Associazione Italiana di Oncologia Medica
SEZIONE REGIONE LAZIO

POST SAN ANTONIO BREAST CANCER SYMPOSIUM 2018

28 Gennaio 2019

POLICLINICO UMBERTO I - ROMA

Aula Bignami (Patologia Generale)
Viale Regina Elena 324

SAN ANTONIO BREAST CANCER SYMPOSIUM

DECEMBER 4-8 HENRY B. GONZALEZ CONVENTION CENTER, SAN ANTONIO, TEXAS, USA 2018

SAN ANTONIO BREAST CANCER SYMPOSIUM®

UT Health San Antonio · AACR American Association for Cancer Research · Baylor College of Medicine · DAN L DUNCAN COMPREHENSIVE CANCER CENTER

Session 1

HOW TO BETTER KNOW
THE "SOUL" OF BREAST CANCER

Heterogeneity: TNBC and other subtypes

Gemelli

Fondazione Policlinico Universitario Agostino Gemelli IRCCS
Università Cattolica del Sacro Cuore

Dr. Armando Orlandi
Dh di Oncologia Medica

DISCLOSURE

I love molecular biology, but I'm just a clinician.

OUTLINE

- Heterogeneity: Background
- Heterogeneity in TNBC and other: SABCS 2018
  - *N. Navin* : DCIS
  - *N. Navin*: Neoadjuvant TNBC
  - *A. Costa*: Fibroblast heterogeneity in BC
- Heterogeneity: Food for thought

OUTLINE

- Heterogeneity: Background
- Heterogeneity in TNBC and other: SABCS 2018
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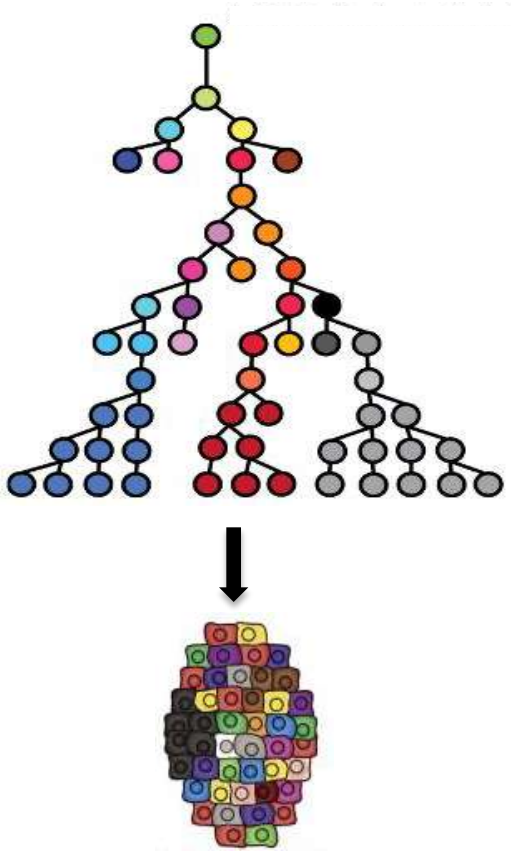
Heterogeneity: Background

How does a ***single cell*** evolve into a complex mass of milignant tissue so ***heterogeneus***?

What is the **role of clonal diversity** in:

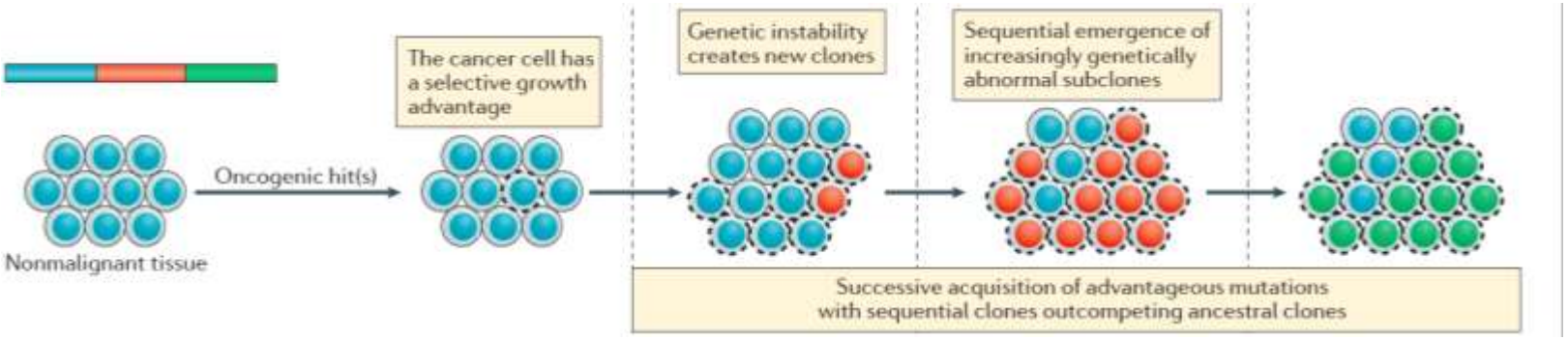
- Tumor initiation and invasion?
- Metastasis?
- Therapy resistence?



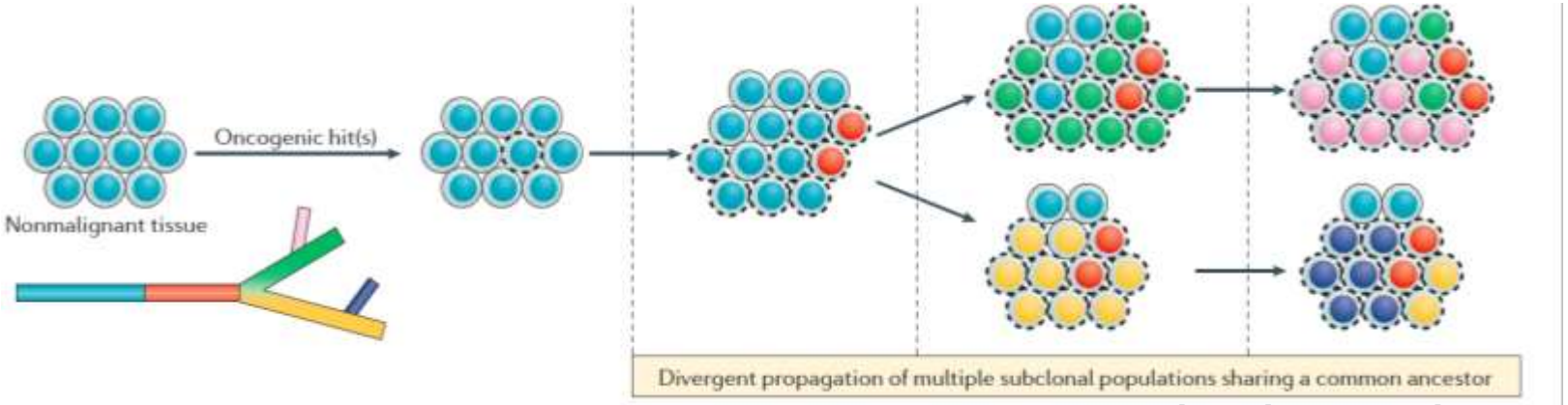
N.E. Navin, *SABCS* 2018

Heterogeneity: Background

Linear evolution model

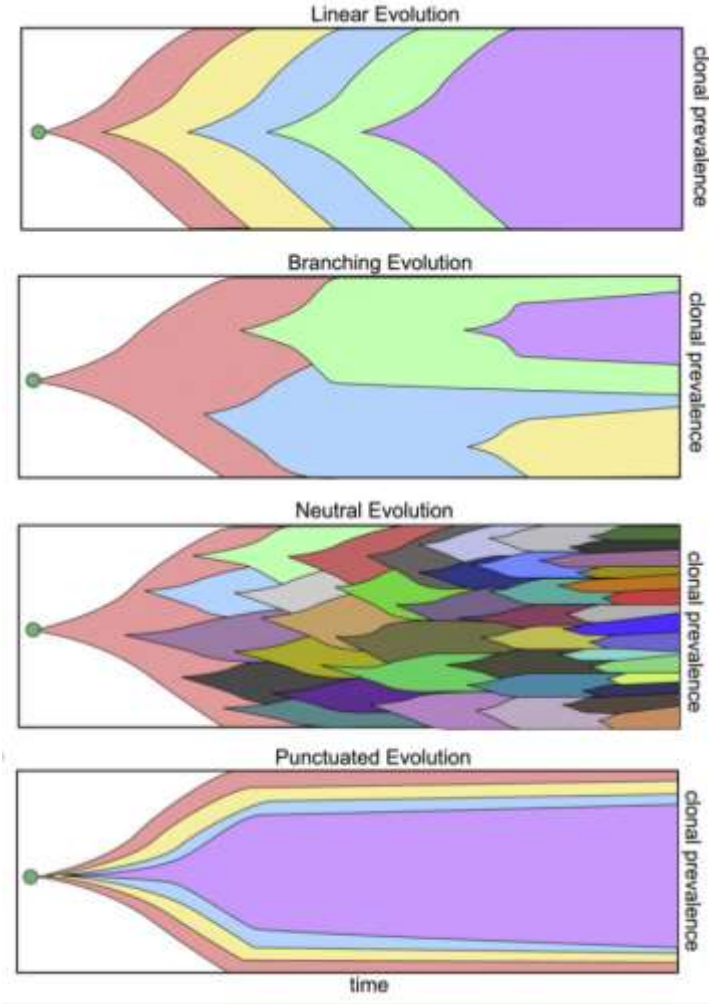


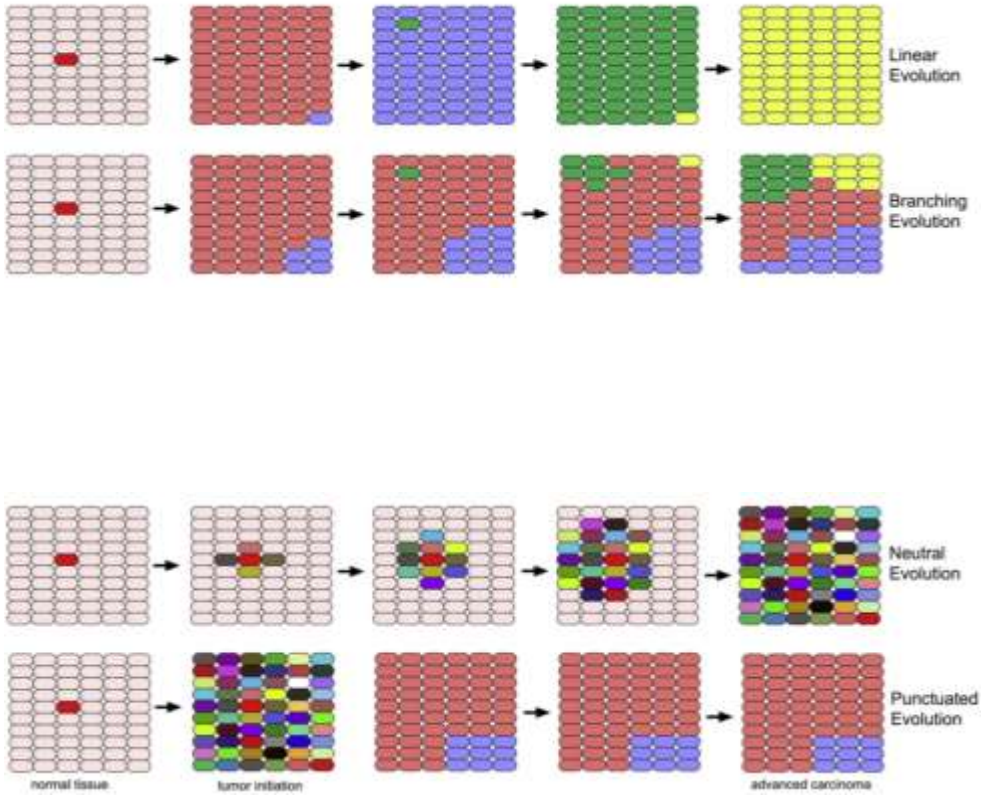
Branched evolution model



I. Dagogo-Jack *et al, Nat Rev Clin Onc* 2018

Heterogeneity: Background





A. Davis *et al,* Bio. et Bio. Acta 2017

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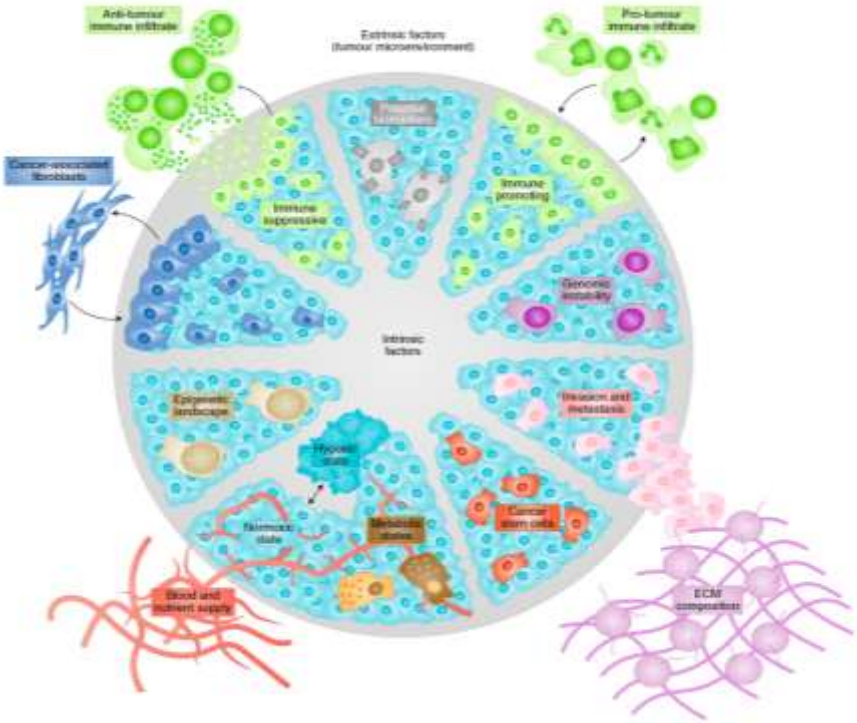
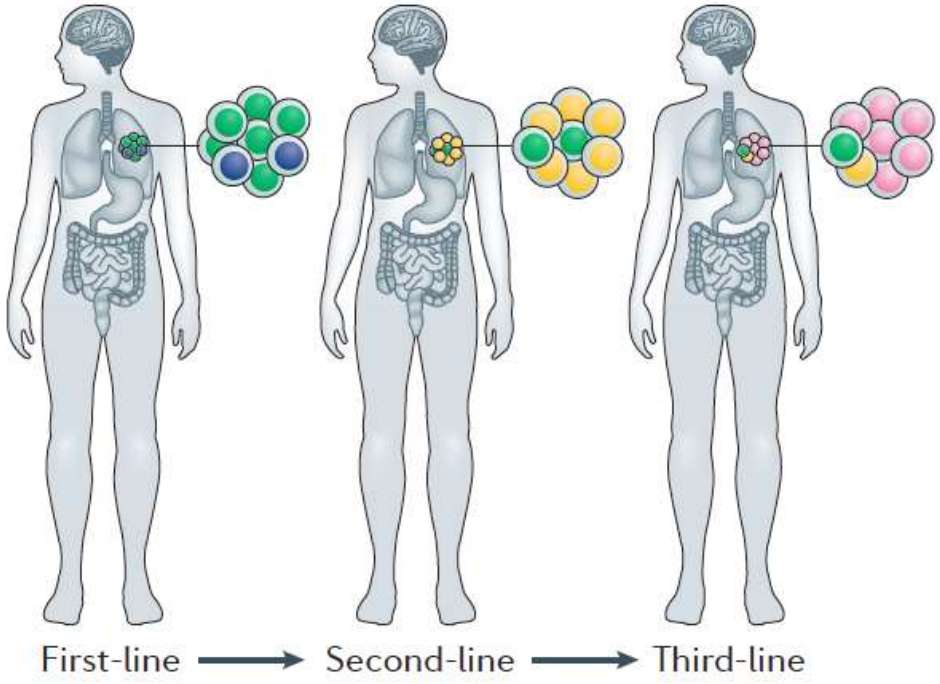
Heterogeneity: TNBC and other subtypes

Heterogeneity: Background

Spatial heterogeneity



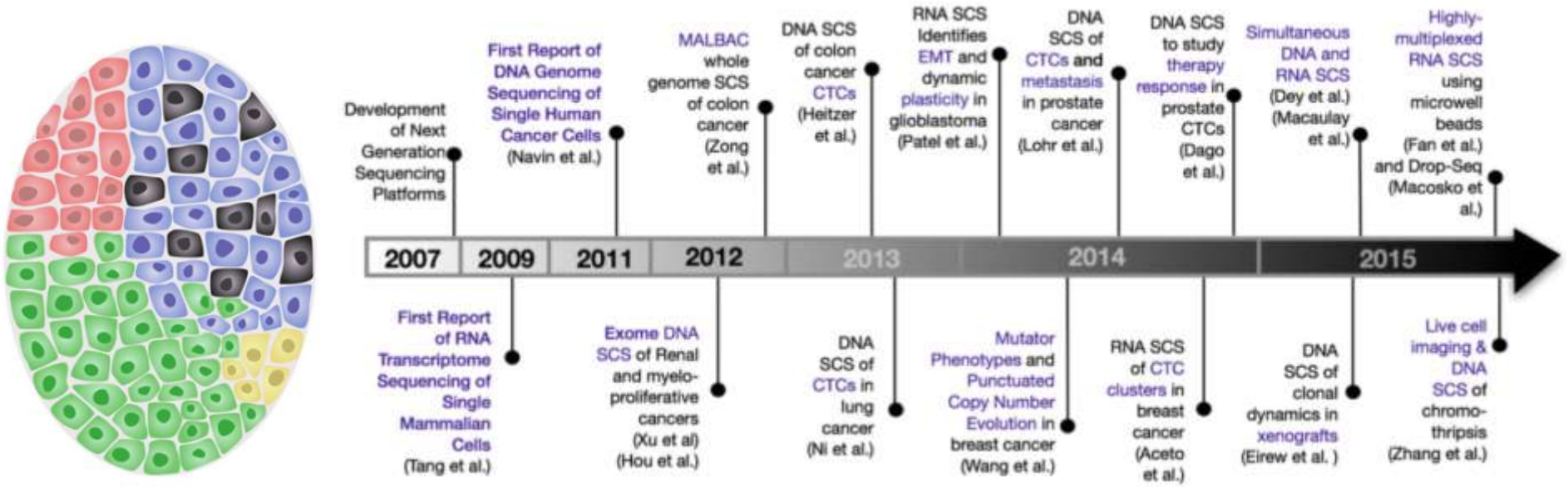
Temporal heterogeneity



I. Dagogo-Jack *et al, Nat Rev Clin Onc* 2018

D. A. Lawson *et al, Nat Cell Bio* 2018

Heterogeneity: Background



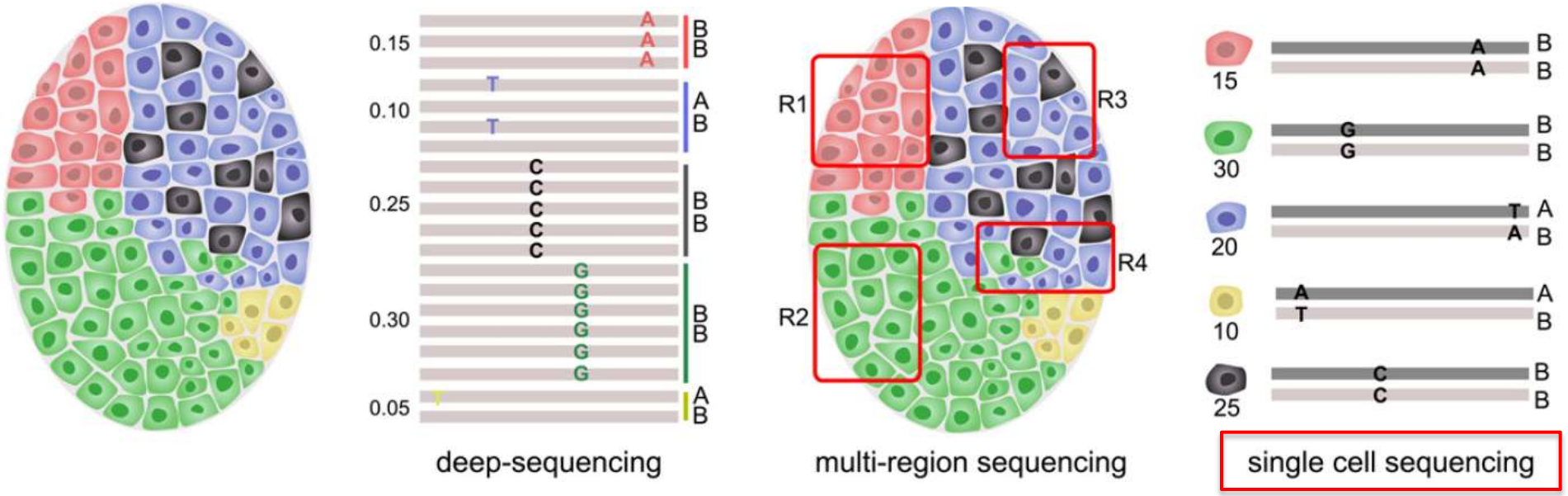
N. E. Navil, Genome Research 2015

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity: Background

NGS method for resolving intratumor heterogeneity



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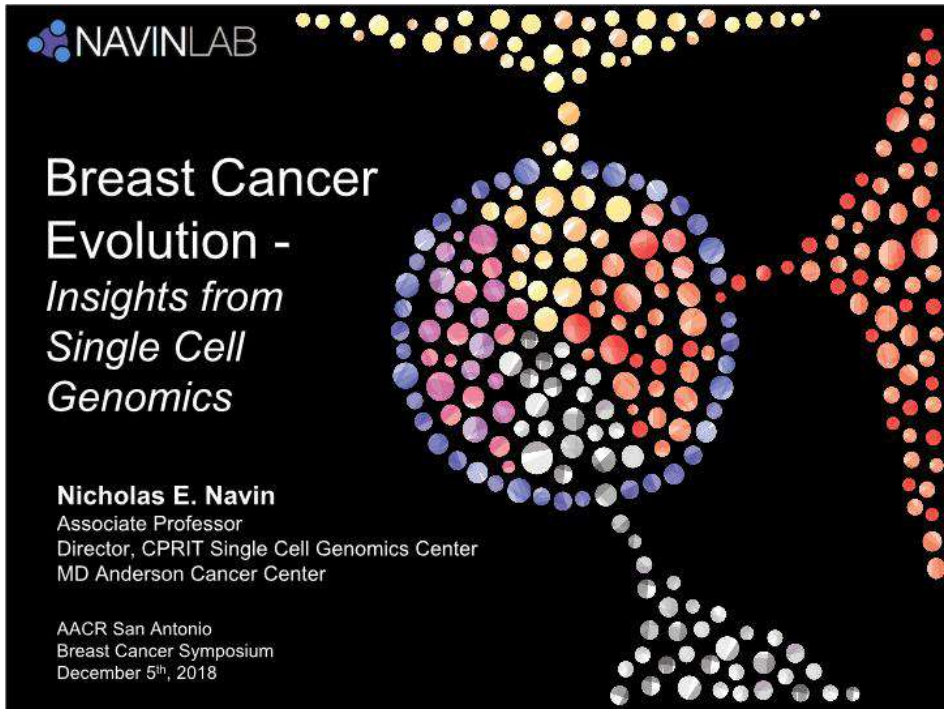
Heterogeneity in TNBC and other: SABCS 2018



N.E. Navin, *SABCS* 2018

Multiclonal Invasion in Breast Tumors Identified by Topographic Single Cell Sequencing

Anna K. Casasent, Aislyn Schalck, Ruli Gao, Emi Sei, Annalyssa Long, William Pangburn, Tod Casasent, Funda Meric-Bernstam, Mary E. Edgerton, and Nicholas E. Navin

C. Kim *et al,* Cell 2018

Chemoresistance Evolution in Triple-Negative Breast Cancer Delineated by Single-Cell Sequencing

Charissa Kim, Ruli Gao, Emi Sei, Rachel Brandt, Johan Hartman, Thomas Hatschek, Nicola Crosetto, Theodoros Foukakis, and Nicholas E. Navin

A. K. Casasent *et al,* Cell 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Roma, 28 gennaio 2019

Dr. Armando Orlandi

Heterogeneity in TNBC and other: SABCS 2018

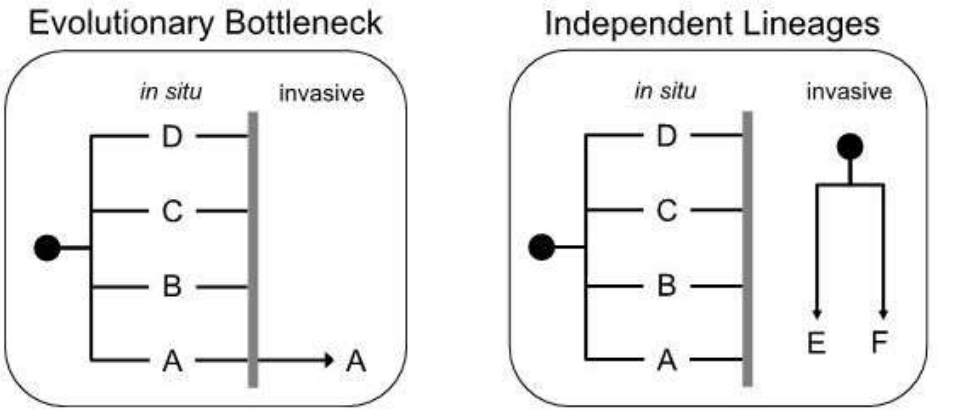
Multiclonal Invasion in Breast Tumors Identified by Topographic Single Cell Sequencing

Anna K. Casasent,[1,2] Aislyn Schalck,[1,2] Ruli Gao,[1] Emi Sei,[1] Annalyssa Long,[1] William Pangburn,[1] Tod Casasent,[3] Funda Meric-Bernstam,[4] Mary E. Edgerton,[5,*] and Nicholas E. Navin[1,2,3,6,*]

A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

Background



Hernandez et al. 2010 *J Path*
Cowell et al. 2013, *Mol Onc*
Kim et al. 2015, *Oncotarget*
Allred et al. 2008, *Clinc Canc Res*

Miron et al. 2010, *Cancer Res*
Allred 2010, *J National Canc*
Pape-Zambito et al. 2014 *Plos One*

Other Evolutionary Models?

N.E. Navin, *SABCS* 2018

Heterogeneity in TNBC and other: SABCS 2018

Methods and Results

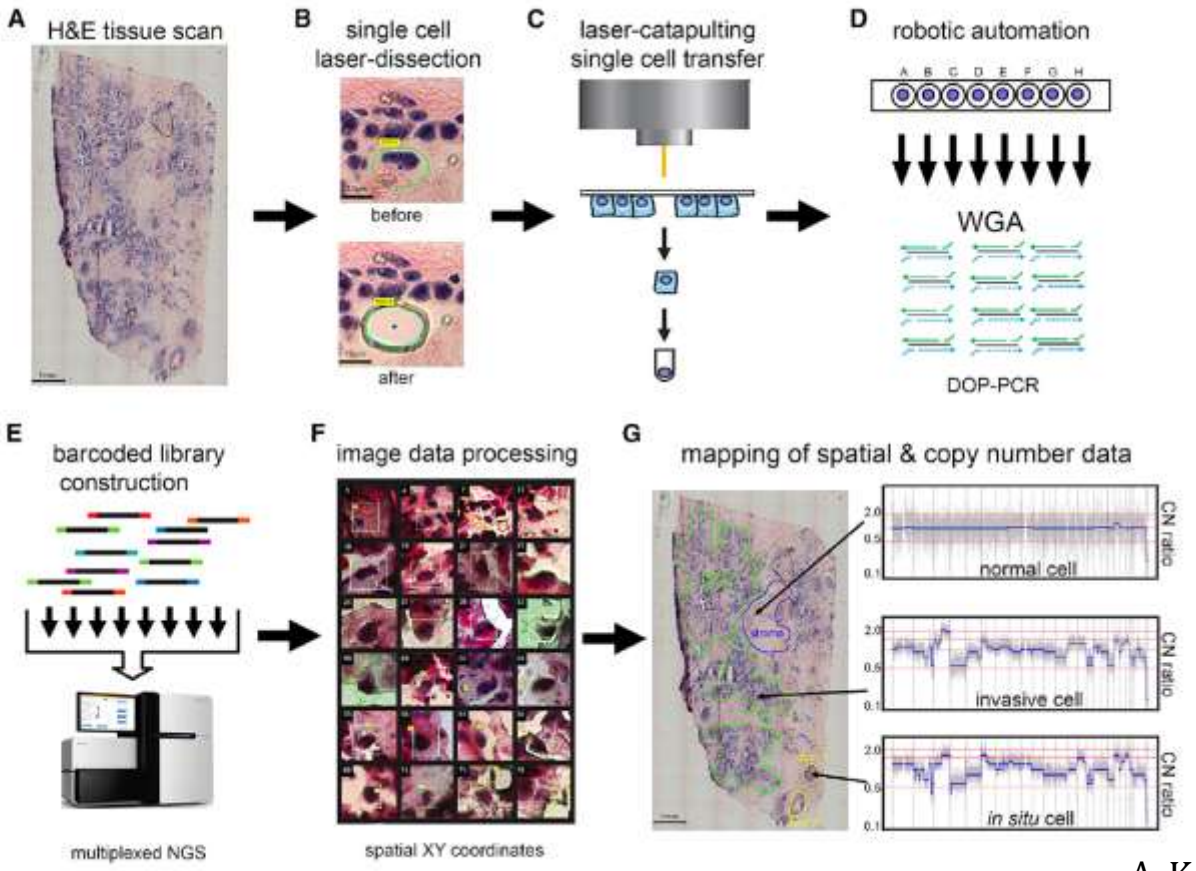
- Selected 10 patients with *synchronous* DCIS-IDC regions in the same tissue sections from the MD Anderson Cancer Center
- Most patients were high-grade (3) and five patients were TNBC
- All patient samples were treatment-naive
- TSCS was used to profile an average of N=129 cells per patient

Patient	Age	TNBC	ER	PR	HER2	grade	Stage	Cells
P1	57	Y	-/-	-/-	-/-	3/3	IIB	57
P2	36	N	+/+	+/+	+/+	3/3	IIB	114
P3	64	N	+/+	+/+	-/-	1/1	IV	102
P4	66	N	+/+	+/+	-/-	2/2	IIIC	104
P5	47	N	+/+	-/-	-/-	3/3	IIA	148
P6	77	Y	-/-	-/-	-/-	3/3	IIA	204
P7	66	N	-/+	-/-	-/-	3/3	IIIC	112
P8	62	Y	-/-	-/-	-/-	3/3	IIA	235
P9	49	Y	-/-	-/-	-/-	3/3	IIA	96
P10	48	Y	-/-	-/-	-/-	3/3	IIA	122

N.E. Navin, *SABCS* 2018

Heterogeneity in TNBC and other: SABCS 2018

Methods and Results



N.E. Navin, *SABCS* 2018

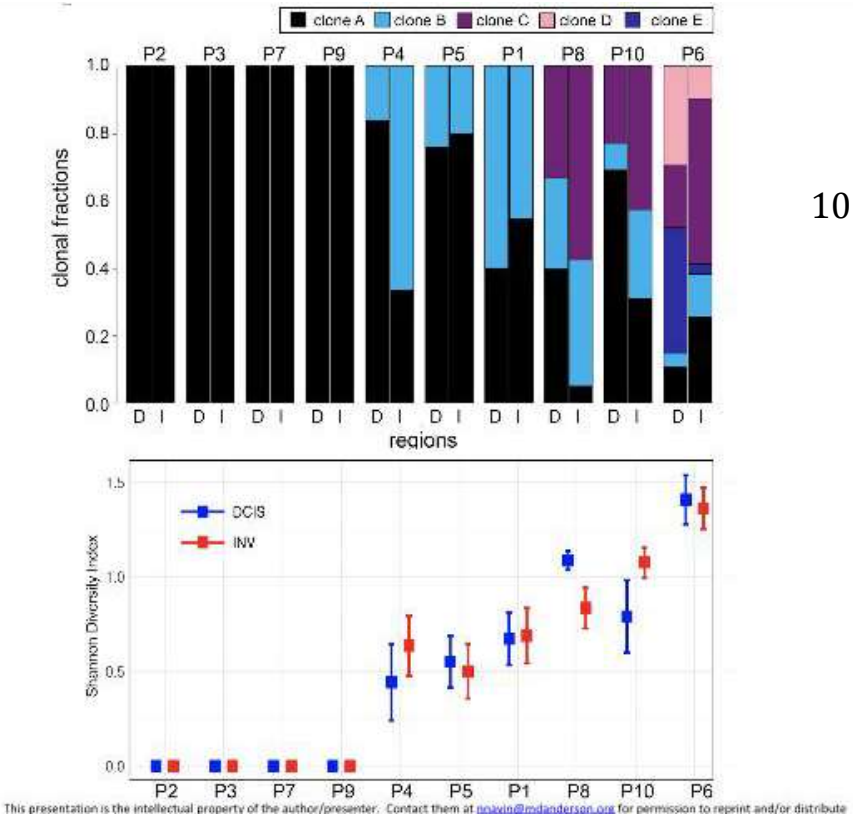
A. K. Casasent *et al,* Cell 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity in TNBC and other: SABCS 2018

Methods and Results

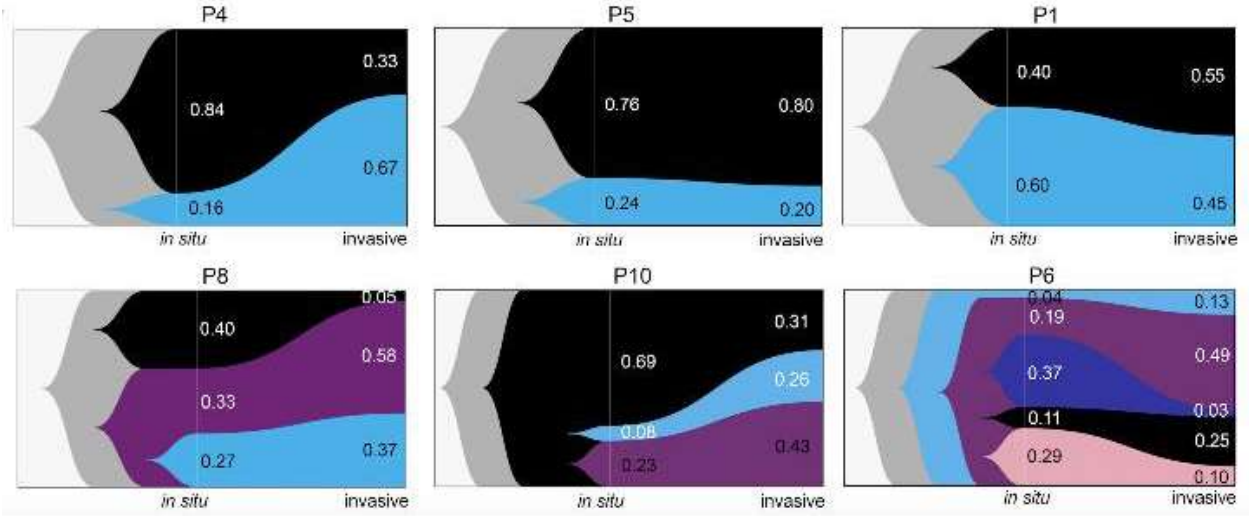


10 patients examined:
4 monoclonal
6 were polyclonal

N.E. Navin, *SABCS* 2018
A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

Methods and Results



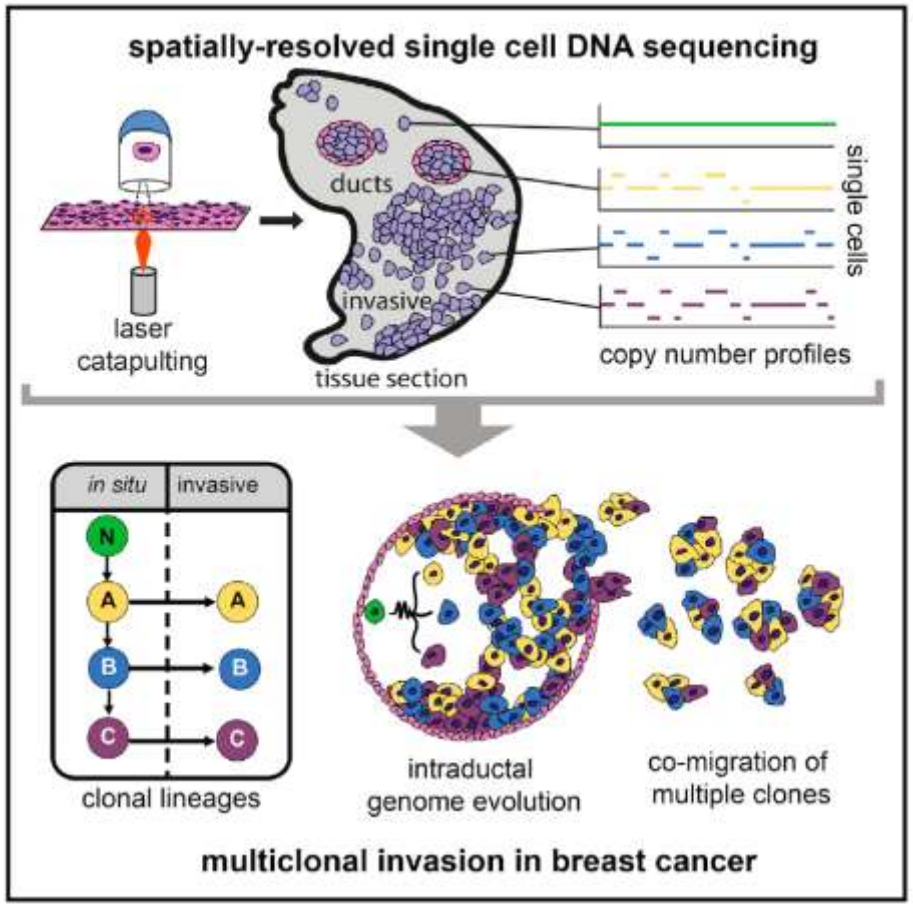
- In the 6 multiclonal patients, 1-5 clones were found to be present in both the ducts and the invasive regions
- In some patients (P4, P8, P6) the clones frequencies changed during invasion
- All clonal subpopulations shared a common genomic lineage and evolved from a single common ancestor: a single cell in the ducts, not multiple initiating cells

N.E. Navin, *SABCS* 2018

A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

Take home Results



- CNA clones derived from a common ancestor.
- CNA subclones detected before invasion through duct.
- The evolutionary model is: Multiclonal invasion model.

A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

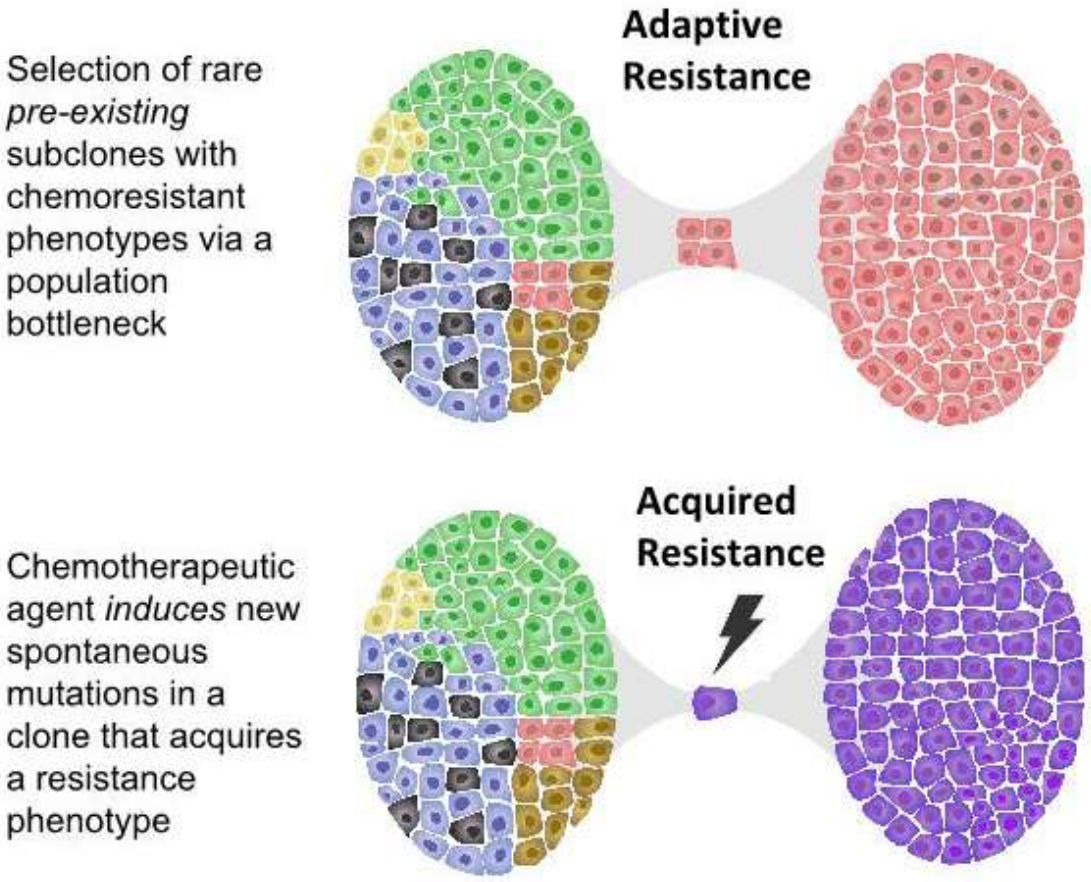
Chemoresistance Evolution in Triple-Negative Breast Cancer Delineated by Single-Cell Sequencing

Charissa Kim,[1,2,6] Ruli Gao,[1,6] Emi Sei,[1] Rachel Brandt,[1] Johan Hartman,[3] Thomas Hatschek,[3] Nicola Crosetto,[4] Theodoros Foukakis,[3,*] and Nicholas E. Navin[1,2,5,7,*]

C. Kim *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

Background



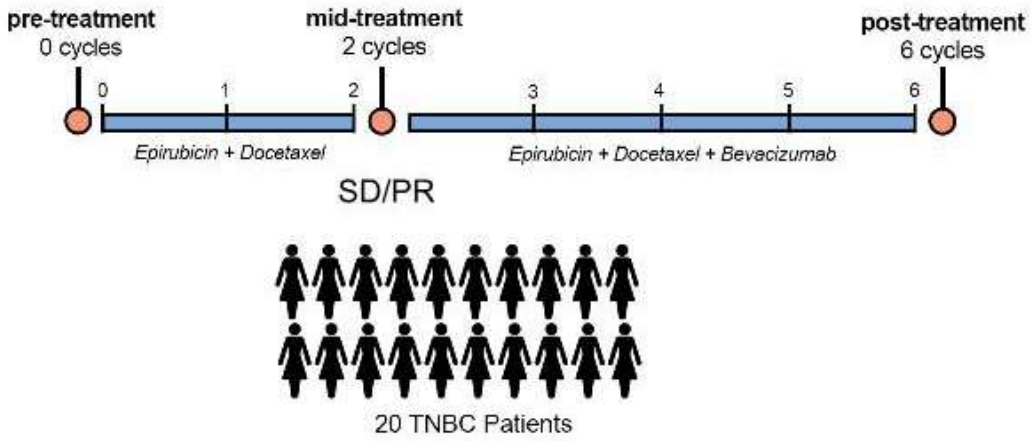
N.E. Navin, *SABCS* 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity in TNBC and other: SABCS 2018

Methods and Results

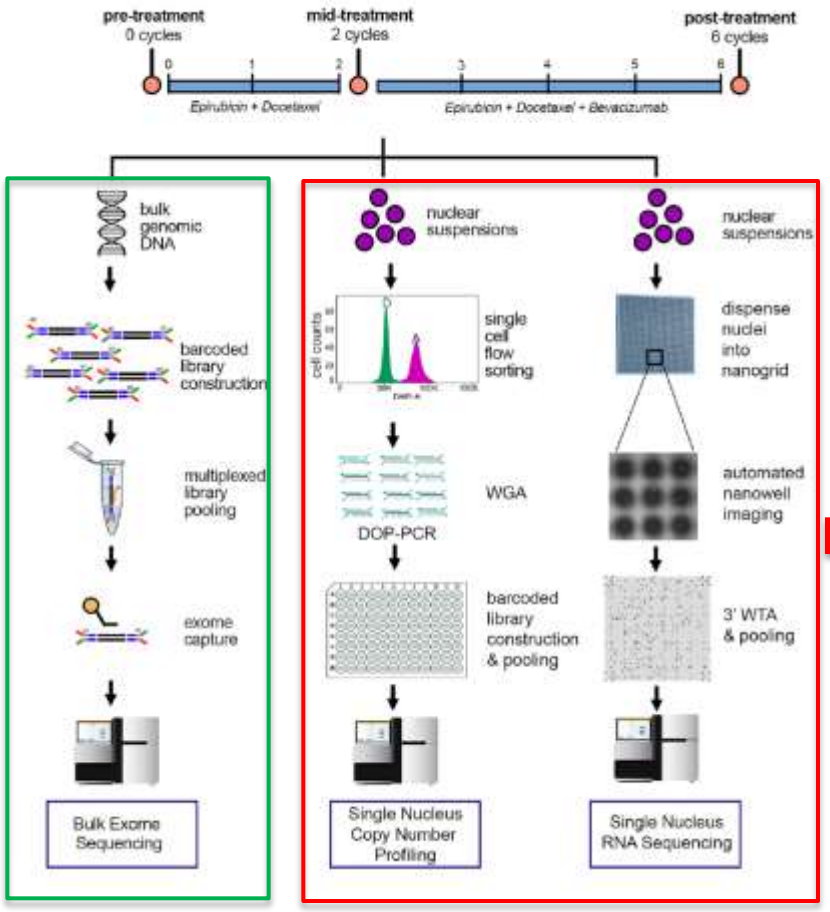


- PROMIX enrolled newly diagnosed TNBC patients with local disease
- Patients were treated with an anthracyclin (Epirubicin), taxane (Docetaxel) and an angiogenesis inhibitor after 2 cycles (Bevacizumab).
- 20 TNBC patients showed partial response (PR) or stable disease (SD) and developed resistance to NAC
- Collected 2-3 matched time points samples per patient, including frozen core biopsies (0,2 cycles) or frozen surgical samples (6 cycles)

N.E. Navin, *SABCS* 2018
C. Kim *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

Methods and Results



Deep-sequencing

Single Cell Sequenting

N.E. Navin, *SABCS* 2018
C. Kim *et al,* Cell 2018

Post San Antonio 2018

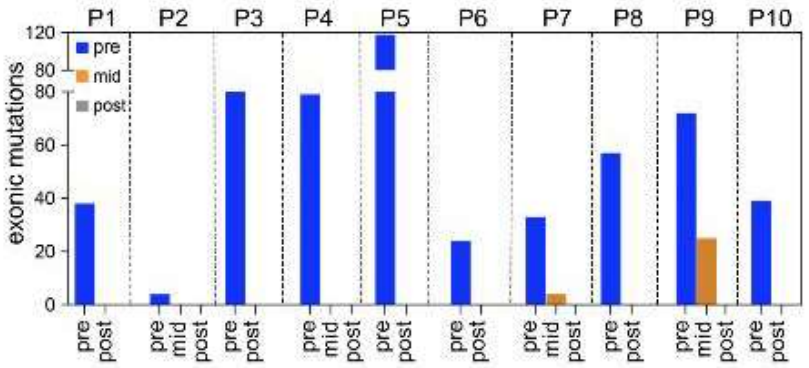
Heterogeneity: TNBC and other subtypes

Heterogeneity in TNBC and other: SABCS 2018

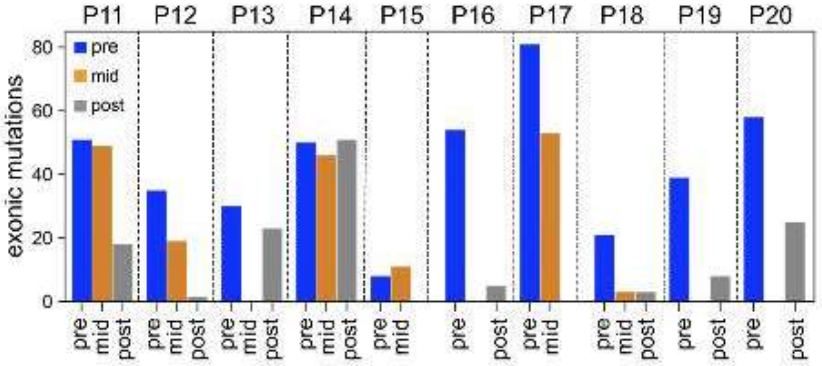
Methods and Results

Deep-sequencing of pre-, mid- and post- treatment samples

10 TNBC patients had a complete elimination of mutations in response to NAC



10 TNBC patients had residual mutations that persisted in the post-treatment tumors after NAC



N.E. Navin, *SABCS* 2018

Heterogeneity in TNBC and other: SABCS 2018

Methods and Results

Single cell Analysis of 8 TNBC patients

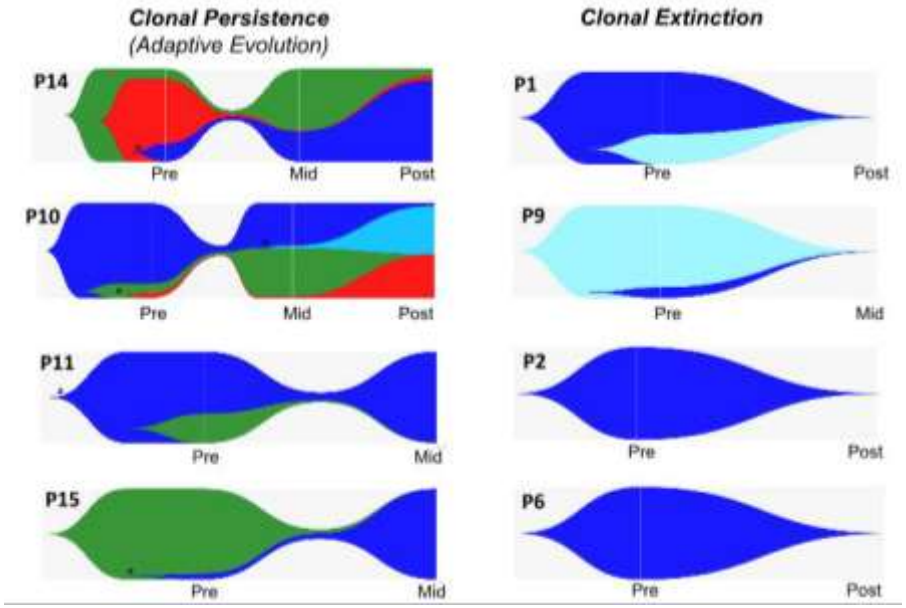
4 clonal extinction

- Single cell copy number profiling
- single cell RNA sequencing

4 clonal persistence

- Single cell copy number profiling
- single cell RNA sequencing

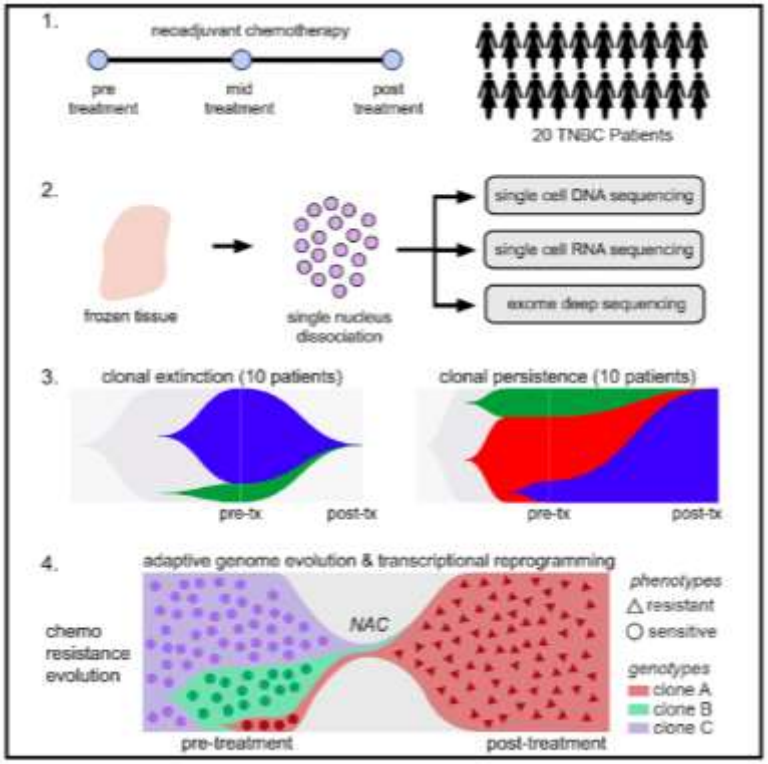
- Based on the exome data, 4 clonal extinction patients and 4 clonal persistence patients were selected for a more detailed analysis using single cell sequencing methods
- ***Genotypic evolution*** during chemotherapy was measured using single cell DNA copy number profiling (Navin et al. 2011, *Nature*) of N=900 cells
- ***Phenotypic evolution*** during chemotherapy was measured using nanowell single cell RNA sequencing (Gao et al. 2016, *Nature Comm.)* of N=6862 cells



N.E. Navin, *SABCS* 2018
C. Kim *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

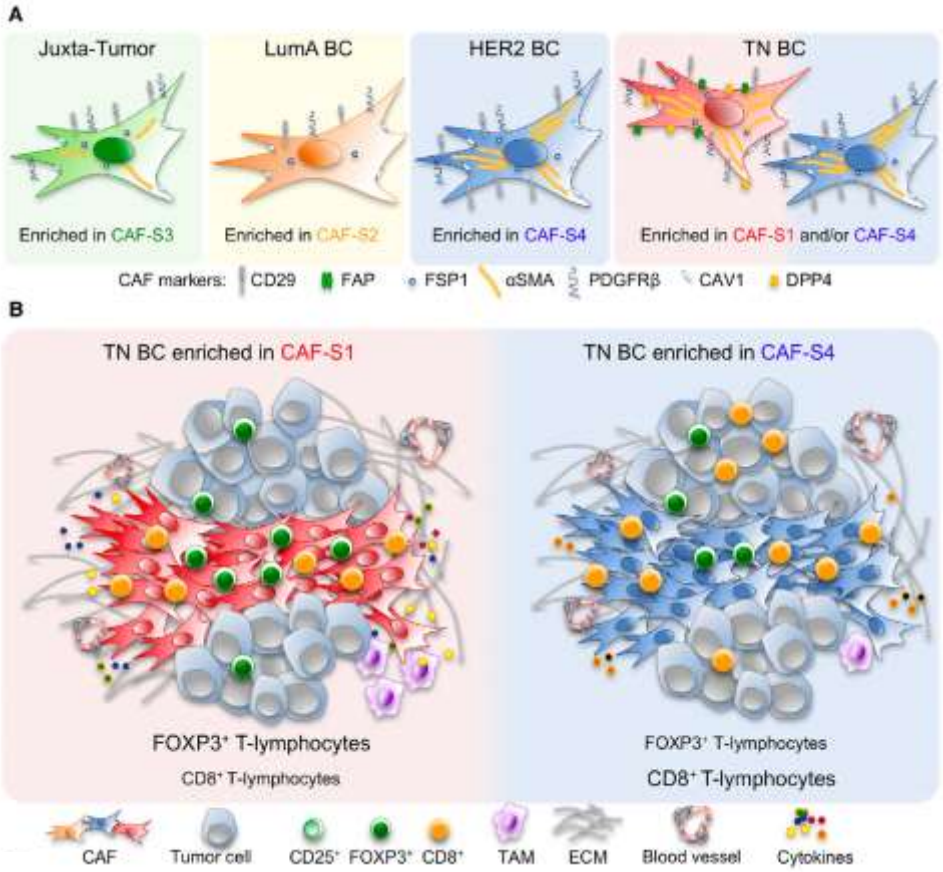
Take home Results



- 50% patients examined, clones of aneuploid/mutated cells were extingued post treatment.
- In the other 50%, pre-existing clones persisted post therapy, adaptive rather than acquired.
- Transcriptional reprogramming leads to «acquired» resistance to therapy (AKT signalling, EMT, hypoxia signature NOT found generally in the pre-therapy nuclei RNA seq).
- Several patients (3) with pCR had residual mutations detected suggesting that single cell DNA or deep-exome sequencing may provide more sensitive methods for detecting residual disease.

C. Kim *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018



Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer

Ana Costa, Yann Kieffer, Alix Scholer-Dahirel, Floriane Pelon, Brigitte Bourachot, Melissa Cardon, Philemon Sirven, Ilaria Magagna, Laetitia Fuhrmann, Charles Bernard, Claire Bonneau, Maria Kondratova, Inna Kuperstein, Andrei Zinovyev, Anne-Marie Givel, Maria-Carla Parrini, Vassili Soumelis, Anne Vincent-Salomon, and Fatima Mechta-Grigoriou

Take home results

- Expression of CD29, FAP, alpha-SMA, PDGFR-beta, FSP1 and CAVI identifield four Cancer Associated Fibroblast (CAF) subsets (CAF-S1-S4).
- CAF subsets show different distribution across breast cancer subtypes.
- CAF-S1 is associated with immunosuppression.
- CAF-1 enriched TNBC had increased recruitment of T cells, survival of CD4+CD25+ T cells that differentiate into FOXP3+ T cells and reduced CD8+ R cells.
- CAF-S1 enhance the ability of Tregs to inhibit effector T cell proliferation.

A. Costa *et al,* Cancer Cell 2018

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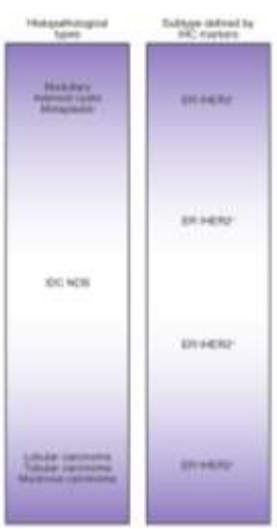
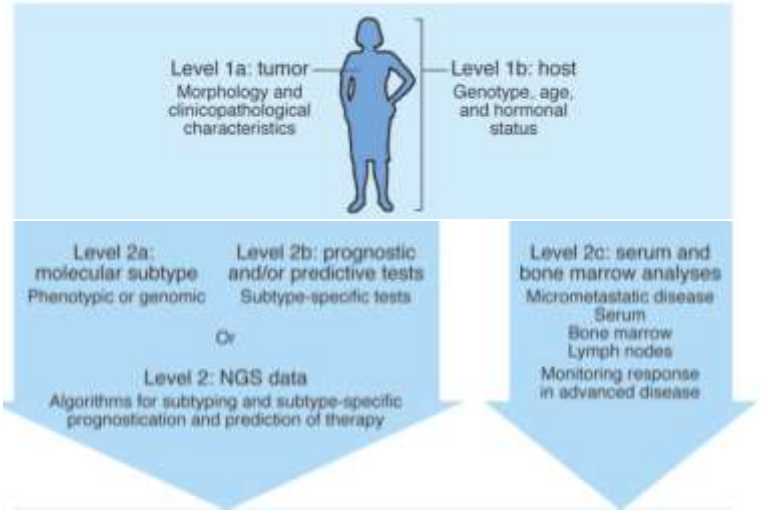
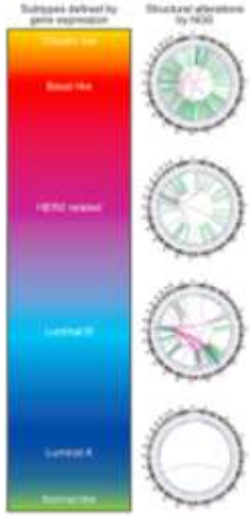
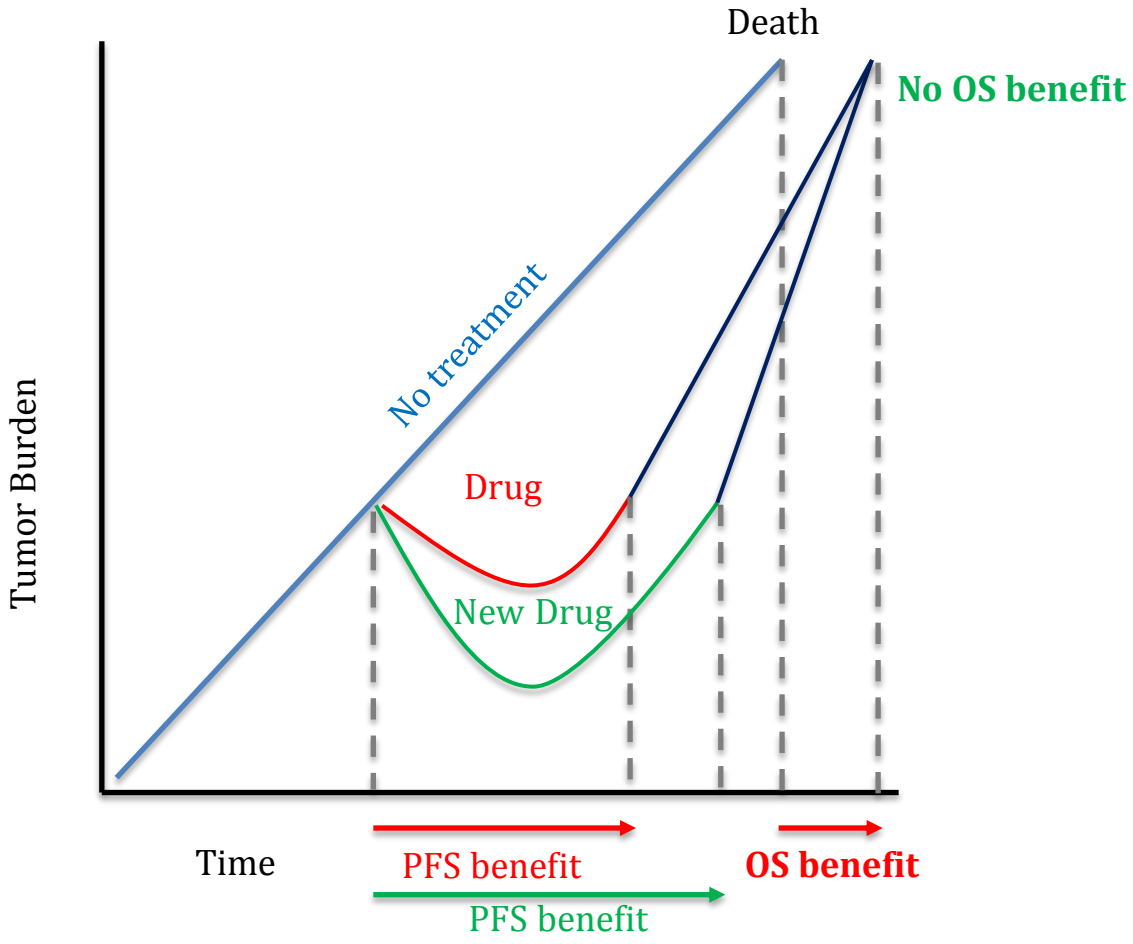






C. Kim *et al*, 2018 Cell 2018
A. Davis *et al,* Bio. et Bio. Acta 2017
H. G. Russnes *et al,* JCI 2011

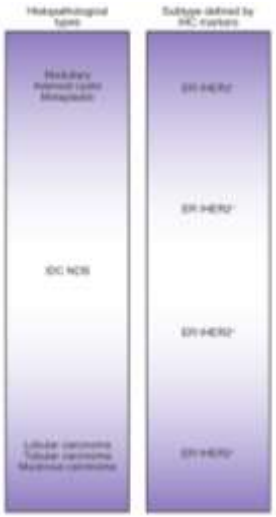
Heterogeneity: Food for thought

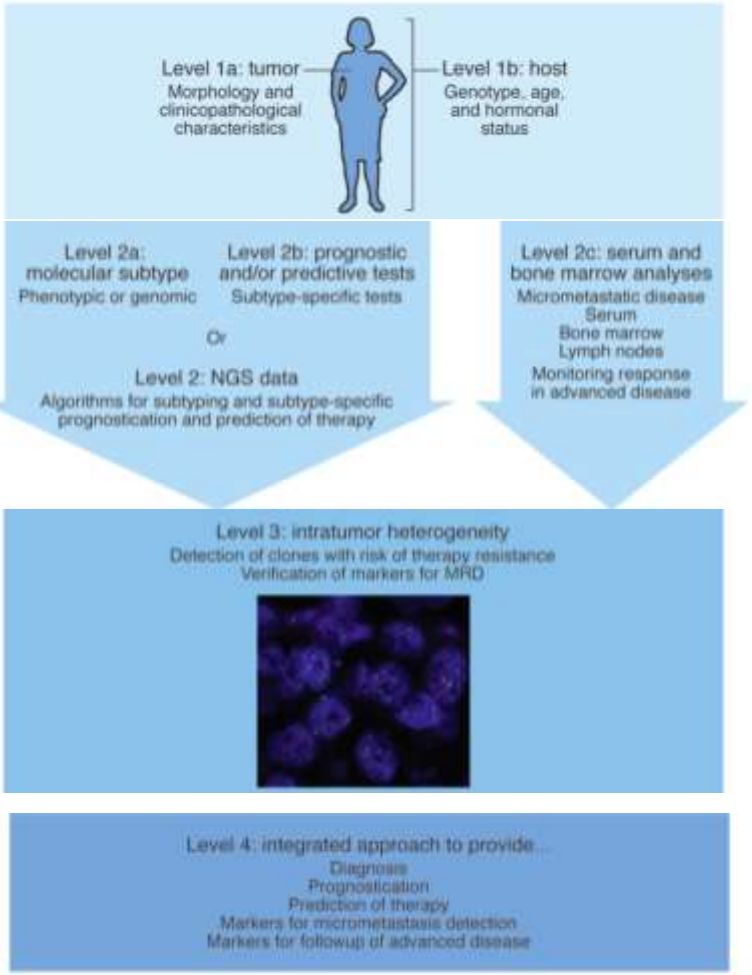


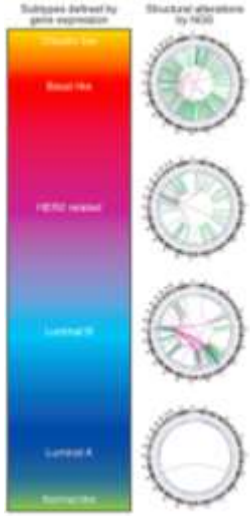
Post San Antonio 2018

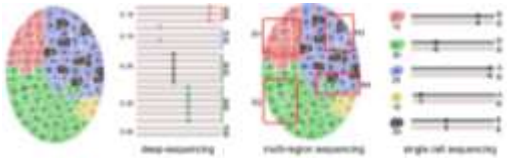
Heterogeneity: TNBC and other subtypes

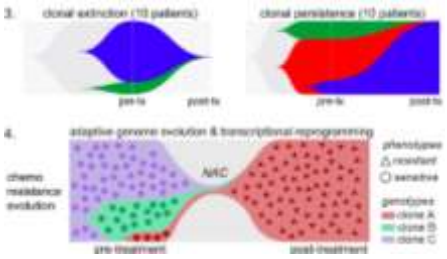
Heterogeneity: Food for thought











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Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

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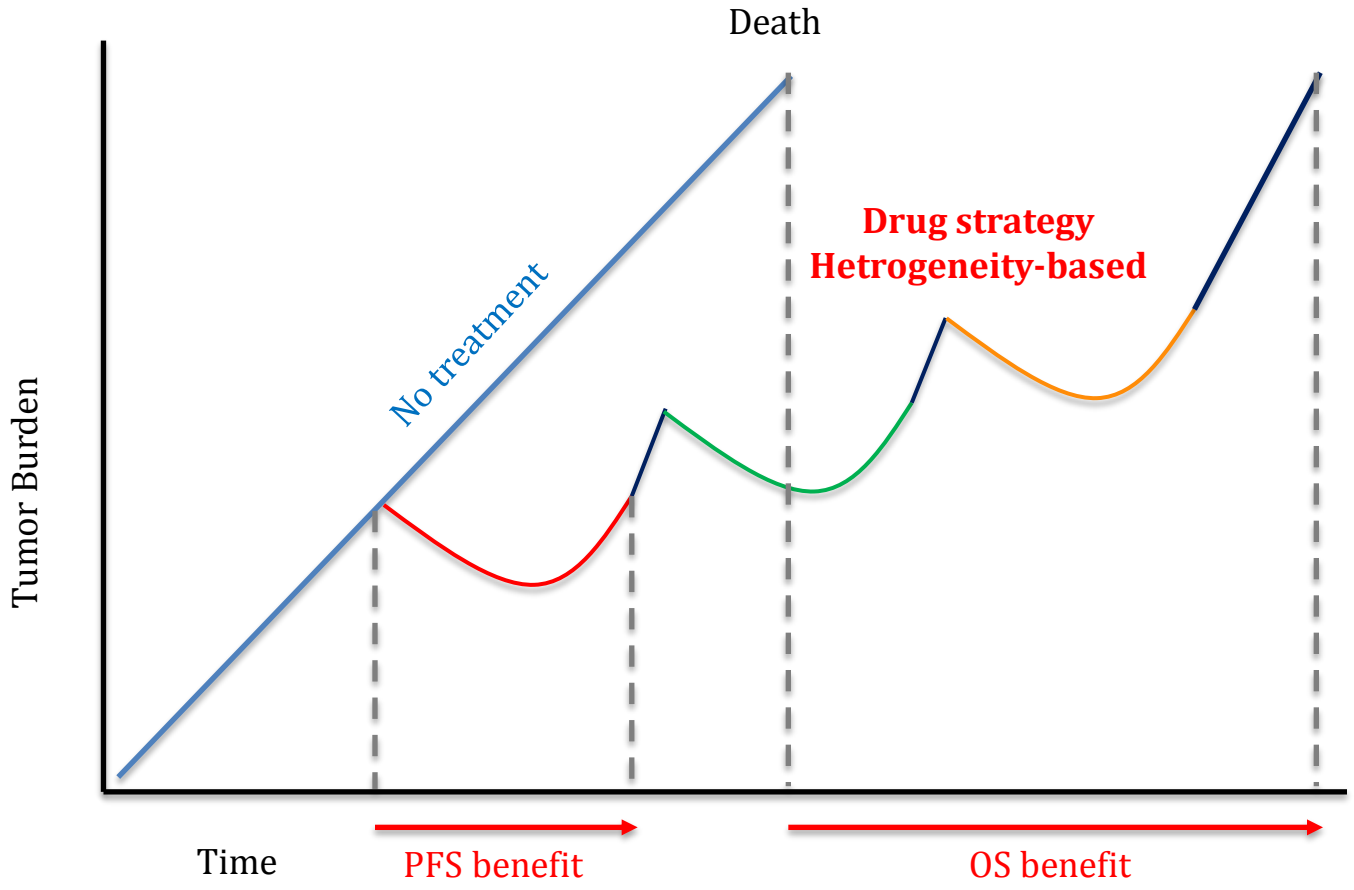


Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

GRAZIE DELL'ATTENZIONE

armando.orlandi@policlinicogemelli.it

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

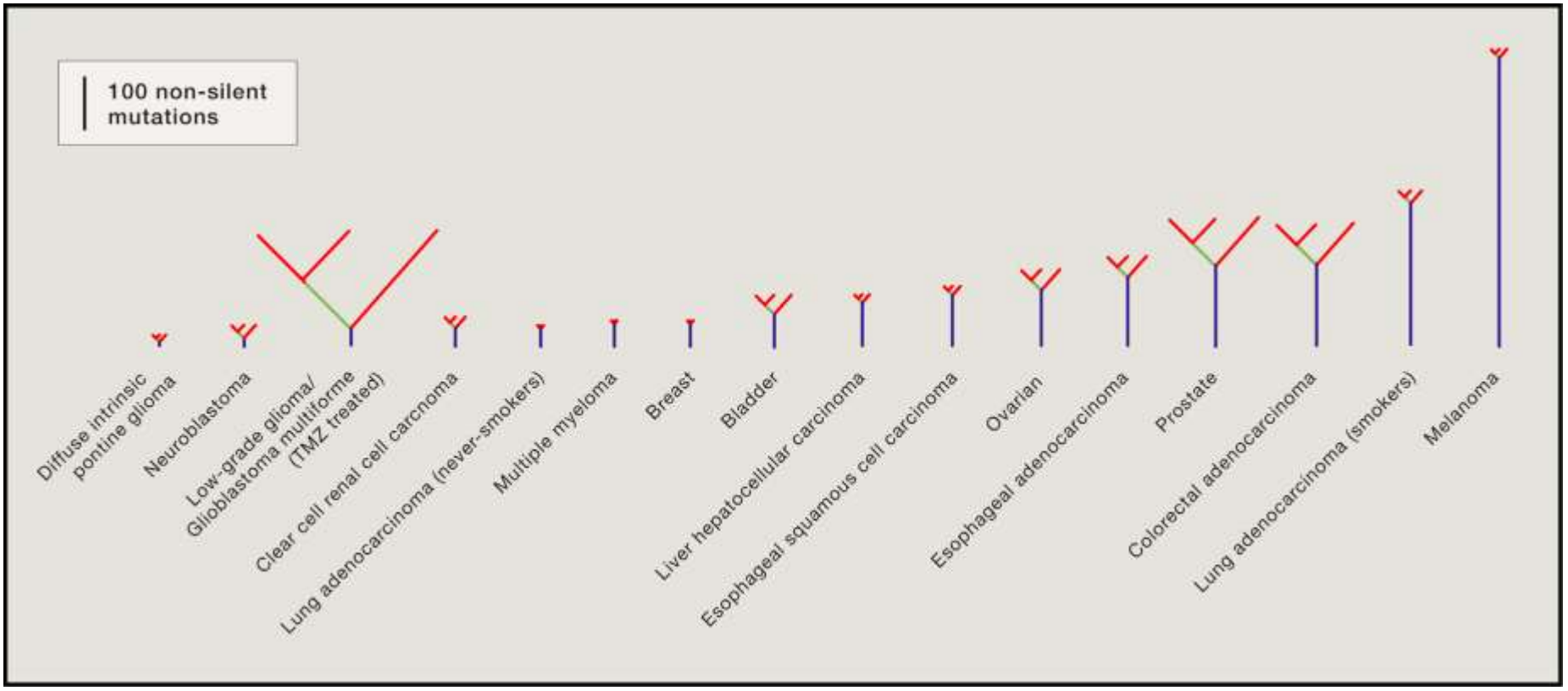
Roma, 28 gennaio 2019

Dr. Armando Orlandi

DISCLOSURE

I love molecular biology, but I'm just a clinician.

Heterogeneity: Background



N. McGranahan *et al,* Cell 2018

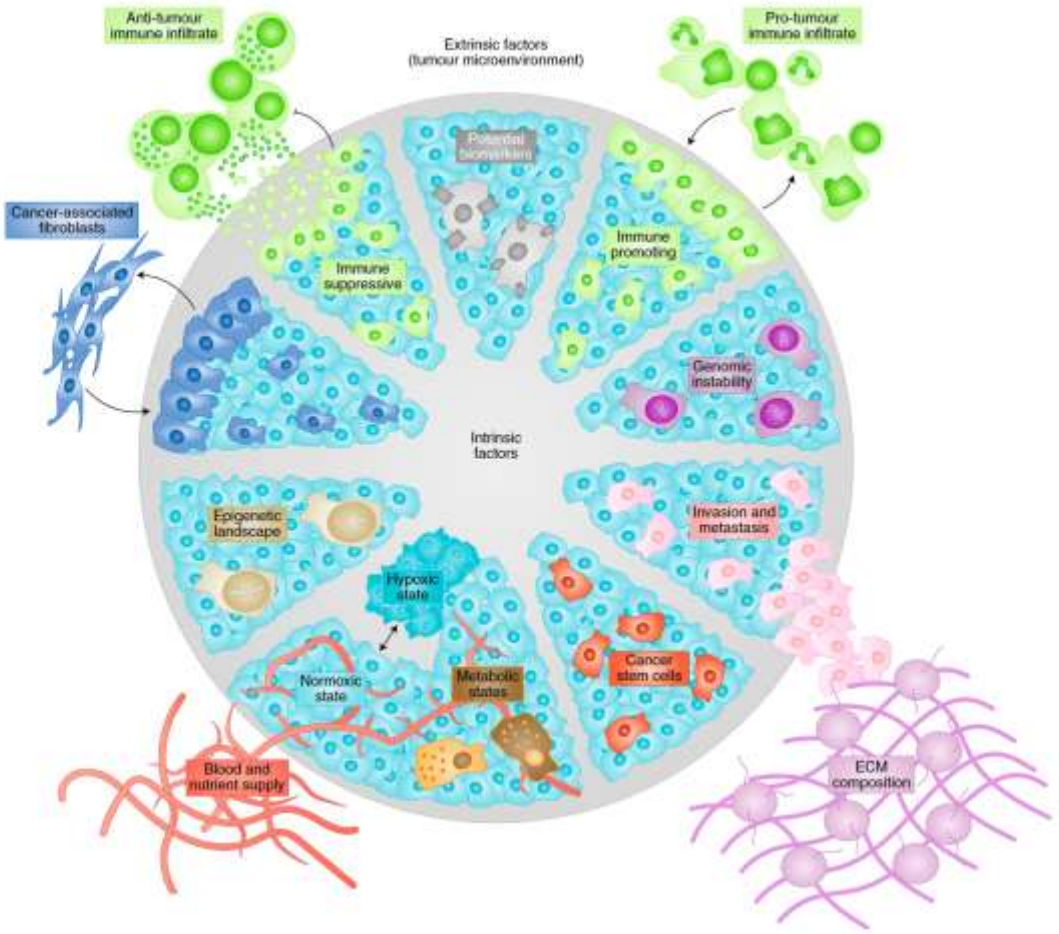
Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Roma, 28 gennaio 2019

Dr. Armando Orlandi

Heterogeneity: Background



D. A. Lawson *et al, Nat Cell Bio* 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity: Background

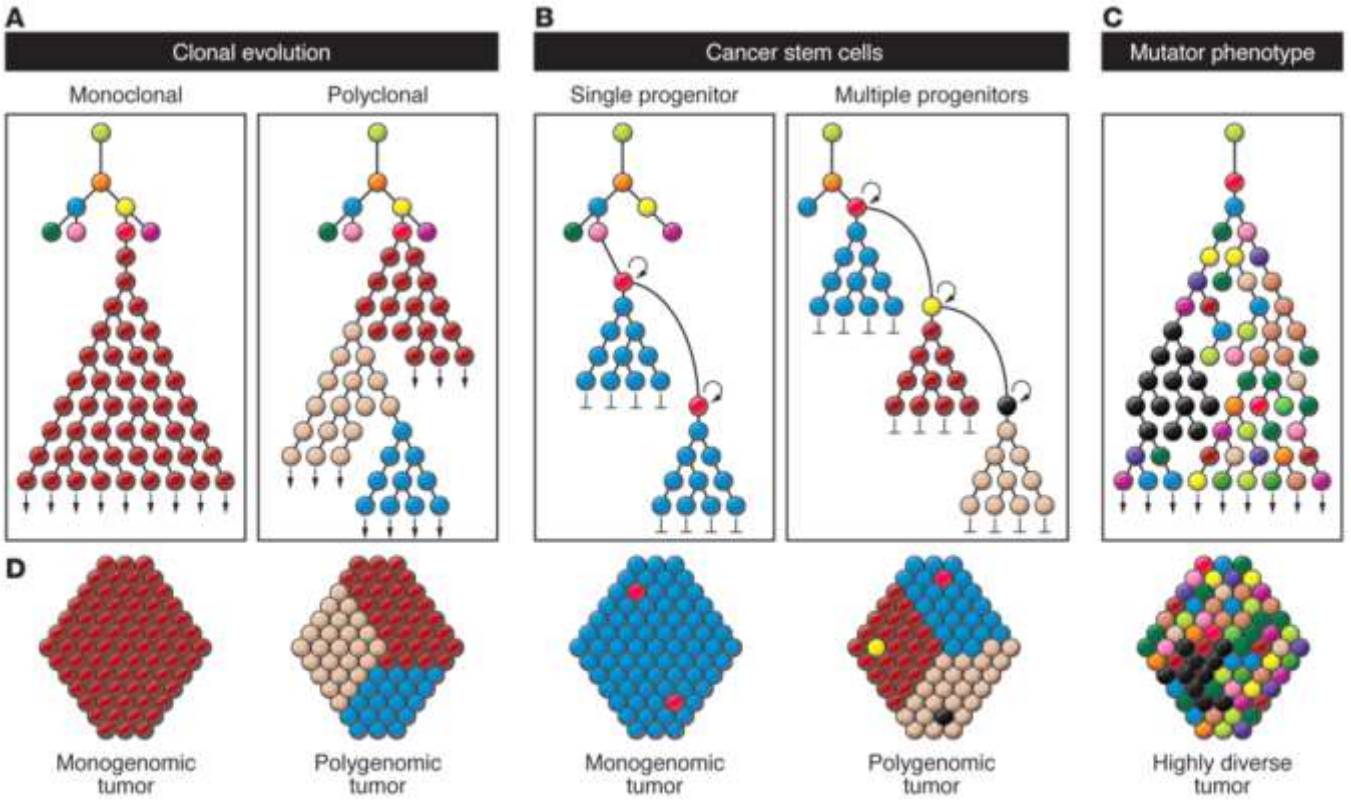
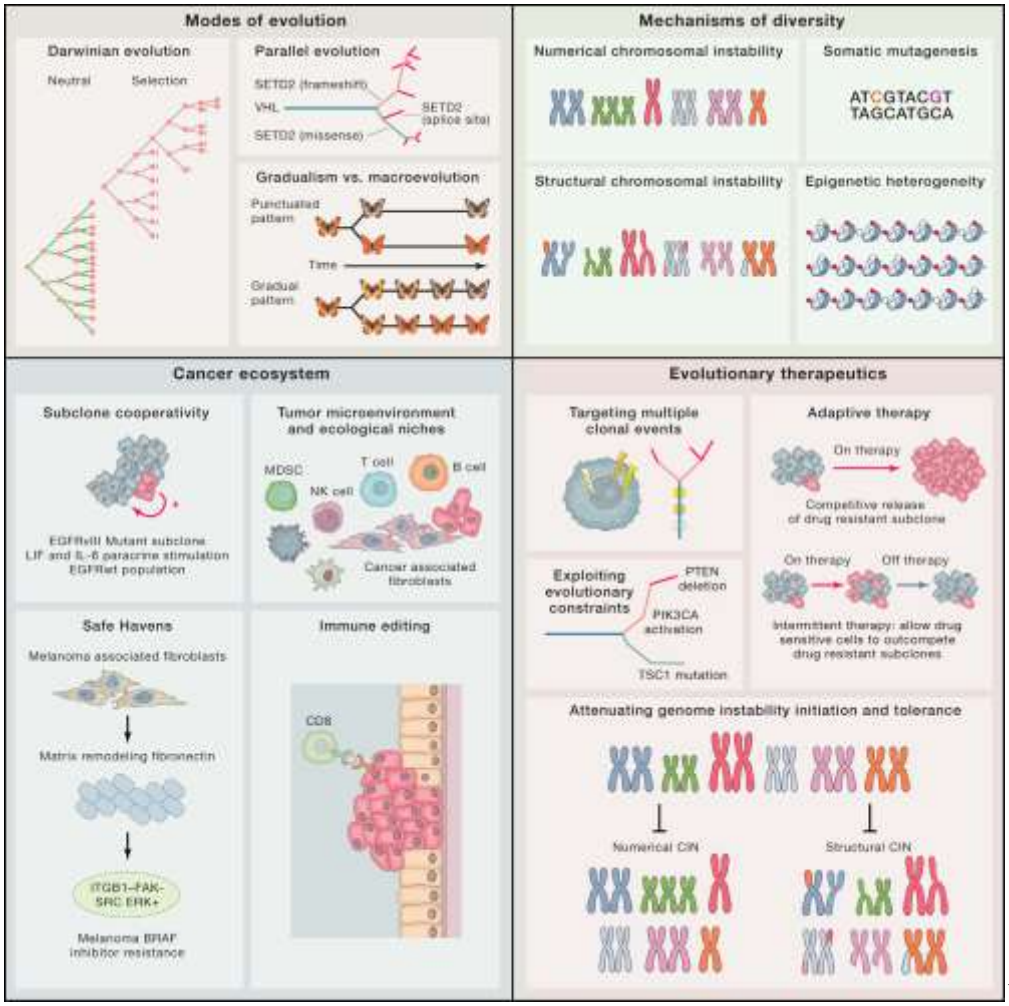


Figure 3
Hypothetical models explaining intratumor heterogeneity. (**A**–**C**) Different models of tumor progression can give rise to distinct types of intratumor heterogeneity, exemplified here by the clonal evolution (**A**), the cancer stem cell (**B**), and the mutator phenotype (**C**) models. (**D**) The different models can result in distinct spatial distributions of subpopulations.

H. G. Russnes *et al.,* JCI 2011

Heterogeneity: Background



N. McGranahan *et al,* Cell 2018

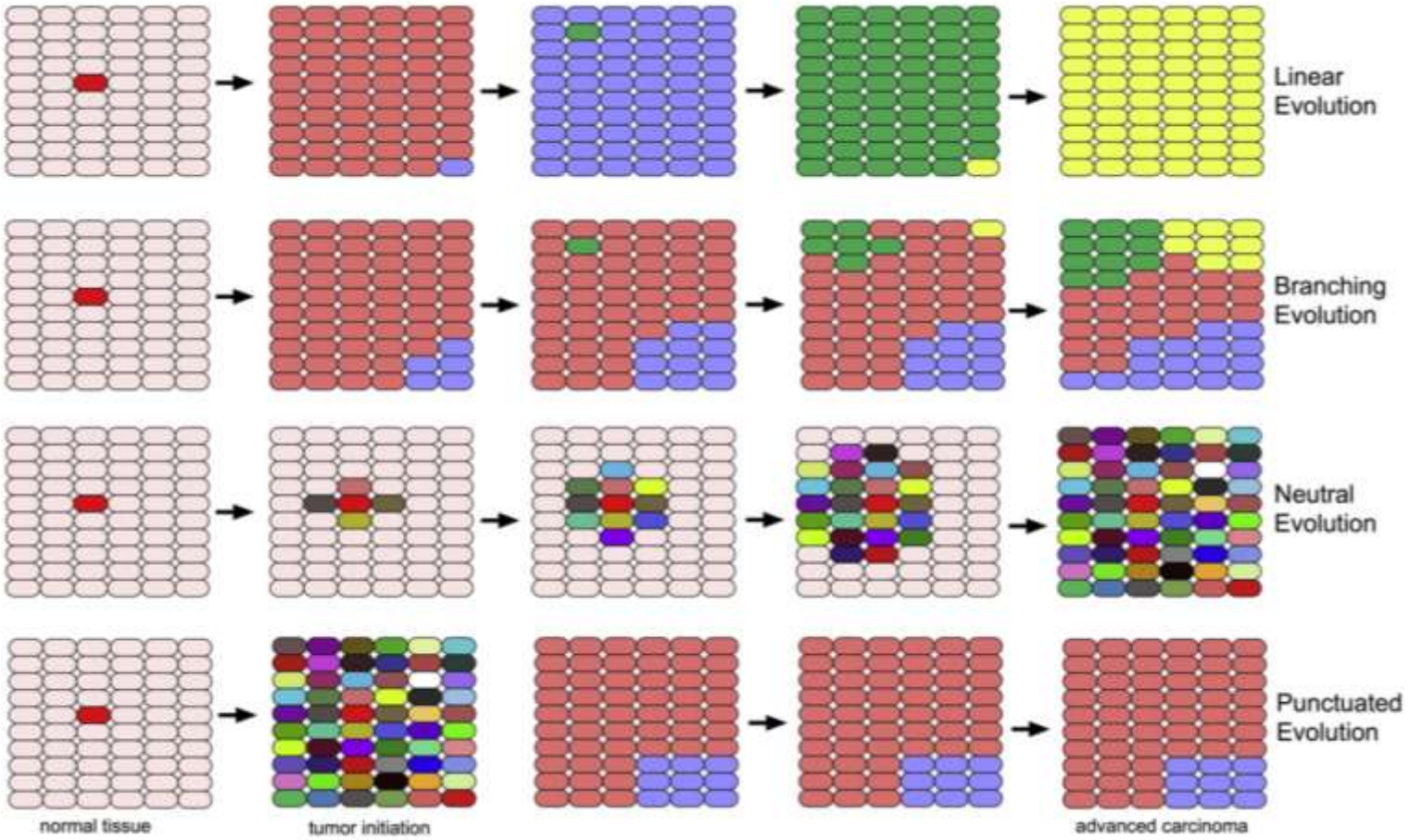
Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Roma, 28 gennaio 2019

Dr. Armando Orlandi

Heterogeneity: Background

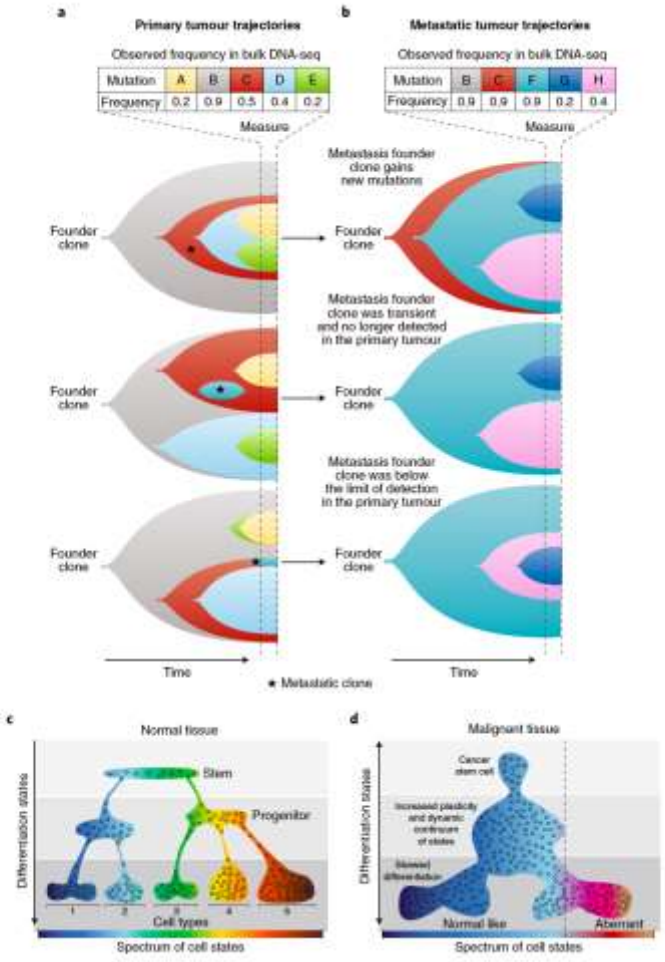


A. Davis *et al,* Bio. et Bio. Acta 2017

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity: Background



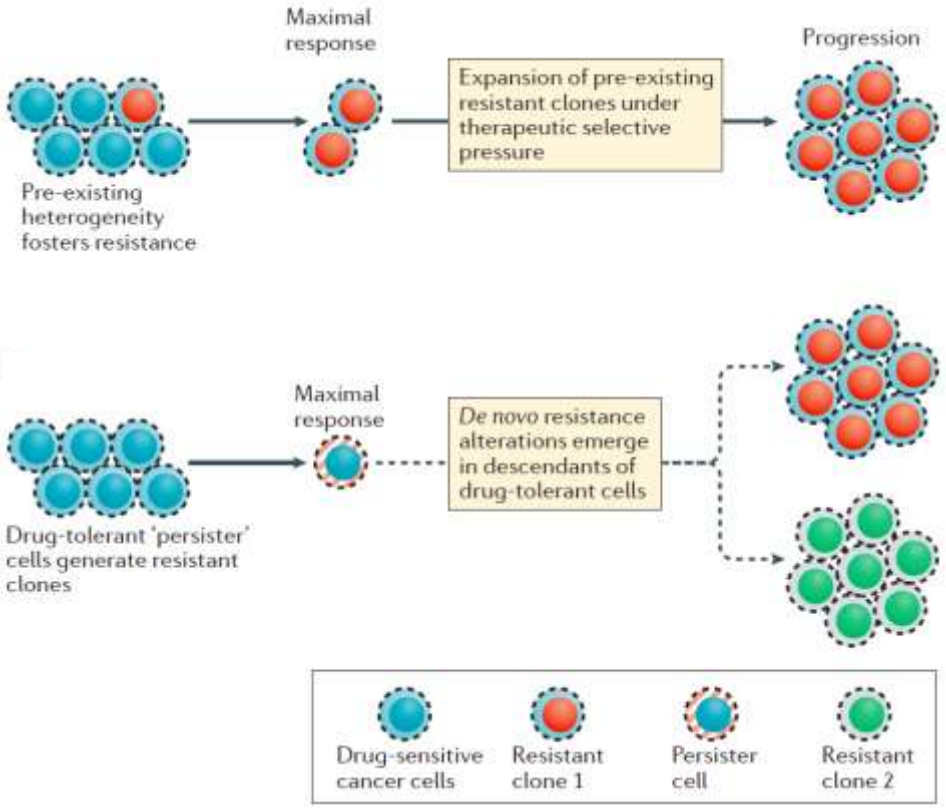
D. A. Lawson *et al, Nat Cell Bio* 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity: Background

Evolutionary pathway of therapy resistance

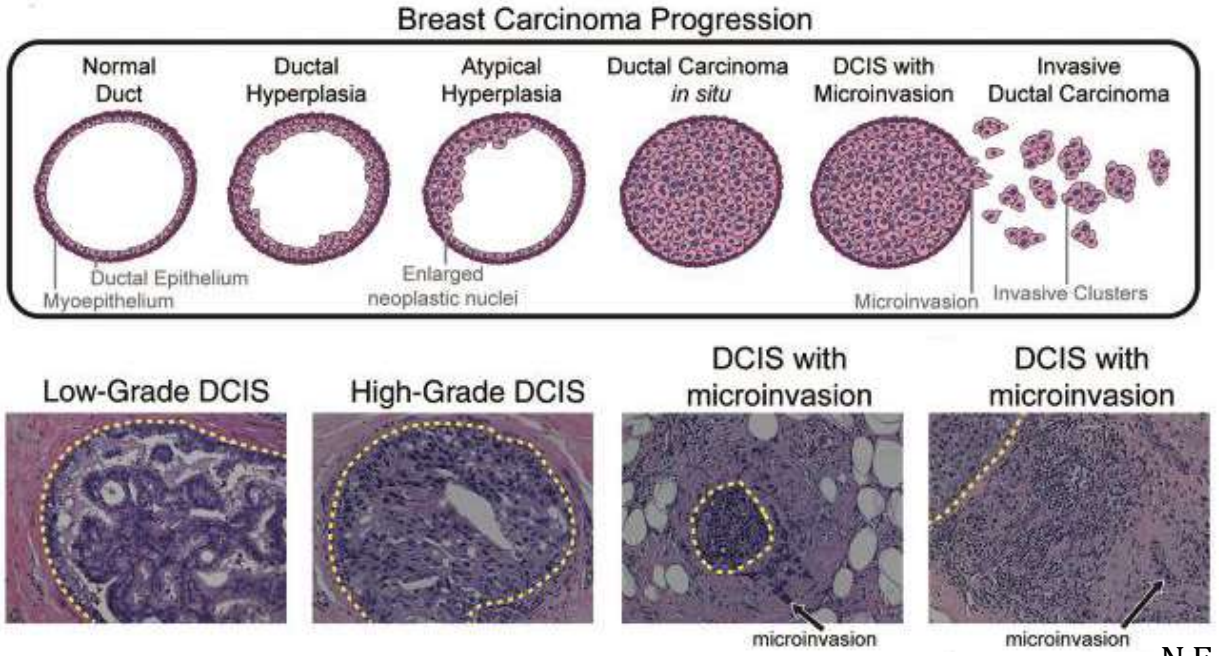


I. Dagogo-Jack *et al, Nat Rev Clin Onc* 2018

Heterogeneity in TNBC and other: SABCS 2018

Background

- Ductal carcinoma In Situ (DCIS) is the most common form of early stage breast cancers and is frequently detected during routine mammographic imaging
- Only 10% of low-grade and 30% of high-grade DCIS patients progress to invasive ductal carcinomas (IDC), making it difficult to determine which patients to treat

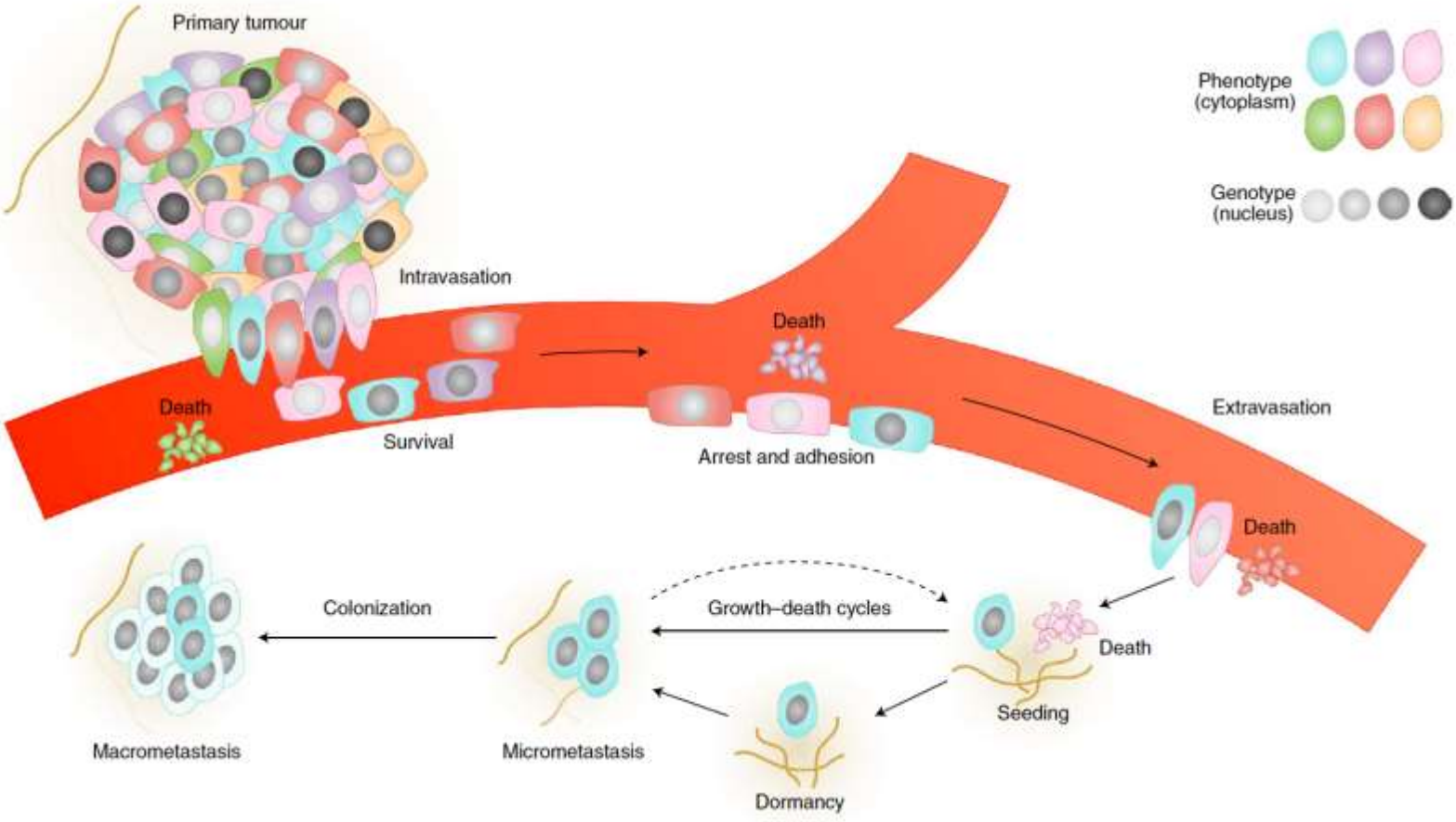


N.E. Navin, *SABCS* 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity: Background

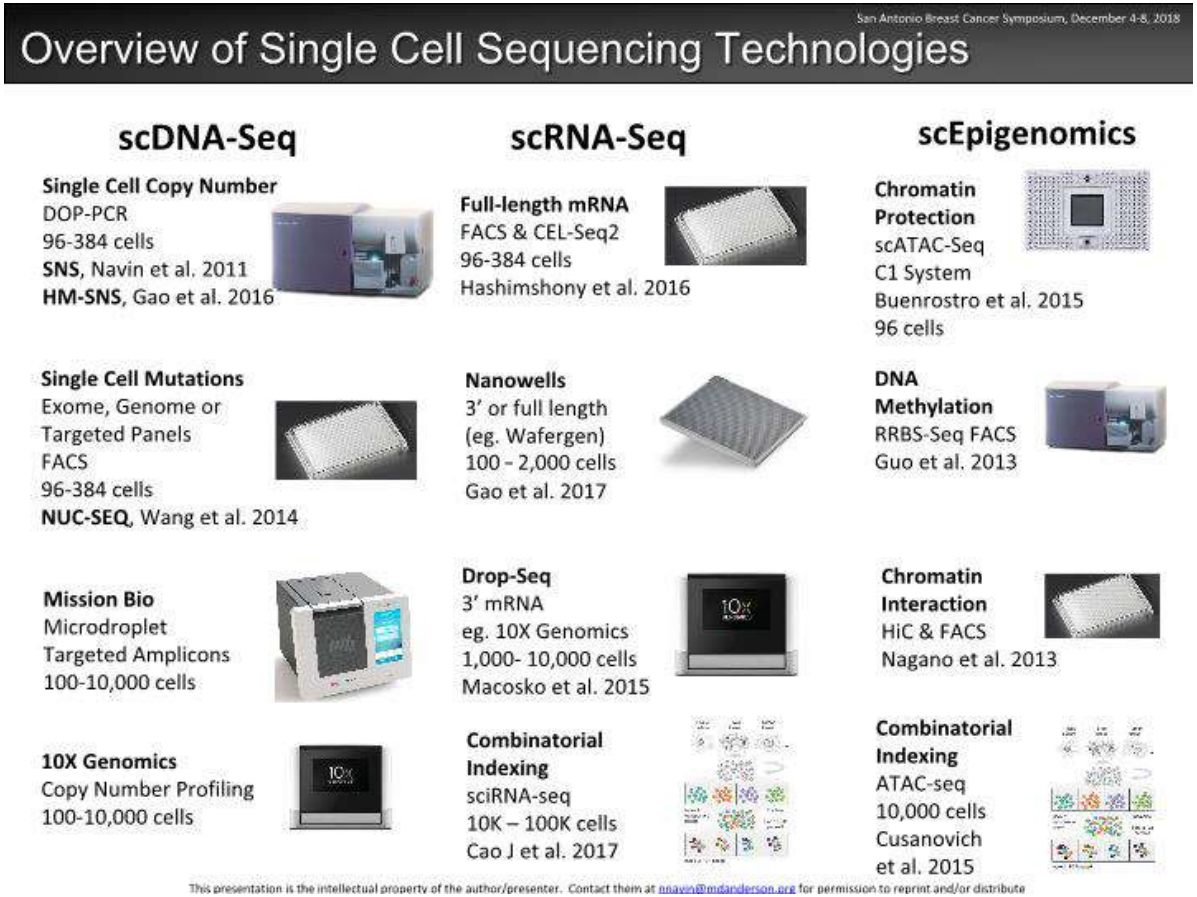


D. A. Lawson *et al, Nat Cell Bio* 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity: Background



N. E. Navil, Genome Research 2015

Post San Antonio 2018

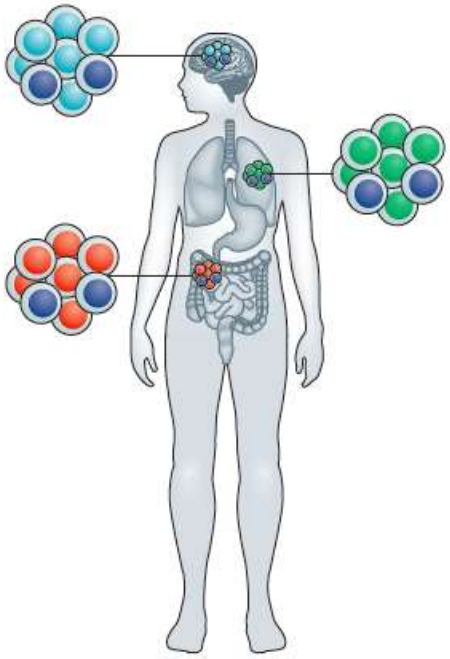
Heterogeneity: TNBC and other subtypes

Roma, 28 gennaio 2019

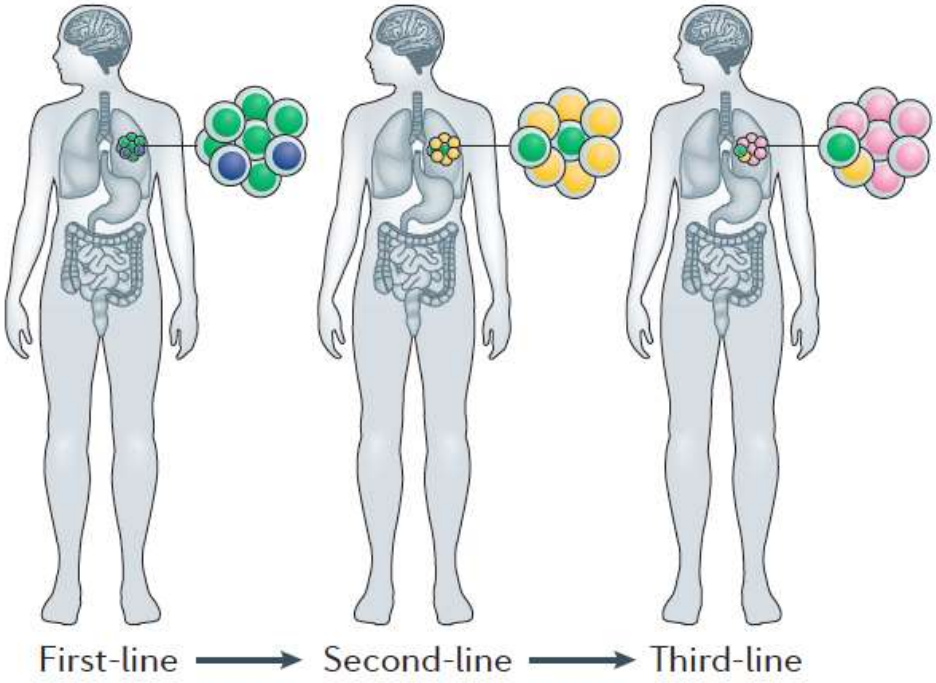
Dr. Armando Orlandi

Heterogeneity: Background

Spatial heterogeneity



Temporal heterogeneity

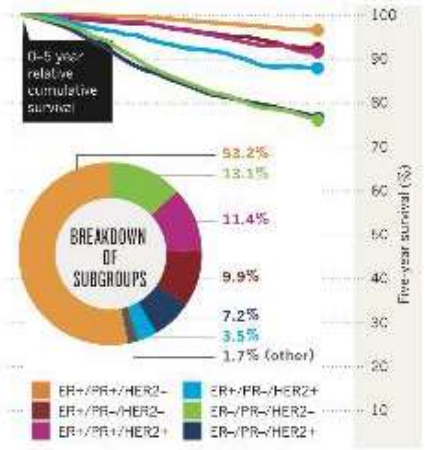


I. Dagogo-Jack *et al, Nat Rev Clin Onc* 2018

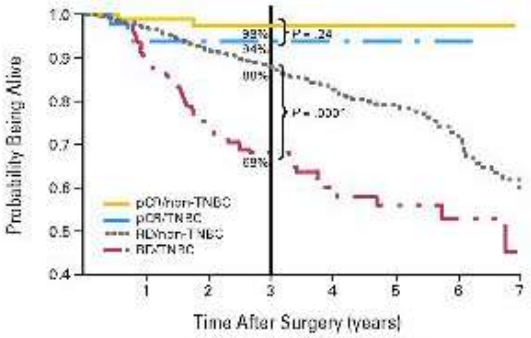
Heterogeneity in TNBC and other: SABCS 2018

Background

- Triple-Negative Breast Cancer (TNBC) is an aggressive subtype of breast cancer that is characterized by a lack of estrogen, progesterone and Her2 receptors by histopathology
- 83% of TNBC patients have *TP53* mutations
- Genomic data has shown that TNBC patients display extensive intratumor heterogeneity within the tumor mass (Shah et al. 2014; Wang et al. 2014)
- Standard of care is neoadjuvant chemotherapy for TNBC patients includes taxanes and anthracyclins (e.g. paclitaxel FAC).
- However 48% of TNBC patients who have non-pathological complete response (non-pCR) developed resistance within 1-2 years and often progress to metastatic disease and morbidity



Maxman (2014) *Nature*



Liedtke et al. JCO 2008

N.E. Navin, *SABCS* 2018

Post San Antonio 2018

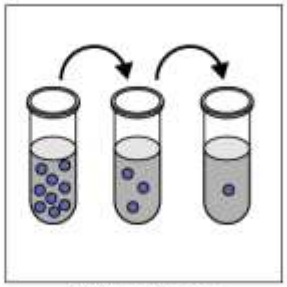
Heterogeneity: TNBC and other subtypes

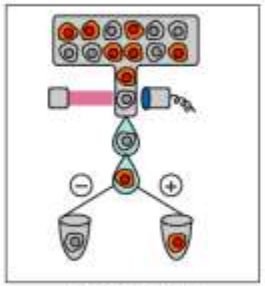
Heterogeneity: Background

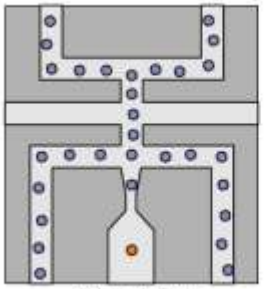
Single **C**ell **S**equencing

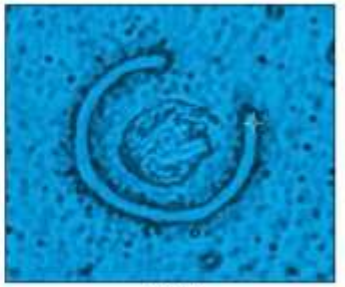
From ***abundant cellular populations***


Micromanipulation

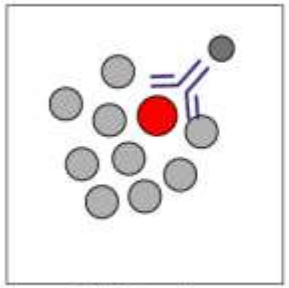

Serial dilution

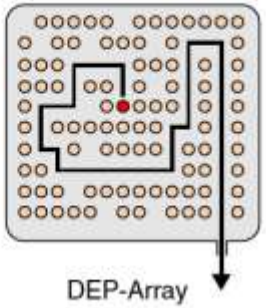

Flow-sorting

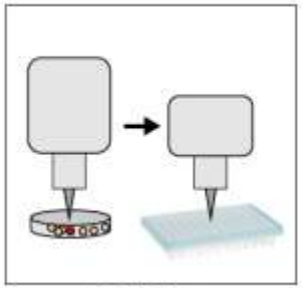

Microfluids

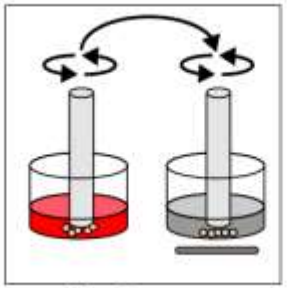

LCM

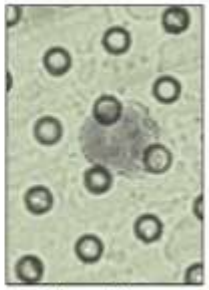
From ***rare cellular populations (CTC, DTC)***


CellSearch


DEP-Array


CellCelector


MagSweeper


Nanofilters

N. E. Navil, Genome Biology 2014

Heterogeneity: Background

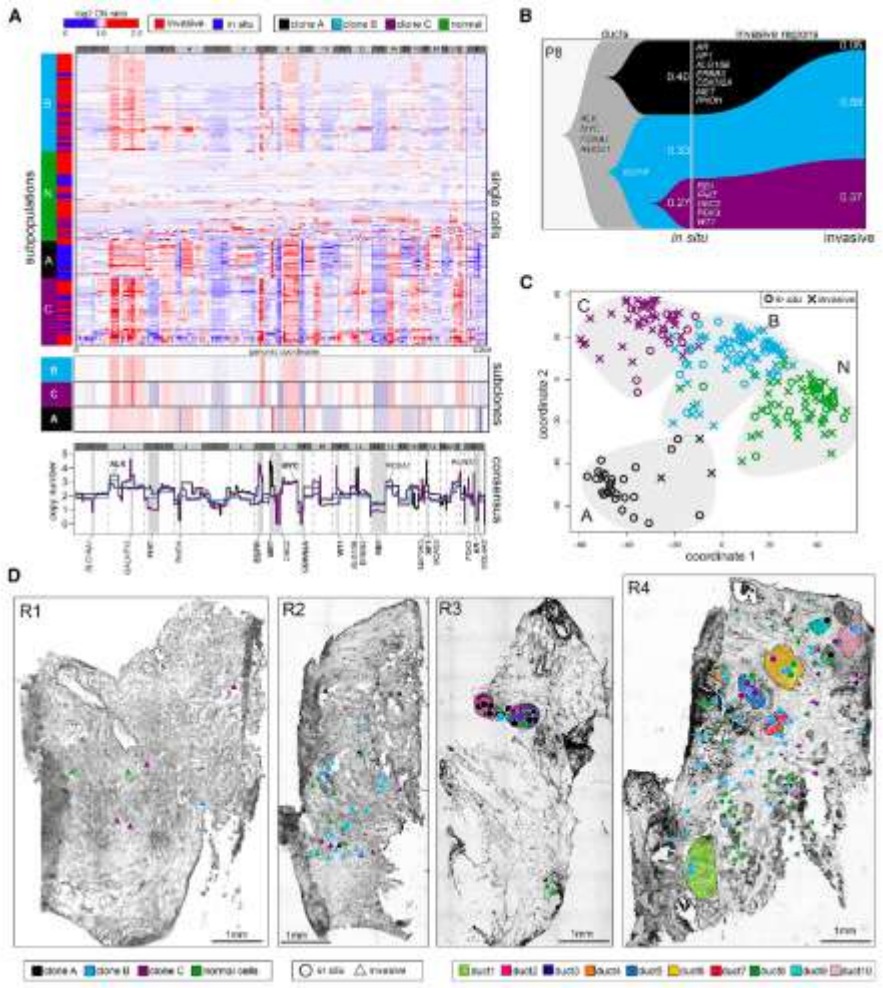
Heterogeneity in TNBC and other: SABCS 2018

A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

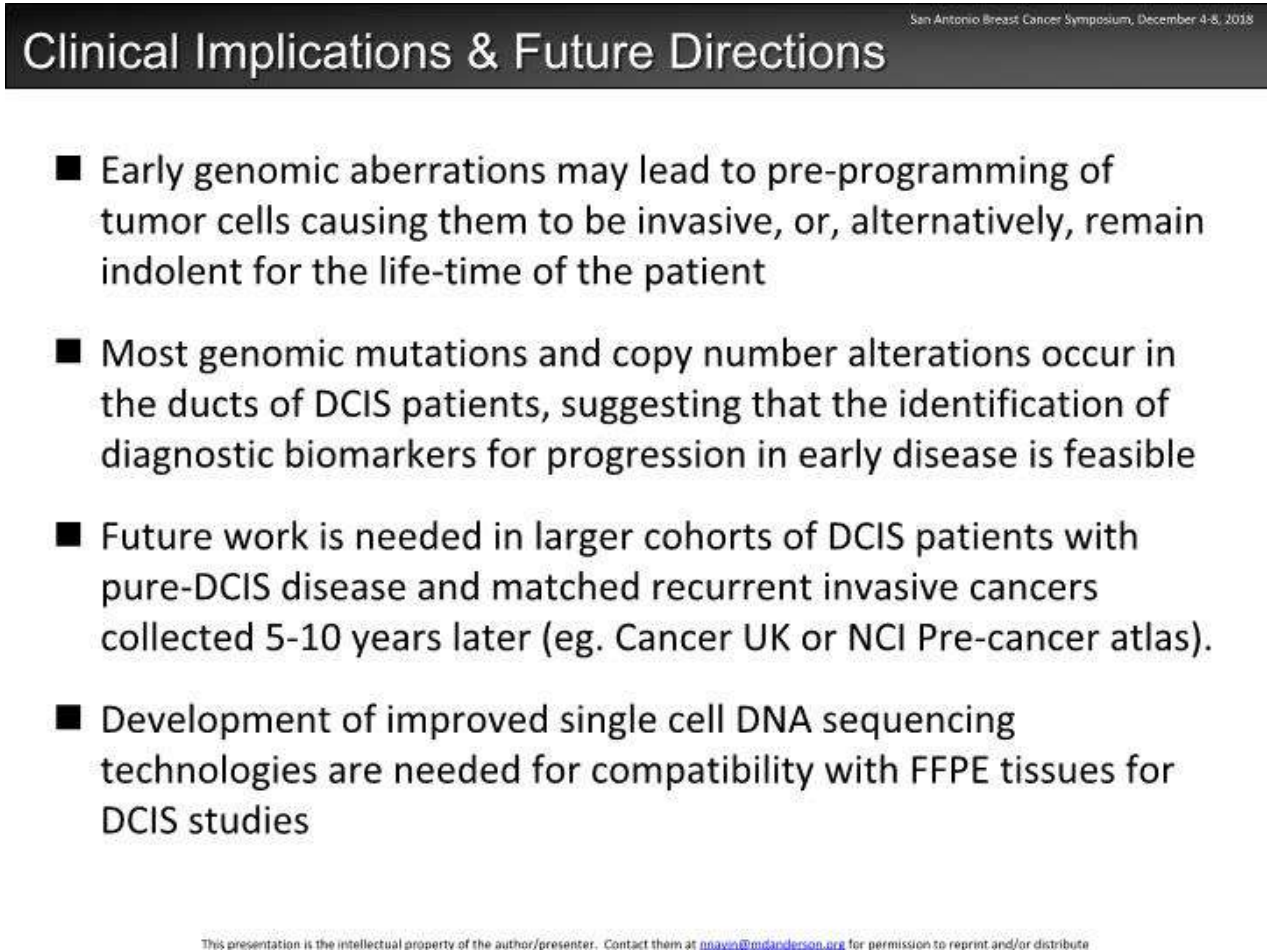


Take home results

- 10 patients examined, 6 were polyclonal, 4 monoclonal
- CNA clones derived froma a common ancestor
- CNA subclones detected before invasion thru duct
- Even in CNA monoclonals, diversification at mutational level seen befor invasion

A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

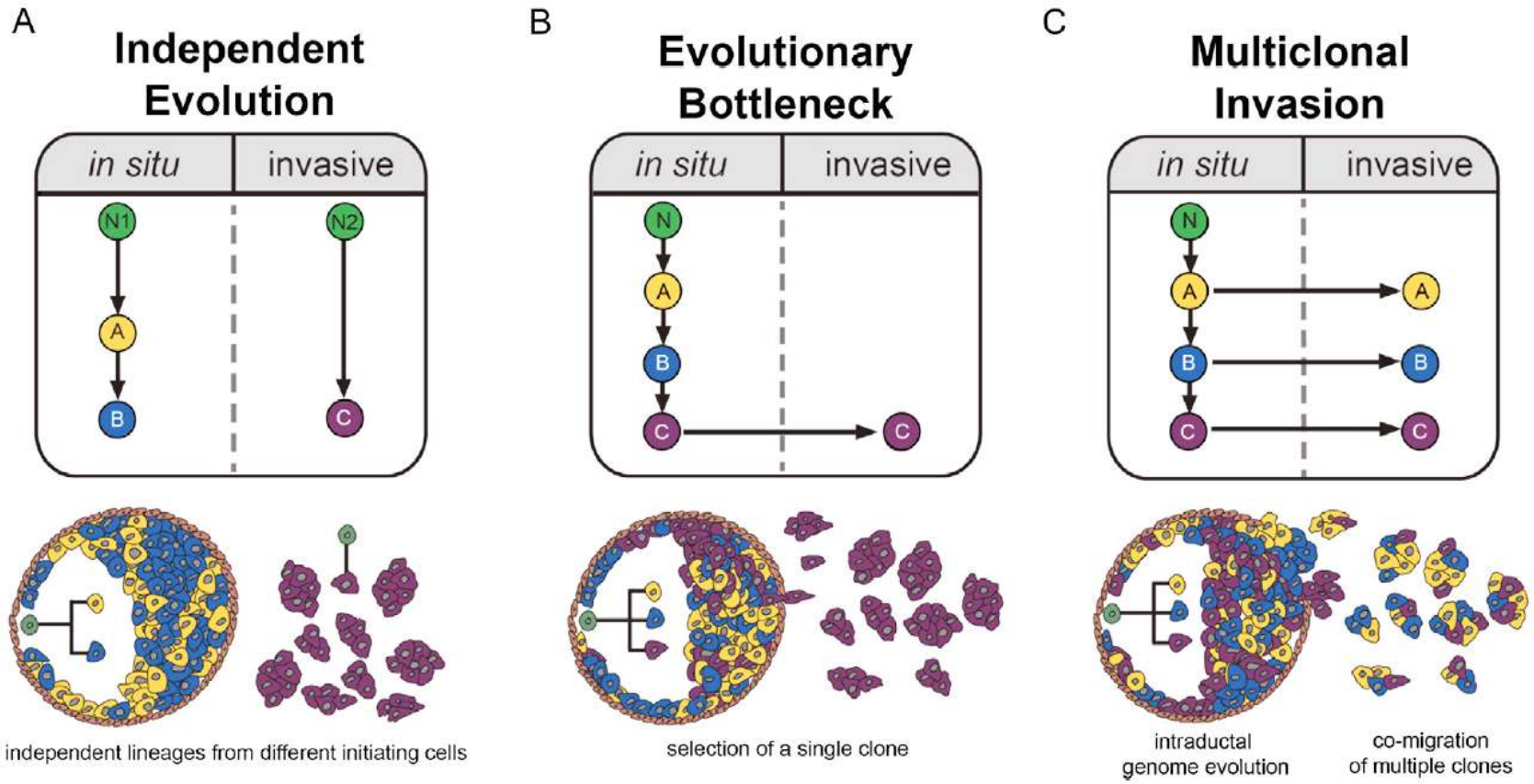


A. K. Casasent *et al,* Cell 2018

Post San Antonio 2018

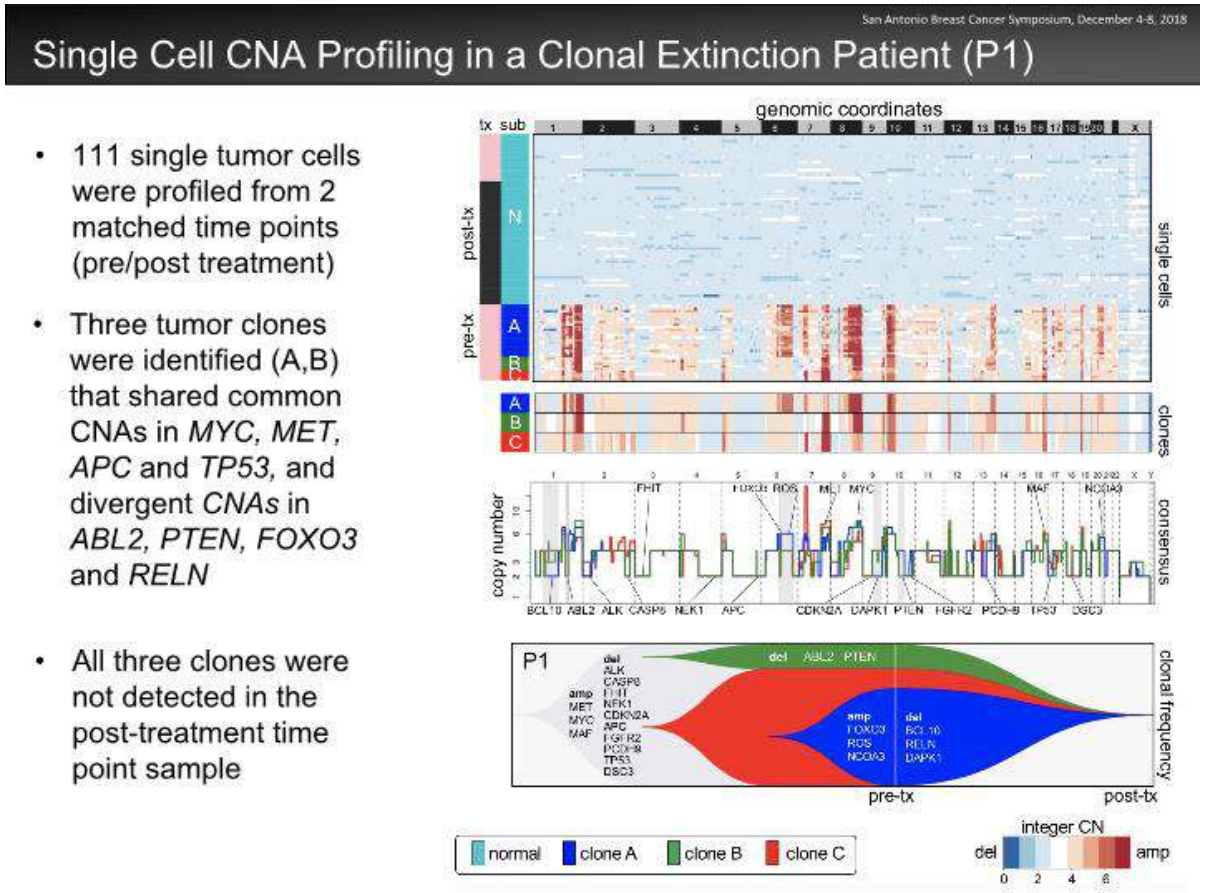
Heterogeneity: TNBC and other subtypes

Heterogeneity in TNBC and other: SABCS 2018



A. K. Casasent *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018

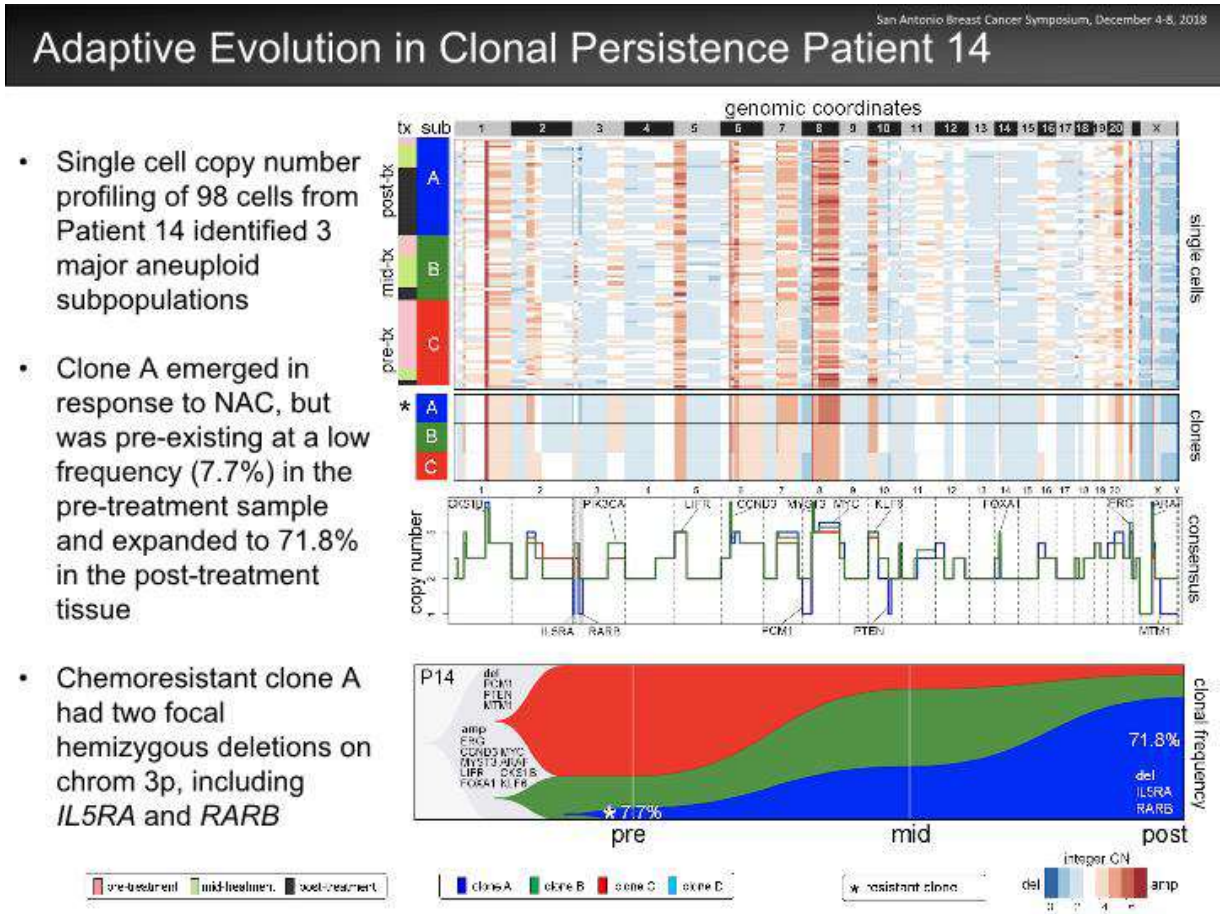


A. K. Casasent *et al,* Cell 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity in TNBC and other: SABCS 2018

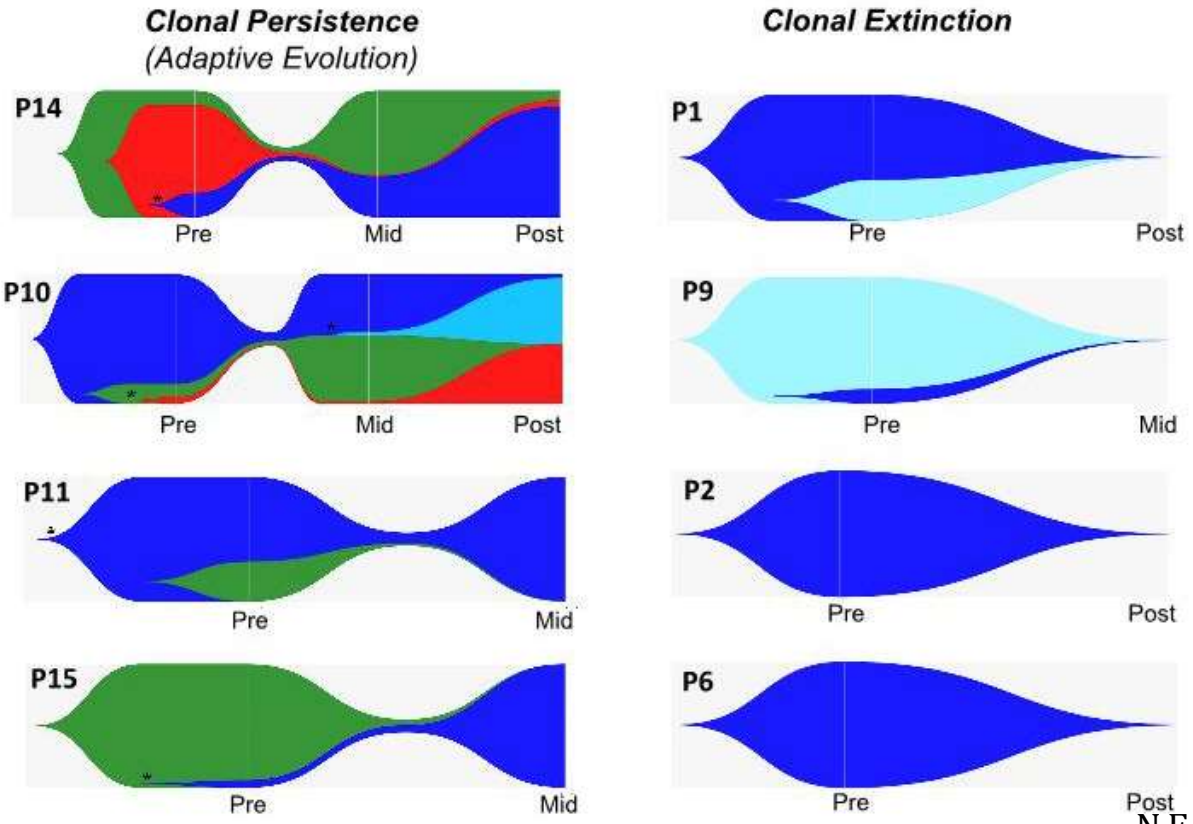


- Single cell copy number profiling of 98 cells from Patient 14 identified 3 major aneuploid subpopulations
- Clone A emerged in response to NAC, but was pre-existing at a low frequency (7.7%) in the pre-treatment sample and expanded to 71.8% in the post-treatment tissue
- Chemoresistant clone A had two focal hemizygous deletions on chrom 3p, including *IL5RA* and *RARB*

A. K. Casasent *et al,* Cell 2018

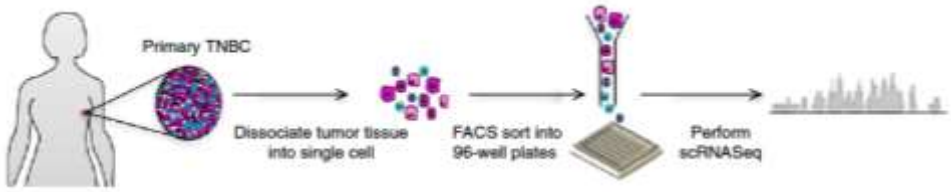
Heterogeneity in TNBC and other: SABCS 2018

Methods and Results



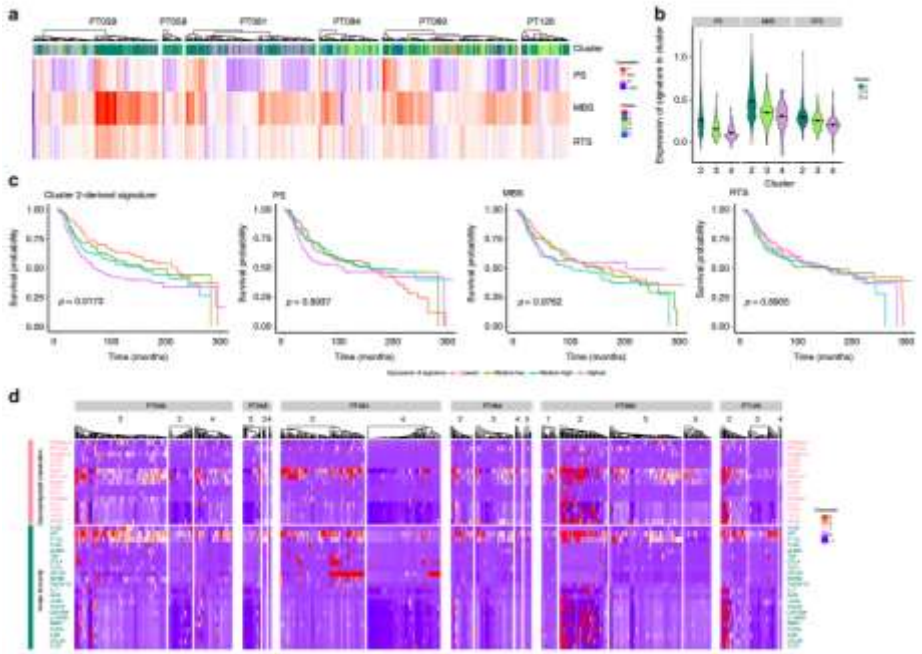
N.E. Navin, *SABCS* 2018
C. Kim *et al,* Cell 2018

Heterogeneity in TNBC and other: SABCS 2018



Unravelling subclonal heterogeneity and aggressive disease states in TNBC through single-cell RNA-seq

Mihriban Karaayvaz[1], Simona Cristea[2,3,4], Shawn M. Gillespie[1,5], Anoop P. Patel[6], Ravindra Mylvaganam[1,5], Christina C. Luo[1,5], Michelle C. Specht[7], Bradley E. Bernstein[1,5,8,9], Franziska Michor[2,3,4,8,9,10] & Leif W. Ellisen[1]

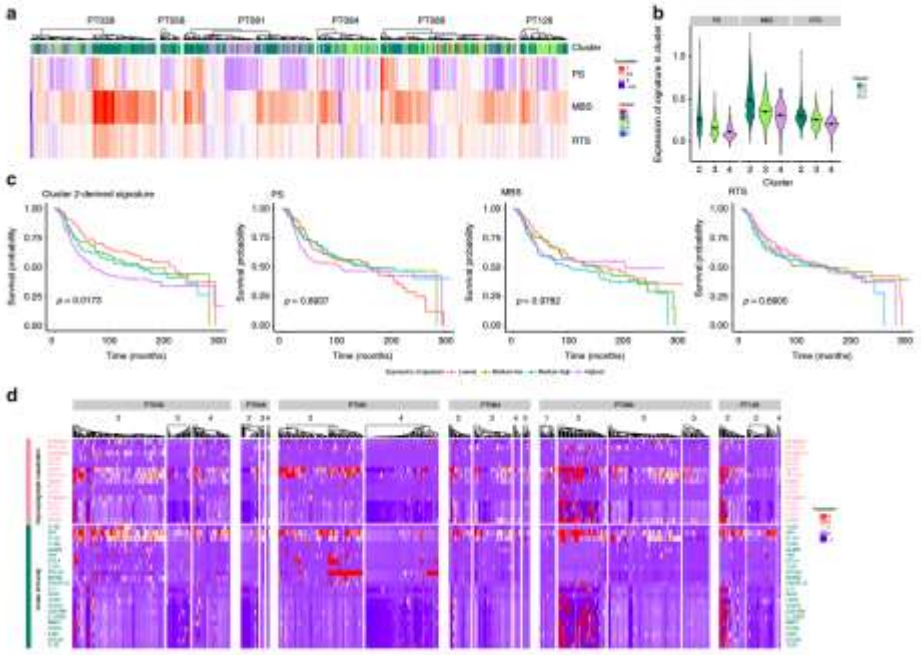


Take home results

- Single cell RNA sequencing of 1189 cells from 6 primary TNBC patients
- Identified a subpopulation of malignant cells shared between tumors, cluster 2
- Malignant subpopulation was associated with metabolism and immunity
- The glycosphingolipid metabolic pathway collelated with outcome: mediates GF signalling, EMT, stem-like behavior
- Glycosphingolipids also module innate and adaptive immunity
- Minor subpopilations shared between patients can determine patient outcomes

M. Karaayvaz *et al,* Natu Comm 2018

Heterogeneity in TNBC and other: SABCS 2018



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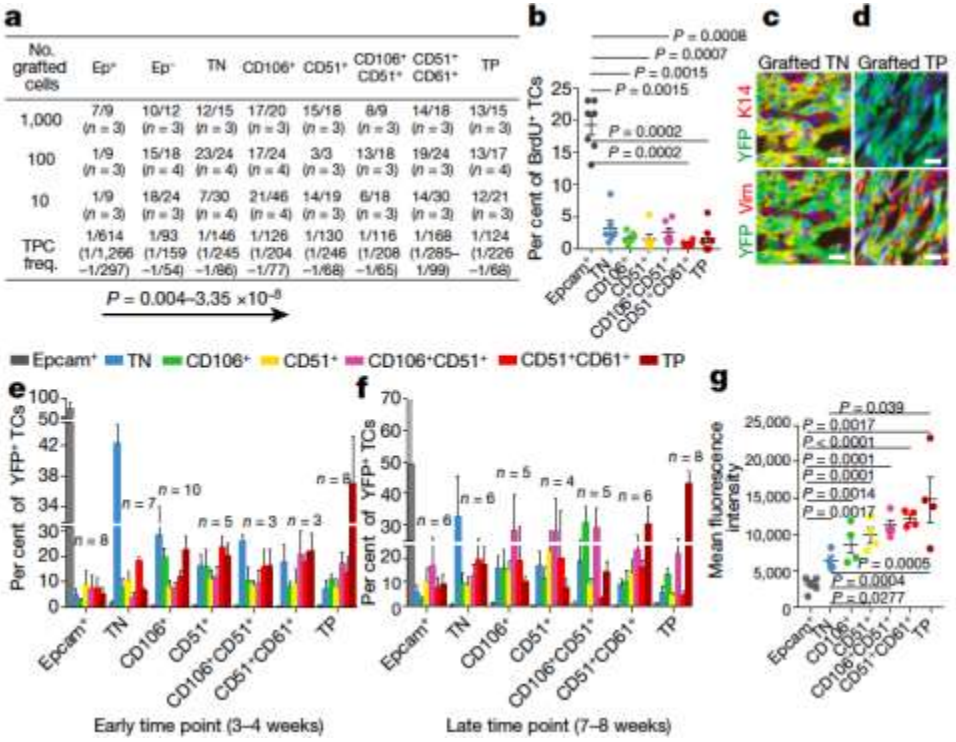
M. Karaayvaz *et al,* Natu Comm 2018

Heterogeneity in TNBC and other: SABCS 2018

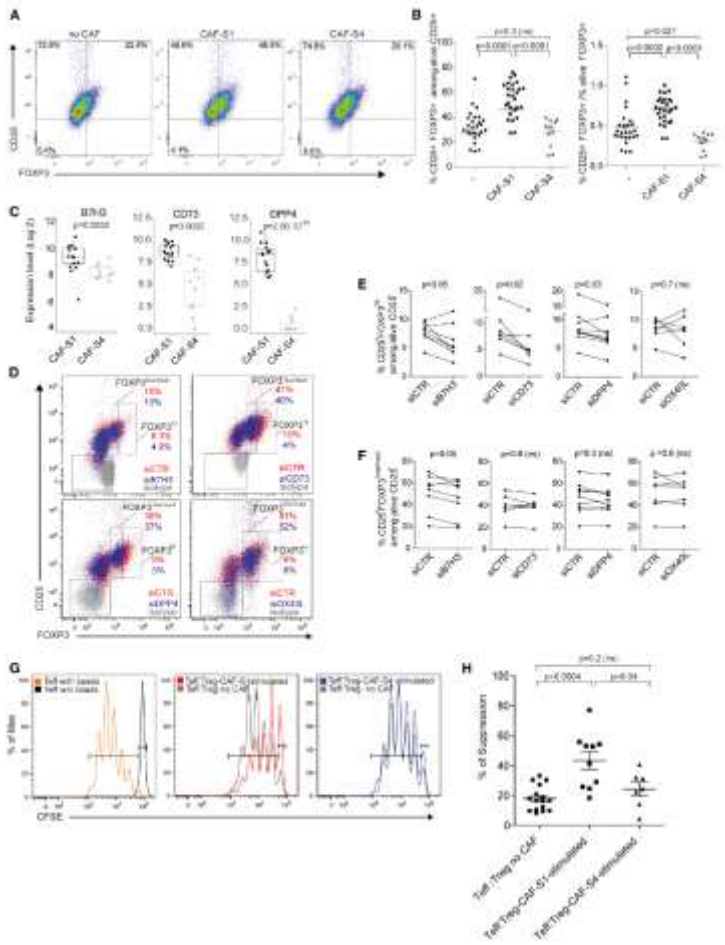


Take home results

- Epcam+ to Epcam – yielded higher stemness
- CD106, CD51 and CD61+ define EMT transition states
- Triple positive (TP) preferential yields TPs
- Itermediate states show grater plasticity
- Higher EMT states more invasive

I. Pastushenko *et al,* Nature 2018

Heterogeneity in TNBC and other: SABCS 2018

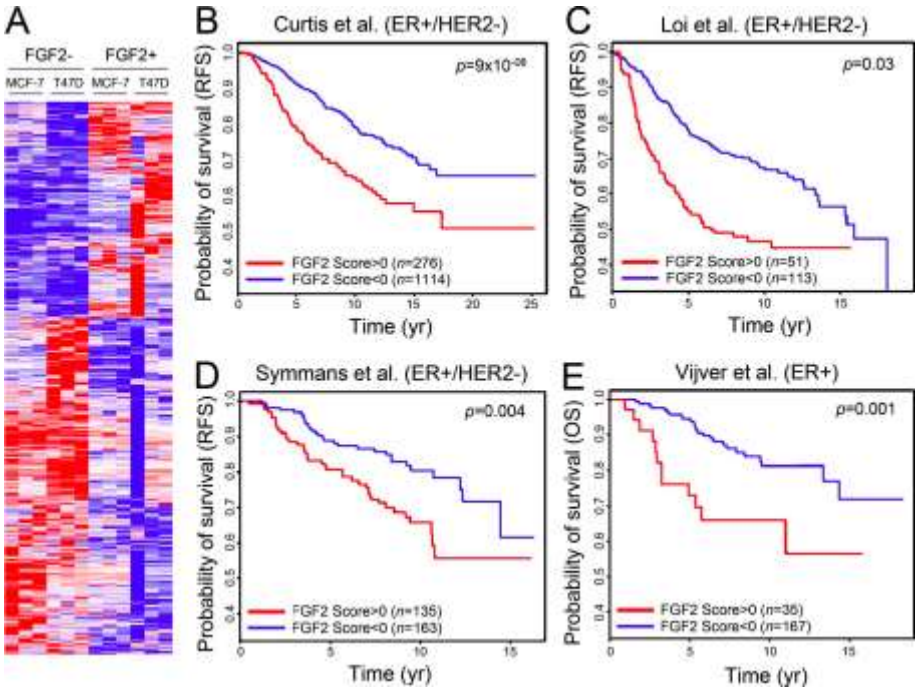


Take home results

- Expression of CD29, FAP, alpha-SMA, PDGFR-beta, FSP1 and CAVI identifield four Cancer Associated Fibroblast (CAF) subsets (CAF-S1-S4)
- CAF subsets show different distribution across breast cancer subtypes
- CAF-S1 is associated with immnosuppression
- CAF-1 enriched TNBC had increased recruitment of T cells, survivall of CD4+CD25+ T cells that differentiate into FOXP3+ T cells and reduced CD8+ R cells
- CAF-S1 enhance the ability of Tregs to inhibit effector T cell proliferation

A. Costa *et al,* Cancer Cell 2018

Heterogeneity in TNBC and other: SABCS 2018



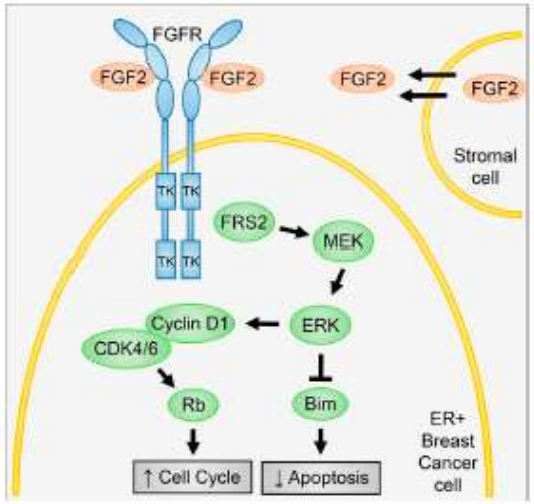


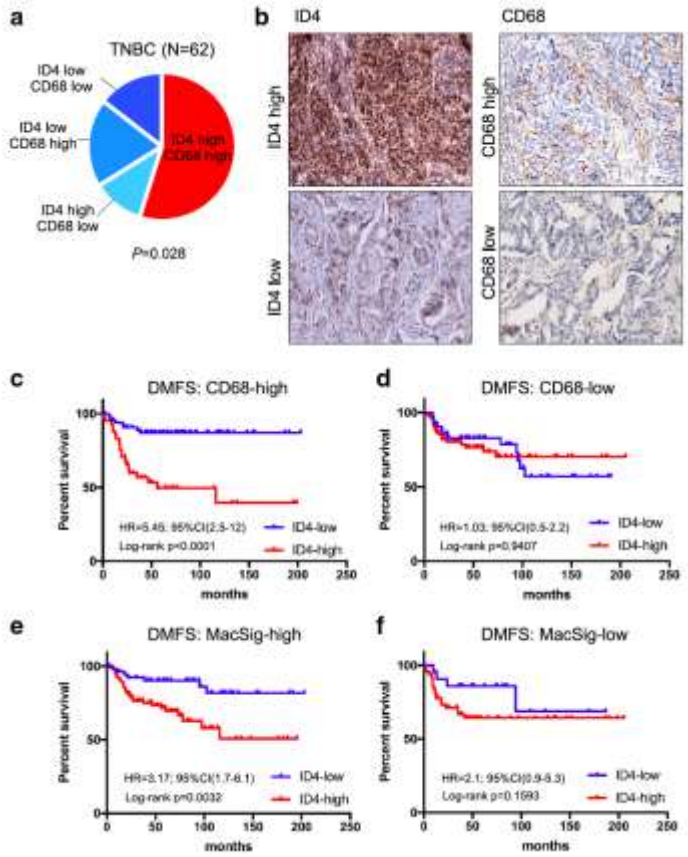
Figure 7. **Model of microenvironmental FGF2–mediated resistance in ER+ breast cancer.**

K. Shee *et al,* JEM 2018

Take home results

- Screening of a library of secreted proteins in tumor microenviroment.
- Fibroblast growth factor 2 mediates drug resistances to drug in ER+ BC.
- RNA sequencing in ER+ BC cell lines revealed a FGF2 response signature that correlates with shorter recurrence free survival.

Heterogeneity in TNBC and other: SABCS 2018



Take home results

- ID4 expression in TBC cells correlates with macrophage recruitment and predicts poor survival in tumors highly infiltrated by macrophages
- TAMs express pro-angiogenic genes and downregulate anti-angiogenic miRNAs in response to ID4 expression in BC cells and this interaction is mediated by secreted VEGFA.

S. Donzelli *et al, BCR* 2018

Food for Thought

- It's importante to integrate the lesson learned from patients and preclinical model to better predict the evolutionary trajectory of cancer evolution to allow to design more effective anticancer therapy

Heterogeneity: Background

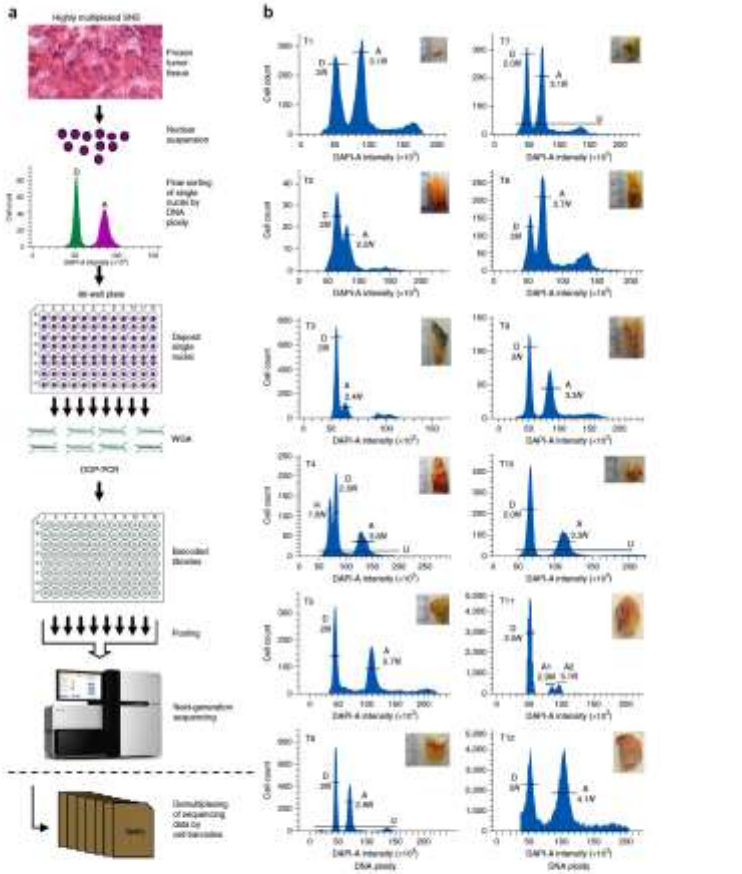


Figure 1 Highly multiplexed single-nucleus sequencing of patients with TNBC. (a) The highly multiplexed single-nucleus sequencing (HM-SNS) method. Tumor tissues are dissociated into nuclear suspensions and stained with DAPI for flow sorting by DNA ploidy. Single nuclei are deposited into 96-well plates and subjected to whole-genome amplification (WGA) by DOP-PCR. Single-cell libraries are barcoded with unique 8-bp identifiers, and 48–96 libraries are pooled for sparse next-generation sequencing. Sequence reads are demultiplexed using cell barcodes after sequencing is completed for copy number profile calculations. (b) FACS plots of DAPI intensity showing the ploidy distribution for each patient with TNBC. Single cells were isolated from different ploidy groups that were gated as diploid (D), hypodiploid (H), aneuploid (A) or universal (U).

R. Gao *et al,* Nature Genetics 2016

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity: Background

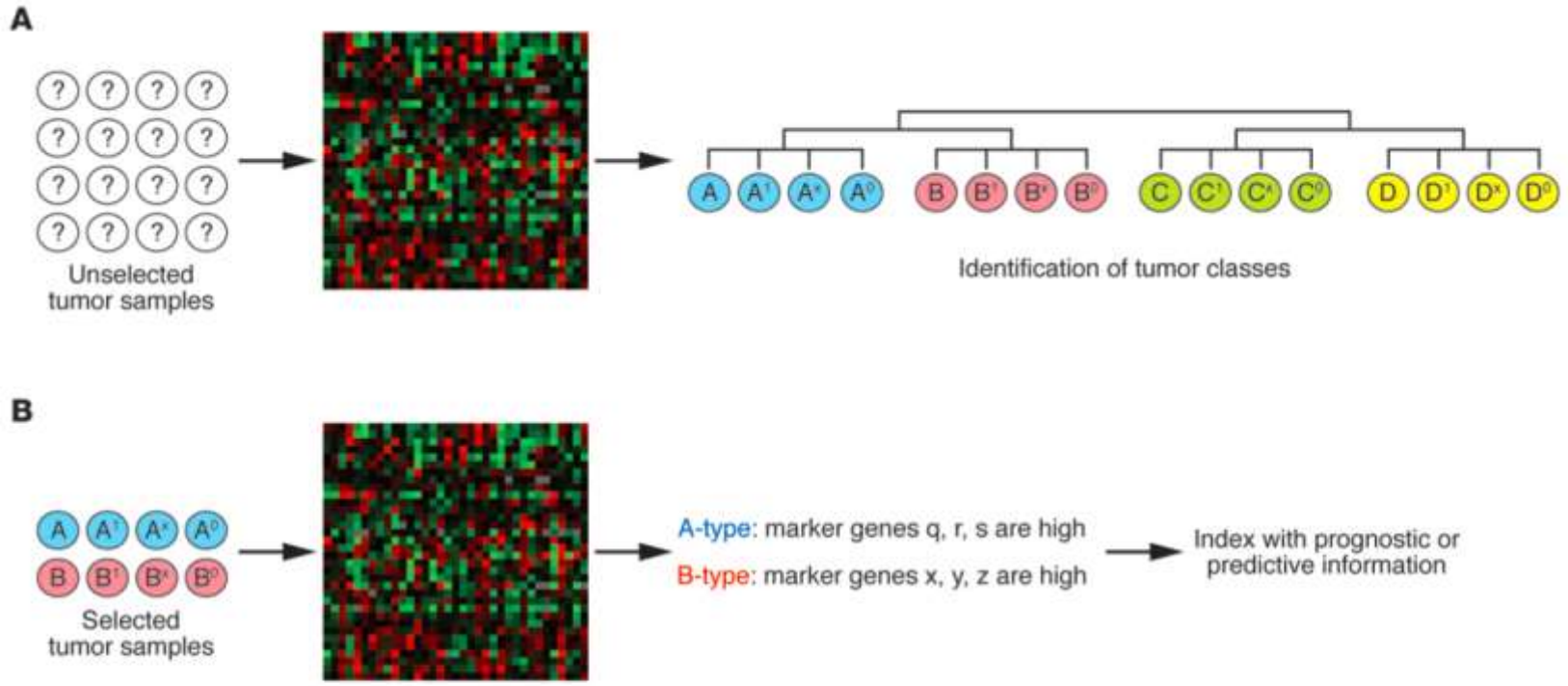
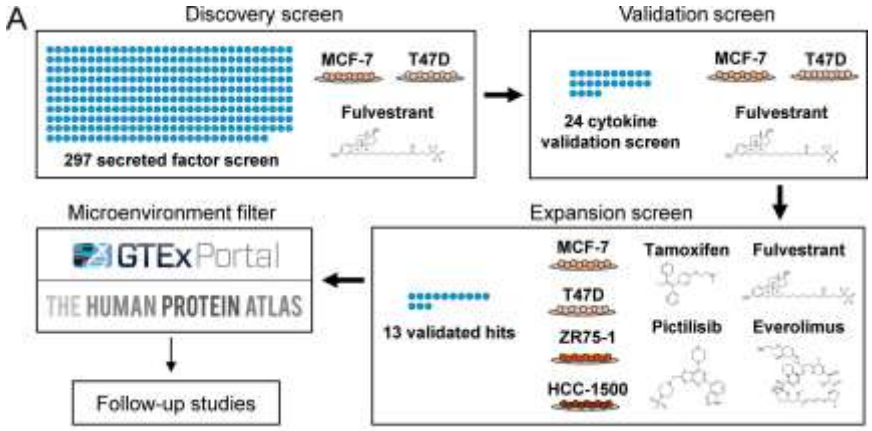


Figure 1
Different study designs for array-based gene expression studies. (**A**) Studies aimed at identifying different subgroups investigate a mixed population of patients to group tumors with similar alterations together, and markers that recognize each type can then be identified. (**B**) This in contrast to studies that search for markers for prediction of therapy response or outcome; here, selected groups of patients are analyzed to identify the most discriminating alterations.

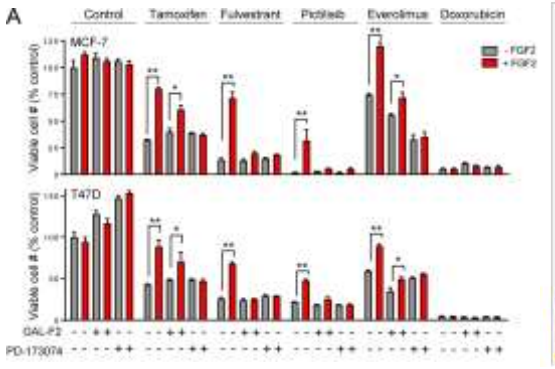
H. G. Russnes *et al.,* JCI 2011

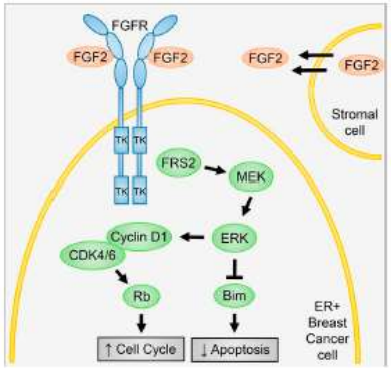
Heterogeneity in TNBC and other: SABCS 2018



Therapeutically targeting tumor microenvironment–mediated drug resistance in estrogen receptor–positive breast cancer

Kevin Shee,[1] Wei Yang,[1] John W. Hinds,[1] Riley A. Hampsch,[1] Frederick S. Varn,[1,3] Nicole A. Traphagen,[1] Kishan Patel,[1] Chao Cheng,[1,3] Nicole P. Jenkins,[2] Arminja N. Kettenbach,[2] Eugene Demidenko,[3] Philip Owens,[5,6] Anthony C. Faber,[7] Todd R. Golub,[8] Ravid Straussman,[9] and Todd W. Miller[1,4]



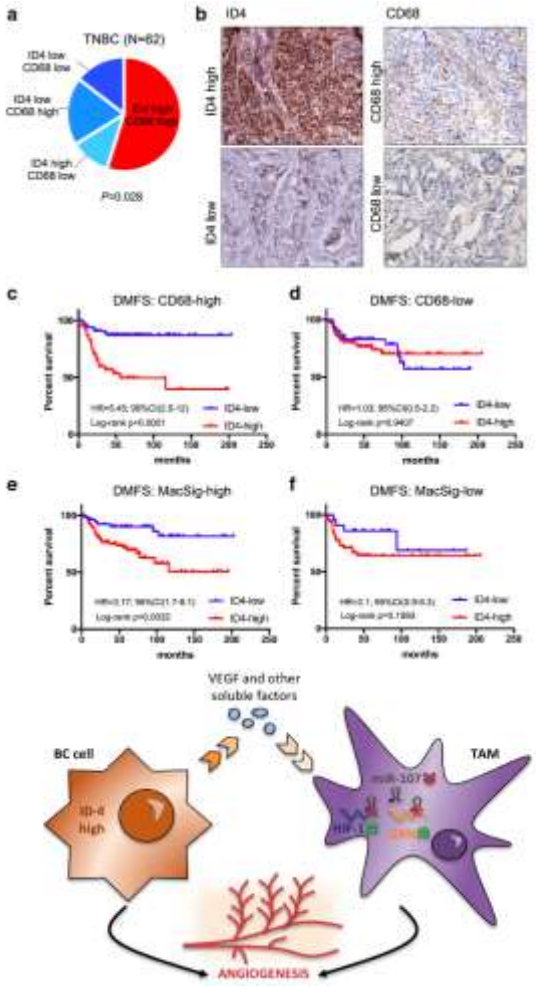


Take home results

- Screening of a library of secreted proteins in tumor microenviroment.
- Fibroblast growth factor 2 mediates drug resistances to drug in ER+ BC.
- RNA sequencing in ER+ BC cell lines revealed a FGF2 response signature that correlates with shorter recurrence free survival.

K. Shee *et al,* JEM 2018

Heterogeneity in TNBC and other: SABCS 2018



RESEARCH ARTICLE Open Access

Expression of ID4 protein in breast cancer cells induces reprogramming of tumour-associated macrophages

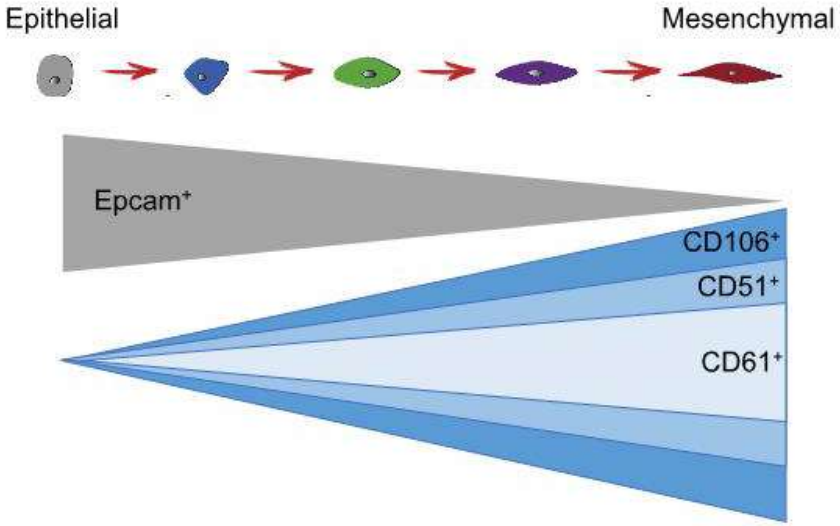
Sara Donzelli[1†], Elisa Milano[1†], Magdalena Pruszko[2], Andrea Sacconi[1], Silvia Masciarelli[3,4], Ilaria Iosue[3,4], Elisa Melucci[5], Enzo Gallo[5], Irene Terrenato[6], Marcella Mottolese[5], Maciej Zylicz[2], Alicja Zylicz[2], Francesco Fazi[3,4*], Giovanni Blandino[1*] and Giulia Fontemaggi[1*]

Take home results

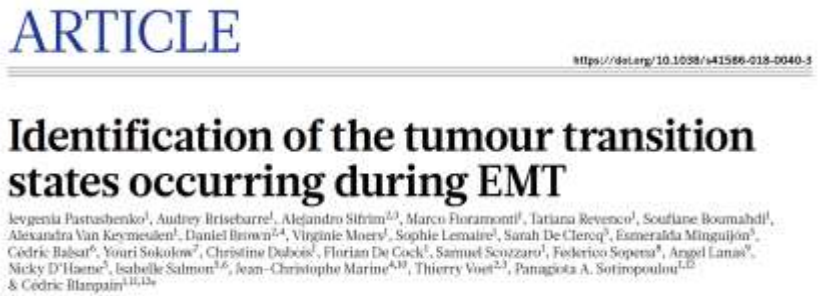
- ID4 expression in TBC cells correlates with macrophage recruitment and predicts poor survival in tumors highly infiltrated by macrophages
- TAMs express pro-angiogenic genes and downregulate anti-angiogenic miRNAs in response to ID4 expression in BC cells and this interaction is mediated by secreted VEGFA.

S. Donzelli *et al, BCR* 2018

Heterogeneity in TNBC and other: SABCS 2018



- CD106: VCAM1 (vascular adhesion molecule 1), expressed on lymphocytes, endothelial cells, myeloid progenitors, bone marrow stromal cells.
- CD51: Integrin alpha 5, expressed on activated T cells, granulocytes, platelets, blastocysts, osteoclasts.
- CD61: Integrin beta 3, expressed on fibroblasts, platelets, osteoclasts and tumor cells.
- CD51 interacts with CD61.



ARTICLE

https://doi.org/10.1038/s41586-018-0040-3

Identification of the tumour transition states occurring during EMT

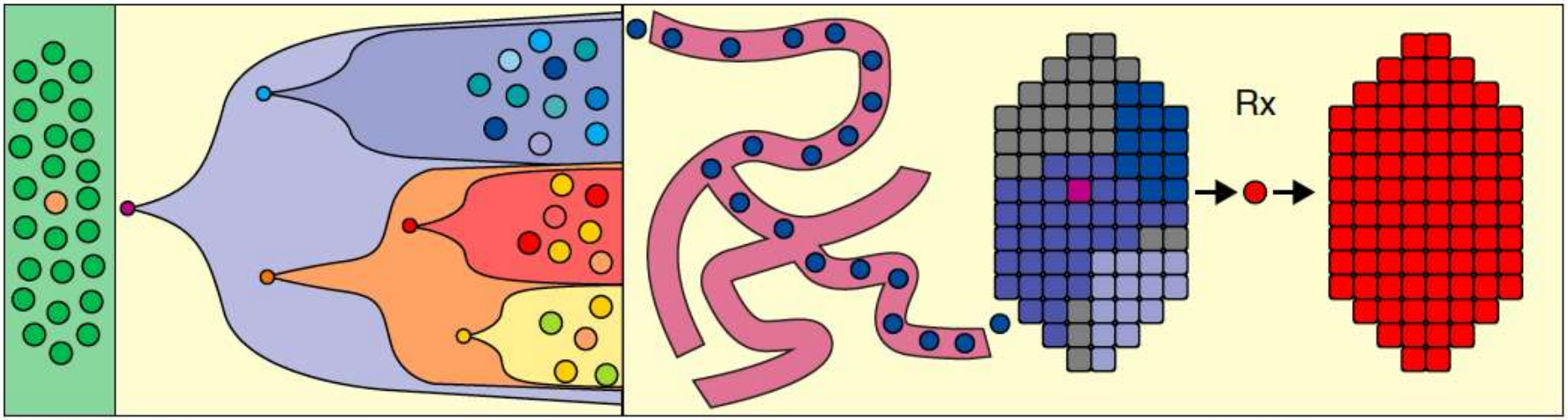
Ievgenia Pastushenko[1], Audrey Brisebarre[1], Alejandro Sifrim[1,2], Marco Fioramonti[1], Tatiana Revenco[1], Soufiane Boumahdi[1], Alexandra Van Keymeulen[1], Daniel Brown[3,4], Virginie Moers[1], Sophie Lemaire[1], Sarah De Clercq[5], Esmeralda Minguijón[5], Cédric Balsat[6], Youri Sokolow[7], Christine Dubois[1], Florian De Cock[1], Samuel Scozzaro[1], Federico Sopena[8], Angel Lanas[9], Nicky D'Haene[5], Isabelle Salmon[5,6], Jean-Christophe Marine[4,10], Thierry Voet[2,3], Panagiota A. Sotiropoulou[1,12] & Cédric Blanpain[1,11,12]*

Take home results

- Epcam+ to Epcam – yielded higher stemness
- CD106, CD51 and CD61+ define EMT transition states
- Triple positive (TP) preferential yields TPs
- Itermediate states show grater plasticity
- Higher EMT states more invasive

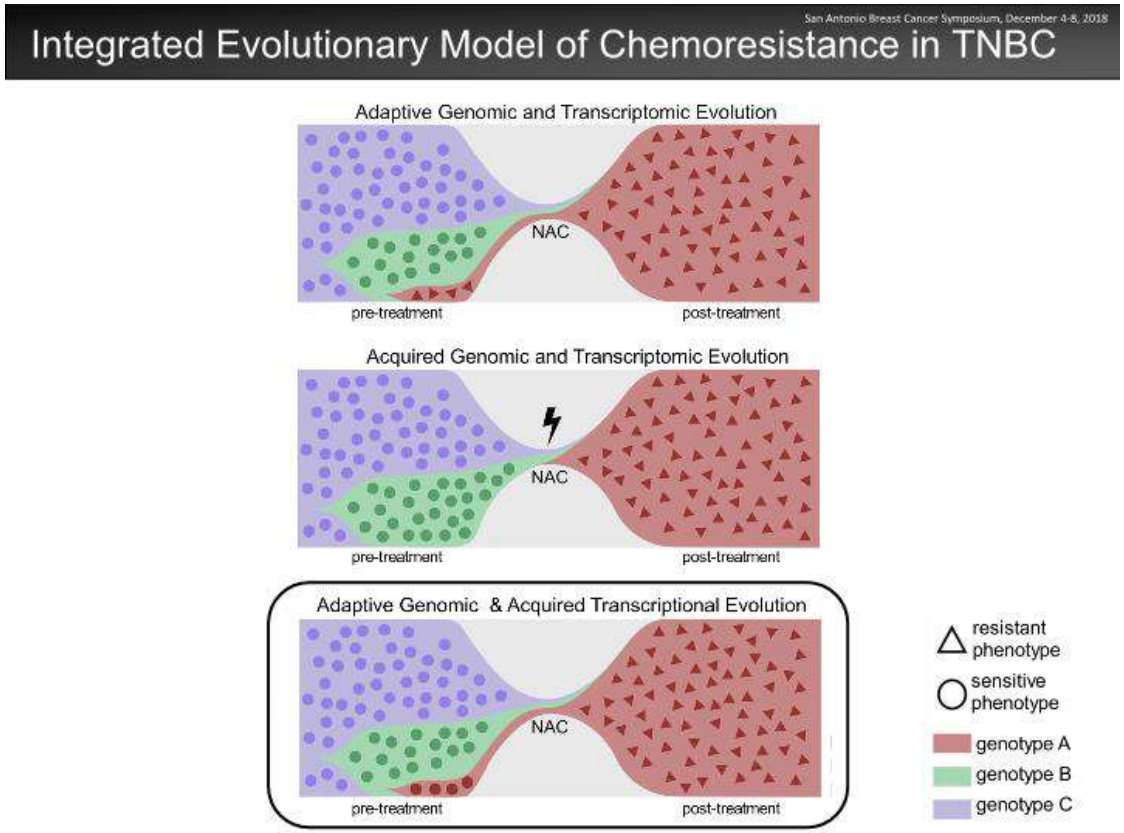
I. Pastushenko *et al,* Nature 2018

Heterogeneity: Background



N. E. Navil, Genome Biology 2014

Heterogeneity in TNBC and other: SABCS 2018



A. K. Casasent *et al,* Cell 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes

Heterogeneity in TNBC and other: SABCS 2018

Clinical Implications

San Antonio Breast Cancer Symposium, December 4-8, 2018

- Diagnostic modalities can potentially be developed to detect copy number aberrations associated with chemoresistance that are pre-existing in TNBC patients prior to treatment to determine which patients will benefit most from NAC
- Therapeutic opportunities include targeting pathways such as *AKT1* signaling, ECM degradation, EMT, *CDH1* targets, hypoxia, angiogenesis to overcome chemoresistance in TNBC patients.
- However study was limited to a detailed analysis of only **4 TNBC patients** that developed chemoresistance, and larger studies are needed to confirm initial results and determine generalizability of findings.
- Several patients (N=3) with pCR had residual mutations detected suggesting that single cell DNA or deep-exome sequencing may provide more sensitive methods for detecting residual disease

: *et al,* Cell 2018

Post San Antonio 2018

Heterogeneity: TNBC and other subtypes