Next generation sequencing-based genomic profiling of high-grade serous ovarian cancer. Natural history and response to chemotherapeutic agents

Introduction

High-grade serous ovarian cancer (HGSOC) is the most lethal gynecological cancer in the world and the 5th most common cancer related cause of death for women between 50 and 69 years in Italy. Overall it represents the 35% of all cancer of the female genital organs [1].

Current HGSOC treatment entails surgical resection of the tumor followed by adjuvant platinum-based chemotherapy, with or without paclitaxel. Despite an acceptable initial response to chemotherapy, most women eventually develop chemotherapy resistance and die of recurrent disease [2]. A major hurdle to improving treatment choices for ovarian cancer is the extreme genomic heterogeneity observed between cases and within the same tumor [3]. Understanding the mechanisms that drive the heterogeneous chemotherapy responses is critical to prolonging ovarian cancer patients' survival.

A large pool of potential targets for anti-cancer therapy resulted from basic research on ovarian cancer, nonetheless, the development of new therapies has been slow. The current and future generation of anti-cancer drugs are developed to specifically activate or deactivate deregulated gene products or to signal pathways in cancer cells. The development of such “targeted” agents is an exciting new opportunity that promises to deliver more anti-cancer efficacy and less toxicity, by interacting with a specific signaling pathway instead of dealing with non-specific phenomena, such as proliferation or metabolism.

The greatest success in identifying a target for ovarian cancer therapy has resulted from studies with inhibitors of the DNA repair enzyme, poly(ADP)ribose polymerase (PARP). Patients with germline mutations of BRCA 1 or 2 have impaired mechanisms to repair DNA damage. In these patients, their tumors are more sensitive to PARP inhibitors (PARPi) as they compound the defective repair of DNA damage [4]. PARPi appeared to be effective also on in BRCAwt patients with tumors expressing homologous recombination deficiency (HRD)[5].

However, despite some new insights in pathway directly involved in the ovarian cancer response to therapy, we do not have many clear targets to aim for. Therefore, there is an urgency to investigate and develop new strategies and technologies to select patients for targeted therapy based on molecular aberrations in individual tumor and in its natural history. One solution could be to start focusing on the mutational genetic profile of the individual tumor and its metastatic counterpart and not on the origin organ of the primary tumor.

Due to the increased affordability of the reading and sequencing of significant parts of the genome, the study of the human cancer genome is a promising field of research that will lead to important innovation in the next years. In particular, Next Generation Sequencing (NGS) platforms are transforming our ability to read and understand the alterations in the genome of cancer cells [6]. The use of such technology for patient care could become cost-effective and is potentially superior in predicting therapy response compared to our standard diagnostic tests mainly focusing on specific mutations in a single gene or protein.

A comprehensive insight of the cancer cell genetic profile is essential in determining whether the targeted drug is inhibiting a crucial component of the signaling cascade. The advent of NGS platforms enables us to assess a significant proportion of the cancer cell genome and thus give the chance to develop a more realistic scenario of the complex genetic changes that occur in ovarian cancer cells during metastatic process, under chemotherapy, and along their evolution. We aim to use NGS platforms to evaluate the genetic profile of patients’ ovarian cancer cells in a prospective study and thus following-up those cells after systemic treatment and recurrence. My long term goal with this research project is to give a substantial contribution to expand the scientific knowledge on ovarian cancer natural genetic history and to identify new possible targeted agents.

Objectives

Primary objective

The main goal of this study is to analyze the individual cancer genome in HGSOC patients at baseline, after treatment, and after relapse to develop future predictors for recurrence and response to systemic treatment.

Secondary objectives

Secondary objectives of this study are:

- To determine the amount of biopsy samples with sufficient DNA for analysis
- To determine the amount of biopsy samples with an adequate mutational profile
- To collect and anonymously interpret all mutational profiles obtained using this protocol
- To determine changes in the mutational profile under the influence of systemic treatment
- To explore and analyze the individual microRNA, proteomic profiles in patients with ovarian cancer to develop future predictors for response to systemic treatment
- To explore the correlation between mutational profiles in solid tumor biopsies and liquid biopsies
- (circulating tumor DNA)

Primary endpoint

The primary endpoint is the number of patients with adequate mutational profiles of their cancer genome and adequate follow up of systemic treatment efficacy. We aim to determine the mutational profile cancer genome in 50 HGSOC patients a year.

Secondary endpoints

Secondary endpoints are:

- Percentage of samples with sufficient DNA for sequencing analysis
- Percentage of samples with an adequate mutational profile that allows biomarker discovery efforts.
- Database of all (anonymized) data obtained using this protocol
- Differences in mutational profile pre, post and during treatment
- Number of samples with an adequate microRNA, proteomic that allows biomarker discovery efforts.
- Number of samples with a clear correlation between mutational profiles in solid and liquid
- biopsies
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Materials and Methods

Study design

My goal is to set up a multicenter study combining histological biopsy of tumor material with DNA sequencing using Next Generation Sequencing (NGS) platform. The study aims to deliver a more accurate pre-treatment stratification of HGSOC patients by getting fresh tumor biopsies for next-generation sequencing to obtain a mutational profile and to follow-up patients along treatment and relapse to monitor mutational profile of cancer cells. In order to achieve the most positive outcome from this research, I already ensured the collaboration of the main stakeholders in the field both at a local and national level. In fact, this multicenter study will be carried out at the University Hospital Policlinico Paolo Giaccone of Palermo and will include the participation of other academic hospitals, including the Gynecology and Obstetric Unit of the Cervello Hospital of Palermo directed by Prof. Perino. At a national level, I already secured the collaboration of the Gynecologic Oncology Unit of the Catholic University of the Sacred Heart in Rome directed by Prof. Scambia, where I am working now.

Patient selection

Selection criteria, defined as inclusion criteria, are:

- Patients age > 18 years, willing and able to comply with the protocol as judged by the investigator with a signed informed consent.
- Pathologically diagnosed ovarian cancer, fallopian tube cancer, or primary peritoneal cancer with a serous histopathological profile with high-grade features.

Baseline screening

All patients will provide written informed consent before any study-specific procedures are performed. Required baseline screening investigations after study enrollment (i.e. after written informed consent) are to be performed within 28 days before the biopsy. Baseline screening investigations include:

- Written informed consent
- Eligibility Screening Form
- History
- Medical history (with special interest in previous malignancies and previous chemotherapy) and demographics
- Pre-biopsy laboratory and physical examinations as per local guidelines.
- Radiological tumor assessment (contrast-enhanced CT or MRI)

Study related procedures

Tumor Biopsy

Complete cytoreduction at the time of primary debulking surgery is currently considered as the goal to be pursued in the upfront management of women with advanced ovarian cancer disease (National Comprehensive Cancer Network guidelines) [7]. In patients with unresectable disease, platinum (Pt)-based neoadjuvant chemotherapy (NACT) has emerged as a reliable therapeutic strategy.

Laparoscopy plays an important role to adequately evaluate the possibility to achieve complete cytoreduction and to obtain biopsy both of tumor and peritoneal metastasis both in the upfront management and at the time of interval debulking surgery (IDS).

In patients undergoing NACT, after three or four chemotherapeutic courses a second surgical evaluation will be performed at the time of IDS and a second tumor biopsy may be obtained depending on the response to therapy. This may allow us to determine direct effects of the systemic treatment on the mutational profile and to observe new mutations that may relate to the response to systemic treatment.

Histological biopsy of the tumor and/or metastatic implants for DNA sequencing are obligatory at baseline (pre-treatment), optional during and after treatment (on-treatment and post-treatment biopsies).

Patients safety issues are limited since tumor biopsy are taken during the surgical procedure according to accepted surgical protocol for ovarian cancer.

Blood samples for DNA sequencing

Blood sample for determining patient's germline DNA background variation: obligatory at baseline only. Blood samples are necessary to assess each patient's germline DNA background variation and compare it with tumor genetic profile to focus on relevant somatic mutations (avoiding for example SNPs) which will be correlated to the response to therapy.

A baseline blood sample for DNA sequencing will be taken preferably combined with baseline laboratory examinations after obtaining histopathology diagnosis. The blood sample will be used to determine the patient's germline DNA background variation.

Blood samples for liquid biopsy (circulating tumor DNA)

Blood sample for liquid biopsy (circulating tumor DNA) at baseline, during treatment and at disease progression. Blood sample for sequencing of circulating tumor DNA (ctDNA) will be taken at baseline after obtaining histopathology diagnosis, and optionally every 8-12 weeks during treatment and at relapse. Samples are preferably combined with baseline or routine laboratory examinations.

Tissue biopsy of healthy tissue

Tissue biopsy of healthy tissue in selected patient groups (early stage ovarian cancer) for correlation analyses between healthy and tumor tissue. Patients with apparent I stage ovarian cancer are supposed to adequately receive appropriate surgery that should comprehend random peritoneal biopsy. If the diagnostic pathological assessment has been completed, a part of remaining tissue may be used for DNA sequencing, serving as a control of healthy tissue in patients with ovarian cancer.

Next generation sequencing (NGS)

The utility of the Sanger DNA sequencing is limited when analyzing multiple genes from several patients simultaneously because tests for targeted genes often need to be conducted serially instead of simultaneously.

Next-generation sequencing (NGS) platforms are based on a single reaction amplification of single molecules and subsequent massive parallel sequencing of millions of molecules. Use of NGS enables assessment of many genes associated with increased cancer risk at once, providing results in less time than required for several Sanger sequencing analyses to be conducted serially. Additionally, with this technique we are able to isolate specific regions of interest from genomic DNA samples before the sequencing reaction, thereby excluding the analysis of irrelevant genes or repetitive regions. NGS application has already been employed with good results in ovarian cancer, although tumors were not followed along their natural history [8].

Obtained sequence data are processed using a publicly available algorithm to identify genetic alterations. Additional filtering steps are applied to focus on those variants most likely affecting protein function, like non-synonymous-, splice-site- and nonsense mutations together with small insertions and deletions. We will also perform a signaling pathway analysis to identify those pathways with aberrant activity, opening up potential intervention strategies.

The research will be focused on but not limited to the following biomarkers and pathways: BRCA somatic mutation, homologous recombination deficiency (HRD), RAD51, NF1, HER2, PI3K/RAS Cyclin E1 (CCNE1), c-Myc, miRNA of the miR-200 family.

Follow-up

Patients will be followed-up every 6 months for five years according to hospital protocols. At relapse, a new surgical evaluation will be performed and tumor biopsy will be obtained for DNA sequencing.

Feasibility of research project and work plan

The project will be developed over the course of three years [Figure 1]. During the first 3 months, I will carry out a bibliographic research to select the proper panel of genes to assess by NGS. In the meantime, I will also write the protocol that will be submitted for approval to the Hospital's ethics committee. In the first Half of the first year I will build a database (using Access) that will help me to manage the case report forms (CRF) and to ensure a homogeneous data collection across all the hospitals involved in the project. At the same time, I will select collaborators in other Hospitals and test NGS platforms.

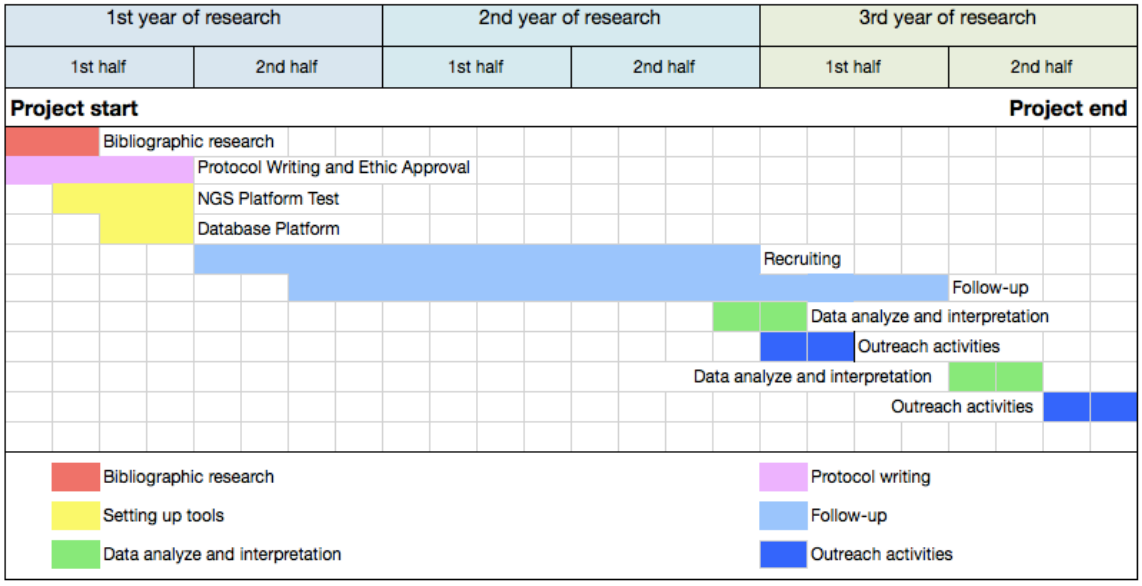

Figure 1. Gantt chart of research project work plan

After 6 months I expect the ethics approval process to be successfully concluded. At this point, I will be able to start recruiting. An early data analysis together with a preliminary publication of the data will be carried out after 1 year of recruiting, when I expect that I will have already collected 40 to 50 cases (out of which 20% with a second biopsy at IDS). The recruiting will continue until the end of the second year, while the follow-up, the IDS biopsy, and the relapse biopsy

will be carried out until the second half of the third year. During the last six months, I will focus on analyzing and interpreting the entire batch of results. This last activity will be partially overlapped and linked to outreach activities such as preparation of scientific publications and actions of knowledge diffusion.

Conclusions

The main outcome of this research will be a substantial contribution to the understanding of the genetic processes that occur in ovarian cancer cells. I will achieve such results by focusing on ovarian cancer mutational profile compared to the patient's germline DNA background variation, to the same cancer cells at relapse or under the selective pressure of chemotherapeutics agents.

The fortunate circumstance that NGS has become increasingly accessible to researchers represents a significant advance in the democratization of human genome sequencing techniques and expands the research borders of ovarian cancer investigation. Such circumstance, together with the increased numbers of publication in this field of research of the last years, suggests that important advances will be achieved in the next 5-10 years. A deeper investigation of on ovarian cancer genome profiles through NGS, such as the one here proposed, will help the Department of Surgical, Oncology, and Dentistry of the University of Palermo to o take part in an international debate by publications of pioneering works.

Furthermore and at a higher level, apart from assessing the predictive value of the mutational profile, generating a more comprehensive insight of ovarian cancer cell molecular portrait may give access to possible targeted agents, tracing the way for developing innovative anti-cancer therapies.

I matured a sound interest and useful experiences in this field during my attendance in gynecology oncology at the Catholic University of the Sacred Heart in Rome where I now work as a surgeon. I also had the chance of establishing personal relations with leading researchers in the field, both at a local and a national level, that help me to successfully accomplish this research project.

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