Integrative Genomic and Transcriptomic Analysis Associated with Neoadjuvant Therapy Response in HER2-Positive Breast Cancer

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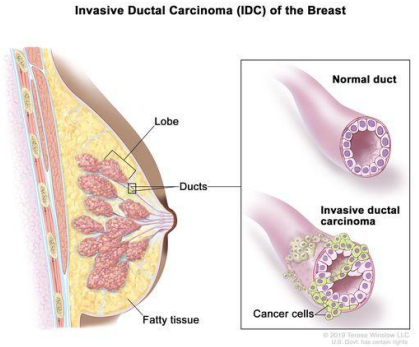
Date: 2024-5-14

01

Introduction

Breast Cancer Histological Subtypes



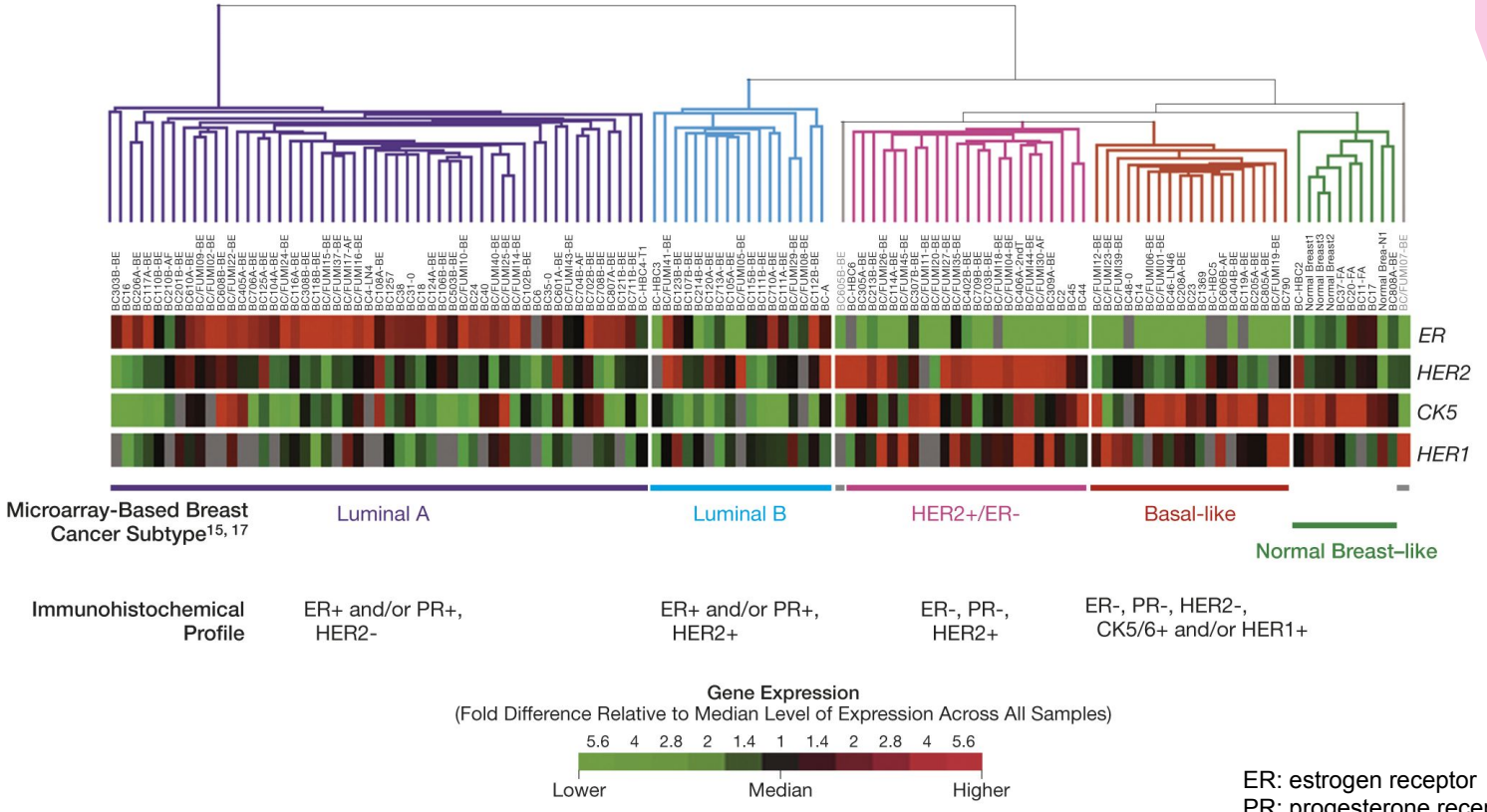
Adapted from NCI Dictionaries

- Tumor
  - **In Situ Carcinoma** (Limited to the epithelial component)
    - **Ductal Carcinoma in Situ** (DCIS)
    - **Lobular Carcinoma in Situ** (LCIS)
  - **Invasive Carcinoma** (Invaded the stroma)
    - **Invasive Ductal Carcinoma** (IDC)
    - **Invasive Lobular Carcinoma** (ILC)

Clin Med Insights Pathol. 2015 Dec 21;8:23-31. doi: 10.4137/CPath.S31563.

Molecular subtypes

The subtypes of breast cancers recognized by their gene signature

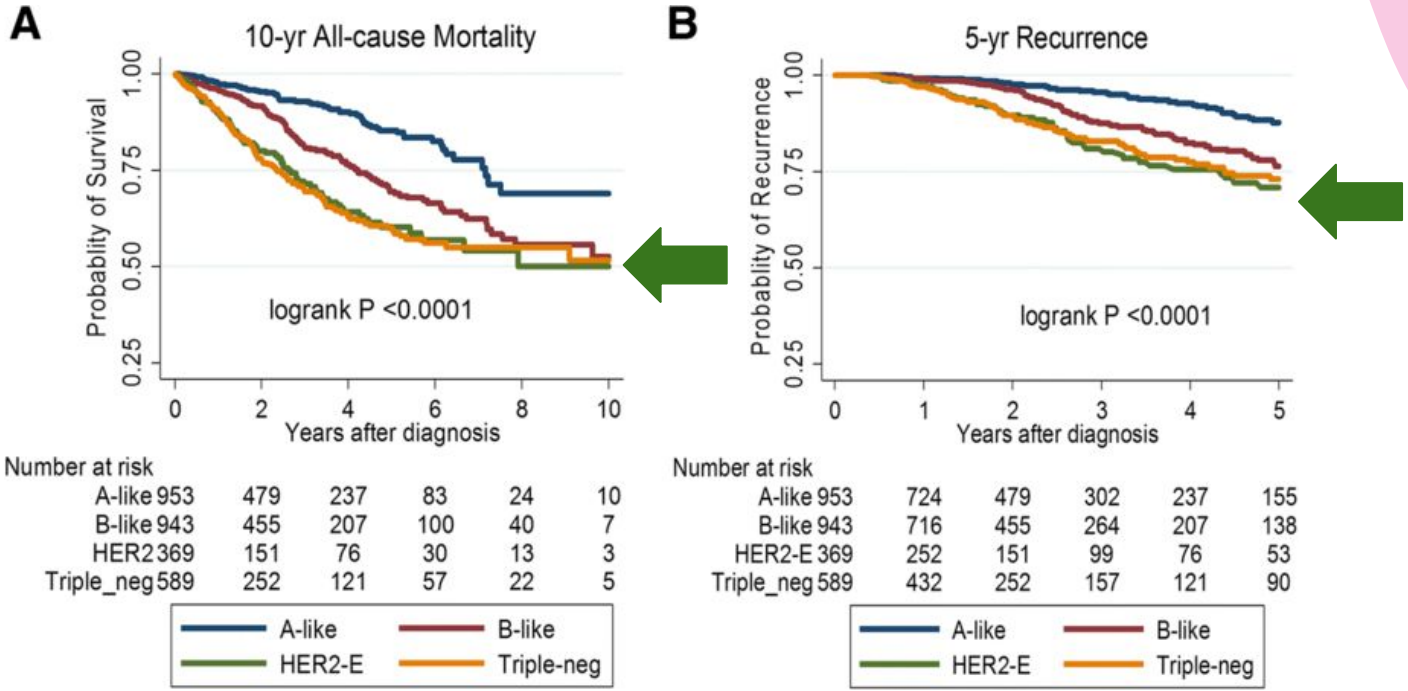


ER: estrogen receptor
PR: progesterone receptor

JAMA. 2006;295(21):2492-2502.

Molecular subtypes

The subtypes of breast cancers recognized by their gene signature



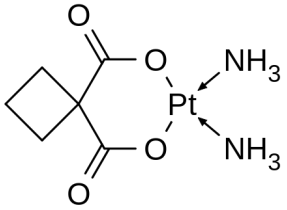
Abubakar M., et al., *Breast Cancer Research*, 2018

Treatment for breast cancer

Neoadjuvant chemotherapy

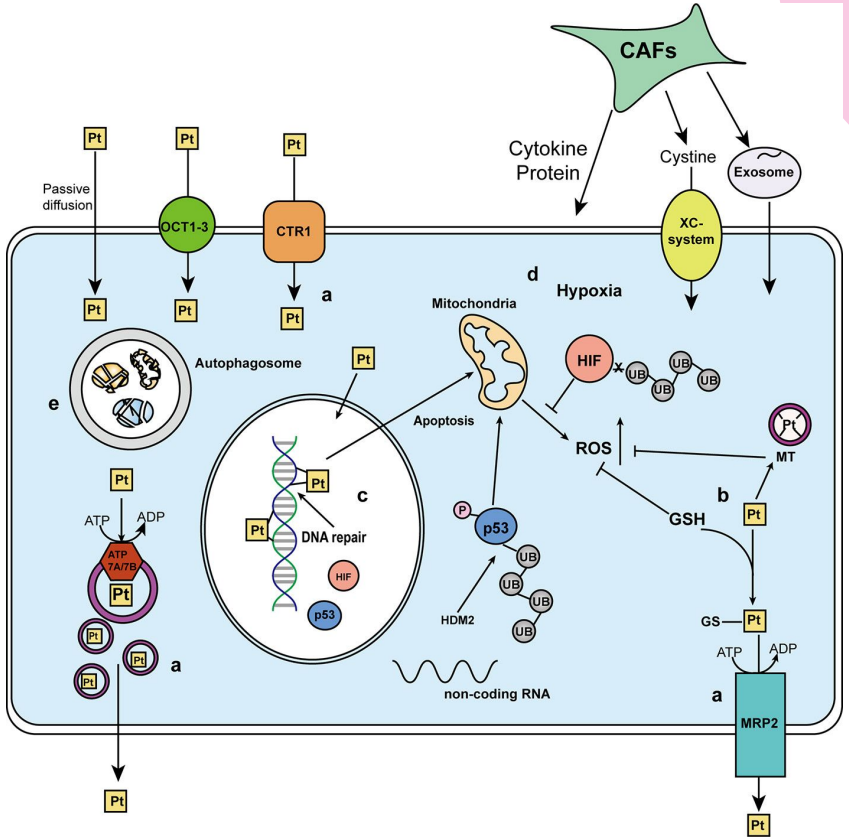
1. Before surgery
2. Reducing tumor size
3. Improving overall survival by treating undetected micrometastasis
4. Accelerating the development and application of new combination therapy drugs for breast cancer.
5. Chemotherapy Resistance occurring

Treatment for breast cancer



Carboplatin

1. Chemotherapy
2. Mechanism of action :
   Inhibits DNA synthesis
3. FDA approval :
   Breast, ovarian, lung, brain cancer, neuroblastoma ...
4. Resistance :
   Efflux, Detoxification system, DNA repair, Apoptosis

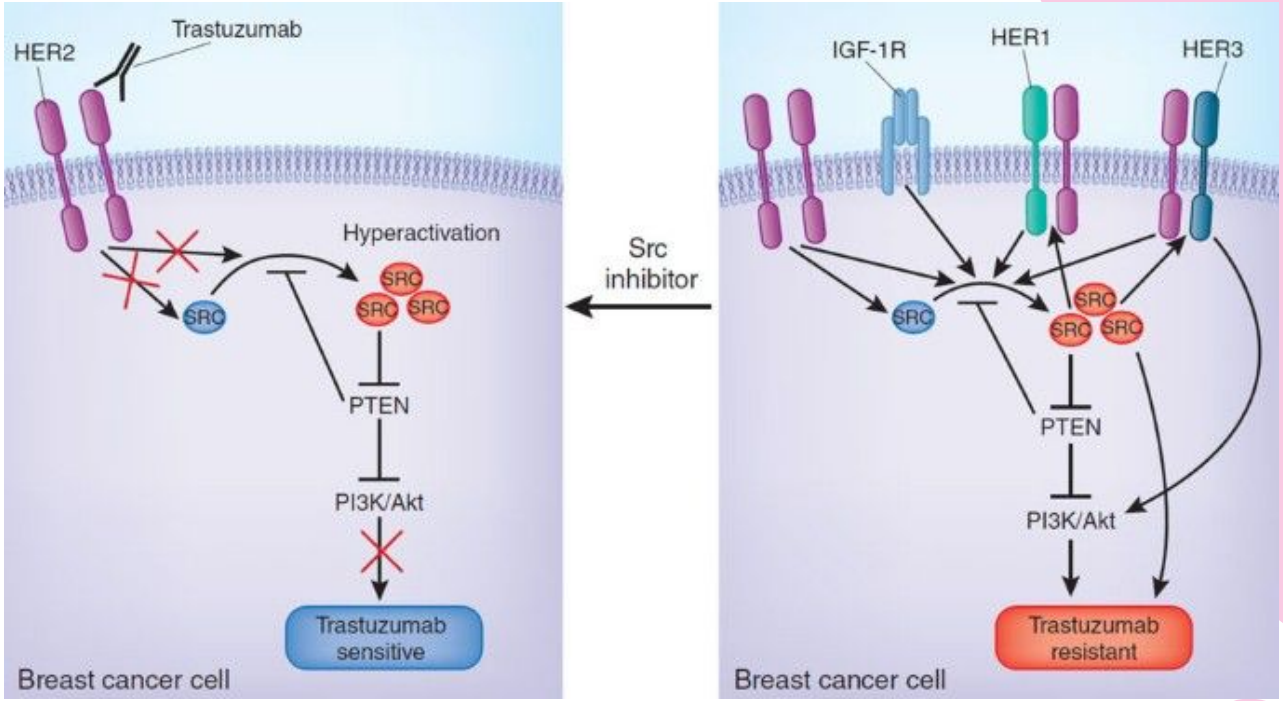


Zhou J, Kang Y, Chen L, Wang H, Liu J, Zeng S, Yu L. The Drug-Resistance Mechanisms of Five Platinum-Based Antitumor Agents. Front Pharmacol. 2020 Mar 20;11:343.

Treatment for breast cancer

Trastuzumab

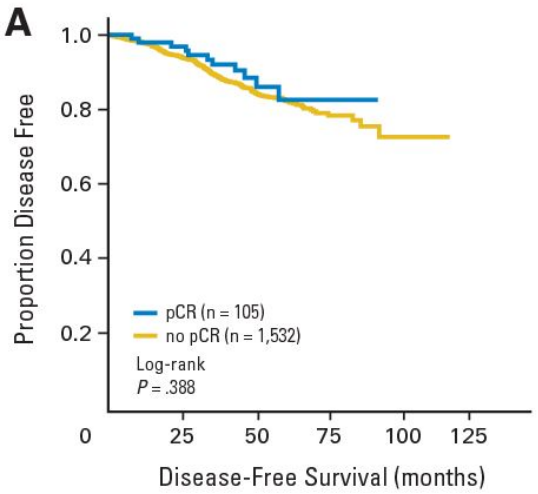
1. Monoclonal antibody
2. Mechanism of action :
   Block HER2
3. FDA approval :
   Breast, gastric cancer
4. Resistance :
   SRC



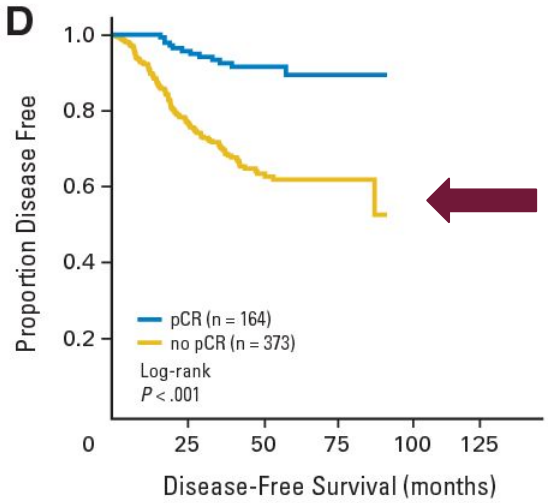
Muthuswamy SK. Trastuzumab resistance: all roads lead to SRC. Nat Med. 2011 Apr;17(4):416-8.

Treatment for breast cancer

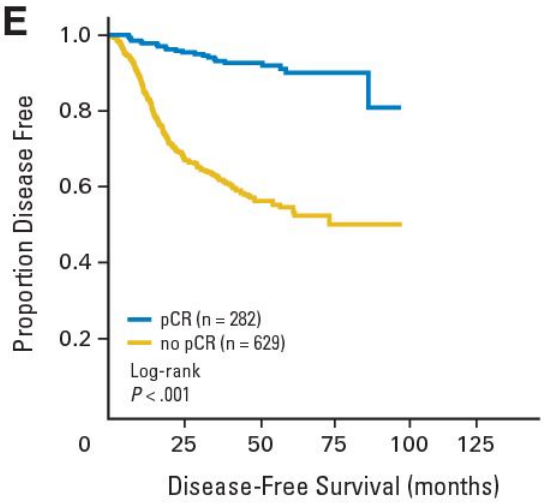
Luminal A (HER2-Low)



HER2+ (non luminal)



Triple-negative



Von Minckwitz, et. al., *J Clin oncol*, 2012.

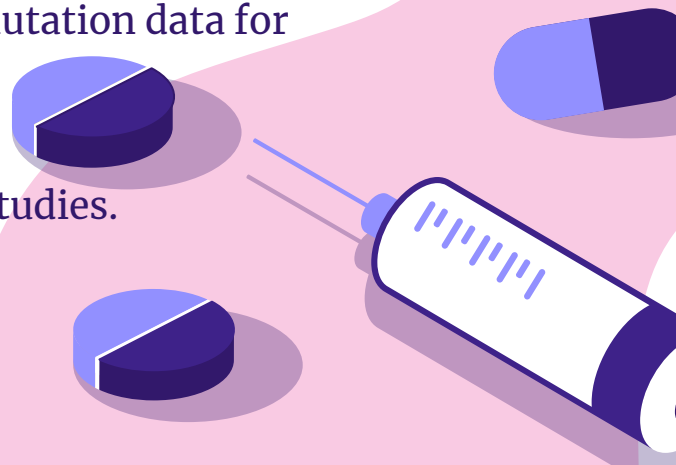
Pathologic complete response (pCR) :
absence of invasive/ in situ cancer in the breast and/or axillary lymph node.

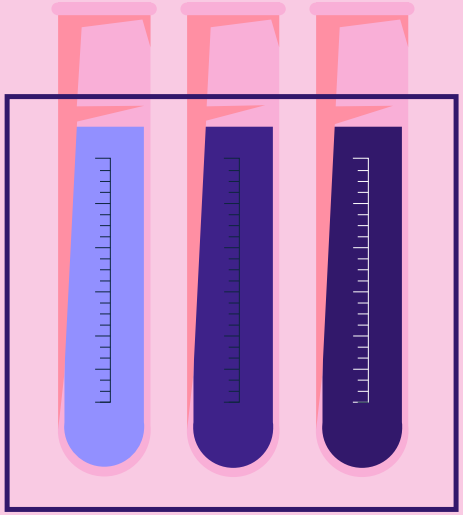
Specific aims

01 To analyze **differential gene expression** between the two patient groups using RNA-seq data, and validate the findings with an independent dataset.

02 To identify **drug resistance-associated gene variants** from two RNA-seq datasets, and integrate with DNA-level mutation data for comparative analysis.

03 To compare and discuss the results with previous studies.

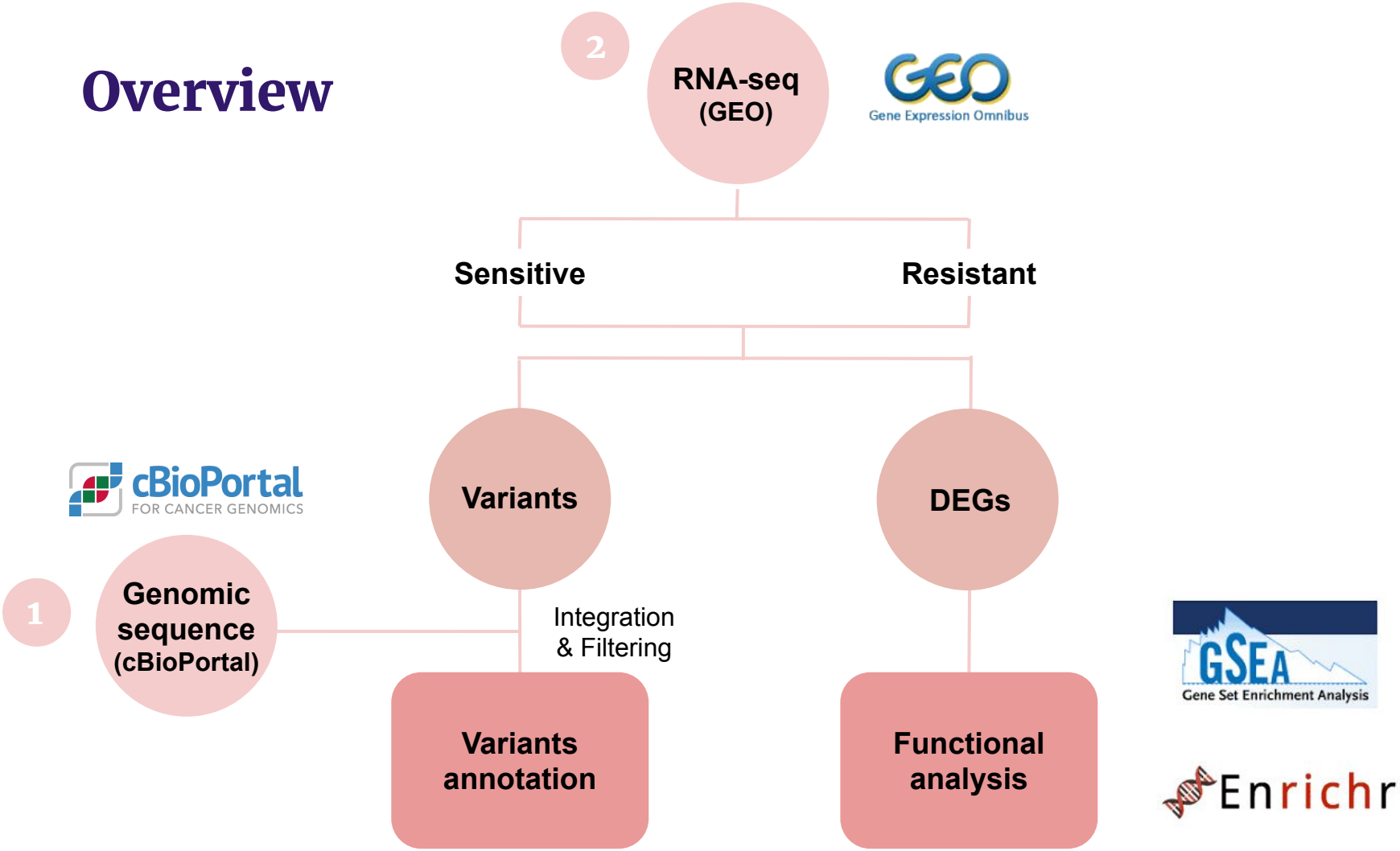




02

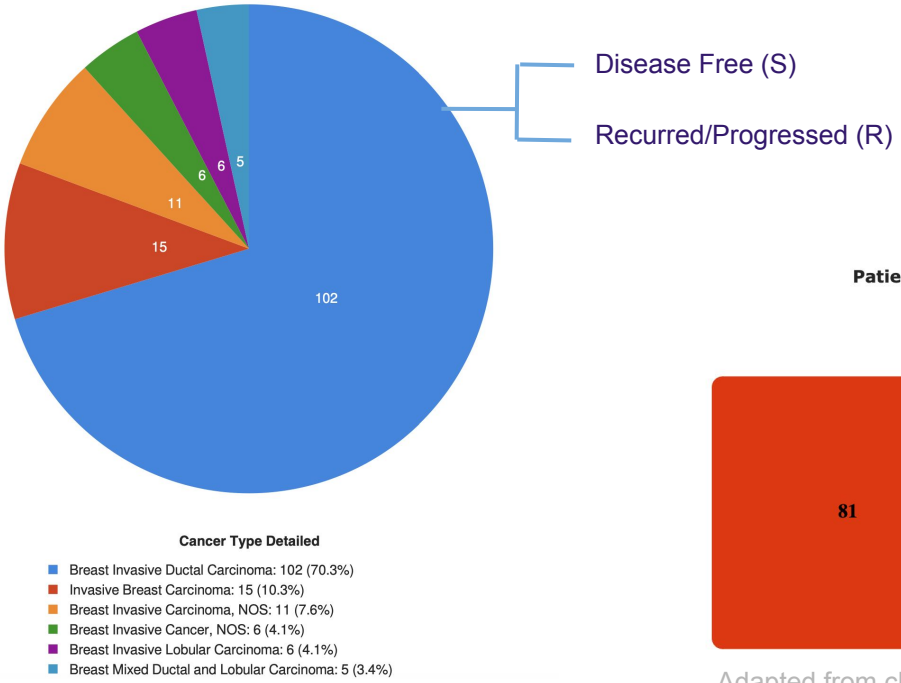
Method

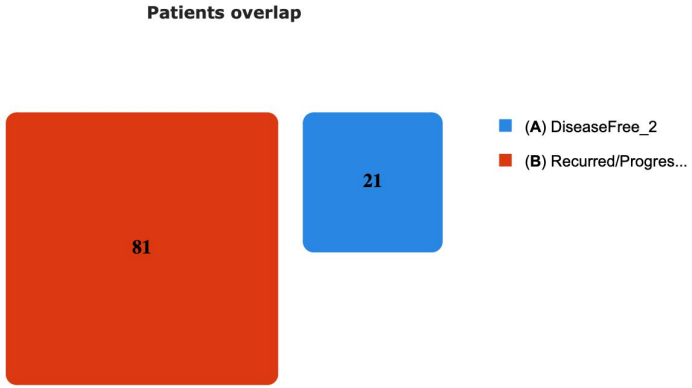
Overview


2
RNA-seq (GEO)
Gene Expression Omnibus
Sensitive
Resistant
cBioPortal FOR CANCER GENOMICS
Variants
DEGs
1
Genomic sequence (cBioPortal)
Integration & Filtering
GSEA Gene Set Enrichment Analysis
Variants annotation
Functional analysis
Enrichr

Dataset 1

MAPK on resistance to anti-HER2 therapy for breast cancer (MSK, Nat Commun. 2022)





nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-27093-y OPEN

HER2 + breast cancers evade anti-HER2 therapy via a switch in driver pathway

Alison E. Smith[1,2], Emanuela Ferraro[1,3], Anton Safonov[1,3], Cristina Bernado Morales[4], Enrique J. Arenas Lahuerta[4], Qing Li[1], Amanda Kulick[5], Dara Ross[6], David B. Solit[1,2], Elisa de Stanchina[5], Jorge Reis-Filho[1,6], Neal Rosen[7], Joaquín Arribas[4], Pedram Razavi[1,2,3] & Sarat Chandarlapaty[1,2,3]✉

Adapted from cBioPortal database

Dataset 2

Gene Expression Omnibus

Transcriptomic analysis of breast cancer patients sensitive and resistant to chemotherapy: Looking for overall survival and drug resistance biomarkers (GSE162187)

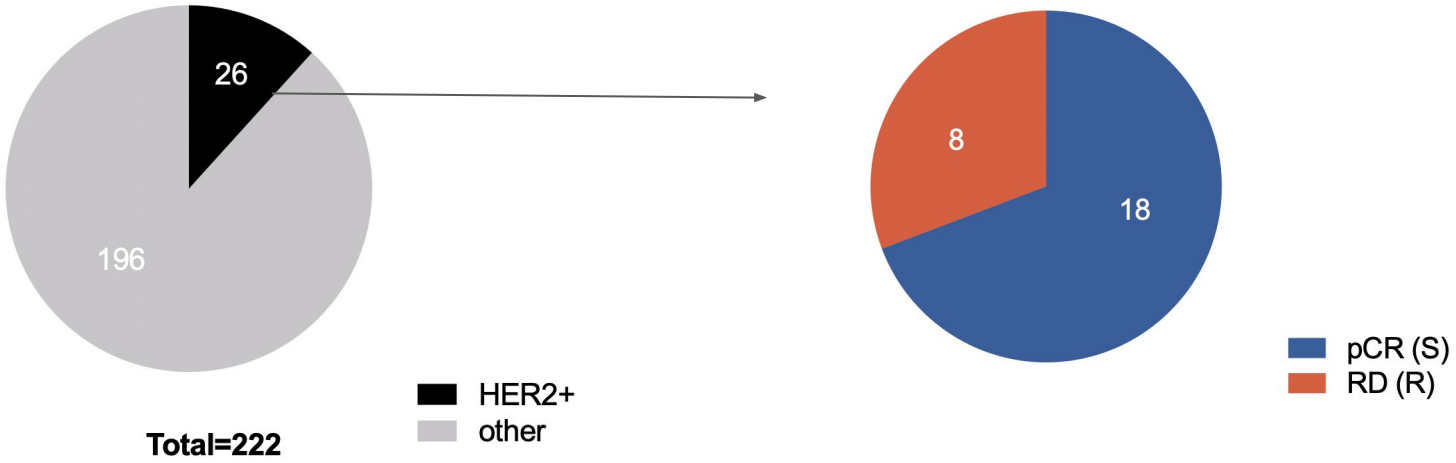
	Treatment	Response	Age	molecular subtype	histological type	histological grade
Breast_cancer_44R	Carboplatin-Docetaxel-Trastuzumab6 cycles	R	58	HER 2	Invasive ductal carcinoma	SBRII
Breast_cancer_30S	Carboplatin-Paclitaxel weekly 4 cycles + Epirubicin-Cyclophosphamide 3 cycles	S	NA	NA	Invasive ductal carcinoma	NA
Breast_cancer_9S	Carboplatin-Paclitaxel weekly 4 cycles + Epirubicin-Cyclophosphamide 4 cycles	S	31	Luminal B	Invasive ductal carcinoma	SBRII
Breast_cancer_41S	Carboplatin-Paclitaxel weekly 4 cycles + Epirubicin-Cyclophosphamide 4 cycles + Trastuzumab6 concomitant cycles	S	52	Luminal B	Invasive ductal carcinoma	SBRII
Breast_cancer_24S	Carboplatin-Paclitaxel weekly 4 cycles + Epirubicin-Cyclophosphamide 4 cycles + Trastuzumab6 concomitant cycles	S	67	HER 2	Invasive ductal carcinoma	SBRIII
Breast_cancer_28R	Carboplatin-Paclitaxel weekly 4 cycles + Epirubicin-Cyclophosphamide 4 cycles + Trastuzumab8 concomitant cycles	R	33	HER 2	Invasive ductal carcinoma	SBRIII
Breast_cancer_21S	Carboplatin-Paclitaxel weekly 4 cycles + Epirubicin-Cyclophosphamide 4 cycles + Trastuzumab8 concomitant cycles	S	48	Luminal B	Invasive ductal carcinoma	SBRIII
Breast_cancer_5R	Carboplatin-Paclitaxel weekly 4 cycles + Epirubicin-Cyclophosphamide 4 cycles	R	44	Triple Negative	Invasive ductal carcinoma	SBRIII
Breast_cancer_8R	Doxorubicin-Cyclophosphamide 2 cycles	R	53	Luminal B	Invasive ductal carcinoma	SBRII
Breast_cancer_32S	Doxorubicin-Cyclophosphamide 4 cycles + Docetaxel 4 cycles	S	55	HER 2	Invasive ductal carcinoma	SBRIII
Breast_cancer_7S	Doxorubicin-Cyclophosphamide 4 cycles + Docetaxel 4 cycles + trastuzumab 3 concomitant cycles	S	64	HER 2	Invasive ductal carcinoma	SBRII
Breast_cancer_25R	Doxorubicin-Cyclophosphamide 4 cycles + Docetaxel 4 cycles	R	54	Luminal B	Invasive ductal carcinoma	SBRII
Breast_cancer_20S	Doxorubicin-Cyclophosphamide 4 cycles + Docetaxel 4 cycles	S	45	Luminal B	Invasive ductal carcinoma	SBRIII
Breast_cancer_22R	Epirubicina-Cyclophosphamide 4 cycles + Docetaxel 4 cycles	R	62	LUMINAL A	Invasive ductal carcinoma	SBRII
Breast_cancer_40R	Epirubicin-Cyclophosphamide + Paclitaxel weekly 4 cycles	R	54	Triple Negative	Invasive ductal carcinoma	SBRIII
Breast_cancer_2R	Epirubicin-Cyclophosphamide 4 cycles + Docetaxel 1 cycle	R	29	LUMINAL A	Invasive ductal carcinoma	SBRII
Breast_cancer_3R	Epirubicin-Cyclophosphamide 4 cycles + Docetaxel 4 ciclo + Trastuzumab3 concomitant cycles	R	49	HER 2	Invasive ductal carcinoma	SBRII
Breast_cancer_6R	Epirubicin-Cyclophosphamide 4 cycles + Docetaxel 4 cycles	R	55	LUMINAL A	Invasive ductal carcinoma	SBRI
Breast_cancer_39S	Epirubicin-Cyclophosphamide 4 cycles + Docetaxel 4 cycles	S	50	Triple Negative	Invasive ductal carcinoma	SBRIII
Breast_cancer_27R	Epirubicin-cyclophosphamide 4 cycles, docetaxel 4 ciclo and trastuzumab 3 cycles	R	56	HER 2	Invasive ductal carcinoma	NA
Breast_cancer_11R	Epirubicin-Cyclophosphamide 6 cycles	R	51	Triple Negative	Invasive ductal carcinoma	SBRIII
Breast_cancer_12R	Fluorouracil-Epirubicin-Cyclophosphamide 6 cycles	R	65	HER 2	Invasive ductal carcinoma	SBRII

HER2+: 8

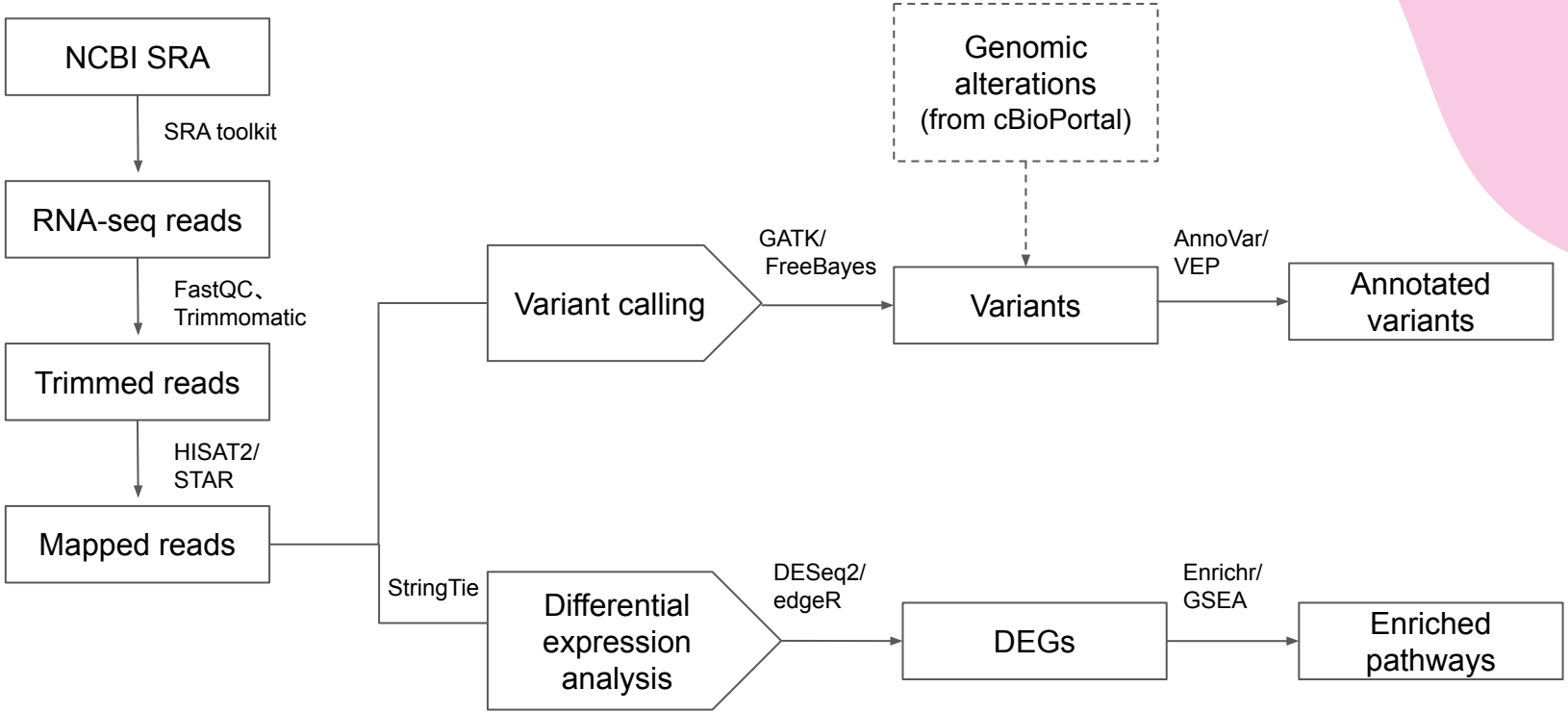
- Sensitive (S): 3
- Resistant (R): 5

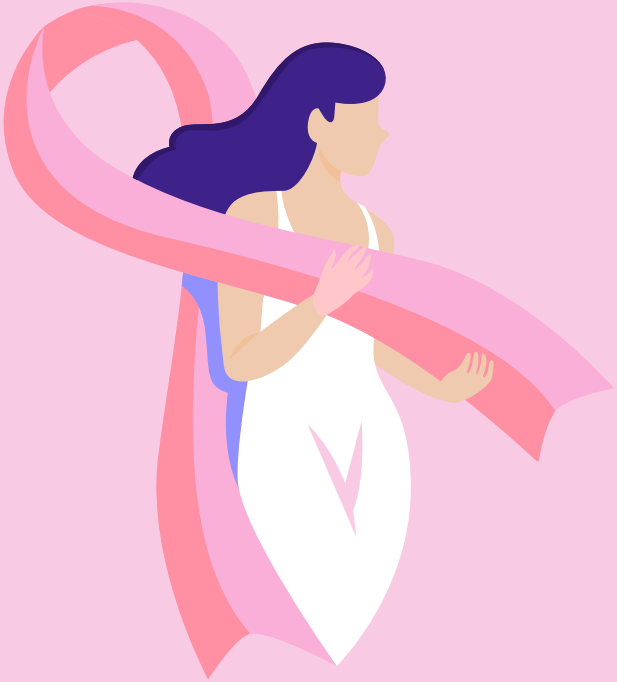
Dataset 3

Multi-center retrospective evaluation of a RNA expression classifier to predict pathological complete response to neoadjuvant chemotherapy in breast cancer biopsies (GSE163882)



Pipeline

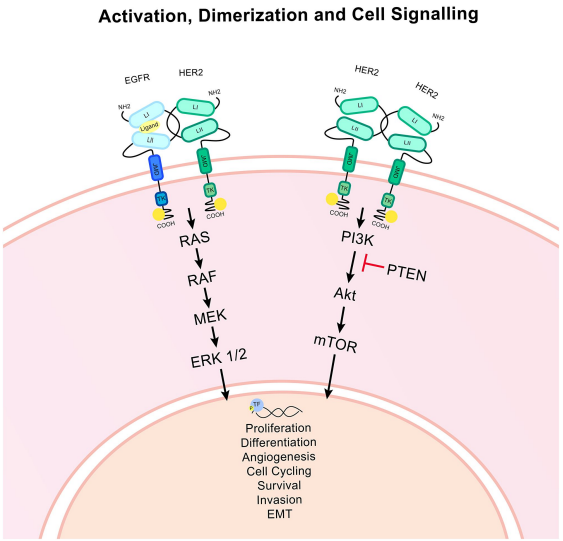

NCBI SRA
SRA toolkit
RNA-seq reads
FastQC、
Trimmomatic
Trimmed reads
HISAT2/
STAR
Mapped reads
Genomic
alterations
(from cBioPortal)
Variant calling
GATK/
FreeBayes
Variants
AnnoVar/
VEP
Annotated
variants
StringTie
Differential
expression
analysis
DESeq2/
edgeR
DEGs
Enrichr/
GSEA
Enriched
pathways

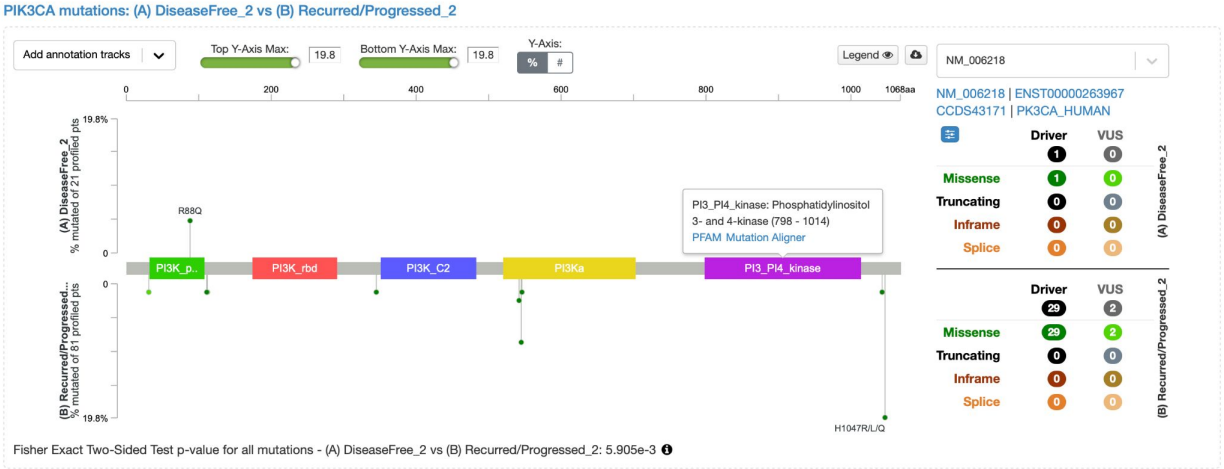


03

Expected outcomes

1. Identify DEGs that are associated with neoadjuvant treatment resistance in HER2+ breast cancer patients.
2. Explore potential drug resistance-associated genetic variants.
3. Combine the identified drug resistance-associated variants and the underlying pathways to gain a comprehensive understanding of treatment resistance mechanisms in HER2+ patients.

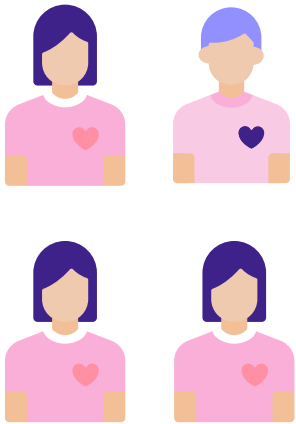




Hart V., et. al., Oncotarget, 2020

Adapted from cBioPortal database

Work distribution



構思問題	黃以慧、黃宣瑜、王瀅茜
搜集資料	黃以慧、黃宣瑜、王瀅茜
設計方法	黃以慧
撰寫程式	林東甫
詮釋結果	黃以慧、黃宣瑜、王瀅茜
進行報告	黃以慧、黃宣瑜、王瀅茜、林東甫

Thank you for your listening!

Hormone Receptor Status
After breast cancer diagnosed…

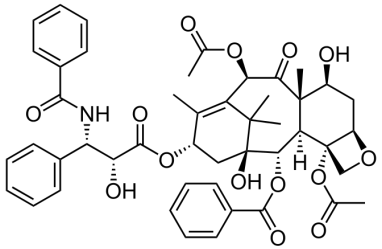
Status	Treatment	Frequency
ER and PR	Adjuvant endocrine therapy	80%
HER2	HER2-directed therapy	23% (32% HR-; 67% HR+)
Triple Negative (ER, PR, HER2-)	Chemotherapy, Immunotherapy, Target therapy	13%

ER: estrogen receptor
PR: progesterone receptor
HR: hormone receptor

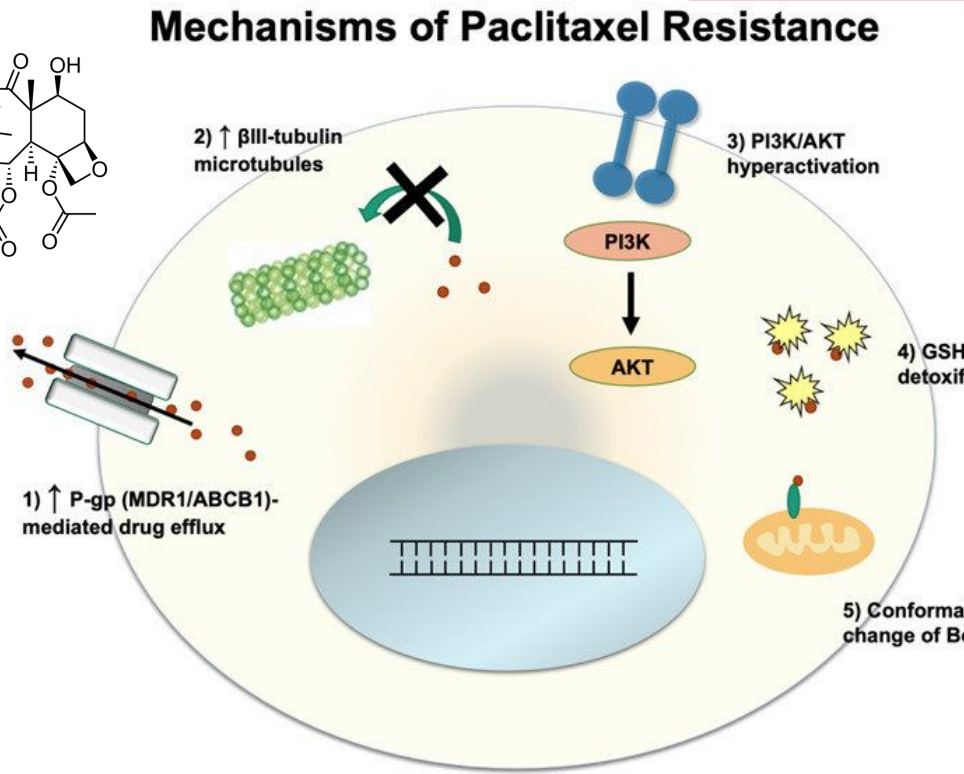
Breast J. 2009;15(6):593-602. doi:10.1111/j.1524-4741.2009.00822.x

Treatment for breast cancer

Paclitaxel & Docetaxel



1. Chemotherapy
2. Mechanism of action :
   targets microtubules, cell cycle arrest
3. FDA approval :
   Breast, ovarian, lung, pancreatic, cervical cancer...
4. Resistance :
   Efflux, Detoxification system, tubulin isotype



Ortiz M, Wabel E, Mitchell K, Horibata S. Mechanisms of chemotherapy resistance in ovarian cancer. Cancer Drug Resist. 2022 Apr 3;5(2):304-31

Treatment for breast cancer

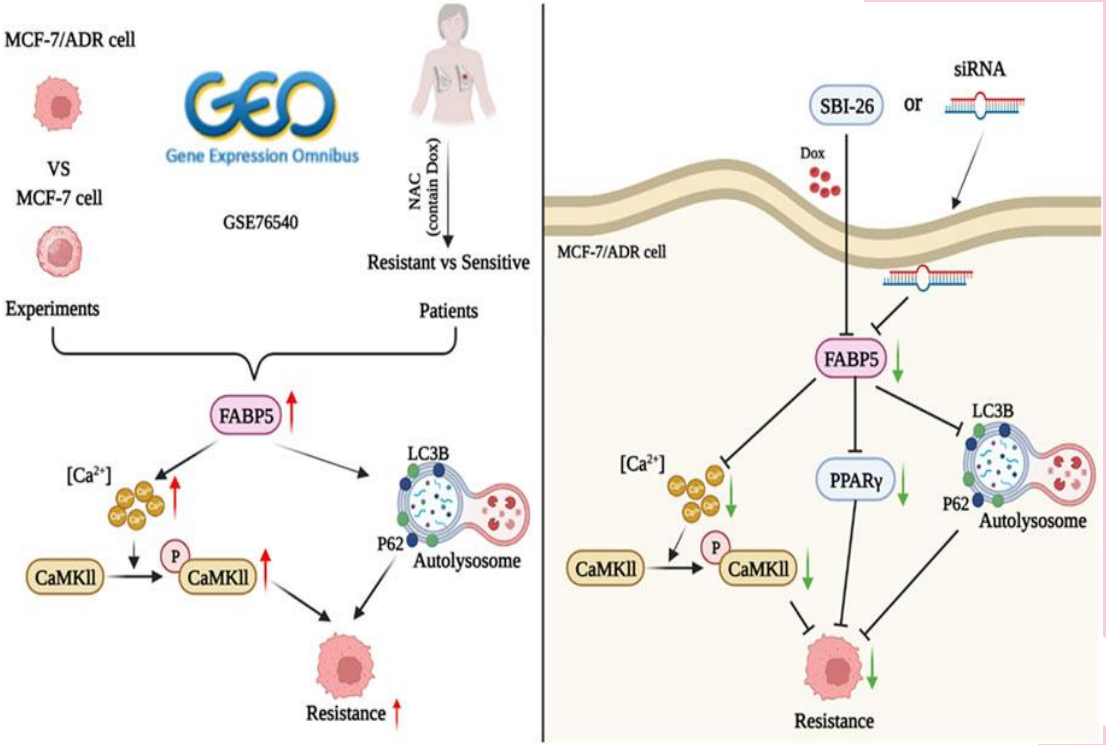
Epirubicin & Doxorubicin

1. Chemotherapy
2. Mechanism of action :

   DNA topoisomerase inhibitor
3. FDA approval :

   Breast, ovarian, lung, brain cancer, neuroblastoma ...
4. Resistance :

   FABP5/PPARγ and CaMKII



Chen NN, Ma XD, Miao Z, Zhang XM, Han BY, Almaamari AA, Huang JM, Chen XY, Liu YJ, Su SW. Doxorubicin resistance in breast cancer is mediated via the activation of FABP5/PPARγ and CaMKII signaling pathway. Front Pharmacol. 2023 Jul 19;14:1150861.

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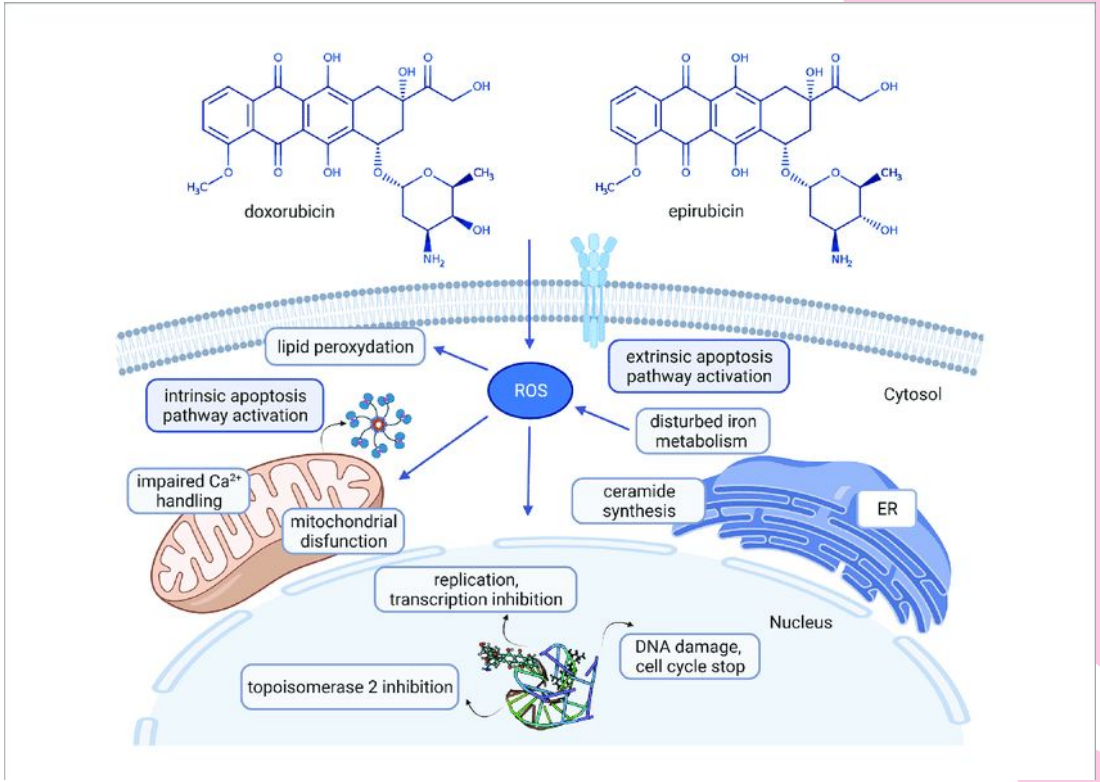

A.M. Tőkés et al. Tumor Glucose and Fatty Acid Metabolism in the Context of Anthracycline and Taxane-Based (Neo) Adjuvant Chemotherapy in Breast Carcinomas, Frontiers in Oncology, 12, 2022.

Treatment for breast cancer

Cyclophosphamide

1. Chemotherapy
2. Mechanism of action :

3. FDA approval :

   Breast cancer , multiple myeloma, sarcoma

4. Resistance :

   Detoxification, DNA repair

-cyclophosphamide
Trastuzumab

fluorouracil

Chen NN, Ma XD, Miao Z, Zhang XM, Han BY, Almaamari AA, Huang JM, Chen XY, Liu YJ, Su SW. Doxorubicin resistance in breast cancer is mediated via the activation of FABP5/PPARγ and CaMKII signaling pathway. Front Pharmacol. 2023 Jul 19;14:1150861.

Dataset 1

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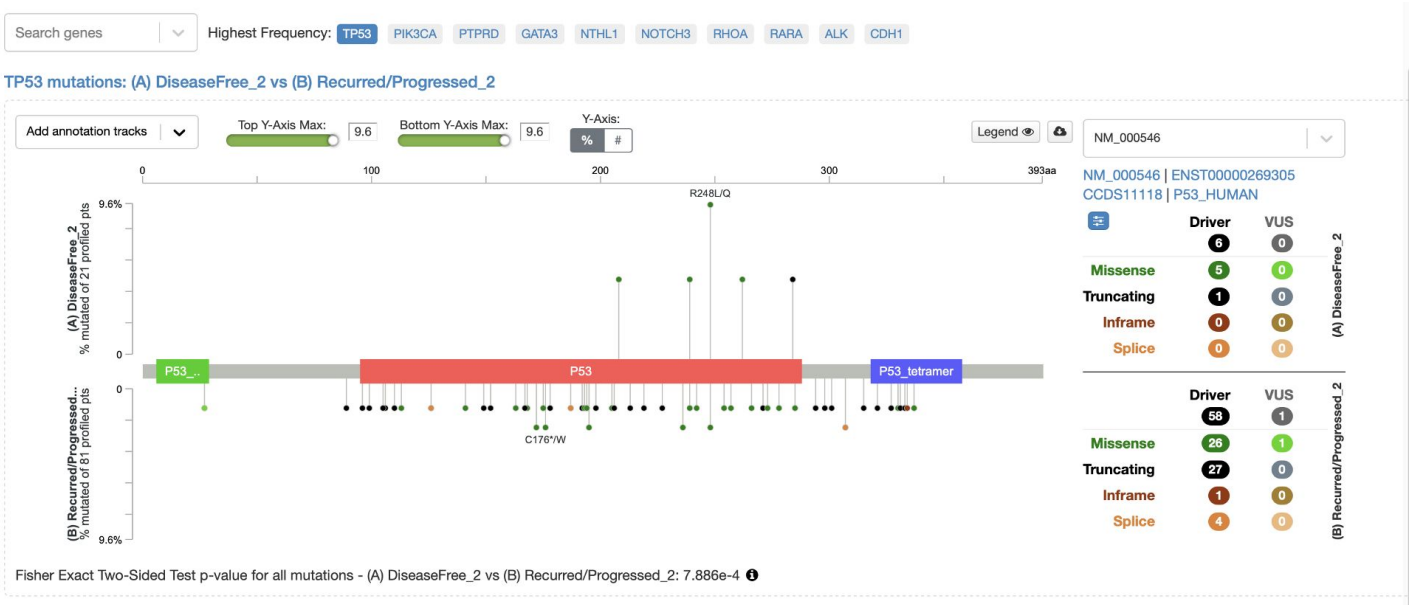
(MSK, Nat Commun. 2022)



Adapted from cBioPortal database

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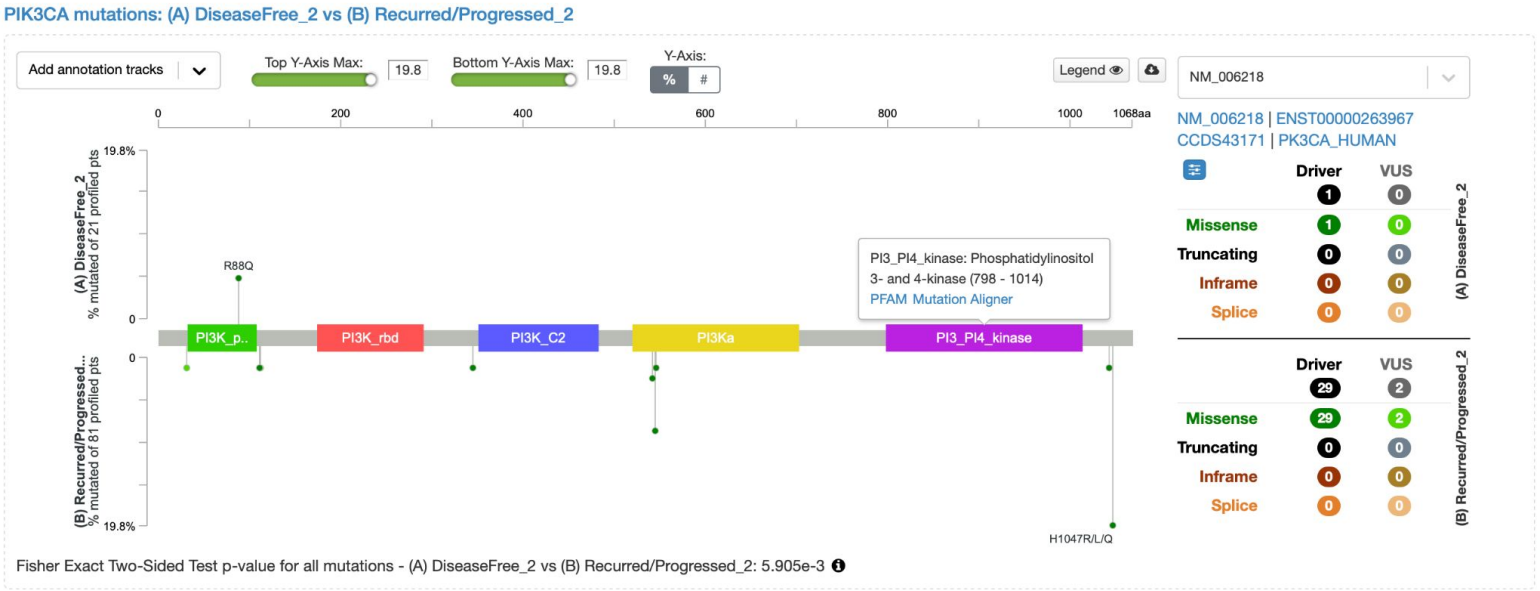

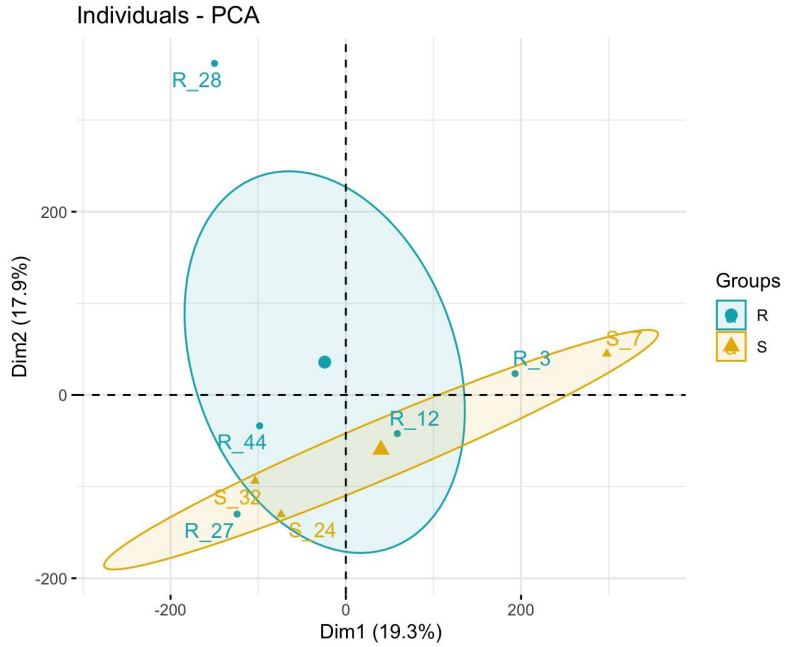

From the plot, we can observe the following:

1. The Resistant (R) and Sensitive (S) groups are clearly separated along the first principal component (Dim1, accounting for 19.3% of the variance), indicating that there are gene expression differences between the two groups.
2. The Resistant group shows more variation or spread along both principal components compared to the Sensitive group, suggesting higher heterogeneity within the Resistant samples.
3. Some overlap exists between the two groups, indicating that while there are overall differences in gene expression, some individual samples may exhibit similarities across groups.