

Gene Expression Profiling and Treatment Decision-Making in Breast Cancer

Mohammad R. Akbari, M.D., Ph.D. Scientist, Women's College Research Institute Associate Professor, Dalla Lana School of Public Health University Of Toronto

akbarilab.utoronto.ca

mohammad.akbari@utoronto.ca

January 30, 2022

Breast Cancer

- 13% of all cancers diagnosed in Iran in 2020 (~17,000 cases)
- 25% of all cancers diagnosed in Canada in 2020 (~27,000 cases)
- A heterogenous disease (molecular, histological and clinical)
- The goal of breast cancer classification:
 - To determine the optimal treatment plan for the patients
- Current BC classification:
 - IHC of ER, PR, and HER2
 - ER+, HER2+ and TNBC subtypes



Gene Expression Profiling Tests

- PAM50 (Prosigna)
- MammaPrint
- Oncotype
- EndoPredict
- Breast Cancer Index
- GEP tests are recommended by:
 - American Society of Clinical Oncology (ASCO)
 - National Comprehensive Cancer Network (NCCN)
 - St. Gallen International Experts Consensus
 - European Society of Medical Oncology
 - National Institute for Health and Care Excellence (NICE)

Oncotype

Oncotype: First Report

- NSABP B14 Trial
- 668 ER+/HER2- node BC patients treated with Tamoxifen





Paik et al., NEJM 2004

Oncotype: First Clinical Trial

- NSABP B20 Trial
- 651 ER+/HER2- node- BC patients randomly assigned to chemo and chemoendocrine therapy



Paik et al., JCO 2006

Oncotype: Intermediate RS

- TAILORx Trial
- 6711/9719 ER+/HER2- Node- BC patients with intermediate RS (16-25)
- Randomized for endocrine or chemoendocrine therapy



Oncotype: Node+ patients

- RxPONDER Trial
- 5018 ER+/HER2- Node+ BC patients with low or intermediate RS (0-25)
- Randomized for endocrine or chemoendocrine therapy



Kalinsky et al., NEJM 2021

PAM50 (Prosigna)

Start of Gene Expression Profiling

Luminal A

Her2-Enriched

Basal-Like

Luminal B



ate de de la Se tit Se de de de de

B-CELL CLULYMPHOMA 2 ESTS, WEAKLY SIMILAR TO MEMBRANE GLYCOPROTEIN

INCOVERTE DNA SEQUENCE FROM CLONE 167A19 ON CHROMOSOME 1P32,1-33 PROLACTIN RECEPTOR

470216 N-ACETYLTRANSFERASE 1 ARVLAMINE N-ACETYLTRANSFERASE CONA DKF2P434A091 FROM CLONE DKF2P434A091 554034

358900 SEVEN IN ABSENTIA DROSOPHILA HOMOLOG 2 HEPSIN TRANSMEMBRANE PROTEASE, SERINE 1

170585 HUMAN SECRETORY PROTEIN PL8 MRNA, COMPLETE COS MERTICOLTE NUCLEAR RACTOR 2, ALPHA ESTICOLTA RECEIPTOR 1 ESTICOLTA RECEIPTOR 1 ESTICOLTA RECEIPTOR 1 GATA-SBROING PROTEIN 3 GATA-SBROING PROTEIN 3 GATA-SBROING PROTEIN 3 CATA-SBROING PROTEIN 3 CATA-

ANNEXIN XXXI BREAST CANCER, ESTROGEN REGULATED LIV-1 PROTEIN LIV-1 345321

IUMAN CHRONOSOME 16 BAC CLONE CIT9875K-254P9

271989 ESTS SMILAR TO INOSITOL POLYPHOSPHATE 4-PHOSPHATASE

CTROT IN LAR TO RESIDE FOR TROOTING ENTRY AND TRANS TYPE I I THE LEWERA VIRAL BAIL ONCOGENE HOMOLOG MURINE ELEWERA VIRAL BAIL ONCOGENE HOMOLOG MURINE ELEWERA VIRAL BAIL ONCOGENE HOMOLOG HOMO SAPERS PAO GENE MINA, 3' END TROST

TODAD SIMPLING Y HID OLER MININ, 3 EINO ACTI-COERCYNYL A GENYTROGOLASS, SHORTISHANCHED CHAIN CARWINNE PALMITOYTTARAMINERARE I BER AS CYTCOENNOW FAOS, SUBFANLY NA ANGOYTENSIN RECEPTOR I ANGOYTENSIN RECEPTOR I HUMAN MININA Y ON KIAASSI STELATED TO AF4 HUMAN MININA Y ON KIAASSI STELATED TO AF4 EPORDE HYDROLASE 2, CYTOPLASMIC GAUL SPECIFICITY PHOSPHATASE 4

ERBB-2 RECEPTOR PROTEIN-TYROSINE KINASE PRECURSOR ERBB2-POLYA ERB-32 ERB-32 ERB-32 ERB-35 ERB-30 ERB-30 ERB-30 ERB-30 ERB-30 ERB-30 ERB-30 ERB-30 ERB-30 ERB-32 ERB-SWISINF RELATED ACTIN DEPENDENT REGULATOR OF CHROMATIN INF RECEPTOR-ASSOCIATED FACTOR 4

FLOTILLIN 2 TGFB1-INDUCED ANTI-APOPTOTIC FACTOR 1 DUAL SPECIFICITY PHOSPHATASE 6

DUALS SPECIFICITY INTO PHATASE 6 MATRIX INTELLOPROTENDAS 14 MEMBRANE-INSERTED COLLAGEN, TYPE XVII, ALFHA 1 CALPONIN, IL BASIC, SINOCOTH MUSCLE PLEIDTROPHIN HEPARIN BINDING CROWTH FACTOR 8 PLEIDTROPHIN HEPARIN BINDING CROWTH FACTOR 8 PLEDTROPHIN HEPAINIB BUDING CIROVITH FACTOR 5 192788 BULLOUS PERMINISON ANTIGEN 1220/2000 BWALL RECORDER CYTOKINE SUBPARILY D CY5-X3-CY5 1 KERATIN 17 KERATIN 5 ESTS. MICHAY SIMELAR TO KERATIN KS. SIKT VYE'B, EPIDERMAL ESTS. MICHAY SIMELAR TO KERATIN KS. SIKT VYE'B, EPIDERMAL ESTS. MICHAY SIMELAR TO KERATIN KS. SIKT VYE'B, EPIDERMAL ESTS. MICHAY SIMELAR TO KERATIN KS. SIKT VYE'B, EPIDERMAL ESTS. MICHAY SIMELAR TO KERATIN KS. SIKT VYE'B, EPIDERMAL ESTS. MICHAY SIMELAR TO KERATIN KS. SIKT VYE'B, EPIDERMAL ANIEZINI, LUPCOTTINI DISTUMPTION SIMELAR DE CONTROLMENT DUCHENKE AND BECKER TYPES EXPREMIA COUNTS LEVTER DEPICETOR UNIT DIMONSTRANSPORTS CARE & ENDERMAL GONTH FACTOR RECEPTOR GROI ONCOGENE MELANOMA GROWTH STMULATING ACTIVITY, ALPHA. HUMAN DNA-BENDROR PROTEIN ABP/27 MIRIA, COMPLETE COS ANTILEUKOPROTEINAGE FATTY ACID BEDINGO PROTEIN 7, BRAIN

PATTY ACID BILONIC PROTEIN 7, BRAIN ONTITIASES LIKE 2 TRANSBERMINANE 4 SUPERAMILY MEMBER 1 HONDO SAPERES MITHA FOR GALPARI-LIKE PROTEASE CANPX KERATIN 7 LAONIN 1, P.CADHEINN FLACKOVIL PROTEIN TYNOSINE PHOSPHATASE. RECEPTOR TYPE, K STY GEX OFFERMING REGION Y-BOX 5 CAMPOMELIC OVERLASIA KERATIN 1 SECOND TYNOSINE PHOSPHATASE. RECEPTOR TYPE, K STY GEX OFFERMING REGION Y-BOX 5 CAMPOMELIC OVERLASIA KERATIN 1 HORONIN, IETA 4 HITGOPOINI, 1 SCALETAL FAST

Perou et al., Nature 2000

Gene Expression Profiling and Outcome



Sorlie et al., PNAS 2001

PAM50: Prediction Analysis of Microarray 50



Parker et al., JCO 2009

PAM50: Risk of Recurrence Score (ROR)

- Molecular Subtype
- Tumour Proliferation Score
- Tumour Size (=<2 or >2 cm)



PAM50: First Study

- ATAC Trial
- 1017 ER+/HER2- BC patients treated with Tamoxifen/Anastrozole



Dowsett et al., JCO 2013

PAM50: Second Study

- ABCSG Trial
- 1478 ER+/HER2- BC patients treated with Tamoxifen/T+Anastrozole



PAM50: Second Study

- ABCSG Trial
- 1478 ER+/HER2- BC patients treated with Tamoxifen/T+Anastrozole



PAM50: Node-Positive Patients

- ABCSG and ATAC Trials
- 331 ER+/HER2- 1 Node+ BC patients with 5-year hormone therapy



PAM50: Node-Positive Patients

- ABCSG and ATAC Trials
- 212 ER+/HER2- 2-3 Node+ BC patients with 5-year hormone therapy



PAM50: Danish Study

• 1163 ER+/HER2- Node- BC patients with 5-year hormone therapy



Lankholm et al., JCO 2018

PAM50: Danish Study

• 1395 ER+/HER2- Node+ BC patients with 5-year hormone therapy



1 node+

2 nodes+

3 nodes+

Lankholm et al., JCO 2018

PAM50: Danish Study

• 1395 ER+/HER2- Node+ BC patients with 5-year hormone therapy



1 node+

2 nodes+

3 nodes+

Lankholm et al., JCO 2018

PAM50 vs Oncotype

- ATAC Trial
- 1017 ER+/HER2- BC patients with 5-year Tamoxifen/Anastrozole



Dowsett et al., JCO 2013

- ATAC Trial
- 1017 ER+/HER2- BC patients with 5-year Tamoxifen/Anastrozole



Sestak et al., JNCI 2013

• 774 ER+/HER2- BC patients with 5-year hormone therapy

Table 1. Univariate HRs and C Indexes for All Prognostic Signatures According to Nodal Status During Years O to 10

	Patient Group										
Gene	Node-Negative Disea (n = 591)	se	Node-Positive Disease (n = 227)								
Signature	HR (95% CI) ^a	C Index (95% CI)	HR (95% CI) ^a	C Index (95% CI)							
CTS	1.99 (1.58-2.50)	0.721 (0.668-0.774)	1.63 (1.20-2.21)	0.640 (0.554-0.726)							
IHC4	1.95 (1.55-2.45)	0.725 (0.665-0.785)	1.33 (0.99-1.78)	0.601 (0.511-0.690)							
RS	1.69 (1.40-2.03)	0.667 (0.585-0.750)	1.39 (1.05-1.85)	0.603 (0.513-0.693)							
BCI	2.46 (1.88-3.23)	0.762 (0.704-0.820)	1.67 (1.21-2.29)	0.652 (0.566-0.739)							
ROR	2.56 (1.96-3.35)	0.764 (0.707-0.821)	1.58 (1.16-2.15)	0.636 (0.552-0.719)							
EPclin	2.14 (1.71-2.68)	0.765 (0.716-0.814)	1.69 (1.29-2.22)	0.671 (0.590-0.752)							

Sestak et al., JAMA Oncology 2018

- 100 ER+/HER2- BC patients with 5-year hormone therapy
- 29% Chemotherapy and 57% Radiotherapy

	Oncotype Recurrence Score							
	Low (<18)	Intermediate (18–30)	High (>31)	Total				
Prosigna risk groups								
Low (<40)	43	22	2	67				
Intermediate (40–60)	8	8	1	17				
High (61–100)	6	9	1	16				
Total	57	39	4	100				

Agreement in risk group assignment between ODX RS and Prosigna ROR.

Abdelhaka et al., Can Treat and Research Com 2021

- 100 ER+/HER2- BC patients with 5-year hormone therapy
- 3/100 had recurrence

ODX categ	RS scoreAnd ory	scoreAnd Prosigna ROR Ki67(%) and score and proliferative category labeling index		(%) and ferative ing index	Recurrence regional and distant (site)	Time to recurrence	Age	Grade	Node	Menopause	Surgery	Hormonal	Chemotherapy	Radiotherapy	
17	Low	66	High	30	High	(chest wall)	6 years	51	2	N	Peri-menopausal	Mastectomy	Y	N	Y
11	Low	70	High	30	High	(left breast)	17 years	70	2	Ν	Postmenopausal	Lumpectomy	Y	Y	Ν
20	Intermediate	80	High	30	High	(liver)	5 years	68	3	Ν	Postmenopausal	Mastectomy	Y	N	Ν

ODX, Prosigna, and Ki67 scores in three cases with confirmed recurrence at different sites and their clinical characteristics.

Abdelhaka et al., Can Treat and Research Com 2021

- 12 ER+/HER2- BC patients recently diagnosed
- All had Oncotype test (Genomic Health Inc.)

Sample ID	Age at Diagnosis	ER Status	PR Status	HER2 Status	Tumour Size	Lymph Node Status	Oncotype RS	Oncotype 10 years Distance Recurrence Risk	PAM50 Molecular Subtype	PAM50 ROR	PAM50 10 years Distance Recurrer	nce Risk
1	44	Positive	Positive	Negative	>2cm	Negative	16	4%	Luminal A	25	4%	
2	59	Positive	Positive	Negative	<=2cm	Micromets	11	13%	Luminal B	55	24%	
3	48	Positive	Positive	Negative	>2cm	Negative	7	3%	Luminal B	54	11%	
4	47	Positive	Positive	Negative	<=2cm	Negative	18	5%	Luminal B	55	11%	
5	41	Positive	Positive	Negative	<=2cm	Negative	18	5%	Luminal A	28	4%	
6	48	Positive	Positive	Negative	<=2cm	Negative	11	3%	Luminal A	44	11%	
7	63	Positive	Positive	Negative	<=2cm	Negative	20	6%	Luminal A	34	4%	
8	30	Positive	Positive	Negative	>2cm	Negative	41	29%	Luminal B	72	22%	
9	34	Positive	Positive	Negative	>2cm	Negative	22	8%	Luminal A	23	4%	
10	52	Positive	Positive	Negative	<=2cm	Negative	12	3%	Luminal A	28	4%	
11	53	Positive	Positive	Negative	<=2cm	Negative	17	5%	Luminal A	26	4%	
12	37	Positive	Positive	Negative	<=2cm	Negative	20	6%	Luminal A	19	4%	

Test Reports

Oncotype Report



Quantitative Single-Gene Scores¹



Oncotype Report



Decision on individual treatment especially around the RS 25 cutoff may consider other clinical factors.

TAM = Tamoxifen CI = Confidence Intervals *For estimated CT benefit for individual RS results, see page 2.

Quantitative Single-Gene Scores¹

9.7 ER Positive			3.2 P	R Negati	ve	9.3 HER2 Negative		
<3.7	6.5	≥12.5	<3.2	5.5	≥10.0	<7.6	10.7 11.5 ≥13.0	

PAM50 Report: Node-Negative



PAM50 Report: Node-Positive



Molecular Subtypes Implications

Discordance of HER2+ and HER2-Enriched Subtypes

clinical subtype HER2+ ER-



Based on 1,237 HER2+ patients

Discordance of ER+ and Luminal Subtypes

clinical sutype ER+ HER2-



Based on 4,402 ER+ patients

Discordance of TNBC and Basal-Like Subtypes

clinical subtype TNBC



Based on 3,931 TNBC patients

pCR in Molecular Subtypes of ER+ Patients

- 474 ER+ patients
- pCR rate after neoadjuvant chemotherapy



Whitworth et al., Annals of Surgical Oncology 2017

Response to Anti-HER2 among HER2-Enriched Patients

- CALGB 40601 Clinical Trial
- 305 HER2+ patients
- Neoadjuvant (Taxol, Trastuzumab, Lapatinib)
- pCR among HER2-Enriched vs nonHER2-Enriched: 70% vs 34-36%



Carey et al., JCO 2016

Response to Anti-HER2 among HER2-Enriched Patients

- NCCTG (Alliance) N9831 Clinical Trial
- 1,392 HER2+ patients
- Adjuvant chemotherapy with (908) or without (484) Trastuzumab
- 1,003 (72%) were HER2-Enriched and 97 (7%) were Basal-Like



Perez et al., JNCI 2017

Response to Anti-Her2 in ER+/HER2-Enriched Patients

- EGF30008 clinical trial
- 568 ER+/HER2- patients were randomized on Letrozole with or without Lapatinib
- Median progression-free survival for HER2-Enriched (6.5 m vs 2,6 m, HR : 0.24, p : 0.02)



DOI: 10.1111/cge.13900

REVIEW





Molecular intrinsic versus clinical subtyping in breast cancer: A comprehensive review

Agata Szymiczek¹ | Amna Lone¹ | Mohammad R. Akbari^{1,2,3}

¹Women's College Research Institute, University of Toronto, Toronto, Ontario, Canada

²Institute of Medical Science, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

³Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada

Correspondence

Mohammad R. Akbari, Women's College Hospital, 76 Grenville Street, Room 6421, Toronto, ON, M5S 1B2, Email: mohammad.akbari@utoronto.ca

Funding information Canadian Institute for Health Research (CIHR), Grant/Award Number: 152939

Abstract

Breast cancer is a heterogeneous disease manifesting diversity at the molecular, histological and clinical level. The development of breast cancer classification was centered on informing clinical decisions. The current approach to the classification of breast cancer, which categorizes this disease into clinical subtypes based on the detection of estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, and proliferation marker Ki67, is not ideal. This is manifested as a heterogeneity of therapeutic responses and outcomes within the clinical subtypes. The newer classification model, based on gene expression profiling (intrinsic subtyping) informs about transcriptional responses downstream from IHC single markers, revealing deeper appreciation for the disease heterogeneity and capturing tumor biology in a more comprehensive way than an expression of a single protein or gene alone. While accumulating evidences suggest that intrinsic subtypes provide clinically relevant information beyond clinical surrogates, it is imperative to establish whether the current conventional immunohistochemistry-based clinical subtyping approach could be improved by gene expression profiling and if this approach has a potential to translate into clinical practice.

KEYWORDS

breast cancer, gene expression profiling, hormone receptor, intrinsic subtyping, prognosis, response to treatment



