CLINICAL RESEARCH

Beyond Oncotype Dx® in postmenopausal women with ER+/HER2- breast cancer.

PART 1 - PROJECT PROPOSAL

A. Abstract (to be provided also in Italian)

Circa il 70% dei carcinomi della mammella (BC) possiede il recettore estrogenico (ER), presenta negatività a HER2 e beneficia della terapia anti-ormonale. Tuttavia all'interno delle lesioni ER+/HER- esistono forme aggressive che necessitano anche della chemioterapia (CT). Attualmente, i parametri per identificare forme a rischio elevato di recidiva sono principalmente istologici, come il grado di differenziazione e l'indice proliferativo, ma soffrono di variabilità diagnostiche analitiche e pre-analitiche. Recentemente sono stati introdotti nella pratica clinica i test genomici, costituiti da pannelli genici in grado di classificare le pazienti con ER+/HER2- BC in gruppi di rischio alto e basso di recidiva. Tra questi, quello più utilizzato è l'Oncotype Dx®. L'adozione di questo test ha comportato un abbattimento della CT in circa il 40% delle pazienti, principalmente in post-menopausa. Tuttavia si tratta di un test centralizzato, eseguibile solo in un laboratorio negli Stati Uniti, ha un costo elevato e comporta un tempo diagnostico di 10-15 giorni (da sommare a quello necessario per la diagnostica istologica e molecolare routinaria). Recenti studi affermano che algoritmi di intelligenza artificiale (AI) applicati ad immagini radiologiche ed istologiche (radiopatomica), associati a parametri immunoistochimici classici, possono aiutare a prevedere l'outcome nel BC. Lo scopo del nostro lavoro è la creazione di un modello di radiopatomica, basato su immagini e dati acquisiti routinariamente ed utile a prevedere l'esito del test molecolare Oncotype Dx®. Presso la Breast Unit di Città della Salute e della Scienza verranno valutate 150 pazienti con tumori ER+/HER2-, sottoposte a Oncotype Dx®, di cui 100 retrospettivamente (training set) e 50 in maniera prospettica (validation set). Per ciascuna di loro verranno raccolte immagini relative a mammografia (Mx), ecografia (US), risonanza magnetica (MRI), istologia e dati clinico-patologici. Grazie all'integrazione di queste informazioni ci si propone di creare un modello multi-omico prognostico per la predizione del risultato di Oncotype Dx®. Questo approccio potrebbe offrire un'alternativa più economica e rapida, applicabile anche in centri con accesso limitato ai test molecolari.

Almost 70% of breast carcinomas (BC) shows estrogen receptor (ER) expression and HER2 negativity, being eligible for anti-hormonal therapy. However, within ER+/HER2- lesions, there are aggressive tumours that also require chemotherapy (CT). To date, the criteria for identifying high-risk recurrence lesions are primarily based on histology, such as grade of differentiation and proliferative index. However, these features are affected by analytical and pre-analytical variability. Recently, genomic tests have been introduced into clinical practice. They are gene panels able to classify ER+/HER2- BC patients into high and low-risk recurrence groups. Among these, the most widely used is the Oncotype Dx®. The adoption of this test has led to a reduction in CT in approximately 40% of patients, mainly in post-menopausal status. However, it is a centralized test, only feasible in United States, with a high cost and a diagnostic time of 10-15 days (in addition to the time required for routine histological and molecular diagnostics). Recent studies have suggested that artificial intelligence algorithm (AI) applied to radiological and histopathological images (radiopathomics) together with immunohistochemical parameters can help in predicting outcomes in BC. The purpose of our work is to create a radiopathomic signature based on routinely acquired images to predict Oncotype Dx® results. At the Breast Unit of Città della Salute e della Scienza, 150 patients with ER+/HER2- BC, who have undergone Oncotype Dx®, will be evaluated. In detail, 100 will be collected retrospectively (training set) and 50 prospectively (validation set). For each patients Mammography (Mx), Ultrasound (US) Magnetic Resonance Imaging (MRI), digital histological images, and clinical-pathological data will be collected. The information provided by this multi-omics dataset will be combined into a prognostic biomarker to predict data provided by Oncotype Dx®. This approach could potentially bypass the use of genomic testing, employing a low-cost, rapidly feasible, highly reproducible method, available even in centres where molecular testing is not yet available.

B. Background/state of the art and relevant preliminary results of the proponent in the area

BC is the most common tumour in females worldwide. An increasing rate of BC is diagnosed at an early stage and is characterized by ER+ HER2- immunophenotype (1). This group of neoplasms is quite heterogeneous concerning biological aggressiveness; while some tumours have low aggressiveness and do not require CT, others have the potential for progression and necessitate adjuvant CT (2). Unfortunately, routine histopathological characterization (histotype, grading, lympho-vascular invasion, etc) and immunophenotypic features (expression of ER, progesterone receptor (PR), Ki-67, HER2) allow for an imprecise prognosis for this group of neoplasms. In recent years, to improve prognostic assessment, genomic tests have been developed (3). These assays have been primarily validated in post-menopausal women and they evaluate a variable number of genes, mainly involved in the proliferation and in the ER pathway. To date, among the available assays, Oncotype Dx® is the most commonly used and highly recommended by international guide lines (4). This assay analyses a panel of 21 genes in order to determine a Recurrence Score (RS) (scored as 0–100), corresponding to the 10-year risk of recurrence, and is used to stratify patients in low and high (RS>25) risk groups, a result that also carries predictive implication (5). The implementation of this test has resulted in a notable 40% decrease in the need for CT among patients (6), delivering undeniable advantages to women's lives and bearing significant economic implications. This is attributed to both the diminution in CT prescription and the decrease of costs associated with therapy-induced side effects, leading to substantial cost reduction in patient care. However, major limitations of RS are represented by its high costs (7), which limits its widespread use. In addition, it is performed only in a central laboratory in USA and the results are available after 10 -15 working days, thus increasing diagnostic times, already impacted by the limited resources in our National Health Service. Last but not least, reimbursement of the test by NHS in Italy is only approved in a subset of patients ER+/HER2-. Very recently, some studies proposed new multi omics approaches using artificial intelligence (AI) to predict outcome in BC (8-9). In particular, it has been showed that radiomics, a methodology requiring high-throughput extraction of quantitative features from radiological images, has the potential to predict treatment outcome, long term survival, presence of local and/or distant metastases and response to treatment in different tumour models (10). On the other hand, preliminary data have shown that histological slides can also be potentially used to develop pathomics biomarkers to predict BC outcome (11-12). However, to date, there is a lack of information regarding the prognostic potential of an integrated biomarker, including radiological and pathological images, and genomic data. In line with this, the main objective of this project will be to develop an integrate AI-based multi-omics prognostic signature able to predict Oncotype-Dx®RS in ER+/HER2-BC patients. **Preliminary results**: in our Breast Unit, we already collected 49 patients, with known Oncotype Dx®-RS (RS high and low risk in 6 and 43 women, respectively) and fully characterized with respect to US, Mx, MRI and histopathological data. We observed that RS is related to the expression of PR (P=0.003) and to lymph nodal involvement (P=0.049), suggesting that outcome in luminal BC patients could be estimated using both immunophenotypic data, corresponding to tumour biology, and data regarding tumour burden that imaging could provide. In addition, we observed that low-risk RS BC had generally higher ADC values compared with high-risk lesions, reiterating the idea that imaging could be an indicator of outcome.

C. Hypotheses and aims

Hypotheses: starting from radiological and histopathological images and clinico-pathological data we will be able to develop an integrated AI-based multi-omics signature to predict outcome in post-menopausal women with ER+/HER2- BC. **Aim**: to develop a low-cost, reproducible, sensitive and specific machine learning model using a combined radiological, histological and clinicopathologic variables to predict Oncotype Dx®-RS in BC patients aged over 50 years. In particular, we aim to implement an algorithm able to classify ER+/HER2-BC patients based on the risk of recurrence and/or disease progression with supervision based on gene expression profiles.

D. Project plan including experimental design, methods and ethical aspects

Experimental design: this project will use a multi omics approach to predict outcome in ER+/HER2 BC patients combining different level of information, namely radiological, pathological and genomic data. First, imaging and pathological data will be integrated into a multi-omic signature. Second, the

model will be validated using a real-world dataset of BC patients, prospectively enrolled for whom the Oncotype Dx®-RS will be available.

Methods: *Task 1(Data collection):* we will perform a data lake containing 100 post-menopausal ER+/HER2-BC retrospectively collected. For each patient, Mx, US, MRI data, pathological data such as histotype, histological grade, lympho-vascular invasion, lymph-node status, Ki 67 index, ER, PR and HER2, expression level and Oncotype Dx®-RS will be collected from the Breast Unit of Città della Salute e della Scienza. Inclusion criteria will be the following: 1. Postmenopausal status; 2. ER+/HER2- BC confirmed by histological diagnosis; 3. Availability of Mx, US, MR imaging and histological and immunohistochemical samples; 4. Oncotype Dx®-RS results. For each patient data/images will be retrieved. Pre-operative Mx, US, MRI will be collected for radiomics analyses. At the same time, a representative histological section of the tumour, along with routine immunohistochemical reactions (ER, PR, Ki-67, and HER2), derived from contiguous serial sections, will undergo high-resolution digital scanning (already available in Pathology Unit) for pathomics analyses

Task 2: Development of radiopathomics signatures to predict Oncotype Dx®-RS results. Dataset: the retrospective dataset of 100 patients will be used for this task. Reference standard: Oncotype Dx®-RS results.

PATHOMICS: a pathomics signature will be created using quantitative imaging features from digitalized routine hematoxylin and eosin (H&E). After digitalization of pathological images to create Whole Slide Images, we will apply different features selection and classification methods (both supervised and unsupervised). For features selection different strategies will be exploited including features clustering (e.g., principal component analysis (PCA), embedded models (e.g., LASSO), and filters models (e.g., genetic algorithms, ranking, mRMR). We will then assess the performances of different classification algorithms including logistic regression, Support Vector Machine, decision tree, random forest, XGBoost, ensemble learning methods and neural networks. Finally, we will also exploit deep learning approaches to overcome the features selection step. RADIOMICS: to develop this signature, we will combine information from Mx, US, MRI. To overcome limitations due to the absence of some images that might occur for some patients, we will investigate and apply methods to handle missing data. Features selection and classification algorithms, as described above, will be applied to develop a prognostic biomarker based on radiological images.

MULTI-OMIC SIGNATURE signature: to boost the performance of the individual signatures, we will combine them thus deriving a single multi-omics signature. This will be developed by integrating radiomics and pathomics signature, together with genomic and clinical data, into a Clinical Decision Support System (CDSS). However, other approaches, such as concatenating radiomics and pathomics features into one high-dimensional feature vector that will be fed to several machine learning models will be implemented and tested to provide the most predictive radiopathomics signature.

*Task 3: Validation of the AI-based signatures***:** a real-world dataset of 50 ER+/HER2- BC patients, fully characterized by radiological, histopathological and Oncotype Dx®-RS data will be prospectively collected. The accuracy of the AI signature in predicting Oncotype Dx®-RS results will be evaluated, by computing sensitivity, specificity, negative and positive predictive values.

The expected duration of the study is 24 months and includes: case selection and digital scanning (24 months); Development of the radiopathomics model and application in the training set (16 months); Validation of the signature on a real word data set (8 months).

	Time (Months)																							
Task	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1. **Data collection**	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
2. **radiopathomics signature construction**			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x						
3. **radiopathomics signature validation**																	x	x	x	x	x	x	x	x

Ethical aspect: after ethical Committee approval, all data will be anonymized and written informed consent for the data collection and storage will be obtained from each patient before starting the study.

References
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E. Expected results and contingency plans and impact for the National Health System

Expected results We aim to implement a radiopathomic signature able to predict outcome in ER+/HER2-BC patients. A more precise characterization of this patient group will decrease the need for Oncotype Dx®-RS, leading to a reduction of its costs for our Institution. More importantly, it will save time in diagnostic waiting periods and facilitate quicker initiation of medical therapies. **Impact for the National Health System:** our radiopathomic approach, once developed, is potentially characterized by several advantages compared to classification methods based on the Oncotype Dx®-RS:**1**.low cost: this approach could be implemented in clinical practice without significant additional costs, since it relies on datasets and information that are already available for every BC patient, in any hospital; **2.** reproducibility: the absence of significant pre-analytical and analytical interferences maximizes the reproducibility of the test; **3.**speed of execution; **4**. application in all patients ER+/HER2-. In addition, as future direction, once validated, our radiopathomics model could be applied to pre-operative setting, aiding in selecting patients with adverse prognosis, who will benefit from CT, even before surgery. Finally, our model could be exported in Hospitals without access to genomic tests and/or in developing Countries. **Contingency plans: 1.** Low accrual rate. Solution: if the accrual rate will be lower than expected**,** we will ask to other Breast Units in Turin to implement our case series. Actually, there is already an agreement between the PI Department of Pathology and Cottolengo Hospital for breast pathology. Thus, PI can count on additional cases to reach the present goal**. 2.** Strong variability of features, due to different acquisition protocols. Solution: Robustness of each feature will be assessed in different conditions, i.e., different normalization/standardizations techniques, different pre-processing methods. In case of low generalizability of the predictive radiopathomics models we will define the standardization of imaging acquisition protocols and clinical enrolment to homogenize the radiopathomics analyses.

PART 2

Proponent's CV

A. Position and honors

Isabella Castellano. Role: **1.** Associate Professor of Pathology, Dept. Medical Sciences, University of Turin. **2.** Medical Doctor (Pathologist) at AOU Città della Salute e della Scienza Hospital, Pathologic Service 2U. Department of Laboratory Medicine. She is responsible for Simple Unit of "*Diagnostica senologica ad elevata specializzazione*". She regularly participates to the discussions in the interdisciplinary teams of care for BC at the Breast Unit of the Città della Salute e della Scienza-Molinette Hospital. The scientific activity is focused on mammary gland patho-physiology and in particular on the role of hormone receptors in BC development and prognosis. She is Professor of Surgical Pathology (Degrees: Medicine and Surgery, Medical Biotecnology, Radiology and Radiotherapy) at the Medical School of the University of Torino. Since 2021 she is President at the Degree in Medical Biotechnology, University of Turin. She is **(1)** Member of the European Working Group for Breast Screening Pathology (2017-present); **(2)** Coordinator of the Italian Group of Breast

Pathology (GIPAM) within the Italian Society of Pathological Anatomy and Diagnostic Cytopathology (SIAPEC) (from 2019 to today); **(3)** Italian representative of SIAPEC in the technical committee responsible for the Italian review ("Adolopment") of the guidelines on Mammographic Screening recently produced by the European Commission (from 2019 to today); **(4)** Representative of SIAPEC in SenoNetwork association (from 2016 to today); **(5)** Coordinator of the Breast Cancer Study Group of the Oncology Network of Piedmont and Valle d'Aosta (from 2019 to today); **(6)** Member of the Italian College of Professors of Pathological Anatomy within the "Commission for Didactic Activities".

B: Publications: ***List of 5 most relevant publications in the last 5 years***

-AR/ER Ratio Correlates with Expression of Proliferation Markers and with Distinct Subset of Breast Tumors, Cells, 2020, 9(4), 845: **IF: 6.6.** PI position: last author.

- Targeting IL-3Rα on tumor-derived endothelial cells blunts metastatic spread of triple-negative breast cancer via extracellular vesicle reprogramming. Oncogenesis, 2020 Oct 10;9(10):90 **IF: 7.4** PI position: author in between.

-Integration of Ki-67 index into AJCC 2018 staging provides additional prognostic information in breast tumours candidate for genomic profiling. British Journal of Cancer, 2020, 122(3), pp. 382–38 **IF: 7.6.** PI position: last author.

-Gene expression signatures for tailoring adjuvant chemotherapy of luminal breast cancer: the pathologists' perspective. Annals of Oncology, 2021, 32(11), pp. 1316–1321 **IF: 51.7.** PI position: author in between.

-Impact of Caloric Restriction in Breast Cancer Patients Treated with Neoadjuvant Chemotherapy: A Prospective Case Control Study. Nutrients. 2023 Nov 4;15(21):4677. **IF: 5.9.** Position: first author**.**

Total number of Publication: 121, H-index (Scopus): 30
Total number of publications in the last 5 years: 42, average IF: 6.06

C. Conferences as invited speakers in the last 5 years

2019 (16-19/10): *Turin:* National Congress Siapec-IAP; (25/09): Catania: National congress GISMA
2020 (18/10): *Novara*: Breast cancer and Breast Unit. Must have in 2020. (17-20/11): *Virtual* national congress AIS.
2021 (3-6/10) *Virtual:* European congress of cytology. (8-10/09) *Virtual*: VII incontro nazionale Gruppo italiano di patologia molecolare. (28-30/10) *Rimini:* Congresso nazionale di radiologia medica (SIRM), (10-11/09) Novara: Innovators in Breast Cancer. (16/11) *Virtual:* III Consensus Conference Nazionale Anisc. (4/11) *Firenze:* Attualità e prospettive future in senologia.
2023 (3/09) Basel-Switzerland: European congress of Pathology. (19-11) *Pisa* Masterclass: breast pathology for residents.

D. Current and anticipated grant support: ***Principal Investigator*** in: (1) 2012, 2015, 2016, 2018 MIUR Ex60%/// (2) 2013, 2020 CRT Foundation. ***Co-investigator*** in: (1) 2011-2014 European grant (TASTE) (2) 2014-2016: *LILT* (Lega Italiana Lotta Tumori) ***Collaborator*** *in*: 2010-2013 EUROPEAN SOCIAL FUND ROMANIAN (2) 2010-2013 AIRC (3) 2010-2013 PRIN.

E. Previous experience in collaborative research: PI actively collaborate with national (GIPAM-Siapec, SENONETWORK, GISMA, ANISC) and international group of researchers (European Working Group for Breast Screening Pathology) with different background (pathologists, radiologists, oncologists, surgeons and molecular biologists).

F. Team

- Isabella Castellano PI: pathologist: data collection and supervisor.
- Valentina Giannini, Department of Surgical Sciences (A.U.O. Città della Salute e della Scienza di Torino), University of Turin: supervisor for the development and validation of AI algorithms and data analyses.
- Durando Manuela, Radiologist, Department of Radiology (A.U.O. Città della Salute e della Scienza di Torino): patient's recruitment, data collection
- Jacopo Cumbo MD, surgeon, Department of Surgery (A.U.O. Città della Salute e della Scienza di Torino): patient's recruitment
- Alessandra Beano, oncologist, Department of Oncology (A.U.O. Città della Salute e della Scienza di Torino): patient's recruitment