Allegato/Annex B

PROGETTO DI RICERCA / RESEARCH PROJECT
(max 5 pagine / max 5 pages)

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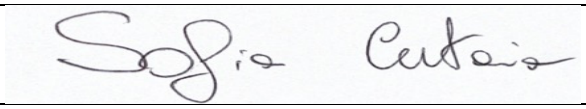
Titolo del progetto / Project title	Radiomics and liquid biopsy in predicting complete pathological response in breast cancer patients undergoing neoadjuvant chemotherapy: multidisciplinarity as the best treatment strategy
Corso di dottorato / PhD	Oncologia e Chirurgia Sperimentali
Firma del candidato/ Applicant's signature	

ABSTRACT:
Breast cancer (BC) is the most common cancer in women of all ages, but thanks to screening campaigns it is now frequently diagnosed at an early stage.
In relation to the anatomopathological characteristics, there are 5 different subtypes with different treatments and prognoses. The locoregional instrumental imaging used in BC involves the use of different methods as well as multiple systemic treatments that can be administered before (neoadjuvant chemotherapy, NACT) and/or after (adjuvant chemotherapy). Scientific progress in the last years has made it possible to identify by means of liquid biopsy various surrogate biomarkers of response to treatments such as cell-free DNA (cfDNA) and to extract new information on the characteristics of tumors thanks to radiomics.
In this scenario, this study aims at evaluating whether there is a correlation between changes in CfDNA and radiomic textures in BC patients undergoing NACT to predict pathological response and evaluate the implications of these data on surgical treatment and the risk of relapse of the disease.

BACKGROUND:
Breast cancer (BC) is the most frequently globally diagnosed cancer and is the leading cause of cancer death in women but also affects one percent of men. In Italy, the 5-year survival of 87% is one of the highest recorded in Europe.
Thanks to screening campaigns, most BC are now diagnosed at an early stage, and this has helped to increase survival and reduce mortality: only 6-7% of cases present metastases already at diagnosis [1].
BC can therefore be defined as a heterogeneous disease that originates from the epithelial cells that line the milk ducts or lobules and in relation to the histopathological and biomolecular characteristics expressed (Ki 67, cell differentiation, expression of hormone receptors (OR) for estrogen (ER) and progesterone (PR) and HER2 amplification) can be distinguished 5 subtypes: luminal A, luminal B, luminal/human epidermal growth factor receptor 2 (HER2), enriched HER2 and triple negative (TNBC) [2]. The distinction in these subgroups also has prognostic significance as Luminal A carcinomas have a favorable prognosis, much better than Luminal B, while HER2 positive and Basal-like carcinomas have a worse prognosis among five subgroups [3; 4].
The main instrumental investigations used in the local staging of breast cancer are mammography and ultrasound [5]. Systemic staging depends on the stage of the disease and on the histotype of BC: in patients at high risk for metastatic disease already at diagnosis or in patients with symptoms indicative of the presence of metastases, diagnostic investigations with CT and scintigraphy are indicated [5]. In recent years, the role of Magnetic Resonance Imaging (MRI) of the breast has been more precisely defined as it has greater sensitivity than the traditional breast imaging in locoregional staging of BC for the definition of

lesion size, multifocality, multicentricity, presence of contralateral malignant lesions, involvement of locoregional lymph nodes and infiltration of the pectoral muscle. [6] MRI is also the best tool for assessing the ongoing and termination response of neoadjuvant therapy (NACT) and the potential for breast-conserving therapy [7]. It plays a role in the definition of pericicatricial lesions, in the search for BC in patients with axillary lymph node metastases and negative breast exams, in case of suspected BC in women with breast implants and in other cases [7].
Systemic therapies involve the use of a wide range of drugs such as chemotherapy (most frequently anthracyclines, cyclophosphamide, platinum derivatives and taxanes) combined for Her2 positive disease with biological drugs directed against Her2 and, in the case of tumors expressing receptors hormonal (OR), endocrine therapies used exclusively or, especially in Luminal B and in Her2 and OR positive tumors, in succession to chemotherapy [2;4;8,9].
Placing the chemotherapy treatment before surgery (NACT) has several advantages: the possibility of determining a downstaging of the primary breast tumor allowing for conservative surgical treatment, the possibility of allowing a prognostic evaluation based on the pathological response and establishing a postoperative treatment based on presence residual disease after NACT [10].
Pathologic complete response (pCR) defined as ypT0 ypN0 is the optimal outcome following NACT and is associated with improved survival. Achievement of PCR depends on the histological characteristics of the BC, the size of the nodule, the involvement of the lymph nodes and the chemotherapy administered [11].
In this scenario it is therefore essential to discuss the cases within a multidisciplinary team that includes the radiologist, the pathologist, the oncologist, the radiotherapist and the plastic surgeon right from the diagnosis, to establish the correct diagnostic and therapeutic procedure.
Scientific progress in the oncology field has allowed the identification of response surrogate biomarkers through liquid biopsy which, if integrated with other data such as those inferable from instrumental investigations, can make their contribution in the management of the path of patients with BC.
Liquid biopsy has several advantages over tissue biopsy as it is a non-invasive and low-cost procedure, it is repeatable over time, it can represent the molecular heterogeneity of the disease containing, at least potentially, DNA tumor from different areas of the same tumor and from different possible sites of the disease [12]. One of the biomarkers detectable by liquid biopsy is cfDNA released from healthy and cancerous cells through apoptosis and necrosis. The size of the tumor, the TNM stage, the presence of metastases and the systemic and surgical treatments have an impact on the quantity of cfDNA allowing it to be considered as a surrogate biomarker of response to treatments [13].
From an imaging standpoint, radiomics is emerging as a promising tool for quantitative assessment of tumors, enabling the extraction of further quantitative data known features.
Radiomics is a complex process consisting of several phases: high quality image acquisition, tumor segmentation, features extraction, exploratory analysis, and model building. In the field of breast imaging, all techniques (mammography, ultrasound and MRI) have shown promise in radiomics studies, but MRI may have an important clinical role in evaluating the characterization of breast lesions by predicting histology and BC subtype, provide information on lymph node involvement, predict tumor response to NACT, provide prognostic information [14; 15].
Based on this evidence we hypothesized that: the correlation between the variations of the data extracted from the radiomic analysis and from the cfDNA concentrations of patients with BC undergoing NACT can provide predictive and prognostic information.

MAIN OBJECTIVE

- The aim of this study wants to demonstrate the role of the correlation between changes from radiomic analysis of breast imaging and quantitative changes in cfDNA in patients with BC in the NACT phase as a predictor of pathological response to systemic therapy to guide subsequent therapeutic and follow-up choices

SECONDARY OBJECTIVES

- To Evaluate the role of radiomics and liquid biopsy (cfDNA) as a prognostic factor in relation to the various subtypes of BC
- To Evaluate the role of the correlation between radiomic texture analysis and cfDNA concentrations as a prognostic factor by identifying patients at higher risk of BC recurrence

PRIMARY OUTCOME
- To evaluate cfDNA and radiomic texture as predictor of pCR

SECONDARY OUTCOMES
- To evaluate median disease free survival (mDFS) at 1, 2, 3 years after NACT based on median cfDNA and baseline radiomic texture analysis
- To find a relationship between the analysis of radiomic texture before and after NACT and the rate of positive margins at surgery
- To understand whether particular radiomic data and analysis of cfDNA concentrations during NACT are associated with a higher rate of locoregional, visceral, non-visceral relapses

MATERIALS and METHODS
Inclusion Criteria
- Signed written informed consent
- Naïve patients
- ECOG PS ≤ 2
- Breast cancers luminal B, Triple negative, Her2 positive candidates for NACT
- Stage I, II, III (according to 8th edition of AJCC)
- Availability of breast imaging (MRI and mammography) performed before the start of systemic and/or surgical treatment and at the end

Exclusion Criteria
- ECOG stage ≥ 3
- Stage IV (according to 8th edition of AJCC)
- Prior systemic oncology therapy and/or radiotherapy
- >1h from blood withdrawal and plasma recovery
- Presence of artifacts in the acquired images in MRI

Methods
- *Plasma collection and cfDNA isolation*

Blood samples and cfDNA isolation for each eligible patient, plasma will be collected at baseline (before NACT, T0), after 3 months (halfway through neoadjuvant treatment, T1), after 6 months (at the end of the neoadjuvant treatment, T1), after surgery (T2), at 3 months (T3), at 6 months(T4) and 1 year (T5) after surgical treatment. For every patient included and for each time-point, blood samples will be collected in four 3-mL EDTA-containing vacutainer tubes and processed within one hour for plasma collection. Briefly whole blood sample are centrifugate at 4° C for 10 minutes at 1200g to eliminate cell debris. The obtained supernatant is then centrifuged again for 10 minutes at 3000g at 4° C. If a refrigerated centrifuge is not available the sample can be processed as follow: a first centrifugation at 2300rpm for 10 min, after supernatant has been recovered it will be subjected to a second centrifugation at 2300rpm for 10 min. Plasma is then stored in cryostat tubes at -80°C until next use.
Circulation DNA is isolated using QIAamp Circulating Nucleic Acid Kit (Qiagen) according to manufacturer's protocols for 2 mL plasma. The procedure is composed by 4 steps (lysis, binding, washing and elution).
Circulating tumor DNA is eluted in 55 µL of buffer and stored at -20°C. DNA quantification is performed using both NanoDrop (Thermo Scientific) according to manufacturer's recommendation and Quibit 2.0 Fluorometer (Life Technologies) with the dsDNA HS (High Sensitivity) Assay Kit.
- *Radiomic analysis*

The MRI and mammography performed at the time of diagnosis and at the end of the NACT will be anonymized and will be sent in Digital Images Communication and Management (DICOM) format to a dedicated workstation with integrated radiomic analysis software. The radiomic analysis will involve the implementation of a semi-automatic segmentation algorithm of the lesions in the aforementioned images, under the supervision of a radiologist. The segmentation will be carried out by tracing the use of software in a region of interest that will outline the lesions in all consecutive images that will detect it in order to obtain a necessary quantification. After segmentation, the texture analysis will allow to extract the radiomic data that will allow the analysis of the characteristics of the lesions.

Study Design

This is a prospective cohort study that aims at enrolling 50 patients with BC candidates for NACT in 24 months. This number can be considered appropriate based on both logistical/time issues. They will be asked to sign an informed consent which will contain information about the study and investigations that will be carried out on both blood sampling and radiological imaging.

Plasma cfDNA concentrations and radiomic analysis data at baseline, during and at the end of chemotherapy will be analyzed and integrated to demonstrate whether there is a concordance between changes in plasma cfDNA concentration and radiomic texture analysis useful for predicting pathological response.

The possibility of stratifying patients into four different risk classes (high or low concentrations of cfDNA and different radiomic textures) will be evaluated in order to modulate all subsequent therapeutic interventions, predict BC relapse and modulate the follow up.

The entire biomolecular analysis will be performed at the Laboratory of Prof. Antonio Russo (Medical Oncology Section, Palermo, University Hospital) and the MRI mammary and mammographic images of the patients who have performed these tests at the Institute of Diagnostic Imaging will be analyzed (Palermo, University Hospital).

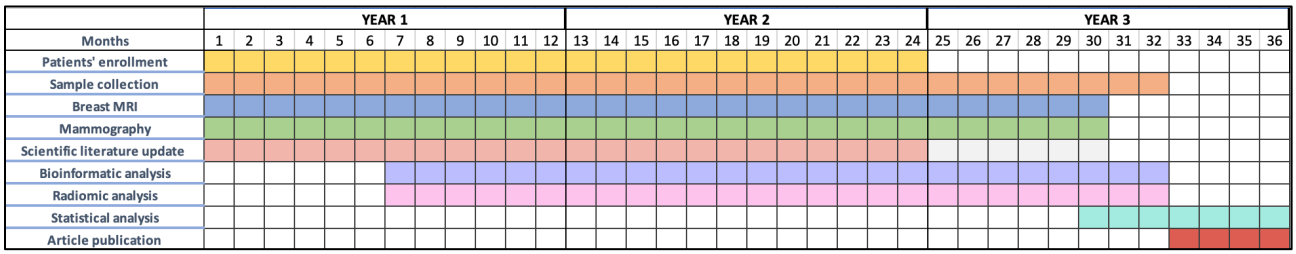
Statistical Analysis

We are planning a single arm, prospective, proof of concept study with an accrual interval of 24 time units, and additional follow-up after the accrual interval of 12 time units. In order to summarize the most relevant features of the clinical variables, descriptive statistics will be performed. In particular the continuous variables will be synthesized as mean and standard deviation or median and interquartile range, depending on the distribution of the data. The distributions of patients in groups for baseline demographic and clinical characteristics will be compared using the Chi-square test for heterogeneity and the Wilcoxon Mann Whitney test for categorical and continuous variables, respectively. Survival analysis will be performed using the Kaplan–Meier method, providing median and p values, with the use of the log-rank test for comparisons. A multivariate regression model (linear or logistic) will be performed to evaluate the impact of the study of cfDNA and radiomics, excluding the impact of other possible parameters that could appear to be confounders during the univariate analysis. Outcomes with p-value < 0.05 will use as a threshold for statistical significance. All the statistical analyses will be performed using SPSS statistics software, version 20 (IBM, Armonk, NY, USA).

The computational statistical analysis will be aimed at identifying the texture parameters at baseline and after NACT to predict the pathological response and evaluate mDFS. The univariate and multivariate analysis of the texture parameters will identify the radiomic characteristics that will allow to achieve these objectives. This statistical analysis will be supported by a computer expert in image analysis and algorithms for the selection of radiomic features.

Statistical evaluation of diagnostic accuracy will include estimating the sensitivity and specificity of each radiomic parameter and their combination. The integration of radiomic parameters with changes in cfDNA concentrations at baseline, during and at the end of NACT, will allow the creation of a predictive system capable of estimating the response to treatment, predicting the pathological response and identifying the group of patients BC risk recurrence.

TIMELINE



WP1: Patient's enrollment
WP2: Sample collection
WP3: Breast MRI
WP4: Mammography
WP5: Scientific literature update
WP6: Bioinformatic analysis

WP7: Radiomic analysis
WP8: Statistical analysis
WP9: Article publication

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