operative stratification of patients, to decide on the extent of the surgery and to plan for further therapeutic approach, but also to prevent overtreatment of patients and associated side effects.

Biomarkers can contribute to early diagnosis in asymptomatic patients and to better tailor post-surgical care to patients for an improved clinical outcome in those women who are likely to develop progressive, recurrent disease and for a decreased overtreatment of EC patients with good prognosis (12, 13). As individual biomarker cannot provide sufficient sensitivity and specificity, identification of biomarker panels is crucial. A biomarker panel consisting of conventional tumor markers is less likely to diagnose early stage cancers with small tumor deposits, thus development of clinically applicable biomarker-based diagnostics requires global “omics” approaches (14, 15).

Metabolomics can be described as the molecular phenotype or biological end points, which culminates in the response creating the current physiological state, reflecting the other omics, including genomics, transcriptomics, and proteomics. By using metabolomics, it is possible to

investigate a large collection of metabolites, which contain several promising biomarkers for risk prediction, diagnosis, and even treatment effects, and end up with certain defined markers related to the outcome of interest, making it very promising for clinical application (16).
We hypothesize that discrete and distinct panels of proteins and metabolites in peripheral blood are associated with early stage cancer and aggressive EC. We expect to find different protein and metabolic profiles in patients with early stages of cancer as compared to controls and in patients with poor prognosis and high risk of tumor progression and recurrence as compared with those with good prognosis.

## 5. Experimental design

### 5.1 Objective

Primary Objectives

- Identification of panels of proteins and/or metabolites and individual clinical data that will serve as a basis for development of diagnostic models with sufficient sensitivity and specificity to distinguish early stage EC in high risk asymptomatic population.
- Development of diagnostic algorithm based on combination of omics derived biomarkers and clinical data of the patients with the best diagnostic characteristics for further clinical validation, translation into clinical application and improved clinical practice.

Secondary Objectives

- Identification of panels of proteins and/or metabolites and individual clinical data that will serve as a basis for development of prognostic models with sufficient sensitivity and specificity to differentiate between EC patients with high and low risk of tumor progression or recurrence.
- Development of prognostic algorithms based on combination of omics derived biomarkers and clinical data of the patients with the best prognostic characteristics for further clinical validation, translation into clinical application and improved clinical practice.

### 5.2 Study Design

This is a prospective observational case/control study. Serum from women diagnosed with EC (n=150) and controls (n=150) will be analyzed using non-targeted and targeted metabolomics and semi- targeted proteomics approaches. Subjects will also fill a life-style questionnaire.
Clinical and epidemiological data, blood metabolome (over 850 metabolites) and proteome (including 900 different cancer-related proteins) will be analyzed with bioinformatics/ biostatistical analysis to derive diagnostic and prognostic algorithms for early diagnosis of EC and to identify EC patients with low risk and high risk for cancer progression and recurrence. The risk of progression will be based on known prognostic criteria (e.g. histology, FIGO stage 1b or higher, high grade) and novel prognostic biomarkers on tissue biopsies like hormone receptor status, ploidy (see 'www.esgo.org/network/enitec/' for a full inventory of potential markers). These markers will be assessed by immunohistochemistry and by global RNA profiling (RNA sequencing). Additionally, patient clinical data will be regularly accessed for five years after inclusion to follow disease status as well as to confirm that control women did not develop only oncologic condition.
The present study is an international collaboration, with different roles in the study: IRCCS Ospedale Policlinico San Martino (Genoa, Italy) will take care of patient recruitment, taking blood samples and endometrial sampling and performing immunohistochemical sample analysis. Maastricht University Medical Centre (MUMC)/ Máxima Medical Centre Veldhoven (MMC) be responsible for bio banking and proteomics/metabolomics and immunohistochemical sample analysis. Each center will obtain its own national approval from the competent ethical authority.

### 5.3 Study Population

Women older than 18 years visiting the outpatient clinic for EC (case group, n = 150) or with benign gynecological disorders (controls, n = 150) will be enrolled.

Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all the following criteria:

***Cases***:

- Being older than 18 year
- Diagnosis of EC (endometrioid, serous, clear cell or mucinous; dedifferentiated EC; high grade or low grade)
- Patients must have signed an approved informed consent

***Controls***:

- Being older than 18 year
- Being diagnosed with a benign uterine disease, e.g. myoma uteri, prolapsed uterus.
- Undergoing hysterectomy as prophylactic measure (Lynch syndrome)
- Controls will be enrolled if they are-matched with cases (benign disorders may occur more frequently than EC at young age)
- Patients must have signed an approved informed consent

Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

***Cases***:

- Being younger than 18 year
- Diagnosis of atypical hyperplasia, other types of cancer, sarcoma uteri
- Previous diagnosis of EC
- Being pregnant at the moment of enrolment
- Inability to approve the informed consent form

***Controls***:

- Being younger than 18 year
- Being diagnosed with a benign ovarian disease
- Being diagnosed with any malignancy
- Previous diagnosis of EC
- Being pregnant at the moment of enrolment
- Inability to approve the informed consent form

Sample size calculation

Samples that enables training of accurate diagnostic and prognostic models was calculated using the inferential approach introduced by Arkin et al (17). We calculated that diagnostic model with a fixed specificity of 0.85 and minimal sensitivity of 0.9 with +/- 0.05 confidence interval, requires at least 138 cases. This is a minimal estimate, but since we are going to employ a nested cross-validation strategy to estimate a realistic generalization performance of our model to unseen data, more samples would be required. Based on our previous experience in biomarker discovery study, approximately 40% of additional samples is needed to account for both layers of nested cross-validation. Overall, according to our calculations 200 case samples is a sufficient number that will enable reasonable performance of our diagnostic model. In the case of prognostic model that would aim to differentiate between high and low risk patients, we naturally cannot have the same number of cases as for diagnostic modelling. Therefore, in order to decrease the required number of samples for prognostic modelling, the expected confidence interval level will have to be relaxed to +/- 0.1 with an expected sensitivity of 0.9 and specificity of 0.85. This would allow us to match the expected number of high-risk EC patients (~40) that will be recruited in our study. As negative outcome individuals 150 controls will be selected in order to one-to-one match the recruited cases. This number is based on an overestimation of

the real controls needed, to account for potential misclassification of controls (a control be found to have endometrial cancer at histology, subject may develop an oncologic condition during the study) or to potential biases in the analyses due to the presence of a (non-oncological) gynecological disease (e.g. that could cause a deviation in the levels of specific metabolites compared to the control levels).
The present study is an international collaboration. A total number of 300 specimens will be reached. An equal number of cases and controls (e.g. 150 cases and 150 controls per center) will be obtained. U.O. Clinica Ostetrica e Ginecologica and U.O. Ostetricia e Ginecologia of IRCCS Ospedale Policlinico San Martino (Genoa, Italy) will be involved in patient's recruitment, following harmonized protocols for sample collection, selection of subjects and timeline of inclusion.

### 5.4 Ethics

The burden for subjects enrolled is minimal, being a blood sampling (6 mL) and giving information about lifestyle via a questionnaire. In addition, there is no involvement of minors or incapacitated subjects. The study will be conducted according to the principles of the Declaration of Helsinki adopted by the 18th World Medical Association (WMA) General Assembly (Helsinki, Finland, June 1964) and amended by the WMA General Assembly 2013. The study will be conducted and in accordance with the Medical Research Involving Human Subjects Act (WMO).
Local center will obtain national approval from the competent ethical authority. The potential study subjects will be directed to one of the local researchers involved in the project for the further steps. Consecutive patients meeting eligibility criteria will be invited to participate in this study. Patients will be informed about the benefits and risks of this study in a detailed discussion on the basis of the patient information form. If the patient confirms to seriously consider participation in this study, the patient will be formally included in the study after having given written informed consent. Local researchers involved in the project will be responsible for informing the patients, and clarifying all possible doubts, indicating the independent physician. All human specimens (blood and endometrial biopsies) will be coded and stored at -80°C in our department facility or in the BioBank facility at the Maastricht University. A part of the endometrial biopsy will also be fixed in formalin and embedded in paraffin for histology and immunohistochemical analyses and one part will be snap frozen for genetic and RNA analyses. Participating subjects will be explicitly asked permission to ship their clinical material aboard for analyses, to keep their material for 15 years after the end of this study and to perform extra investigations.

## 6. Further details on the overall methods that will be used in this project

Specifically, study parameter/endpoint are represented by:

- Targeted blood metabolite analysis (180 metabolites)
- Non-targeted blood metabolite analysis (700 metabolites)
- Blood proteomics (900 cancer related proteins)
- Clinical data (EC, histology, grading, staging)
- Tumors molecular characteristics (immunohistochemical and RNA markers)
- Eventual subject genetic characterization

A blood sample (6 mL) will be obtained from each woman enrolled, together with clinical information (via an electronic case report form, complied by the visiting physician) and a life-style questionnaire investigating ethnicity, dietary and physical activity habits. If hysterectomy is indicated, tumor material will also be obtained and will be immediately frozen or used for immunohistochemistry (formalin fixed).

To analyze the metabolomics profile in 300 samples we will employ two complementary approaches:
**- Non-targeted metabolomics profiling** based on the Metabolon platform (Durham, USA) developed for the discovery of new biochemical biomarkers and that allows relative quantification. This will be performed using ultra performance liquid chromatography - tandem

mass spectrometer (UPLC-MS/MS) UHPLC/MS) (in positive and negative mode) to identify novel biomarkers among a panel of 700 metabolites (water soluble metabolites and lipids). Metabolites will be identified, and relative quantification will be performed (18).
- **Targeted metabolome analysis** based on the Biocrates platform (Innsbruck, Austria) for absolute quantification and validation. This will be performed by electrospray ionization tandem mass spectrometry (ESI-MS/MS). Analysis will be done using the AbsoluteIDQ p180 method (Biocrates AG) validated by the Food and Drug Administration (FDA) to measure concentrations of 186 metabolites, including acylcarnitines, amino acids, biogenic amines, hexoses, glycerophospholipids, lysophosphatidylcholines, phosphatidylcholines, and sphingomyelins. The analytes in plasma samples will be quantified by the use of internal stable isotope-labeled standards (19, 20).
- **The proteomics** will be analyzed with the established *scioDiscover* platform enabling the parallel analysis of **900 cancer-related proteins** for the identification of novel protein biomarker candidates. In this study the most informative targets as well as antibodies will be selected to help build a statistical model of the highest accuracy.
- **Tumors characteristics** will be obtained from the dossier (stage, grade), through immunohistochemistry (for the expression of prognostic markers like L1CAM, steroid hormone receptors, p53) and by global gene expression profiling (RNA-sequencing). Prognostic markers, signaling activation pathway (RNA analyses) will be used to predict patient prognosis at baseline.
- **Modelling** and biostatistics analyses fed with the metabolomics/proteomics/tumor features will be used to build diagnostic and prognostic tools. Since recent studies indicated that the performance of prediction models is improved by including also genetic background information and Mendelian segregation (21), genetic analyses could be performed on the materials. Whether to perform or not this analysis will be decided based on the performance of the model.

Metabolomics, proteomic and genetic analysis will be performed at Maastricht University Medical Centre (MUMC)/ Máxima Medical Centre Veldhoven (MMC) (Netherlands). Immunohistochemistry will be performed at U.O. Anatomia Patologica of IRCCS Ospedale Policlinico San Martino (Genoa, Italy). Materials will be coded and shipped to these centers in a way that the identity of the subjects cannot be retrieved by the local personnel.

Features that will be used to feed the biostatics models and algorithms
Blood metabolomics data; Blood proteomics data; General subject features (age, BMI, medical personal and family history, drug use); Clinical features (diagnosis, grade, stage, molecular markers on immunohistochemistry and RNA expression profiling); Genetic characterization. Depending on the model performance, not all parameters may be deemed necessary to develop the final prediction tools.

*Unsupervised analysis and preprocessing*
Various unsupervised techniques such as principle component analysis and clustering will be used for assessing initial data quality and potential need for normalization, outlier detection etc. Logarithmic transformation will be performed to ensure reasonable performance of statistical models that require data to follow normal distribution. The use of this unsupervised learning/analysis is aimed at obtaining insights into the underlying structure of the data.

*Cross-validation and supervised learning*
Next, supervised learning will be used to get insights into the predictive value of metabolites and proteins. We will make all possible combinations of datasets obtained from three described approaches (targeted and non-targeted metabolomics and proteomics) and clinical data of patients. In order to identify a robust differential set of biomarkers and be able to train and compare performances of different models the nested cross-validation strategy will be used. At the first level of which, we will split the corresponding dataset into train and test sets, and iteratively apply feature selection method to produce a set of features. Each individual machine learning model will be trained on produced features inside another (second) cross-validation loop in order to produce an optimal model.

*Performance evaluation*

We will use sensitivity, specificity, ROC curve shape, AUC and pAUC to assess the quality and performance of generated models and subsets of features on the outer loop of cross- validation. We will use bootstrapping method to calculate 95% confidence intervals for AUC. Both the quality of the gold standard and of our modelling methods will be quantified using the same tools (Sensitivity, Specificity, ROC curves, AUC) so that comparison will be possible using statistical tests. Gold standard for diagnosis will be the histologic diagnosis of EC or the absence of malignancy. Gold standard for prognosis will be a combination of current prognostic markers including grade Ia, Ib or higher, stage, histology, p53 positivity, L1CAM expression plus additional RNA signatures indicative of signaling pathway activation. Follow up data (5 years) will be used to confirm prognosis estimation.

*Building diagnostic and prognostic models*

Once the optimal model structure, algorithm and the set of robust features will be identified both diagnostic and prognostic models will be produced. For this all the available samples will be used. Diagnostic modelling will be applied to the whole population, whereas prognostic modelling will be applied to the case group.

**7. Work carried out and preliminary results**

Andrea Romano, member of our research group, has a great expertise in disrupts of local estrogen metabolism (or intracrinology) in EC, having performing several reviews and original articles on this topic (22-26). With specific regard to metabolomic evaluation, Romano *et al.* studied plasma metabolites in patients with EC with short (n = 20) and long (n = 20) survival, according to FIGO (International Federation of Gynecology and Obstetrics, 2009 criteria) stage, histology, and grade. A multiplex system including 183 metabolites was employed for evaluating plasma metabolites, determined using liquid chromatography-mass spectrometry. Partial least square discriminant analysis, together with hierarchical clustering, was used to identify patterns which distinguished short from long survival. A proposed signature of metabolites related to survival was suggested, and a multivariate receiver operating characteristic (ROC) analysis yielded an area under the curve (AUC) of 0.820–0.965 ($p \leq 0.001$). Methionine sulfoxide seems to be strongly associated with poor survival rates in these patients. In a subgroup with preoperative contrast-enhanced computed tomography data, selected metabolites correlated with the estimated abdominal fat distribution parameters. Metabolic signatures predicted prognosis, showing to be promising supplements when evaluating phenotypes and exploring metabolic pathways related to the progression of EC (27). In another study, Romano *et al.* investigated plasma levels of 19 steroids using liquid-chromatography tandem mass-spectrometry in 38 postmenopausal EC patients, with long (n = 19) and short (n = 19) survival. It was explored if estradiol levels were associated with specific abdominal fat distribution patterns and if transcriptional alterations related to estradiol levels could be observed in tumor samples. The plasma steroid levels for DHEA, DHEAS, progesterone, 21 OH progesterone and E1S were significantly increased (all $p < 0.05$) in patients with long survival compared to short. Estradiol levels were significantly positively correlated with visceral fat percentage ($p = 0.035$), and an increased expression of genes involved in estrogen related signaling was observed in tumors from patients with high estradiol levels in plasma. Overall, the association between increased estradiol levels and a high percentage of visceral fat indicated that visceral fat may be a larger contributor to estradiol production compared to subcutaneous fat in this population of patients (28).

**2. Expected results and relevant corresponding milestones**

Currently there is a lack of any valid noninvasive screening method for EC: biochemical diagnostic and prognostic assays that would identify early cases of EC and women with poor prognosis are needed; moreover, in high-risk populations, such as patients with breast cancer treated with tamoxifen, women who are severely obese, patients with diabetes mellitus, and patients with Lynch syndrome, a cost-effective screening method would be invaluable for early diagnosis; furthermore, there is also a great need for prognostic biomarkers of EC that would help in the stratification of patients with EC preoperatively into high-risk and low-risk categories, thus allowing further personalized treatments. The expected benefits of our research topic are the discovery of

diagnostic biomarker signatures for early diagnosis and for development of screening test, and the discovery of prognostic biomarkers for pre-surgical selection of EC patients with poor prognosis. Thus, this would lead to early diagnosis of EC non-invasively and improved treatment outcomes for the high-risk patients, likely decreasing the cases of under and over treatment. Due to the association of EC with the metabolic imbalances induced by, e.g., diabetes and obesity, it is likely to assume that metabolic features may be associated with patient prognosis. To this regard, we have previously demonstrated an association between a high visceral fat percentage and the reduced survival of patients with EC (28). In addition, as we have shown already for blood steroids, plasma metabolites may also be associated with survival and could serve as biomarkers for better prognostication (27).
Overall, metabolomics, the study of metabolites in body fluids and tissues, is a promising tool which has now become more readily available and has the advantage that it only requires fluids like blood and urine which are easily and non- or minimally-invasively collectable. The feasibility of metabolomics for biomarker discovery is supported by the assumption that metabolites are important players in biological systems and that diseases cause disruption of biochemical pathways. Our findings will aim to investigative putative serum biomarkers useful for detecting EC and predicting recurrence following initial surgery or medical treatment, to ultimately improve patient survival based on better stratification and informed treatment decisions.

**References of the research proposal are available as Supplementary Document 2.**

## 8. References and relevant publications by the research group, already available

8.1 Previous manuscripts about epidemiology, pathogenesis and treatment of EC


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- Konings GF, Cornel KM, Xanthoulea S, Delvoux B, Skowron MA, Kooreman L, Koskimies P, Krakstad C, Salvesen HB, van Kuijk K, Schrooders YJ, Vooijs M, Groot AJ, Bongers MY, Kruitwagen RF, Enitec, Romano A. Blocking 17beta-hydroxysteroid dehydrogenase type 1 in endometrial cancer: a potential novel endocrine therapeutic approach. J Pathol. 2018;244(2):203-14.

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8.2 Previous manuscripts about metabolomics biomarkers for diagnosing and predicting prognosis of EC


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PERSONNEL INVOLVED IN THE RESEARCH

| Name and date of birth | Role on Project | Fellowship required | Effort on project (%) | Present position |
|---|---|---|---|---|
| Simone Ferrero | Principal investigator | No | 25 | Associate Professor, U.O. Clinica Ostetrica e Ginecologica, IRCCS Ospedale Policlinico San Martino, Genoa, Italy |

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|---|---|---|---|---|
| Fabio Barra | Subinvestigator | No | 35 | Resident, U.O. Clinica Ostetrica e Ginecologica, IRCCS Ospedale Policlinico San Martino, Genoa, Italy |
| Valerio Gaetano Vellone | Subinvestigator | No | 5 | Associate Professor, U.O Anatomia Patologica, IRCCS Ospedale Policlinico San Martino, Genoa, Italy |
| Claudio Gustavino | Subinvestigator | No | 5 | Head Physician, U.O. Ostetricia e Ginecologia, IRCCS Ospedale Policlinico San Martino, Genoa, Italy |
| Paolo Sala | Subinvestigator | No | 5 | - Staff Physician, U.O. Ostetricia e Ginecologia, Ospedale IRCCS Ospedale Policlinico San Martino, Genoa, Italy<br>- President, LILT Sezione provinciale di Genova<br>-LILT coordinator, Regione Liguria |
| Sara Stigliani | Subinvestigator | No | 5 | Postdoctoral researcher, UOS Fisiopatologia della Riproduzione Umana, IRCCS Ospedale Policlinico San Martino, |

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|---|---|---|---|---|
| | | | | Genoa, Italy |
| Matteo Tantari | Subinvestigator | No | 5 | Resident, U.O. Clinica Ostetrica e Ginecologica, IRCCS Ospedale Policlinico San Martino, Genoa, Italy |
| Andrea Romano | Subinvestigator | No | 15 | Associate Professor, Faculty of Health, Medicine and Life Sciences, Maastricht University, Netherlands |

CVs of the research group is available as Supplement documents 3-9

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

1 **Simone Ferrero**: presentation of the data collected to the research team; presentation of preliminary results to the research team; presentation of preliminary results in international meetings; publication of final study results in international scientific journals

2 **Fabio Barra**: collection and processing of biological samples; data analysis; presentation of the data collected to the research team; presentation of preliminary results to the research team; presentation of preliminary results in international meetings; publication of final study results in international scientific journals

3 **Valerio Gaetano Vellone**: pathological analysis of biological samples

4 **Paolo Sala**: presentation of preliminary results to the research team; presentation of preliminary results in international meetings; publication of final study results in international scientific journals

5 **Claudio Gustavino**: presentation of preliminary results to the research team; presentation of preliminary results in international meetings; publication of study final results in international scientific journals

6 **Sara Stigliani**: collection and processing of biological samples

7 **Matteo Tantari**: collection and delivery of biological samples

8 **Andrea Romano**: proteomic and genetic analysis of biological samples

Budget Form /year

1. Research costs. 20.000 €
2. Instruments. 12.000 €
3. Indirect costs*. 3.000 €

**Sub-total 35.000** €

4. Overheads*. 5.000 €

**Total 40.000 €**

The entire budget is intended to support the expenses for the Italian center involved in the study protocol.

**DETAILS OF BUDGET COSTS**

- **RESEARCH COSTS**

-Participation to scheduled meetings of
the scientific centers involved in the project 5.000 €
-Samples collection, processing and delivery 6.000 €
-Dissemination of the results obtained 4.000 €
(i.e. publications, organization of meetings and/or events)
-Participation in national and international meeting 5.000 €
for presenting the study results

- **INSTRUMENTS**

-Small equipment 8.000 €
(Purchase/ annual rental of non-refrigerated centrifuge; attached a cost estimate for purchase as supplementary document 10)
-Expenses for materials of use 4.000 €
(Purchase of materials of use for collecting patients' samples; of antibodies and other materials of use for doing immunohistochemical analysis of samples)

**EXISTING/PENDING SUPPORT:** None

**SUGGESTED REVIEWERS (MAX 3)**

- prof. **Fabio Ghezzi**, full professor of Gynecology and Obstetrics
Clinica Ostetrica e Ginecologica, Ospedale F. Del Ponte, Varese, Italy
mail: fabio.ghezzi@uninsubria.it

- dott. George **Angelos Vilos**, full professor of Gynecology and Obstetrics
Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Schulich School of Medicine and Dentistry, Western University, London, UK
mail: george.vilos@lhsc.on.ca

-dott. **Salvatore Gueli Alletti**, oncology staff specialist
Fondazione Policlinico Universitario Agostino Gemelli IRCCS Università Cattolica del Sacro Cuore, Rome, Italy
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**BIOETHICAL REQUIREMENT**

1. Human experimentation YES

The application for study approval to the ethics committee of IRCCS Ospedale Policlinico San Martino has been performed (see Supplementary Document 11).

Maastricht University has obtained the approval from ethics committee (see Supplementary Document 12).

2. Animal experimentation NO

**Declaration**
I shall confirm to the Declaration of Helsinki in its latest version.
I shall also apply the Bioethics Convention of the Council of Europe.
In implementing the proposed research, I shall adhere most strictly to all existing ethical and safety provisions applicable.

Before start of the research, I shall obtain clearance from the competent ethical committee in case of involvement of human subjects in the research and /or in case of other ethical implications.
I shall conform with all regulations protecting the animals used for research purpose.

Date: 10/02/2020 Name of PI Simone Ferrero Signature.

**Principal investigator's signature** .....

**Authorized Administrative Official's signature** .....

Date 10/02/2020

Si autorizza al trattamento dei dati ai sensi del d.lgs. 196/2003