MRO. ART
MODERN RADIATION ONCOLOGY

SESSION 9 OMICS and AI in WOMEN'S CANCER

Omics driven systemic treatments

Luca Visani
SOD Radioterapia
AOU Careggi - Firenze

Background

Breast cancer (BC) is widely recognized as a **heterogeneous disease**, both at the molecular and clinical level

Nowadays, therapeutic options for BC treatment include **surgery**, **radiotherapy**, **chemotherapy** and **targeted therapies**

Parsons J et al. Front Cell Dev Biol. 2019
Masoud V et al. World J Clin Oncol. 2017

Despite recent and important advances in understanding BC biology, diagnosis and treatment, **several significant clinical issues** still remain **unclear**. In particular, these unmet clinical needs are related to **prevention, diagnosis, tumor progression, treatment, therapeutic resistance and metastasis formation**

Polyak K et al. J Clin Investig. 2007
Wood SL et al. Cancer Treat Rev. 2014

Background

In this context, modern systems biology based on **“omics” approaches** can potentially make a major contribution to overcome these problems

In fact, in the era of precision medicine, “omics” strategies and their integration in the study of BC can lead to the identification of **novel biomarker molecules** and **molecular signature** with **a potential in clinical practice**

Wood SL et al. Cancer Treat Rev. 2014

Genomics in BC

Genomics in BC – genetics and genomics

Genetics is the study of **heredity**, primarly focuses on the **likelihood of developing cancer** and finds **mutations**

On the other hand, **genomics** is the study of **how genes interact** and are **expressed as a whole**

Genomics and gene expression profiling tools focus on the cancer itself and can help to determine **how aggressive is the cancer** and what is the likely **benefit from a treatment**

Understanding cancer series: gene testing. National Cancer Institute

Genomics in BC – the role of NGS

The history of BC genomics can be broadly divided into two categories, **before next-generation sequencing (NGS) and after NGS**

Pre-NGS era is mainly characterized by the **study of individual genes associated with BC**, and during the pre-NGS era, the hallmark genes such as BRCA1 and BRCA 2 were discovered

After the advent of NGS, BC study was not limited to only few genes and **several new genes and intergenic interactions** were discovered

Rossi C et al. Oncogenesis 2022

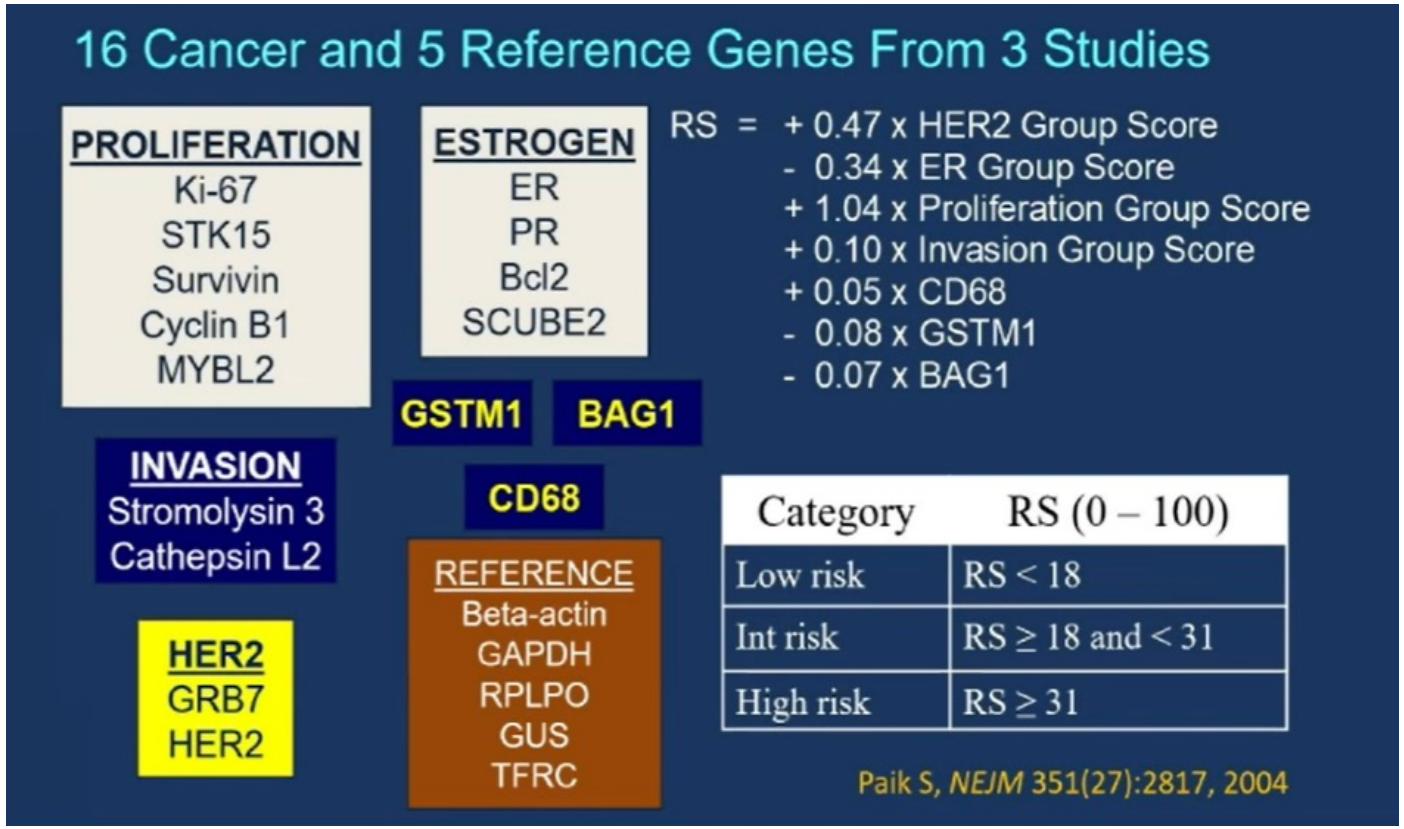
Genomics in BC – the role of NGS

Gene	Change	Gene penetrance	Remark
BRCA1	Mutation	High	Breast cancer risk at the age of 70 is 57% for BRCA1 mutation carriers
BRCA2	Mutation	High	Breast cancer risk at the age of 70 is 49% for BRCA2 mutation carriers
Tp53	Inactivating Mutation	High	Tp53 mutation also causes Li-Fraumeni syndrome
PTEN	Truncating mutation	High	Mutated in Cowden syndrome families. 25-50% lifetime BC risk in women.
BARD1	LOF mutation	High	BARD1-mutated BC patients showed a significantly younger mean age at first diagnosis
CHEK2	Mutation	High	Deletion in CHEK2 (CHEK2*1100delC)
CDH1	Germline mutation	High	CDH1 have been associated with an increased risk of hereditary diffused gastric cancer
ATM	Truncating and exon-skipping mutations	High-moderate	A-T patients do not survive to an age at which BC generally occurs. The penetrance for L1420F mutation is high (85% at age 60)
NF1	LOH	High-moderate	Women with NF1 develop BC at younger ages
STK11/ LKB1	Truncating germline mutation	High-Moderate	LKB1 gene is mutated in patients with Peutz-Jeghers syndrome
PALB2	Biallelic mutations	High-Moderate	L35Pa is a pathogenic missense mutation in PALB2
BRIP1	LOF mutations	Moderate	Increases developing risk of BC at an early age
RAD51C	Mutation/pathogenic variant	Moderate-Low	Risk increases with variant carriers with two first-degree relatives diagnosed with BC
RAD51D	Mutation/pathogenic variant	Moderate-Low	Risk increases with variant carriers with two first-degree relatives diagnosed with BC
SMAD4	Inactivation	Moderate-low	SMAD4 is located on 18q21, a region frequently lost in breast cancers
NBN	Mutation	Moderate-low	A protein-truncating variant, c.657del5, is sufficiently common in some Eastern European populations
MutYH	Mutation	low	p.Tyr179Cys/p.Arg241Trp are pathogenic variants of MutYH
CDK12	Mutation	Low	The penetrance estimates of 39% by age 80 years is a cumulative risk in the absence of other causes of cancer/mortality
MSH2	Mutation	Low	1.1% woman with BC carries MSH2 mutation
APC	Mutation/polymorphism	Low	A single nucleotide polymorphism (SNP), rs2229992 was identified in the APC gene, with an increased risk of breast carcinogenesis
CDKN2A	Mutation	Low	Variant A148T was identified in 5.1% of women with breast cancer, in a Polish study

Genes recognized to be **involved in BC,** categorized as their change and penetrance

Rossi C et al. Oncogenesis 2022

Genomics in BC – Oncotype Dx



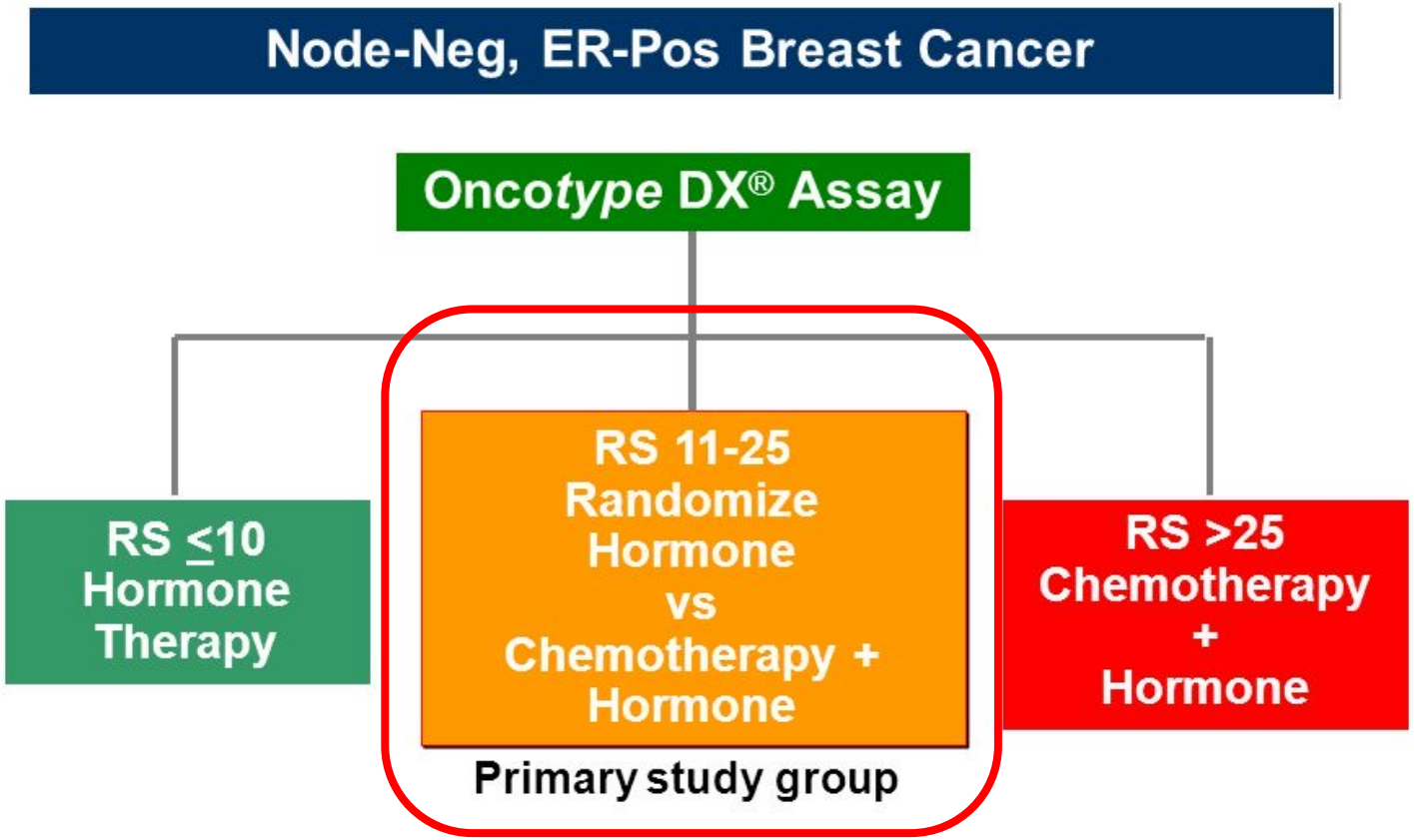
Oncotype DX is a **21-gene recurrence-score assay** that quantify the expression of 21 genes in BC tissue by PCR

The test assigns BC a continuous recurrence score (RS) ranging **from 0 to 100** and is **predictive of chemotherapy benefit**

Formalin-fixed paraffin-embedded samples are used and centrally processed

Paik S et al. NEJM 2004

Genomics in BC – TAILORx trial



ORIGINAL ARTICLE

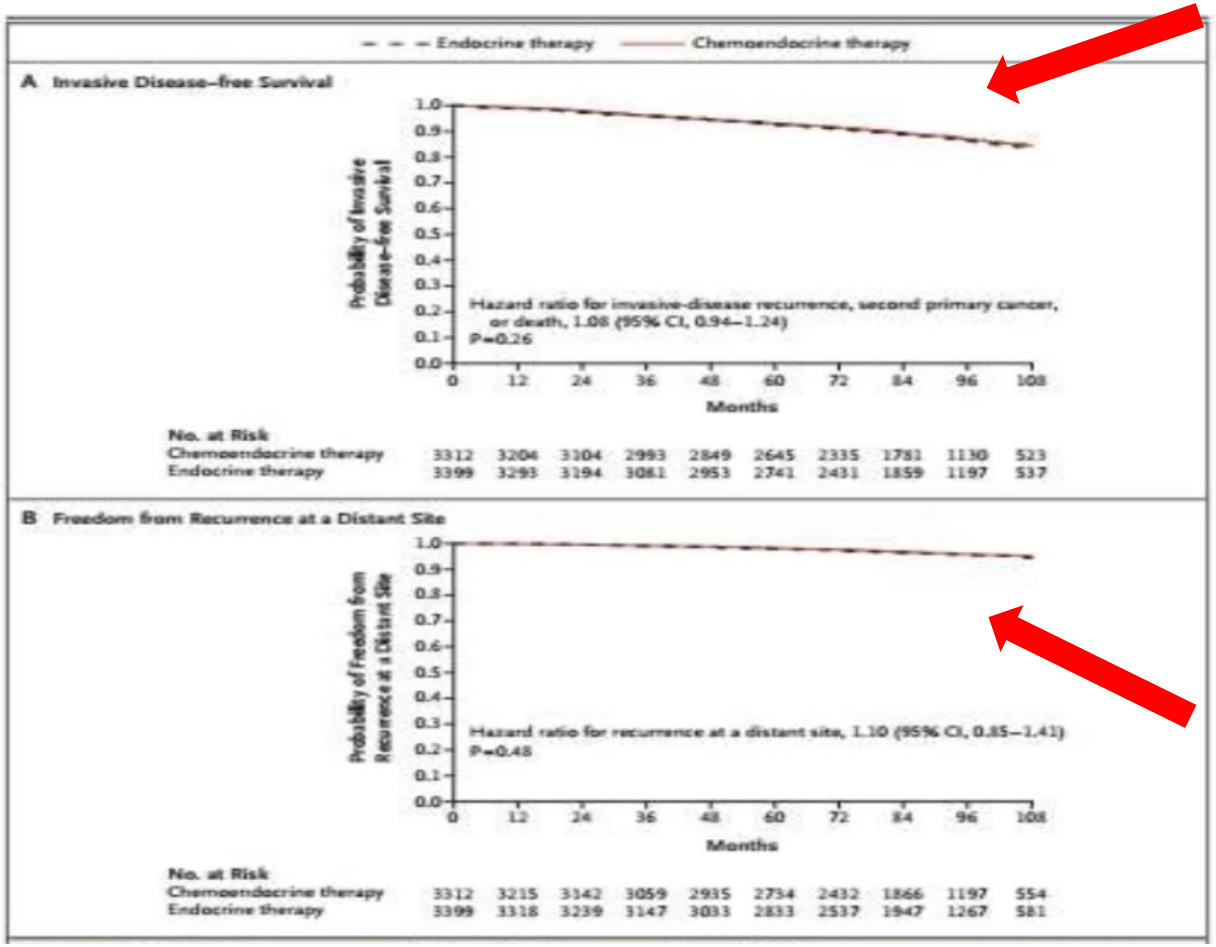
Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer

J.A. Sparano, R.J. Gray, D.F. Makower, K.I. Pritchard, K.S. Albain, D.F. Hayes, C.E. Geyer, Jr., E.C. Dees, M.P. Goetz, J.A. Olson, Jr., T. Lively, S.S. Badve, T.J. Saphner, L.I. Wagner, T.J. Whelan, M.J. Ellis, S. Paik, W.C. Wood, P.M. Ravdin, M.M. Keane, H.L. Gomez Moreno, P.S. Reddy, T.F. Goggins, I.A. Mayer, A.M. Brufsky, D.L. Toppmeyer, V.G. Kaklamani, J.L. Berenberg, J. Abrams, and G.W. Sledge, Jr.

TAILORx trial design

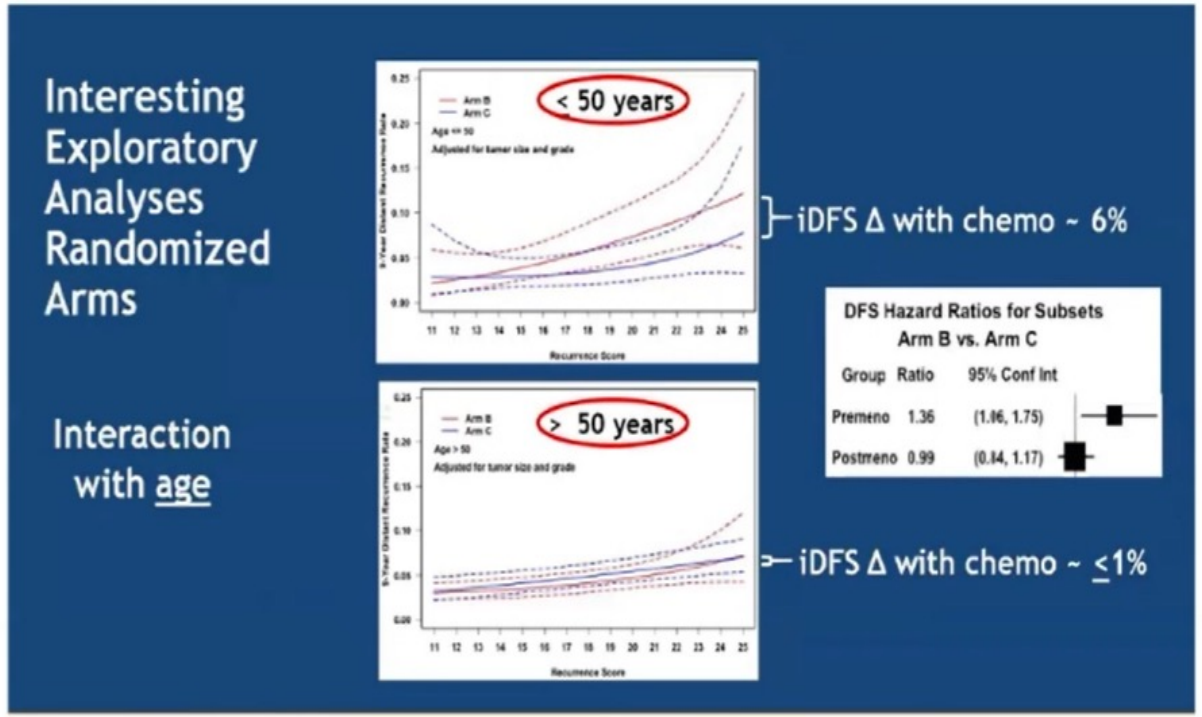
Sparano JA et al. NEJM 2018

Genomics in BC – TAILORx trial



In this trial, among 6711 women with **HR+ HER2-, axillary node-negative BC** and a midrange RS of 11 to 25 on the 21-gene assay, **ET** was **not inferior to chemo-endocrine therapy**, which provides evidence that adjuvant chemotherapy was not beneficial in these patients

Genomics in BC – TAILORx trial

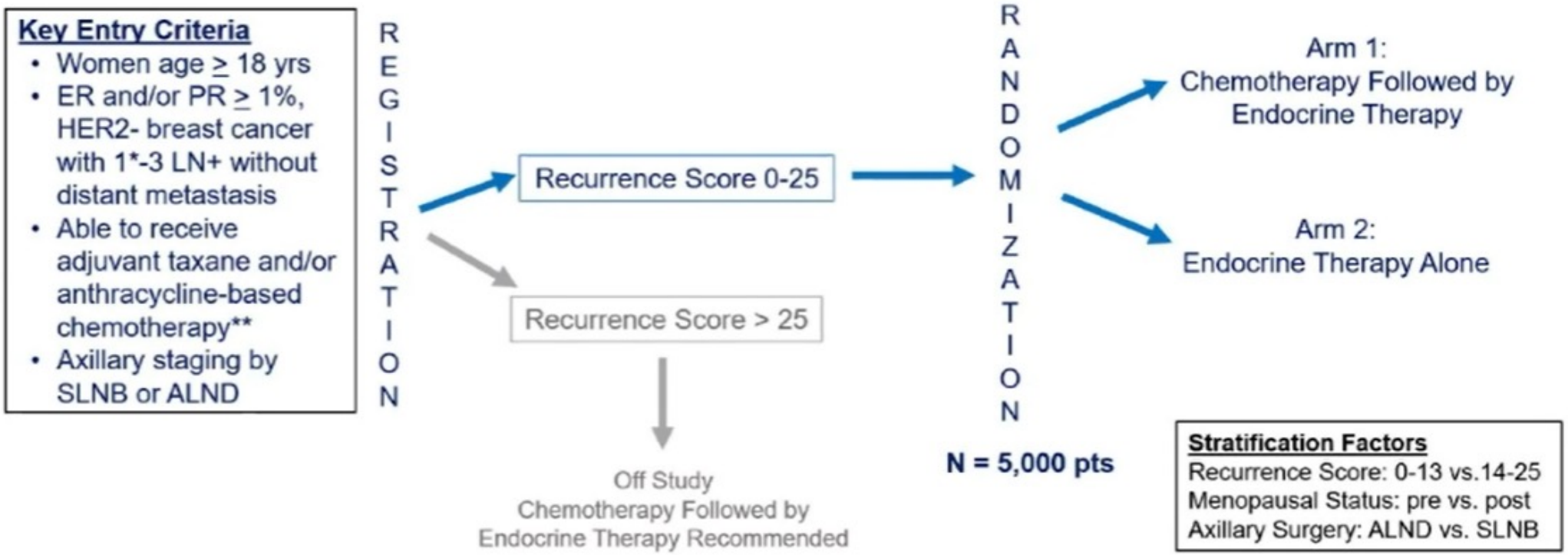


A total of 40% of women who were 50 years of age or younger had a RS of **15 or lower**, which was associated with a **low rate of recurrence with endocrine therapy alone**

Exploratory analysis indicated that **chemotherapy** was associated with **some benefit** for **women ≤50 years with a RS from 16 to 25** (found in the 46% of women in this age group)

Genomics in BC – RxPONDER trial

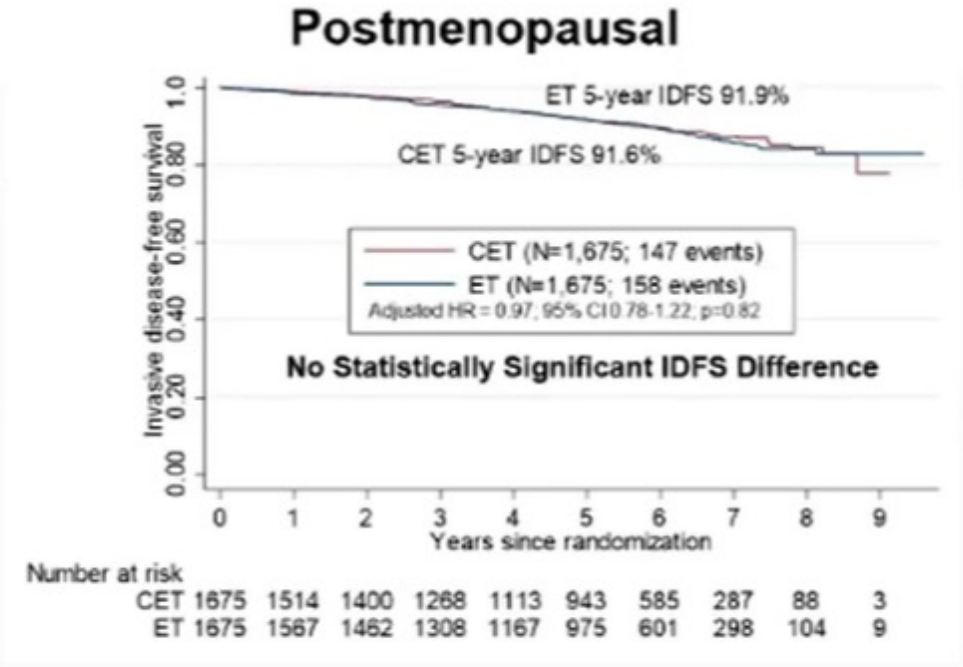
RxPONDER trial design

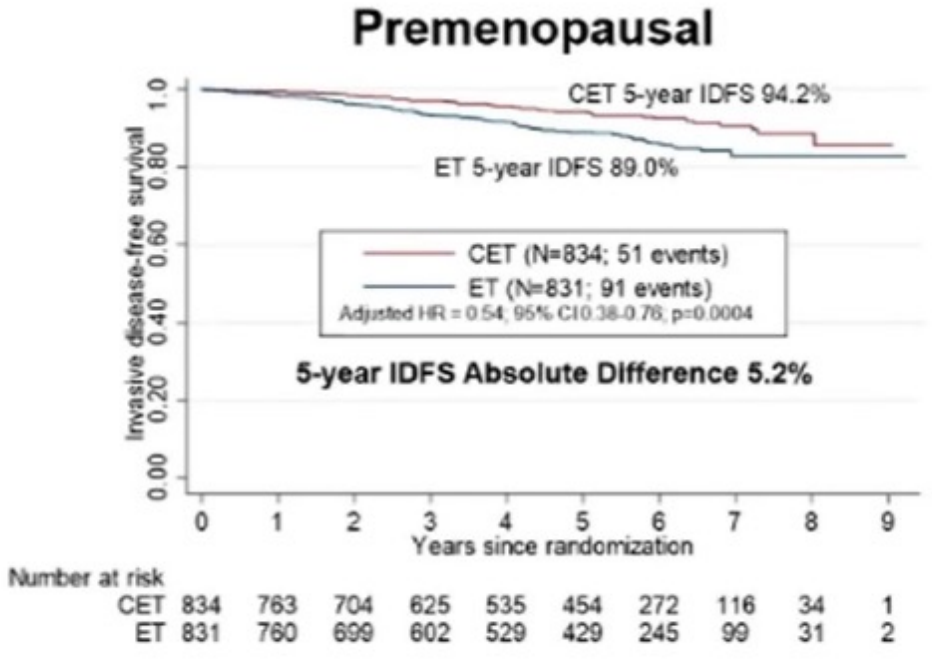


Kalinsky et al. NEJM 2021

Genomics in BC – RxPONDER trial

IDFS stratified by menopausal status

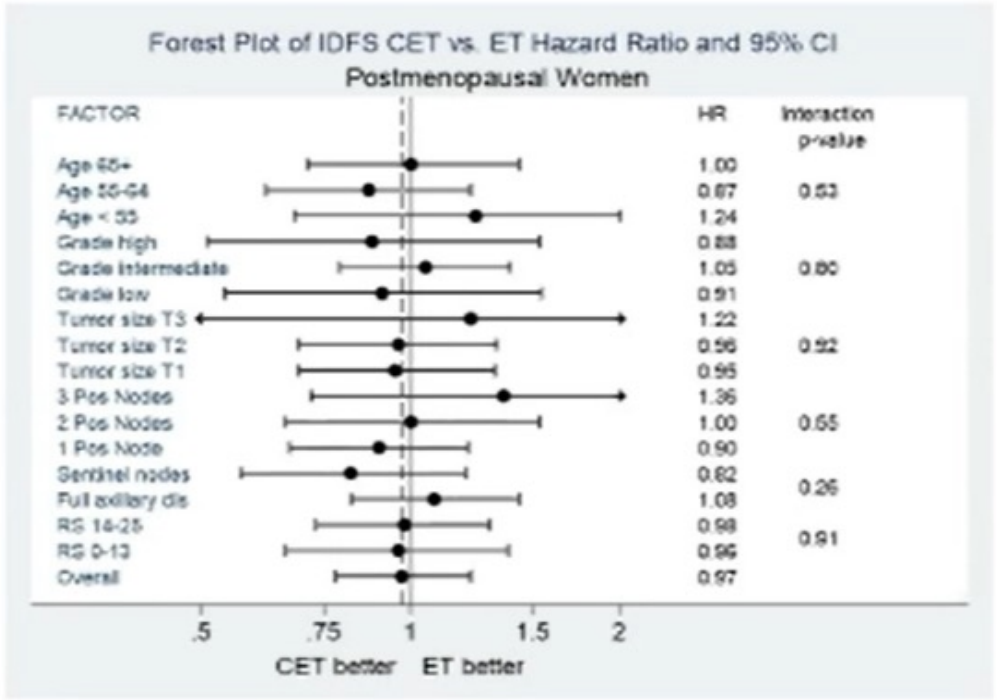


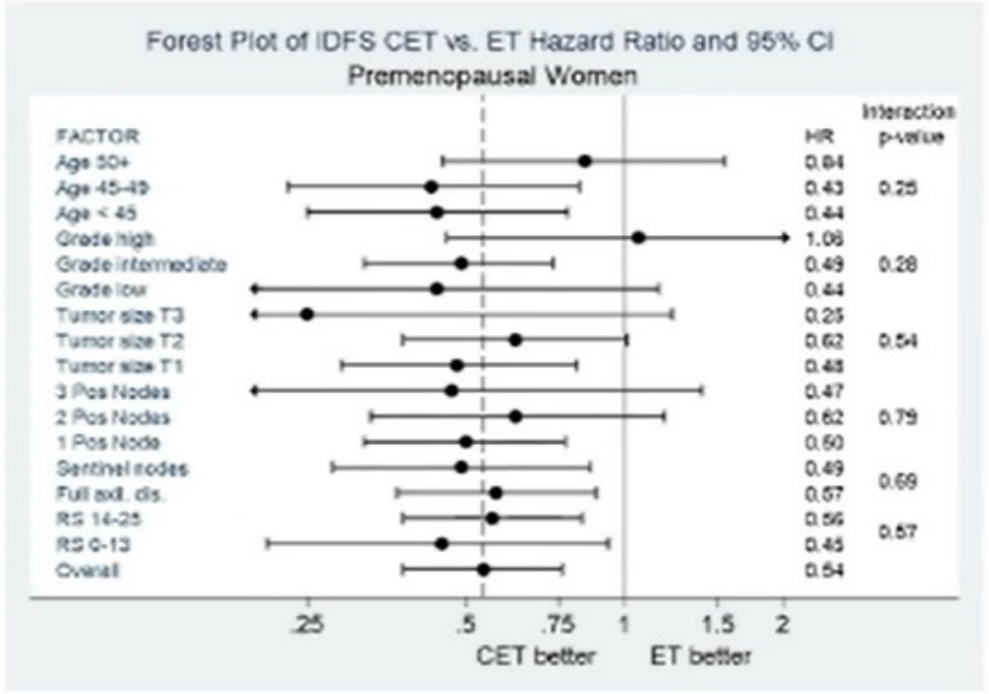


Kalinsky et al. NEJM 2021

Genomics in BC – RxPONDER trial

Forest plots of IDFS by menopausal status





Kalinsky et al. NEJM 2021

Genomics in BC – RxPONDER trial

Postmenopausal women with RS 0-25 did not benefit from adjuvant chemotherapy in any subgroup; on the other hand, premenopausal women with RS 0-25 had benefit from the addition of chemotherapy to ET

Postmenopausal women with 1-3 positive nodes and RS 0-25 can likely **safely forego adjuvant chemotherapy** without compromising IDFS

Premenopausal women with 1-3 positive nodes and RS 0-25 **likely significantly benefit from adjuvant chemotherapy**

Kalinsky et al. NEJM 2021

Genomics in BC – algorythm for adjuvant systemic therapy in HR+ HER2- BC patients

Take home messages

Patient group by LN and RS	Premenopausal/age ≤50 years	Postmenopausal/age >50 years
Lymph node negative		
RS <11	ET alone	ET alone
RS 11-15	ET alone	ET alone
RS 16-25	CT + ET (or OFS+AI)	ET alone
RS >25	CT + ET	CT + ET
Lymph node positive (1-3+ LN)		
RS <11	CT + ET (or OFS+AI)	ET alone
RS 11-18	CT + ET (or OFS+AI)	ET alone
RS 19-25	CT + ET (or OFS+AI)	ET alone
RS >25	CT + ET	CT + ET

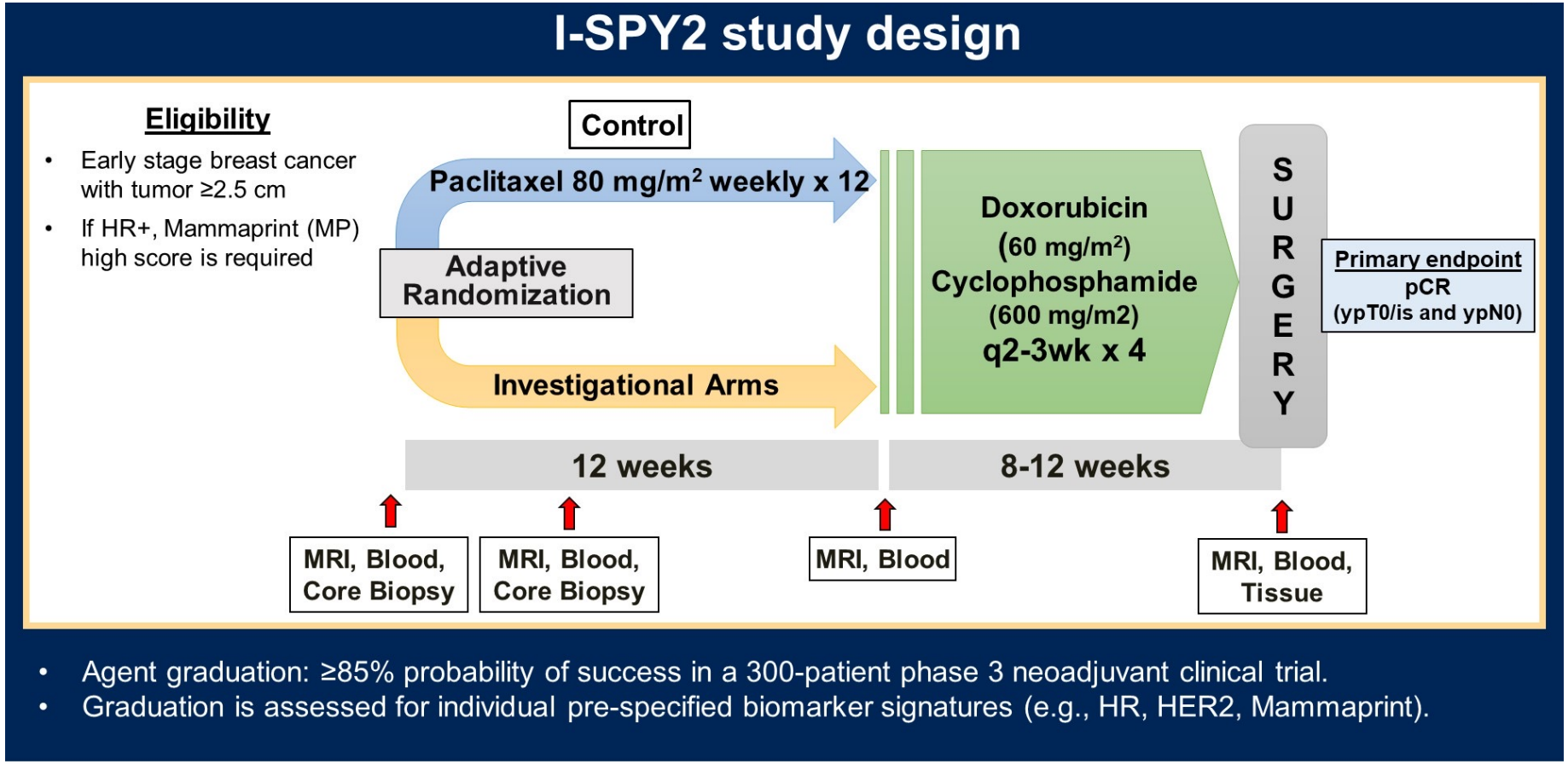
Genomics in BC – new perspectives

I-SPY2 is an **adaptive, multicenter phase II** clinical trial of neoadjuvant therapy for patients with early-stage BC with a **primary endpoint of pCR**

Clinical and molecular characteristics associated with pCR in patients with HR+/HER2- and HER2+ disease were evaluated

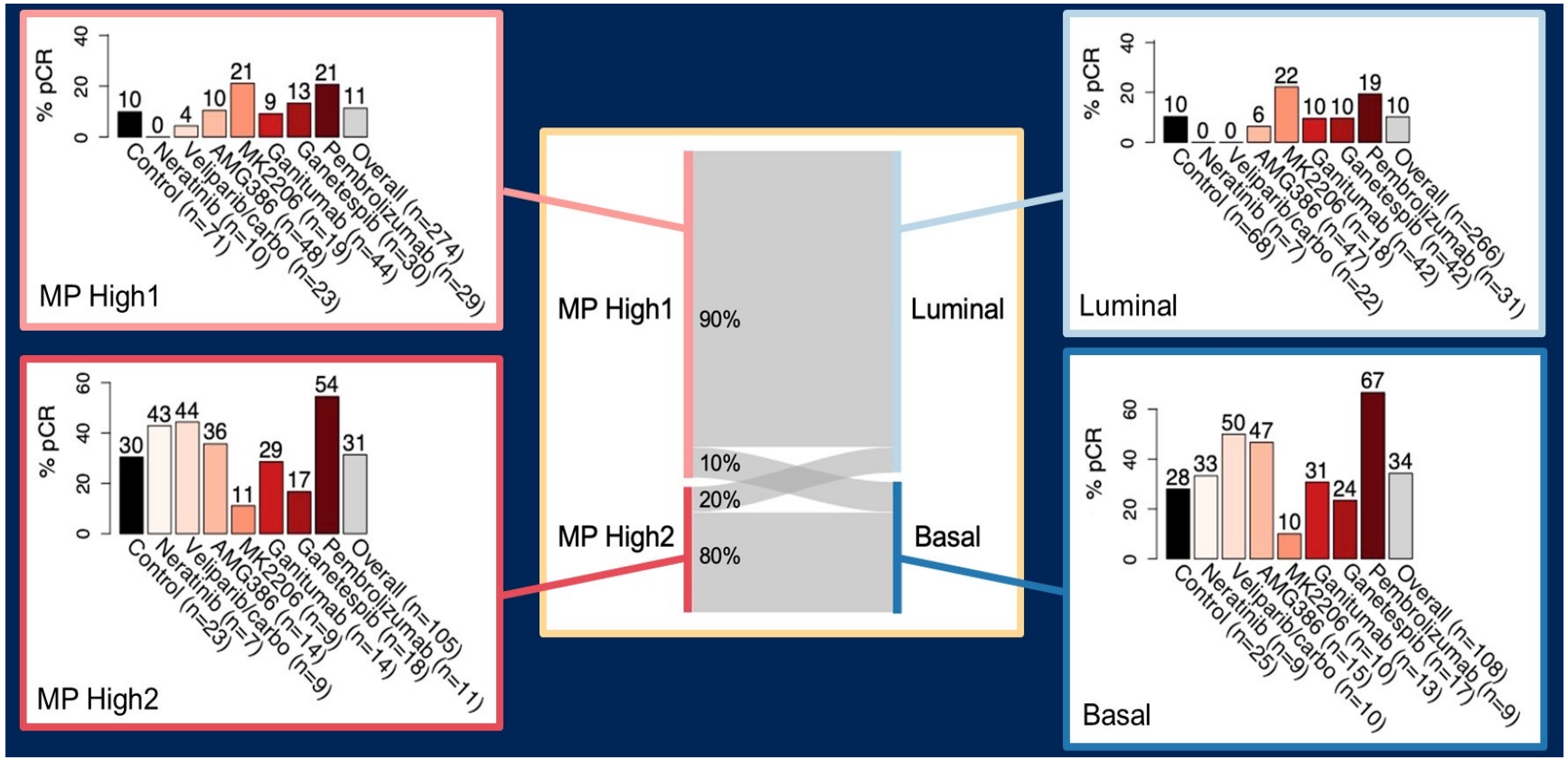
Huppert LA et al. ASCO 2022
Thomas A et al. ASCO 2022

Genomics in BC – new perspectives



Huppert LA et al. ASCO 2022
Thomas A et al. ASCO 2022

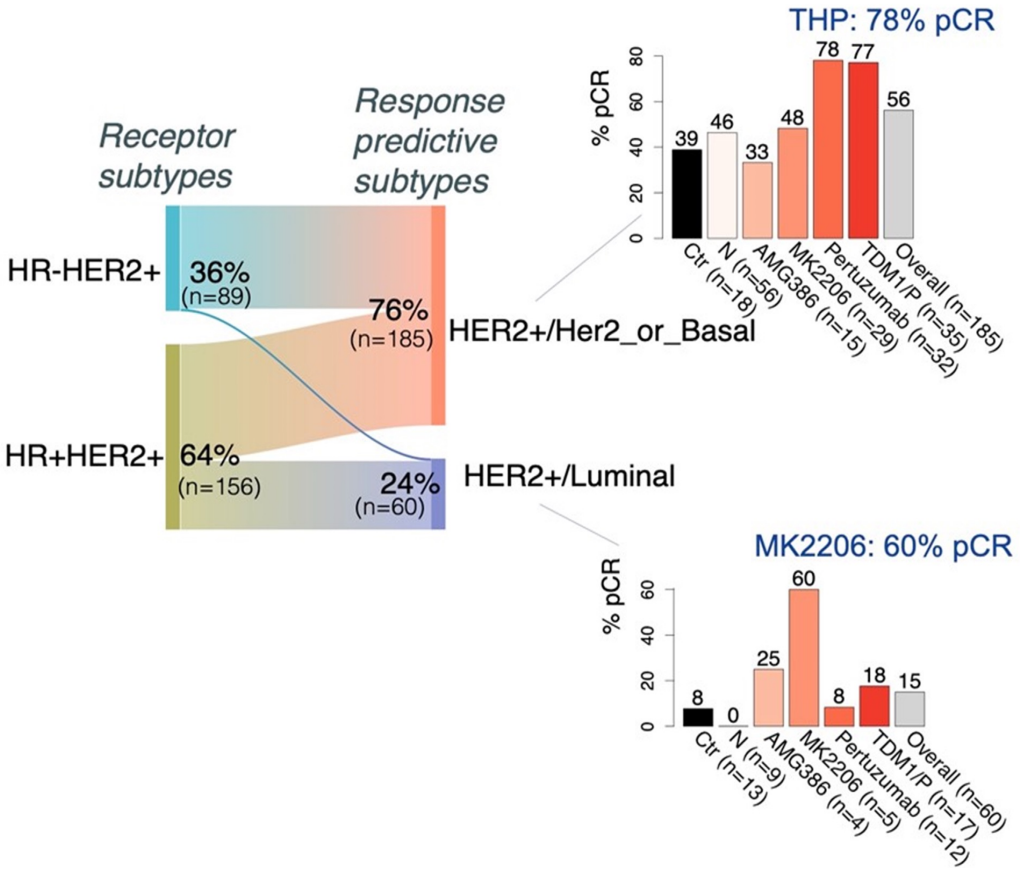
Genomics in BC – new perspectives



Mammaprint High 2 and **Blueprint Basal** signatures were associated with **higher pCR rates** in patients with high risk early stage HR+/HER2- BC receiving neoadjuvant therapy

Huppert LA et al. ASCO 2022

Genomics in BC – new perspectives

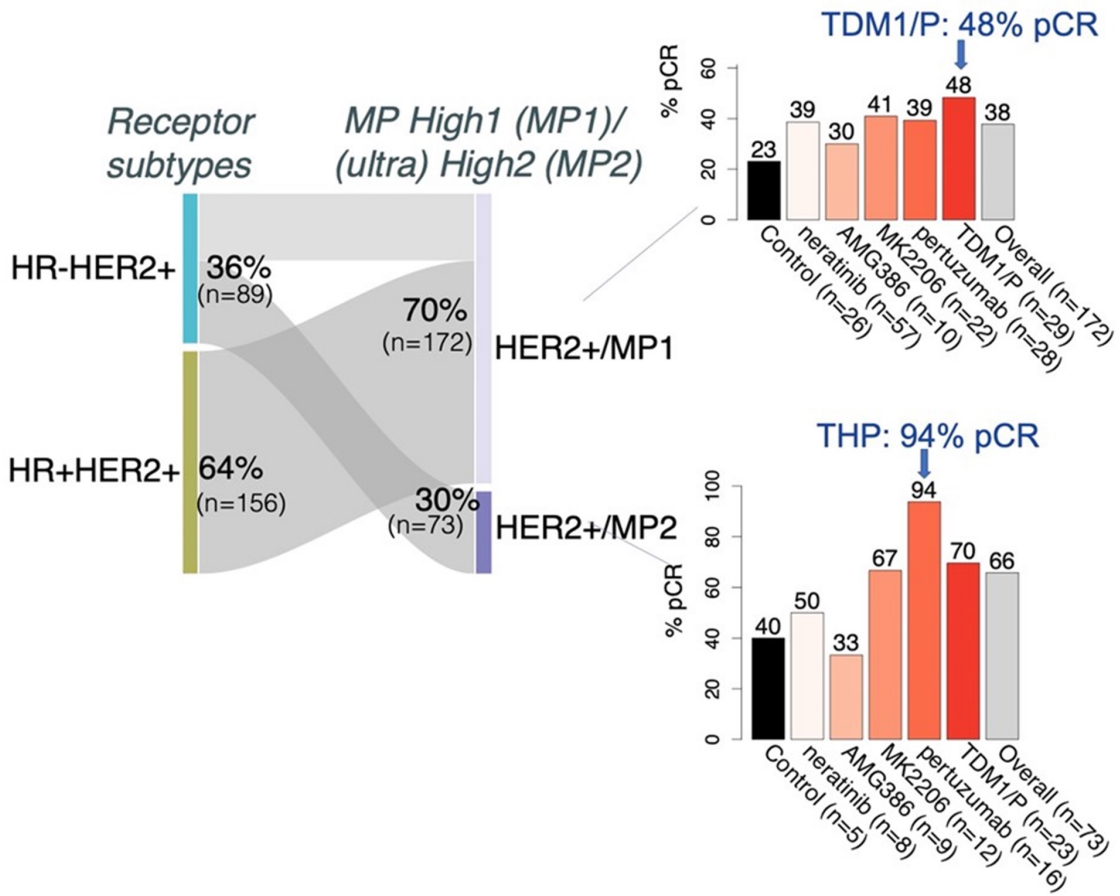


The **HER2+/Luminal** group had **low pCR rates** with **dual HER2-blockade** but may have **higher pCR rate** with the **addition of an AKT inhibitor**

Testing of AKT pathway inhibition and other novel approaches seeking to improve outcomes for HER2/Luminal patients is planned in I-SPY2.2 trial

Thomas A et al. ASCO 2022

Genomics in BC – new perspectives



Dual HER2-blockade with trastuzumab and pertuzumab had a particular **high pCR rate** in **MP-High2 tumors**

Molecular response predictive subtype classification provides insight on how to **better target therapy**

Thomas A et al. ASCO 2022

Proteomics in BC

Proteomics in BC

Why it is important to **identify the pattern of proteins' expression**?

- ✓ There is **not always correlation** between the amount of mRNA and the amount of protein
- ✓ The proteome is a **snapshot of the phenotype** at the biochemical level
- ✓ The proteome accounts for **protein modifications**

Proteomics in BC – correlation with transcriptome

Correlation coefficients vary across different organisms ranging from 0.2 to 0.47 in bacteria, 0.34 to 0.87 in yeast and 0.09 to 0.46 in multi-cellular organisms

Proteomics 2016, *16*, 2533–2544

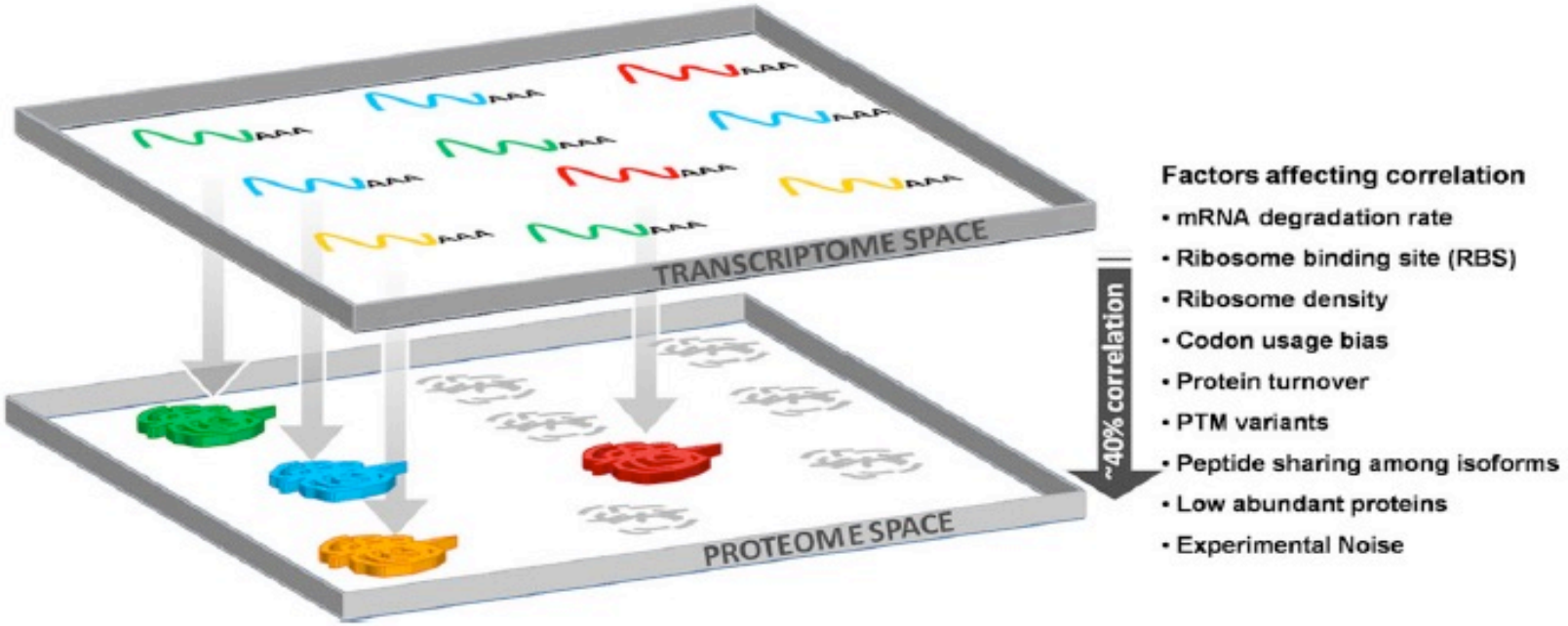


Figure 2. Factors influencing the correlation between mRNA–protein quantities.

Proteomics in BC – post genomic era

The human genoma project provided a **huge amount of information** on the sequence of **single genes**

BUT

It is **not a static system**

A lot of **genes working together**, at the same time, and the expression level of an mRNA often **does not correlate** with the amount of protein

PROTEOME (PROTEins expressed by gen**OME)** is a **dynamic system**

Analysis of proteins and their functions expressed by a biological system

Proteomics in BC – identification of potential biomarkers

The **profiling of tumor tissue proteomics** provides important information on the discovery of **biomarkers**

Brown JE et al. Cancer Informatics. 2019
Yoneten KK et al. Cancer Genomics Proteomics. 2019

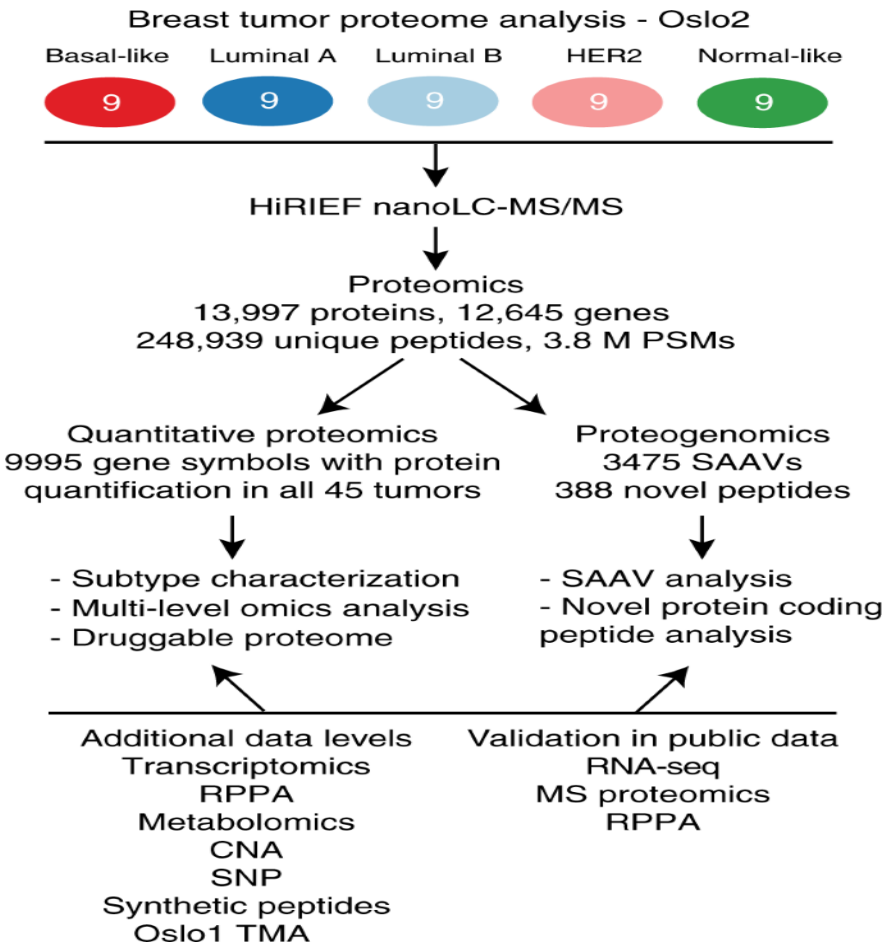
Several studies on protein biomarkers of **prognosis**, **tumor growth** and **aggression** have been conducted on various cellular subtypes of BC

Rossi C et al. Oncogenesis 2022

In addition, in recent years many researchers have shifted their attention from the study of BC cell lines to **cancer steam cells (CSCs)**. As a result, some **protein biomarkers** placed **on the surface of the CSCs** or involved in **self-renewal of CSCs** have been identified

Koh EY et al. Mol Cells. 2020
Mol Cells. 2020 et al. Mol Cells. 2020

Proteomics in BC – comparison to PAM50 subtypes



An unbiased analysis of breast tumor proteomes, inclusive of **9995 proteins** quantified across all tumors, for the first time recapitulates BC subtypes

Nine patients classified into each of the five **PAM50 subtype groups** were selected from the Oslo2 study cohort to ensure tumor diversity is represented

Unsupervised hierarchical clustering of **proteome profiles stratifies tumors largely in agreement with the PAM50 subtypes**

Johansson HJ et al. Nature Communications 2019

Metabolomics in BC

Metabolomics in BC

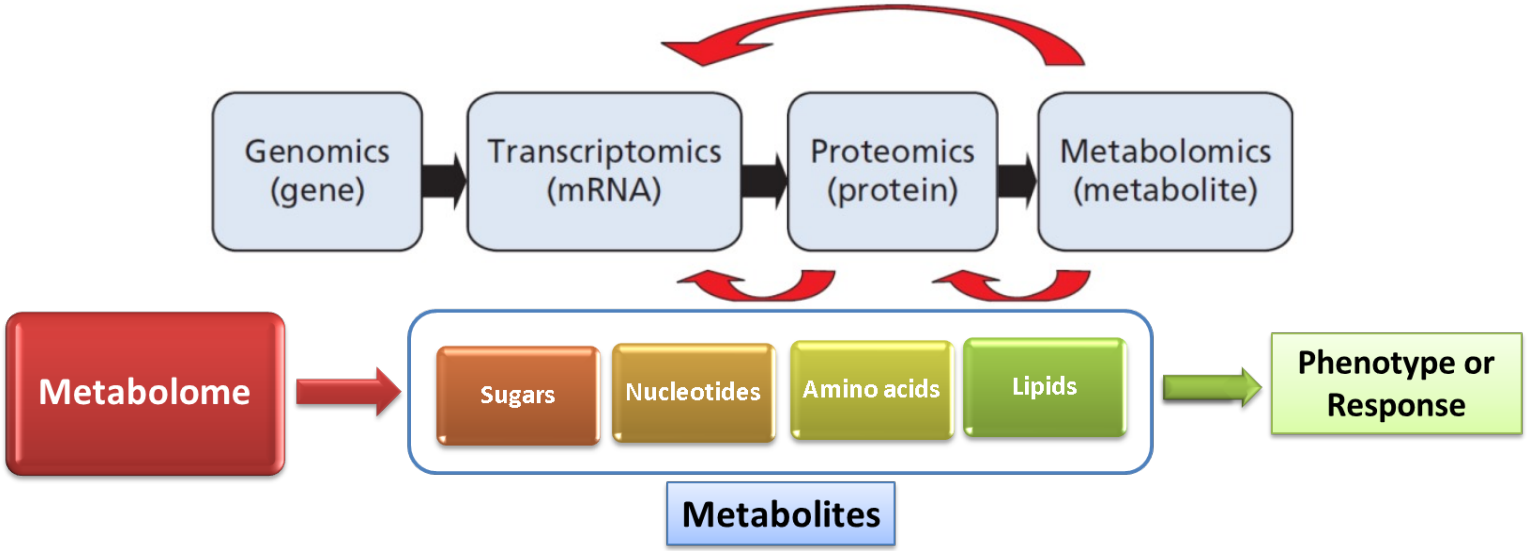
Metabolomics is the study of the **multiparametric metabolic response** of living systems to pathophysiological stimuli or genetic modifications

The **metabolome** is the **quantitative ensemble of metabolites**, and it is established via analysis of **various biological samples** (for example, blood, urine, saliva, tissue) and influenced by both **exogenous and endogenous factors**, such as age, gender, race, diet, presence of disease, and drug exposure

Nicolson JK et al. Xenobiotica 1999
Beger RD et al. Metabolomics 2016

Metabolomics in BC – The relationship between -omics approaches of systems biology

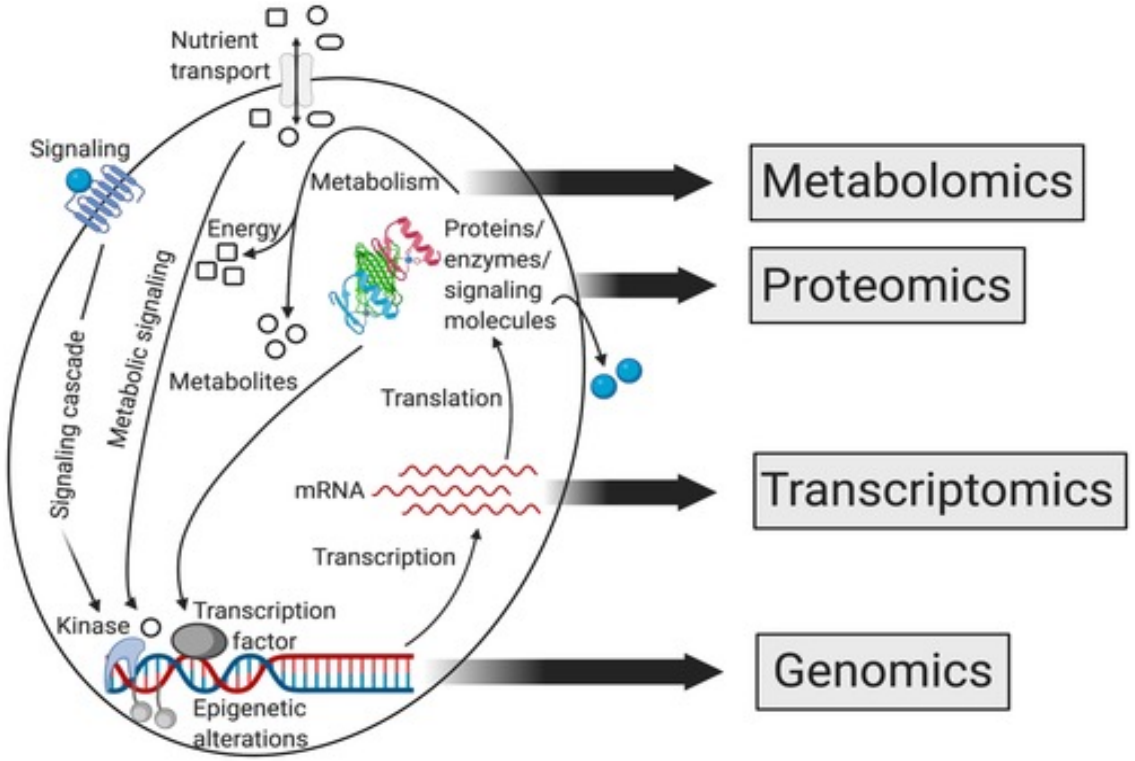
Metabolome Reflects the State of the Cell, Organ or Organism



- **Change in the metabolome is a direct consequence of protein activity changes**
  - **Not necessarily true for genomic, proteomic or transcriptomic changes**
- **Disease, environmental factors, drugs, etc., perturbs the state of the metabolome**
  - **Provides a system-wide view of the organism or cell's response**

Courtesy of Andrea Morandi

Metabolomics in BC – The relationship between -omics approaches of systems biology



Cancer is caused by **changes at the genomic level that result in altered RNA transcription, protein expression, and protein function**

The **metabolome** provides a functional readout of these upstream changes. In turn, **individual metabolites affect protein activity** and thereby **alter RNA transcription and DNA replication**

Schmidt DR et al. Ca Cancer J Clin 2021

Metabolomics in BC – identification of potential biomarkers

Several experiences conducted on relatively small samples showed a potential of metabolomics analysis to **predict treatment response** in advanced and early BC patients or to **refine existing molecular subtypes**

Tenori L et al. Mol Oncol 2012
Wei S et al. Mol Oncol 2013
Choi JS et al. PLoS ONE 2013
Miolo G et al. Oncotarget 2016
Cao MD et al. BMC Cancer 2012
Borgan E et al. BMC Cancer 2010
Aure MR et al. Breast Cancer Res 2017
Haukaas TH et al. Cancer Metab 2016

Perhaps of most interest to clinicians is the potential metabolomics may have in **generating prognostic biomarkers**. In 2010, the first evidence supporting metabolomics as a potential biomarker of recurrent disease was published. A retrospective analysis was performed on 56 patients with eBC, all of whom had **serial serum samples** collected over 6 years. **Eleven metabolite markers** that differentiated between those with recurrent disease and those without were identified

Asiago VM et al. Cancer Res 2010

Radiomics in BC

Radiomics in BC

Radiomics is a **quantitative approach to medical imaging**, aiming to **enhance the existing data** available to clinicians by means of **advanced mathematical analysis**

Van Timmeren JE et al. Educational Review 2020

Radiomics is based on the **hypothesis** that **extracted quantitative data** derives from mechanisms occurring at **genetic and molecular levels**

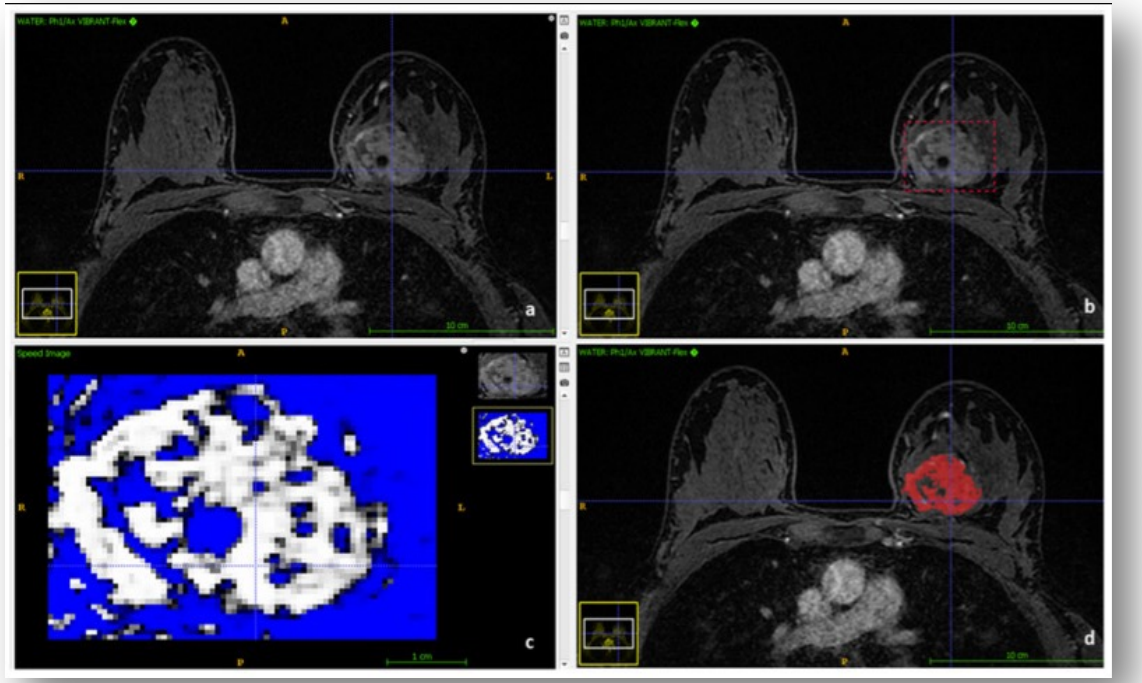
Tagliafico AS et al. Breast 2020

Radiomics in BC – response to NACT

Monocentric retrospective study on 83 patients receiving **neoadjuvant chemotherapy (NACT) and breast MRI**

For each patient, radiomic features were extracted within the biopsy-proven tumor **on T1-weighted** contrast-enhanced MRI performed **before NACT**

The **association of clinical/biological and radiomic features** with response to NACT was evaluated by univariate and multivariable analysis

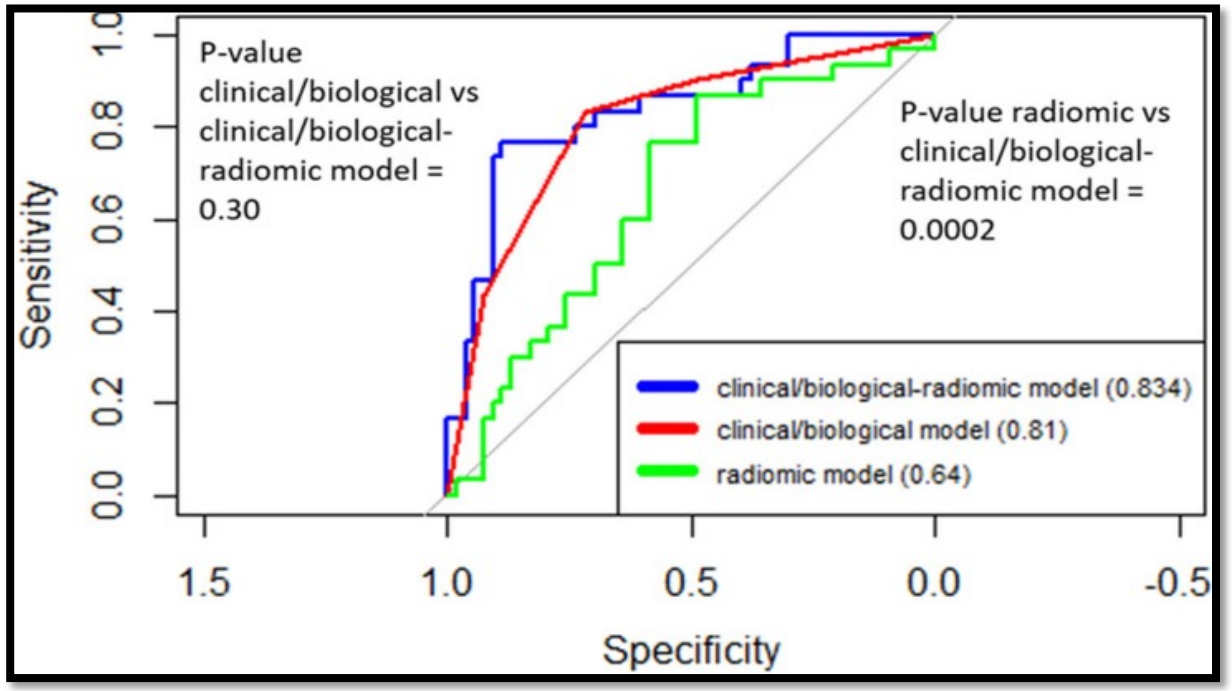


Pesapane F et al. Cancers 2021

Radiomics in BC – response to NACT

Using **136 radiomics features**, a radiomic score was calculated to **predict the response to NACT**, with AUC (95% CI): **0.64** (0.51-0.75). After combining the clinical/biological and radiomics models, the AUC (95% CI) was **0.83** (0.73-0.92)

MRI-based radiomic features **slightly improved the pre-treatment prediction of pCR to NACT**, in addiction to biological characteristics. If confirmed on larger cohorts, it could be **helpful to identify such patients**, to avoid unnecessary treatment



Pesapane F et al. Cancers 2021

Radiomics in BC – prediction of prognosis

A total of **278 patients** with **LABC** from 2010 to 2015 retrospectively reviewed and **radiomics features** were extracted from **enhanced MRI**

Patients were divided into **training cohort** and **validation cohort**. Radiomics score was constructed and **significantly associated with DFS of the patients in training cohort**, **validation cohort and external validation cohort** ($p<0.001$, $p=0.014$ and $p=0.041$, respectively)

The radiomics-based nomogram showed **better predictive performance of DFS** compared with TNM model

Immunophenotype and immune cell composition was **different in each radiomics score group**

Wang X et al. Breast Cancer Research 2022

THANKS FOR YOUR ATTENTION!!!

luca.visani@unifi.it