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

TRANSLATIONAL RESEARCH

Principal investigator's full name and qualification: Professor Laura Ottini, MD
(CV in European format with list of publications, IF and H-index is included as separate file)

Proposal title: Germline and somatic characterization of male breast cancer for new molecular biomarker discovery.

Primary area of Relevance: Prognostic and Predictive biomarkers in Breast Cancer.

Relevance for the National Health System: Breast cancer (BC) in men is a rare and less investigated disease compared with BC in women. To date, male BC (MBC) clinical management has been considered similar to female BC management, but increasing evidence indicates that BC in men and women may behave differently. Thus, there is an urgent need to obtain further data on the genetics and biology of this disease and how to best treat and support MBC patients.
A fundamental goal of cancer precision medicine is to incorporate individualized factors into clinical decision making. Our study, integrating germline and somatic data with clinical-pathologic information, will provide insights into key molecular classification of MBC which will facilitate the characterization of actionable strategies for MBC patients.
The identification of subgroups of patients who deserve specific, individualized clinical management both in terms of screening and treatment, will address an urgent and unmet clinical need in MBC and have profound impact on our approach to this disease.
The multicentric study with national coordination and the collaborative multidisciplinary approach, core of this proposal, will also ensure an increase in MBC understanding and dissemination of information and awareness to the public.

Institution / University: Sapienza University of Rome, Department of Molecular Medicine, Viale Regina Elena 324, 00161 Rome, Italy. Phone 06/49918268; e-mail: laura.ottini@uniroma1.it

Authorized Administrative Official: Sapienza University of Rome, Department of Molecular Medicine, Viale Regina Elena 291, 00161 Rome, Italy. Phone 06/49255681; e-mail: carlo.appetecchia@uniroma1.it

Proponent's signature .....

Authorized Administrative Official's signature .....

IL RESP. AMM.VO DELEGATO
Dott. Carlo Appetecchia

Place and date: Rome, 14/02/20

SELF EVALUATION FORM

1. Investigator's full name: (PI) **Laura Ottini**
2. Total papers **131**; IF (relative to the publication year) **721.55**
3. Total papers (last 10 years) **73**; IF (relative to the publication year) **507.5**
4. Total Papers as first/last author or corresponding author **62**
5. Total H-index **35** (Scopus)

PROPOSAL MAIN BODY

PROPOSAL TITLE: Germline and somatic characterization of male breast cancer for new molecular biomarker discovery.

ABSTRACT

Rationale of the study: male breast cancer (MBC) is a rare and less investigated disease compared with female BC. To date, MBC clinical management has been considered similar to female BC management, however, increasing evidence indicates that BC in men and women may behave differently. Thus, there is an urgent need to obtain further evidence on the genetics and biology of this rare disease and how to best treat and support MBC patients.

A fundamental goal of cancer precision medicine is to incorporate individualized factors into clinical decision making, from risk assessment to prevention and treatment. Our scientific premise is that the identification and integration of germline and somatic alterations will have profound impact on understanding the biology and genetics of MBC and will provide a strong evidence base for the potential clinical translation of findings with a positive impact on individualized medicine for MBC patients.

Preliminary results: we have established the first Italian Multicenter Study on MBC, including thus far 15 centers. Results from the multicenter study showed that in Italy *BRCA1/2* (*BRCA*) germline mutations are responsible for about 15% of all MBCs.[1] Thus, a large percentage of MBCs remains to be assigned to specific genetic factors. In collaboration with the international Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA), we obtained evidence that men with *BRCA* mutations are prone to develop bilateral BC and multiple tumors,[2] including prostate cancer, suggesting the male *BRCA* mutation carriers might deserve specific screening and surveillance program. We also showed that *BRCA*-associated MBCs are likely to represent a subgroup of tumors with a more aggressive phenotype than their sporadic counterpart[3] and may deserve specific clinic management.

Detailed description of the translational value of the research and the expected impact on the NHS: in this project, we aim to characterize a large series of MBC cases for germline and somatic alterations. Specifically, we aim to improve genetic risk assessment in men affected by BC and to identify specific subgroups of MBCs and potential biomarkers with clinical value.

Available samples and data from the ongoing Italian Multicenter Study on MBC will be used and the series will be expanded including new centers. Newly recruited MBC cases will be screened for germline mutations using comprehensive cancer gene panels, in order to better define the fraction of MBCs due to genetic predisposition. Furthermore, breast tumors arising in men with and without germline mutations will be investigated by a comprehensive genomic profiling, including tumor mutation burden (TMB), a promising predictive biomarker to select patients that may benefit from immune checkpoint inhibitor therapy, in order to facilitate the discovery of prognostic and predictive molecular biomarkers.

The proposed extensive analysis of genetic susceptibility in MBC is expected to identify a group of men with inherited predisposition to BC. These results will add important information to the genetic predisposition in MBC and will facilitate the development of gender-specific clinical management guidelines in order to offer more targeted screening and surveillance programs for these men and their families. The discovery of genetic variants associated with MBC may be also useful for the identification of families with high genetic susceptibility to cancer and may improve clinical management of all family members, including female relatives.

The characterization of somatic mutational landscape and TMB in MBCs with and without germline mutations is expected to lead to the identification of subgroups of patients who could benefit from individualized therapeutic approaches, addressing an urgent and unmet clinical need in MBC which has profound impact on our approach to this rare disease.

The collaborative multicentric study with national coordination and multidisciplinary approach, core of this proposal, will also ensure an increase in MBC understanding in the medical and scientific communities and dissemination of information and awareness to the public.

INTRODUCTION, BACKGROUND AND RATIONALE

Male breast cancer (MBC) is a rare disease accounting for about 1% of all BC cases. Inherited mutations in *BRCA1* and, mainly, *BRCA2* genes predispose to MBC and account for up to 15% of MBCs.[1] In Italy, *BRCA2* and *BRCA1* mutations account for 13% and 2% of MBCs, respectively.[1] Our results, based on an extensive genomic screening of a large series of MBCs by a multigene panel, showed that pathogenic variants in DNA repair/genomic instability genes, other than *BRCA*, may account for about 6% of MBCs and, in particular, indicated a relevant role of *PALB2* in MBC predisposition.[4-6] We have shown that, in Italy, more than 1% of non-*BRCA* MBC cases are carriers of *PALB2* germline mutations.[4-5]

Overall, a large percentage of MBCs, particularly MBC cases at high genetic risk, remain to be assigned to specific genetic factors. With this project we aim to expand the spectrum of MBC susceptibility genes and further improve genetic risk assessment in the male population. An extensive genetic screening of a large series of non-*BRCA* MBCs by Next Generation Sequencing (NGS) using a well-designed multi-gene panel, including DNA repair genes and phenotype-recommended genes, based on personal and family history of cancer, will be performed. This strategy may lead to a better characterization of MBC genetic predisposition and help in evaluating pathways with potentially therapeutic implications.

Molecular tests that analyze both germline and somatic alterations may yield detailed characteristics of molecular events for diagnostic, prognostic and predictive purposes, thus facilitating the discovery of actionable mutations. In this project, we plan on integrating matched germline and somatic data in order to enhance prediction of pathogenicity of rare germline variants and provide insight into new, potentially actionable, gene variants. The analysis of the molecular profiles of *BRCA* and non-*BRCA* MBCs for DNA repair variants may lead to the identification of possible co-existing somatic mutations in tumors, associated and non-associated with germline *BRCA* mutations, that might be therapeutic targets. Overall, a comprehensive investigation of the molecular profiles of MBCs characterized for germline mutations by multigene panel sequencing, could provide opportunities for the delivery of precision medicine approaches facilitating the identification of subgroups of patients that may benefit from agents targeting specific pathway defects, as for example PARP inhibitors for DNA repair deficiency.

Pathogenic variants in DNA repair genes have been associated with increased tumor mutation burden (TMB) and neoantigen burden.[7] Based on this observation, BCs with defects in *BRCA* are hypothesized to be more immunogenic than tumors without genetic defects in these genes. However, despite *BRCA1*- and *BRCA2*-associated BCs display similar features of genomic instability, they do not show similar immunophenotype.[8] On the other hand, there are evidence suggesting that some tumors without germline *BRCA* mutations are similar to *BRCA*-associated tumors and display “BRCAness”. Several somatic mutational signatures seem to be linked to pathogenic germline variants in DNA repair genes, including *PALB2*.[9] Currently, based on the hypothesis that the inactivation of *BRCA* genes may give rise to a BC specific immunophenotype, preclinical and clinical studies are investigating whether the immunogenic microenvironment seen in *BRCA*-mutated BC patients could be leveraged with checkpoint blockade.[10] However, the degree of anti-tumor response varies widely and molecular features contributing to this variability remain unknown.

In this context and as a result of NGS technology applications on cancer research, TMB is emerging as a promising predictive biomarker to select patients that may benefit from immune checkpoint inhibitor therapy.[11] However, the role of TMB in tumor immunogenicity is not completely understood in BC. To the best of our knowledge, a comprehensive genome analysis associated to TMB status has not yet been described in MBC. The analysis of the molecular profiles associated to TMB status in MBC cases with and without germline mutations in genes involved in genome maintenance, such as *BRCA* and *PALB2*, may provide further insights into the comprehension and characterization of MBC somatic landscape and lead to a more accurate classification of MBC molecular subtypes, with potential therapeutic implications.

EXPERIMENTAL DESIGN AND OVERALL METHODS

In this project we intend to characterize a large series of MBC cases for germline and somatic alterations. Available samples and data from the ongoing Italian Multicenter Study on MBC will be used and the series will be expanded (***Task 1***). Newly recruited MBC cases will be screened for germline mutations using comprehensive cancer gene panels, in order to better define the fraction of MBCs due to genetic predisposition (***Task 2***). BC arising in men with and without germline mutations will be investigated by comprehensive genomic profiling for the discovery of prognostic and predictive molecular biomarkers (***Task 3***).

Task 1: Study population and data collection

During the past 10 years, we have established the first collaborative Italian Multicenter Study on MBC, recruiting a large series of MBC cases.[12-15] For each case we obtained detailed information on: -personal history, and first- and second-degree family history of cancer at any site; -clinical-pathologic and molecular data, including histology, grade, stage, node status, ER, PR, HER2, Ki67 and *BRCA* mutation status. Survival data have been already obtained in a subset of cases and successfully correlated to genetic and lifestyle factors.[16]
Blood samples and step sections from formalin-fixed paraffin-embedded (FFPE) primary mammary tumor blocks or frozen tumor samples were obtained to perform ad hoc bio-molecular analyses. Overall, 750 Italian MBCs, including 110 *BRCA* mutation carriers, are available. In the present project, we plan on expanding the collection of cases, together with clinical-pathologic features. Survival data and follow up will be collected and kept updated for all cases.

Aims:

- To collect additional MBC cases together with clinical-pathologic and survival data.

Methods:

New MBC cases who refer to Investigation Centers participating in the Italian Multicenter Study on MBC will be collected. Centers which already participated in the Italian Multicenter Study on MBC are the following:

- Sapienza University of Rome, Department of Molecular Medicine (coordinating center), Department of Experimental Medicine
- Cancer Risk Factors and Lifestyle Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Florence
- University of Modena and Reggio Emilia, Department of Oncology and Haematology
- University of Palermo, Department of Surgical and Oncological and Oral Sciences
- University of Napoli “Federico II”, Department of Advanced Biomedical Sciences
- Genome Diagnostics Program, IFOM - The FIRC Institute of Molecular Oncology, Milan
- Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Research, Fondazione IRCCS Istituto Nazionale Tumori (INT), Milan
- Division of Cancer Prevention and Genetics IEO, European Institute of Oncology IRCCS, Milan
- Unit of Functional Onco-Genomics and Genetics, Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Aviano
- Immunology and Molecular Oncology Unit, Veneto Institute of Oncology IOV - IRCCS, Padua
- IRCCS Ospedale Policlinico San Martino, Genoa
- Molecular Genetics Laboratory, Istituto Tumori Giovanni Paolo II, Bari
- Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola
- Department of Pathology, ASST Settelaghi and Centro di Ricerca per lo Studio dei Tumori Eredo-Familiari, Università dell'Insubria, Varese
- Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste

Additional centers will be involved in the multicenter study, including S. Andrea Hospital, Rome (Prof. Andrea Vecchione).
Based on the available estimates, an additional series of about 150 cases will be included. Clinical-pathologic information, including personal history of cancer, first- and second-degree family history of cancer, tumor histology, grade, stage, node status, ER, PR, HER2, Ki67 and *BRCA* mutation status, will be collected for each case. Detailed data on vital status, in terms of overall survival

(OS), will be collected for all recruited cases. Follow-up will be required to each participating center and periodically updated.
To ensure the achievement of statistically significant results in the molecular analysis of tumor samples, the current series of 150 FFPE male breast tumors will be expanded to include a minimum of 200 tumors.

***Task 2*: To screen MBC cases for germline mutations by a large multigene panel**

Although *BRCA1* and *BRCA2* are recognized as the major MBC susceptibility genes, together they account for only about 10-15% of the hereditability of MBC.[1] In our MBC series we observed that about 15% of MBCs are due to *BRCA* mutations and that a large fraction of MBC cases with personal and/or family history suggestive of hereditary BC are not accounted for by mutations in *BRCA* genes. We also reported that mutations in other known/proposed BC genes, mainly involved in DNA repair/genomic instability pathways, are responsible for about 6% of the Italian MBC cases.[5]
In this project, we plan on expanding to the newly recruited cases (***Task 1***), the ongoing extensive genetic screening by NGS, using a well-designed multigene panel, including DNA repair genes and phenotype-recommended genes. This strategy may lead to a better characterization of MBC genetic predisposition and help in improving and facilitate the clinical management of these patients and their relatives of both sexes.

Aims:

- To perform genetic screening of MBCs by a comprehensive multigene panel.

Methods:

All newly recruited MBC cases will be screened by NGS using a custom-designed multigene panel including 50 cancer-associated genes. This panel has been already successfully used to screen more than 500 MBC patients from the Italian Multicenter Study on MBC.[5]
For each new study participant, DNA from blood samples will be extracted using ReliaPrep Blood gDNA Miniprep System (Promega, Madison, Wisconsin, USA), according to the manufacturer's instructions. Genomic regions of interest are prepared in paired-end libraries using the Nextera Rapid Capture Custom Enrichment kit (Illumina, San Diego, California, USA), pooled and loaded into the MiniSeq system (Illumina) for automated cluster generation, sequencing and data analysis, including variant calling.
Results will be viewed, annotated and filtered using Illumina Variant studio software. Variants will be classified as pathogenic based on the American College of Medical Genetics and Genomics Criteria.[17] Furthermore, to assess the clinical relevance for variants of uncertain significance, comparison of germline and corresponding tumor DNA, family co-segregation, and in silico analyses will be performed. Mutation positive samples will be confirmed by double-stranded Sanger Sequencing.

***Task 3*: To screen MBC cases for somatic mutations and Tumor Mutational Burden (TMB)**

There is evidence that BCs with *BRCA* alterations may be unstable at genomic level, with increased TMB. The increased numbers of non-synonymous single nucleotide variants, observed in *BRCA*-associated tumors, is likely to be associated with neoantigen burden leading to increased immunogenicity and making these tumors potential candidates for immune checkpoint blockade therapy.[18-21] Thus, TMB is emerging as a promising predictive biomarker to select patients that may benefit from immune checkpoint inhibitor therapy. *BRCA* wild-type BCs may also show a high degree of genomic instability, suggesting that there may be BCs with defects in other DNA repair genes that may benefit from immune checkpoint blockade therapy. Thus, BCs with defects in *BRCA*, and possibly other DNA repair genes, are hypothesized to be more immunogenic than tumors without genetic defects in these genes.
A comprehensive investigation of the molecular profiles of MBC cases, characterized for germline mutations by multi-gene panel sequencing, could provide opportunities for the delivery of precision medicine approaches facilitating the identification of molecular subgroups that may benefit from agents targeting specific pathway defects. In this context, we plan to analyze the somatic landscape and the TMB status in MBCs characterized for germline mutations, in order to shed light

on mechanisms by which different genomic features may give rise to actionable somatic alterations.

Aims:

- To characterize actionable somatic alterations and TMB status in MBC cases with and without germline mutations in *BRCA* and other DNA repair genes in order to identify clinically relevant molecular biomarkers.

Methods:

MBC cases, previously characterized for germline mutations in 50 cancer related genes (***Task 2***), for which tumor FFPE slides are available, will be evaluated at somatic level. A minimum of 30 MBC cases, 15 with and 15 without germline mutations will be selected.
DNA will be isolated from FFPE tumor sections by using the QIAmp DNA FFPE tissue kit, according to the manufacturer's instructions (Qiagen Inc. Charlesworth, CA). Tumor DNA will be extracted from microdissected specimens to enrich for about 70% of tumor cells.
Archived FFPE sections are a widely available source of nucleic acids for molecular profiling. However, the yield and quality of extracted DNA depends on the type, age of the sample and the conditions used for the fixation. Low quantity and fragmentation of DNA extracted from FFPE tissues may affect somatic molecular characterization. Specific extraction protocols, developed to improve DNA quality and quantity from FFPE samples, have been already validated in our laboratory.[22,23]
We will use DNA sequencing approaches, specifically NGS technologies, that allow for molecular analysis of FFPE samples through a massive scale sequencing, require a low nucleic acids input quantity and enable the molecular profiling of these samples with robust and reproducible qualitative data.[24,25] As shown by our recent publications[22,26] and preliminary results presented here, we have acquired expertise in optimizing DNA extraction from FFPE samples and in subsequent molecular analyses using NGS technologies.
In this study, TruSight™ Oncology 500 (Illumina, San Diego, CA), a commercial NGS panel designed to analyze 523 cancer-relevant genes, particularly appropriate for FFPE samples, will be used for library preparation. Its size (1.94 Mb) allows for the calculation of TMB status. Sequencing will be performed on the Illumina NextSeq System.
The processing of NGS data, including alignment, calling, annotation of somatic alterations and TMB calculation will be carried out by an ad-hoc bioinformatic pipeline integrated within PierianDX (https://www.pieriandx.com/). This clinical software will also provide variant interpretation and reporting, based on its Clinical Genomics Workspace platform and Clinical Genomics Knowledgebase, allowing for rapid identification of actionable alterations, for which targeted therapy is available.
Chi square test or Fisher's exact test and logistic regression models will be performed in order to evaluate the potential associations between actionable somatic mutations, TMB status and clinical-pathologic features. Differences in OS between groups of patients with different set of alterations will be assessed using the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression will be also used. Statistical analyses will be performed with the R software (*www.r-project.org*) and STATA program.

FEASIBILITY/RESOURCES

The Multicenter Study guarantees the availability of significant amount of information, materials and cases, and offers an ideal environment where specific expertise in the field of BC are put together.[12-15] The coordination and centralization of standardized clinical and epidemiological data gathering and of molecular and pathological analyses will assure uniformity and quality control across centers. The specific expertise of the P.I. in molecular epidemiology of MBC and the multidisciplinary approach will assure the success for the proposed studies.
The Research Unit of Cancer Molecular Epidemiology, headed by the P.I., Prof. Laura Ottini, and hosted by the Department of Molecular Medicine, at Sapienza University of Rome (https://web.uniroma1.it/dmm/) is provided with the facilities and equipment needed to carry out the analyses outlined in this proposal. These include: the high-throughput sequencing platform MiniSeq (Illumina), automated Sanger sequencer (ABI-Prism 3130XL genetic analyzer, Applied Biosystems), Pyrosequencing system (PyroMark Q24 System, Qiagen), Real-Time PCR ABI 7500

Fast and 96-well ProFlex Thermal Cycler, Qubit fluorometer (Thermo Fisher), 2100 Bioanalyzer (Agilent Technologies). Illumina MiSeq and HiSeq sequencing platforms, as well as Nanostring nCounter profiler and Covaris sonicator, are available at the core facility of the Italian Institute of Techology. In addition, Illumina iScan, NextSeq and HiSeq platforms are available at Genomix4life, a spin-off of the University of Salerno, providing genomic services, which has already guaranteed a successful support in our previous studies.

WORK CARRIED OUT AND PRELIMINARY RESULTS

This research project benefits from the availability of a significant amount of cases, information and materials collected in the frame of the collaborative Italian Multicenter Study on MBC. The current series of about 750 Italian MBCs, collected from 15 Italian Investigator Centers, represents one of the largest MBC series ever assembled in a single country for which extensive clinic-pathological data, blood samples and FFPE tumor specimens are available.

Overall, in our Italian MBC series we observed that about 15% of MBCs are due to germline *BRCA* mutations.[1] In order to identify additional genes that may contribute to the missing heritability of MBC, we have performed a genomic screening of a well-characterized series of 503 non-*BRCA* MBC cases by a comprehensive multigene custom panel of 50 cancer-related genes, using MiniSeq platform (Illumina). Overall, about 6% of MBC cases were carriers of germline pathogenic variants in genes other than *BRCA*.[5] *PALB2* was the most frequently altered gene (1.2%) associated with high risk of MBC. Results of our national multicenter study support a central role of *PALB2* in MBC susceptibility. We confirmed the role of *PALB2* as an MBC susceptibility gene also in a collaborative international study.[27] Thus, in the present project, we plan to expand the multigene panel testing to the newly recruited MBCs to characterize cases based on their germline mutation status.

We have also investigated the somatic landscape of MBCs by examining gene-specific somatic alterations in about 100 cases.[22,28,29] Our results indicated that copy number variation (CNV) is frequent in MBC, and, specifically, in *BRCA* mutation negative MBCs. As CNV signatures may identify subtypes of BC suggestive of DNA repair deficiency,[30] a more in-depth somatic characterization of MBC will further facilitate the identification of molecular biomarkers and MBC subgroups, with relevant therapeutic implications. Thus, in the present project, we plan to further characterize the somatic landascape in MBC by TruSight™ Oncology 500 panel, a comprehensive genomic approach that allows for profiling of the mutational status of more than 500 cancer-relevant genes and for evaluating TMB, in a large series of MBC cases characterized for germline mutations by multigene panel testing. We have started the characterization of somatic mutational landscape and TMB in a small series of MBC cases obtaining encouraging preliminary results. In this pilot study, the series analyzed showed an adequate quality metrics of the libraries, allowing for obtaining an acceptable coverage metrics achieved for the most targeted exon regions included in TruSight™ Oncology 500 panel. Moreover, the reports generated after analytical pipeline optimization and criteria tuning from the clinical software PierianDX, showed different TMB values for each case, providing necessary preliminary evidence that TMB values may have clinical utility in MBC.

EXPECTED RESULTS

A fundamental goal of cancer precision medicine is to incorporate individualized factors into clinical decision making. Our study, integrating germline and somatic genomic data with clinical-pathologic information, will provide insights into key molecular classification of BC which will facilitate the characterization of actionable strategies for MBC patients.

The proposed extensive analysis of genetic susceptibility in MBC is expected to identify a group of men with an inherited predisposition to BC. These results will add important information to the genetic predisposition in MBC patients and will eventually facilitate the development of gender-specific clinical management guidelines in order to offer more targeted screening and surveillance programs for these men and their families. The discovery of genetic variants associated with MBC may be useful in the identification of families with high genetic susceptibility to cancer and may improve clinical management of all family members, including female relatives.

The characterization of somatic mutational landscape and TMB in MBC with and without germline risk variants is expected to lead to the identification of subgroups of patients who could benefit from specific clinical management, addressing an urgent and unmet clinical need in MBC which has profound impact on our approach to this rare disease.

The collaborative, multidisciplinary approach which forms the basis of this project, ensures an increase in MBC understanding in the medical and scientific communities, and will also result in an increase in dissemination of information and awareness to the public.

MBC represents an ideal "model" to improve knowledge of the biology and genetics of BC in general, since it is not affected by confounding factors related to the high frequency of the disease as in females. Thus, the findings of this research project will be of significant relevance in the clinical management of BC patients of both sexes.

MILESTONES

- To collect additional biological samples, clinical-pathologic features and survival data of MBC cases.
- To perform an extensive genetic screening of a cancer susceptibility gene panel in the newly recruited series of MBCs.
- To screen MBCs for actionable somatic mutations and TMB.

REFERENCES AND RELEVANT PUBLICATIONS

(Relevant publications of the research group are in bold)

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PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	Role on Project	Fellowship required	Effort on project (%)	Present position
Laura Ottini (23/10/1965)	PI	-	30	Full Professor
Valentina Silvestri (27/11/1984)	Biologist, Senior investigator	-	50	Research Fellow
Virginia Valentini (01/07/1989)	Biologist, Experienced researcher	-	50	Research Fellow
Agostino Bucalo (27/04/1992)	Biologist, Early stage researcher	-	90	PhD Fellow
Giorgia Scafetta (18/05/1992)	Biologist, Early stage researcher	YES	100	Fellow

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

Laura Ottini (PI, MD, Full Professor)

Prof. Ottini, as P.I., will be involved in all *Tasks* of the proposal, providing overall supervision, coordination of the work, evaluation of results, writing of scientific reports and publications. The specific expertise of the P.I. in molecular epidemiology of MBC is documented by her relevant publications in the field, most of which as a last author.

Valentina Silvestri (Biologist, Research Fellow)

Dr. Silvestri will be primarily involved in the management of large datasets, analysis of high-throughput sequencing data and statistical support. She has been involved in research project on MBC since 2009 and acquired experience in biostatistics and bioinformatics by attending specific post-graduate courses.

Virginia Valentini (Biologist, Research Fellow)

Dr. Valentini will be primarily involved in the characterization of somatic alterations and TMB in MBC (***Task 3***). During her PhD fellowship, she acquired the necessary experience in FFPE sample preparation and high-throughput sequencing techniques applied to tumor profiling.

Agostino Bucalo (Biologist, PhD Fellow)

Dr. Bucalo will be primarily involved in the characterization of germline alterations in MBC cases by next generation sequencing (***Task 2***). During the preparation of his Master Thesis *entitled "Genetic susceptibility of male breast cancer: a possible role for MUTYH pathogenic variants"* he acquired specific experience in multigene panel testing for the study of cancer genetic susceptibility.

Giorgia Scafetta (Biologist, Fellow)

Dr. Scafetta will be primarily involved in collection of biological samples and clinico-pathologic data, extraction and preparation of DNA from blood samples and paraffin-embedded tumor tissues (***Task 1***). She will also be involved in the characterization of MBC at somatic level (***Tasks 3***).

BUDGET FORM /YEAR

Research costs	62.000,00 euro
Instruments	-
Indirect costs	3.000,00 euro
Sub-total	**65.000,00 euro**
Overheads	6.500,00 euro
Fellowships:	18.500,00 euro
Total	**90.000,00 euro**

JUSTIFICATIONS / ITEMIZED RESEARCH COSTS

Research costs

Consumables:

- Laboratory consumables and plastic disposables;
- Reagents and disposables for DNA extraction and quantification;
- Reagents and kits for genetic screening (germline and somatic) using MiniSeq platform (Illumina): Nextera Rapid Capture Custom Kit and TruSight Oncology500 kit;
- MiniSeq Reagent kits; NextSeq Reagent kits;
- Reagents for PCR and Sanger sequencing.

Meetings and travel costs:

- National coordination, scientific meetings and participation to International meetings to present data.

Publication costs:

- Publications fees on international journals.

Indirect costs

- PCs and workstations for the researchers involved in the project;
- Stationery and office supplies.

Overheads

- Administrative costs of the hosting Institution amount to the 10% of the sum of direct and indirect costs.

Fellowships

- A fellowship is requested to support Dr. Scafetta, a young and brilliant Biologist. Dr. Scafetta has expertise in pathological and molecular analyses of FFPE tumor samples. In the present project, she will be responsible for collection of biological samples, extraction and preparation of DNA from blood samples and paraffin-embedded tumor tissues and she will participate in the characterization of MBC at somatic level.

EXISTING/PENDING SUPPORT

AIRC, IG2018 # 21389

SUGGESTED REVIEWERS (MAX 3)

Dr. Raffele Palmirotta, raffaelepalmirotta@gmail.com

Prof. Eitan Friedman, Eitan.Friedman@sheba.health.gov.il

BIOETHICAL REQUIREMENT

1. Human experimentation: **NOT**

2. Animal experimentation: **NOT**

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 14.02.'20

Name of PI: Laura Ottini

Signature .....

Principal investigator's signature .....

Authorized Administrative Official's signature.....

IL RESP. AMM.VO DELEGATO
Dott. Carlo Appetecchia

Date 14.02.'20

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