VIRTUAL MEETING
Saturday January 30, 2021

Best of SABCS 2020: Basic and translational discoveries

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KU LEUVEN

LTBCR
Laboratory for Translational Breast Cancer Research

Disclaimer

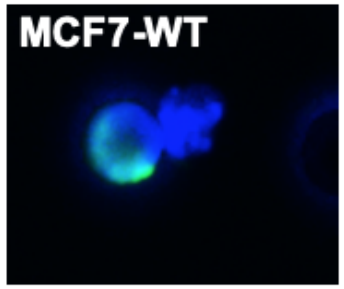
- I had to do a selection- not possible to present everything.
- This is an overview, for more details I invite you to see the original presentations and posters.

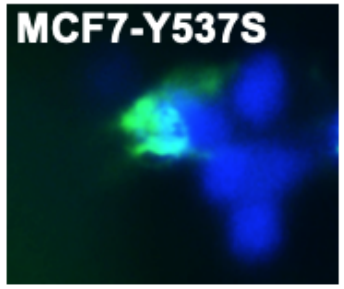
Outline

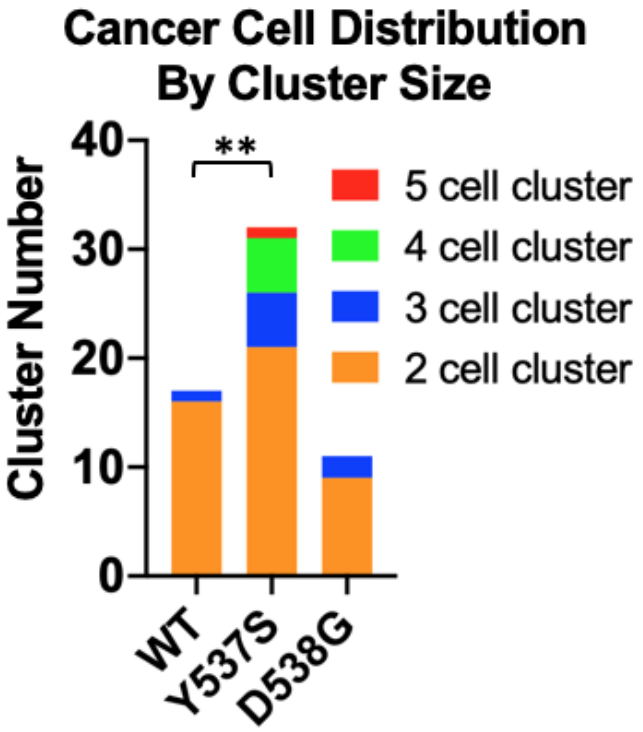
- Prognostic/predictive markers
- Lobular breast cancer
- Genomic testing in plasma
- Studying, monitoring and treating disease progession
- Models (PDTX & PDTOs)
- Ressources to advance research

Prognostic/predictive markers

Li (GS1-05): *ESR1* mutations to facilitate metastases



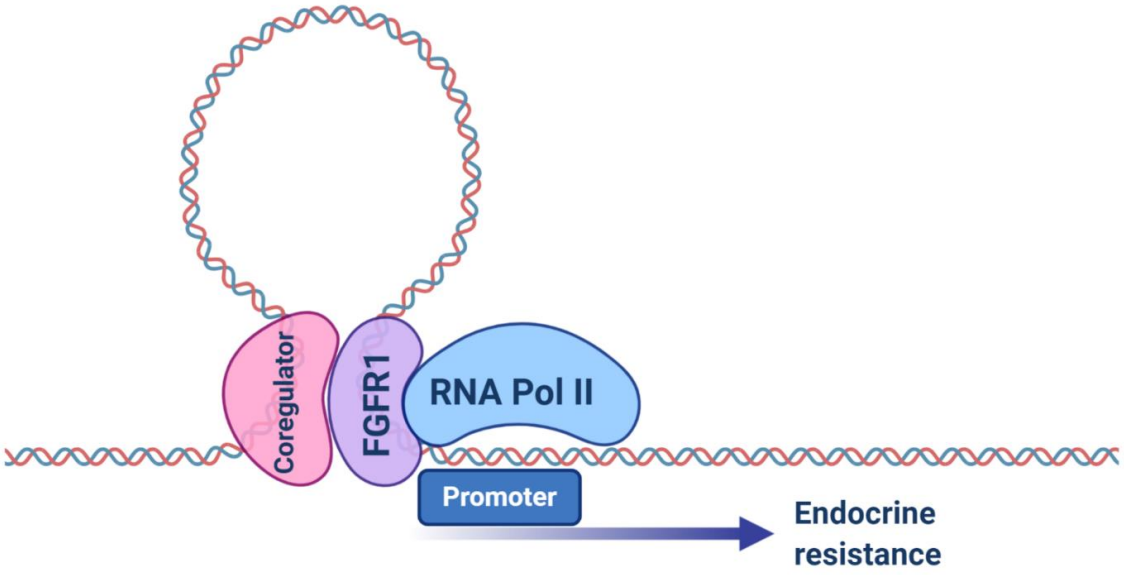




1. *ESR1* mutations mainly found in distant metastases, known to be associated with **endocrine resistance** (mainly to aromatase inhibitors).
2. Various *ESR1* mutant cells models show **increased cell-cell adhesive properties**.
3. Y537S *ESR1* mutant cells form **enlarged CTC cluters** in vivo and promote distant metastasis.
4. **CTC clusters** are significantly enriched in *ESR1* mutant breast cancer patients.

Ccl: Novel role highlighted for *ESR1* mutations beyond endocrine resistance.

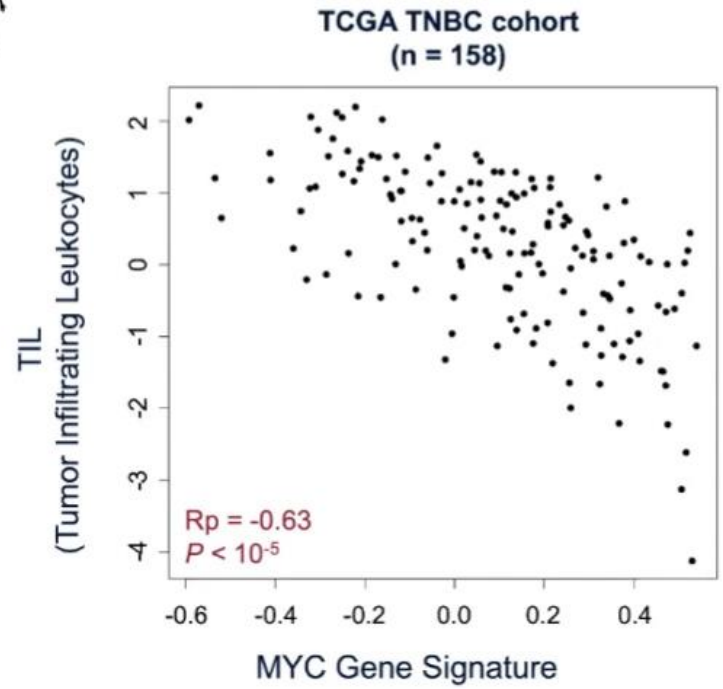
Servetto (GS1-06): FGFR1 & endocrine resistance



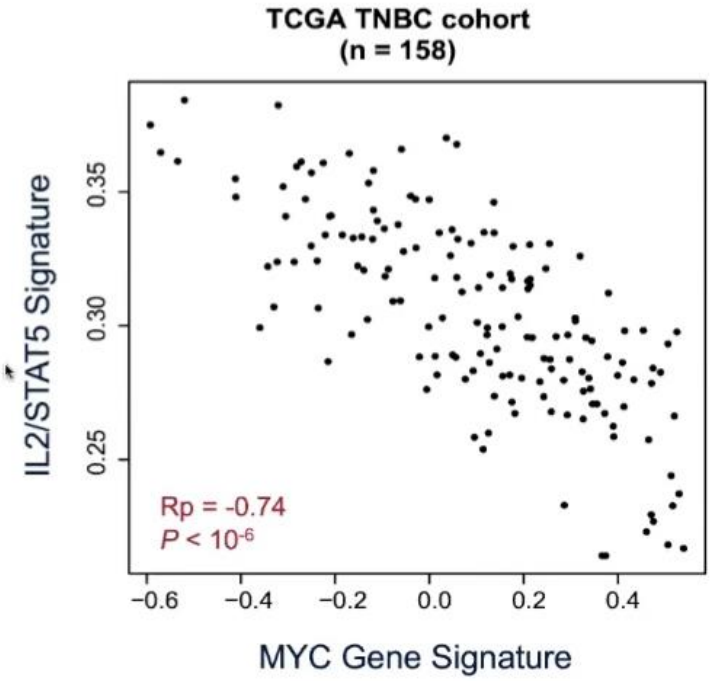
- *FGFR1* amplification is present in ~15% ER+ BC.
- *FGFR1* amplification → nuclear expression of FGFR1.
- Nuclear FGFR1 occupies chromatin at promoter regions of thousands of genes → direct modulation of gene transcription in ER+ BC.
- Need to develop treatment strategies to inhibit nuclear FGFR1 in ER+/FGFR1 overexpressing BC.

Lee (GS1-08): MYC & immune infiltrates

Patient tumors with a high MYC signature have low T-cell activity



Higher MYC Signature = less immune cell infiltration



Higher MYC Signature = less T-cell cytokine signaling

- High MYC expression → low sTILs and low T-cell cytokine signaling
- MYC also downregulates MHC-I in vivo

Ccl: TNBC tumors with MYC amplified/overexpressed tumors might require additional therapies to stimulate the immune system.

Bild (ES3-01): resistance mechanisms in early HR+ BC pts treated with endocrine & CDK inhibitors

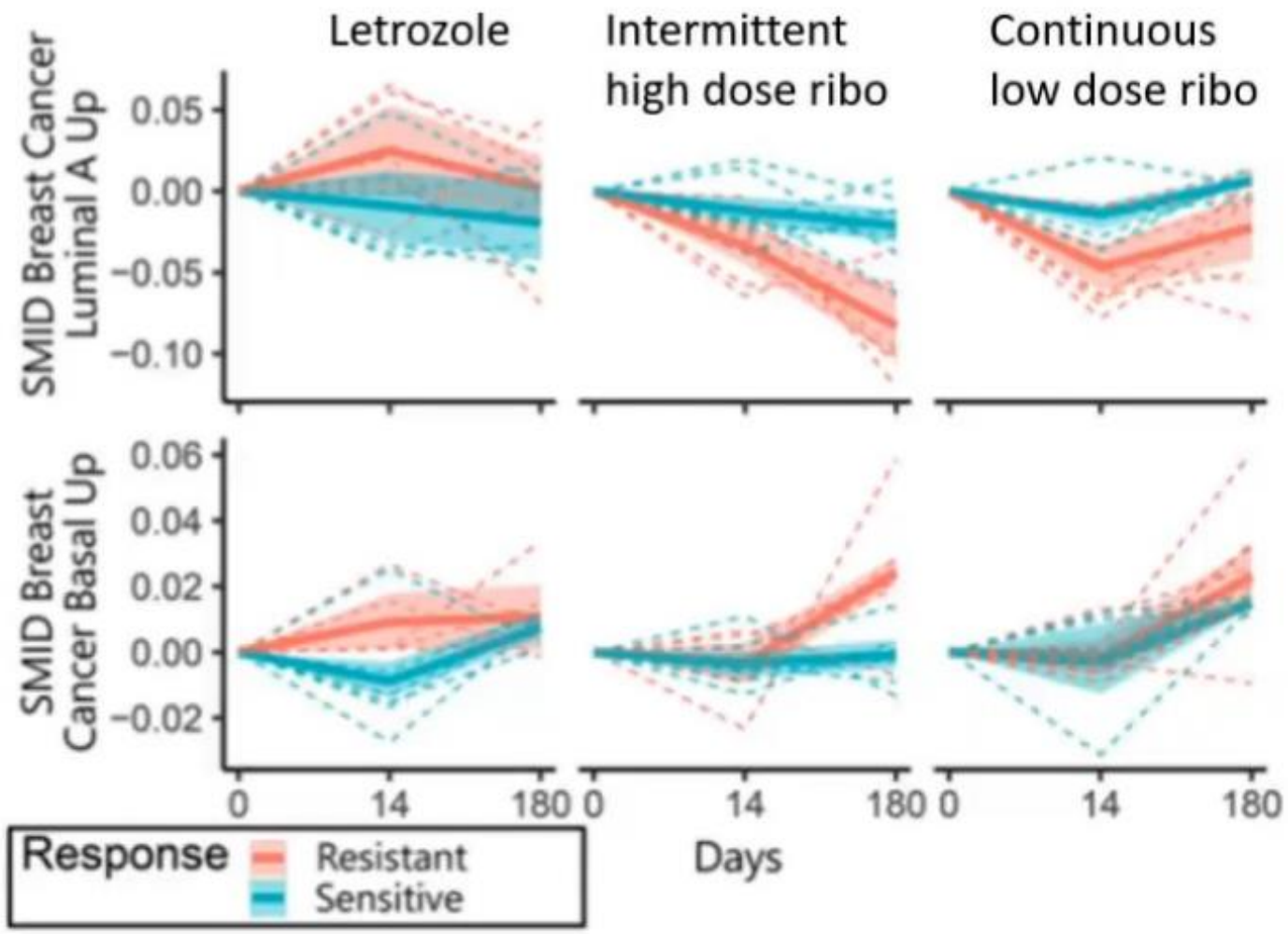
FELINE trial (NCT02712723):
Letrozole Plus Ribociclib or Placebo as Neo-adjuvant Therapy in ER-positive, HER2-negative Early Breast Cancer.

No ≠ in primary endpoint (PEPI score)
~25% of PEPI 0 @ surgery with and without ribociclib*.

Single-nuclei RNA sequencing
On samples taken at 3 timepoints (d0, d14 and d180)

*DOI: 10.1200/JCO.2020.38.15_suppl.505

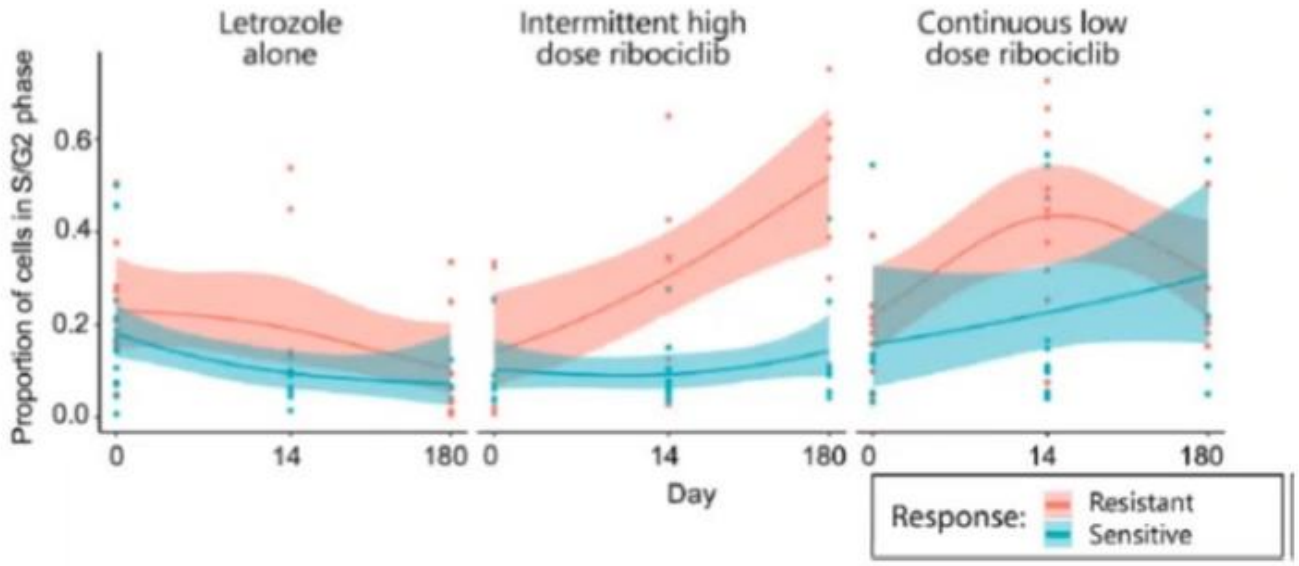
N=45 patients (~15/arm, and half resistant)



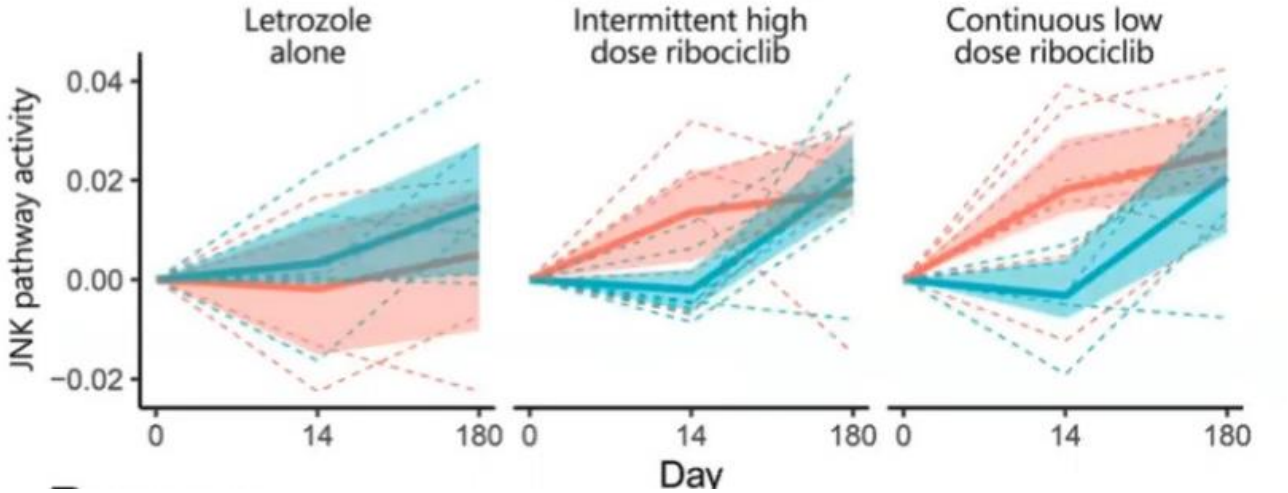
Letrozole alone arm:
Insufficient blocking of estrogen signaling in resistant patients.

Letrozole + ribo arms:
Diminished ER-pathway activity accompanied by a transition from luminal-like to basal-like in resistant patients.

Each hatched line represents a unique patient single cell's pathway average



Proliferative state:
Resistant tumors in combination arms have a higher proportion of proliferative cells

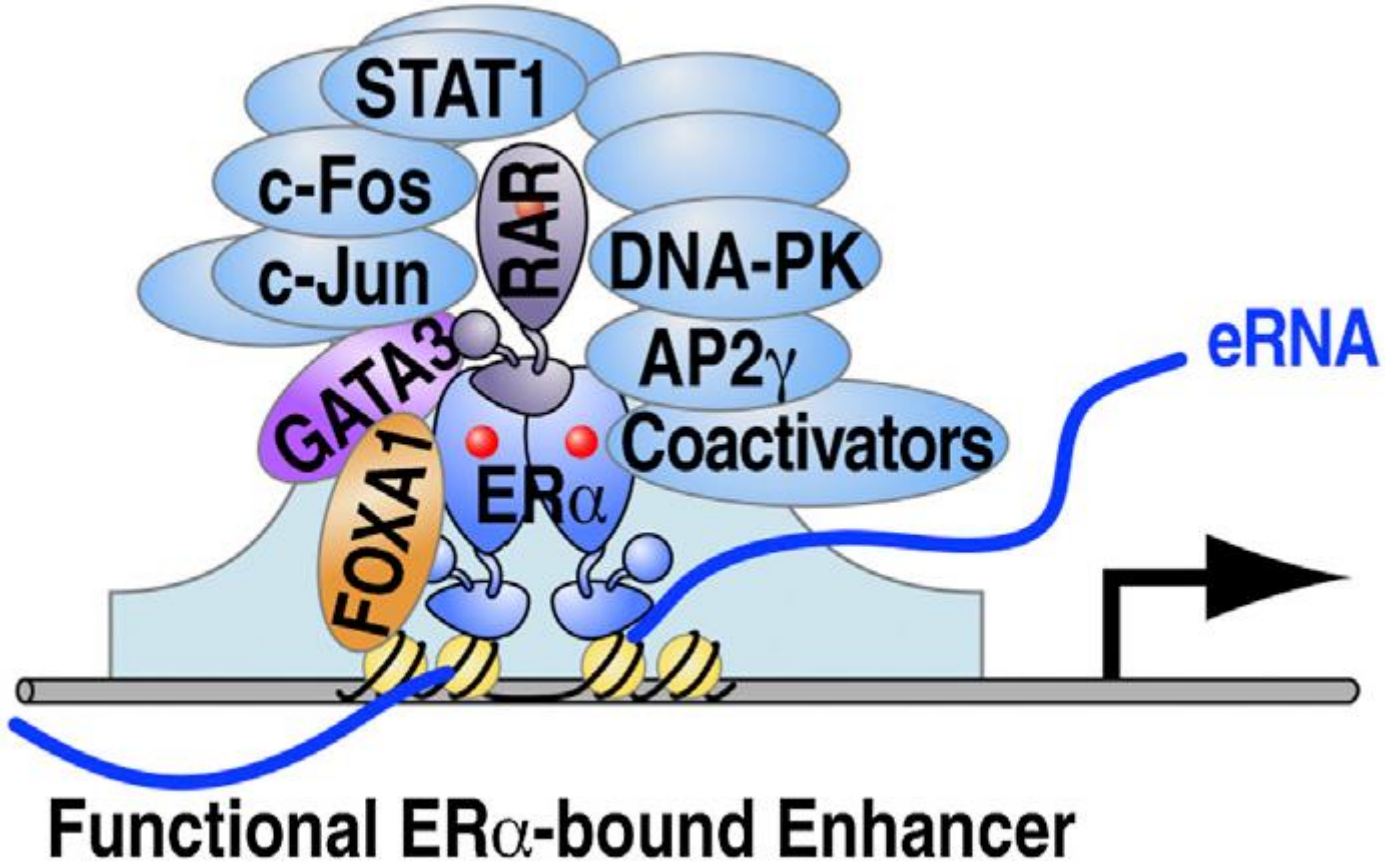


Increase in JNK pathway signaling:
At day 14 in the resistant tumors of the combination arms.

Ccl: 1) excellent **model to study resistance mechanisms** (preoperative setting, molecular analyses @ different time points) even when clinical endpoints are not met; **2) new resistance mechanisms** unraveled to be validated.

Lobular breast cancer

Jeselsohn (ES9-02): FOXA1 & lobular breast cancer



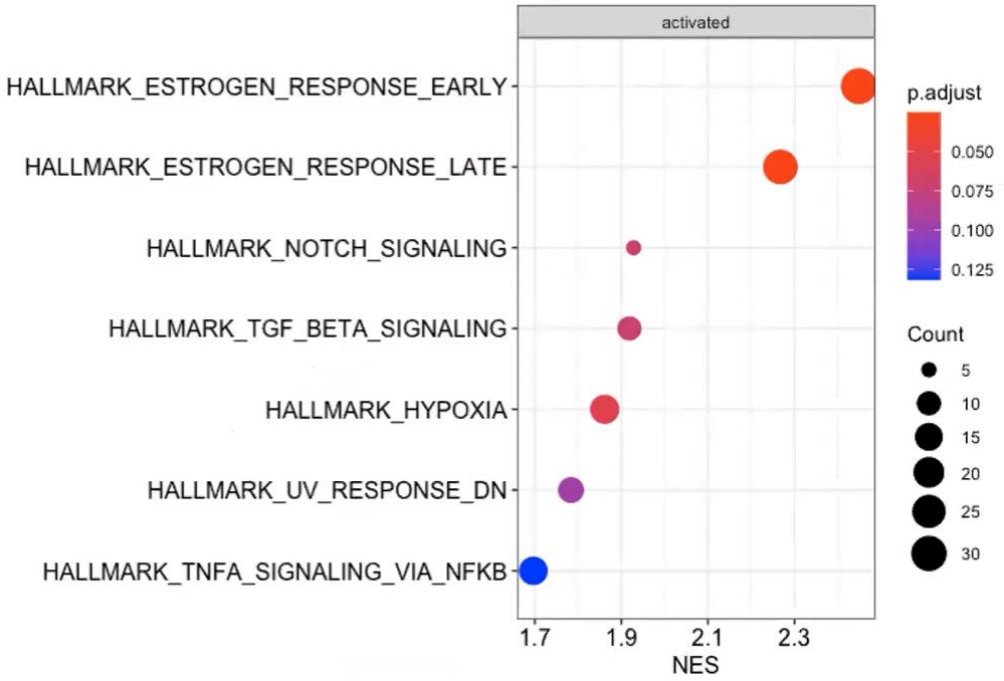
FOXA1 is one of the key regulators of the ER-dependent transcriptional program[1].

***FOXA1* mutations** are more frequent in ILC compared to IDC➔ increased FOXA1 expression & chromatin binding[2]

FOXA1 mutations are associated with resistance to endocrine treatment[3].

1. Liu et al. Cell 2014, 2. Ciriello et al. Cell 2015 & Desmedt et al. JCO 2016, 3. Arruabarrena-Aristorena et al. Cancer Cell 2020

Jeselsohn (ES9-02): FOXA1 & lobular breast cancer

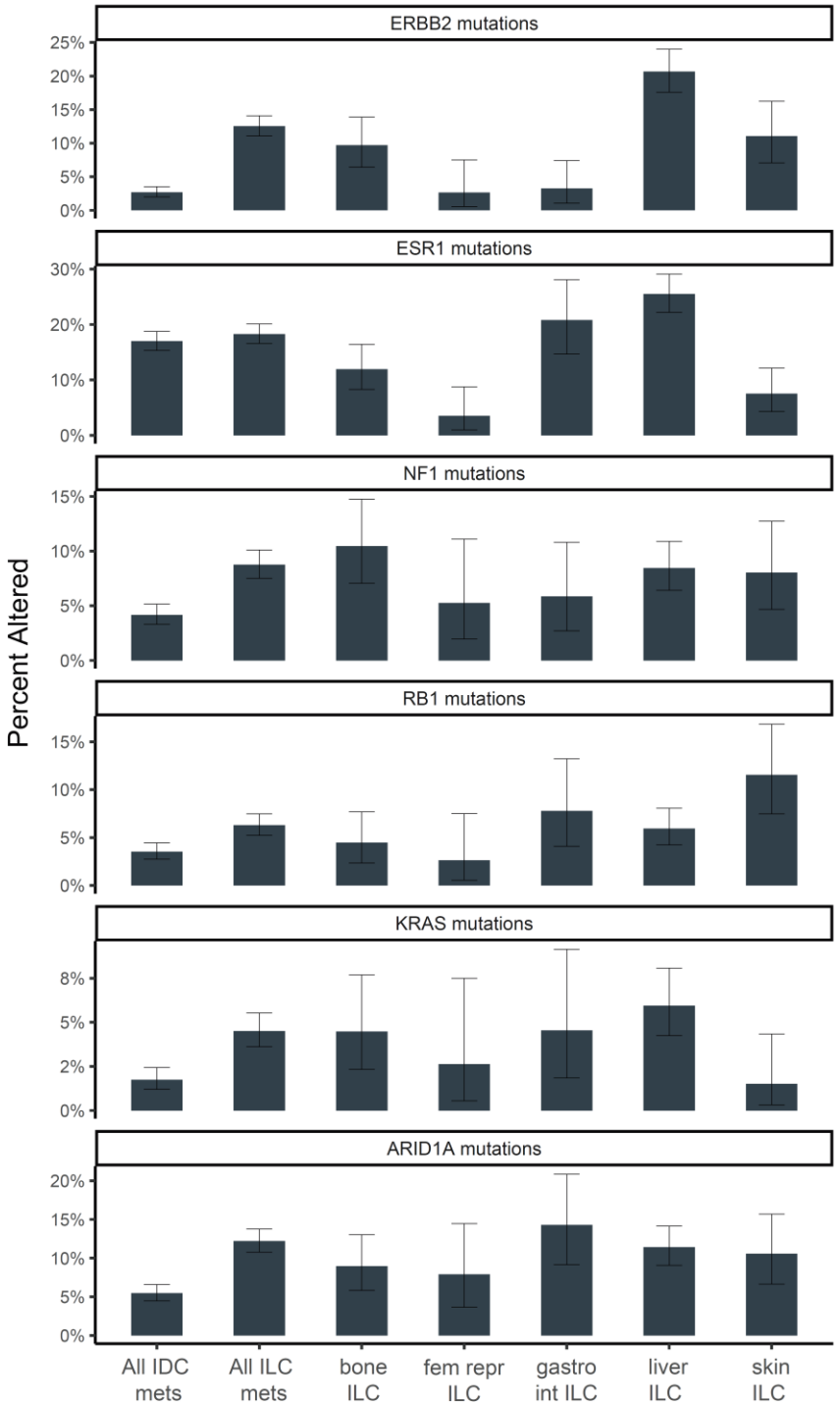


New mechanims for **auto-upregulation FOXA1 expression**

Experiments in ILC models, FOXA1 expression:
Increase ER expression
Unique landscape of chromatin accessible sites ➔ different ER-regulated transcriptional program

FOXA1-associated transcriptional program:
- Associated with worse prognosis in ER+ BC (still to be shown in ILC)
- Associated with resistance to tamoxifen

Ccl: 1) new insights about ILC/IDC differences in ER-transcriptional program ; **2)** more research needed to indivudualize endocrine therapy according to histological subtype.



Sokol (PD8-01): Genomics according to metastatic sites.

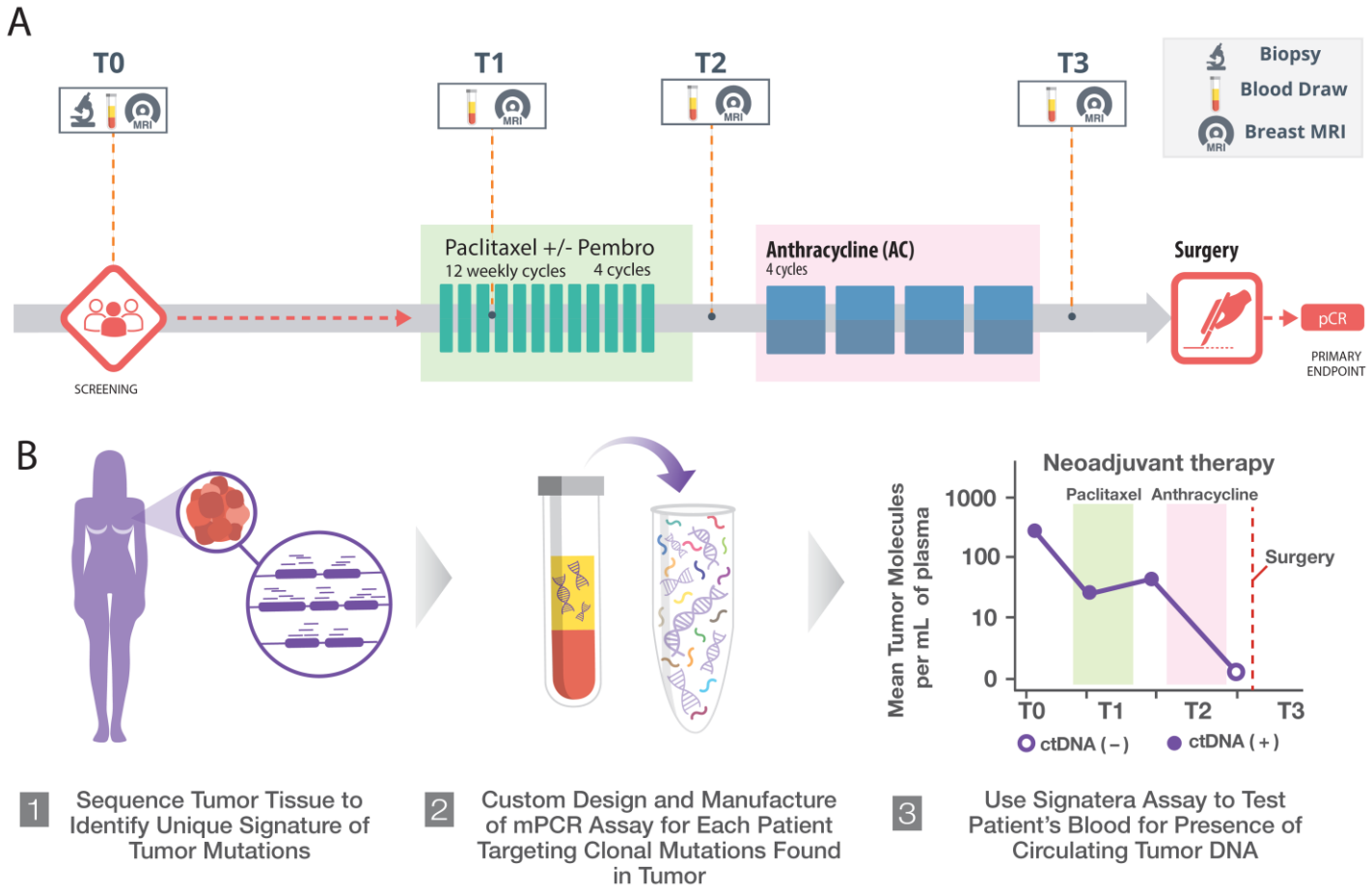
Combined analysis of **1,909 metastases** sequenced by Foundation Medicine (all ILC considered, not only ER+):
154 GI mets
639 liver mets
114 mets reproductive organs
268 bone mets
199 skin mets

- Alterations in *PIK3CA* common in all ILC mets ➔ broad utility for PIK3CA inhibitors.
- *ERBB2* mutations most prevalent in ILC liver mets.
- *RB1* mutations highest in gastrointestinal and skin sites

Ccl: 1) largest analysis of ILC metastases done so far, **2)** incidence therapeutic targets/ markers of resistance might differ according to site.

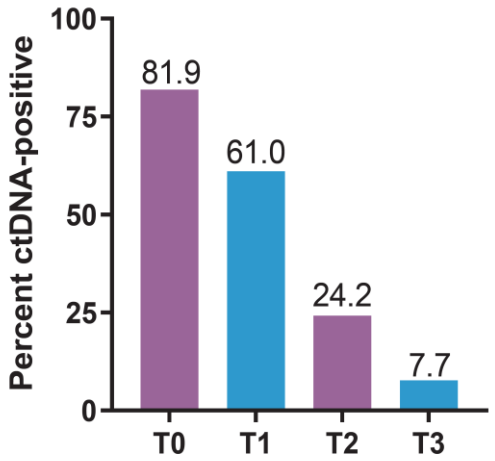
Genomic testing in plasma

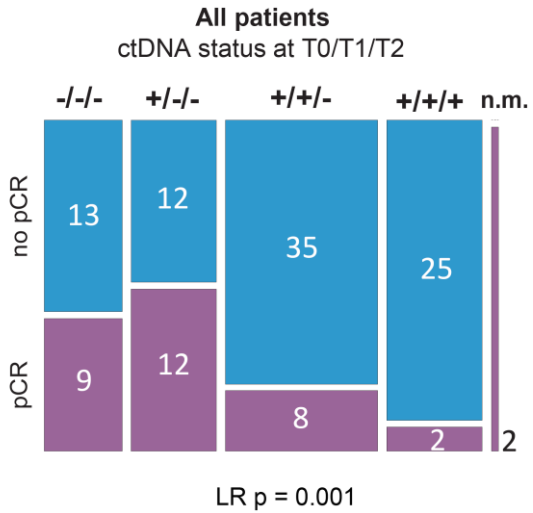
Magbanua (PD9-02): personalized ctDNA monitoring in I-SPY2



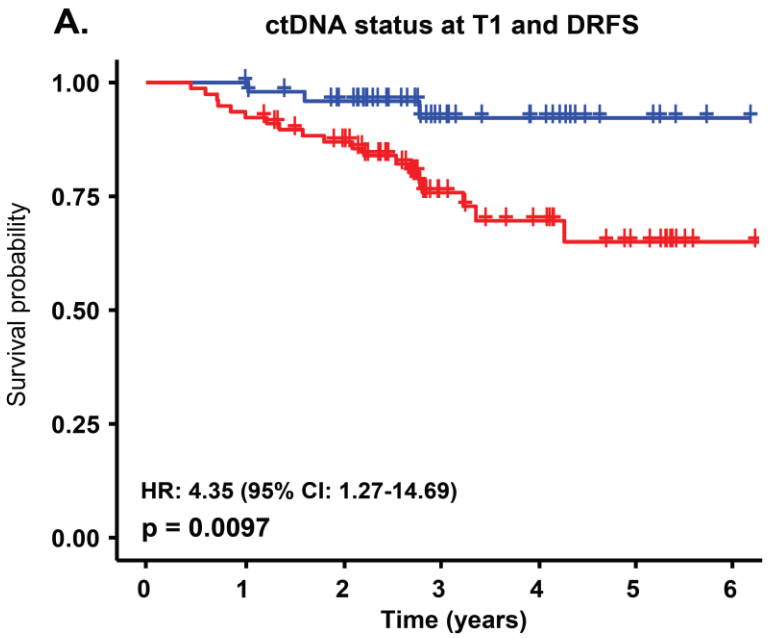
138 patients with high-risk HR+/HER2- (n=77) or TN (n=61) stage II/III EBC.

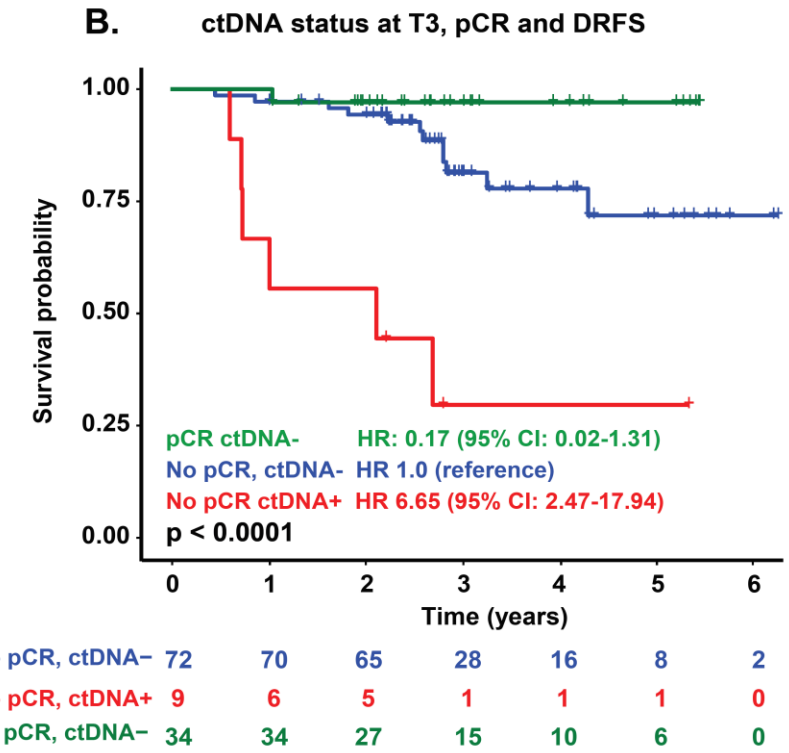
Blood collected at 4 time points and patient specific mutations tested.





- **Decrease in ctDNA positivity over time**
- **pCR rate the highest in patients with early ctDNA clearance** (ctDNA+/-/-: 50% pCR), followed by late ctDNA clearance (ctDNA+/+/-: 19% pCR) and the lowest in patients with lack of ctDNA clearance (ctDNA+/+/+: 7% pCR)

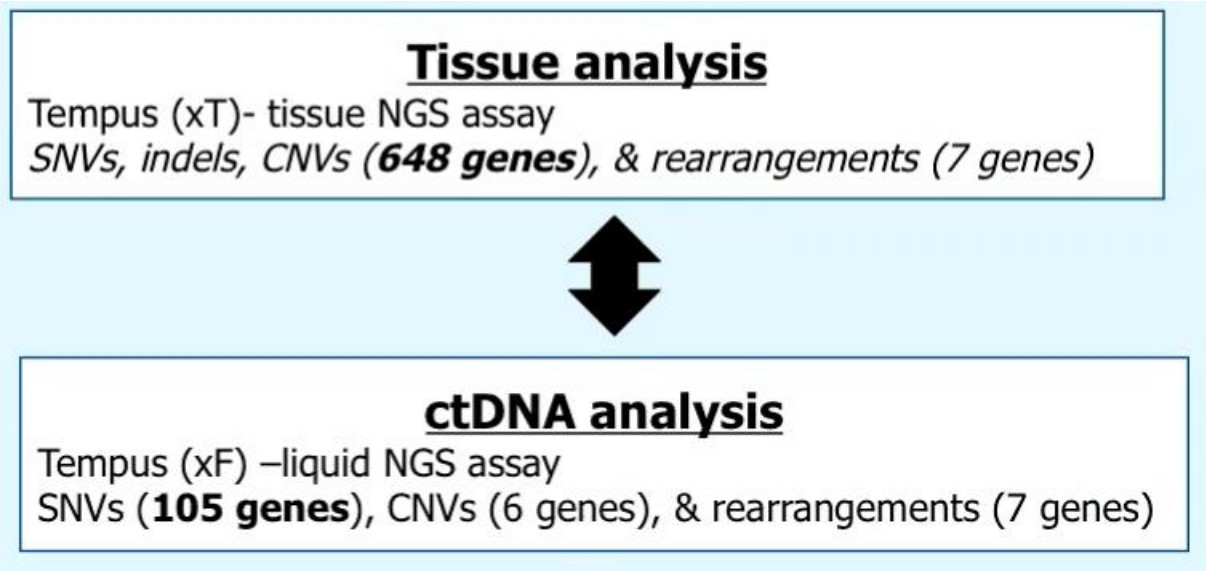




Residual ctDNA after NAC treatment is a significant predictor of metastatic recurrence and death.

Ccl: Personalized monitoring of ctDNA during NAC is feasible and provides information that can be combined with imaging and pathology.

Liu (PD9-12): concordance tissue & ctDNA

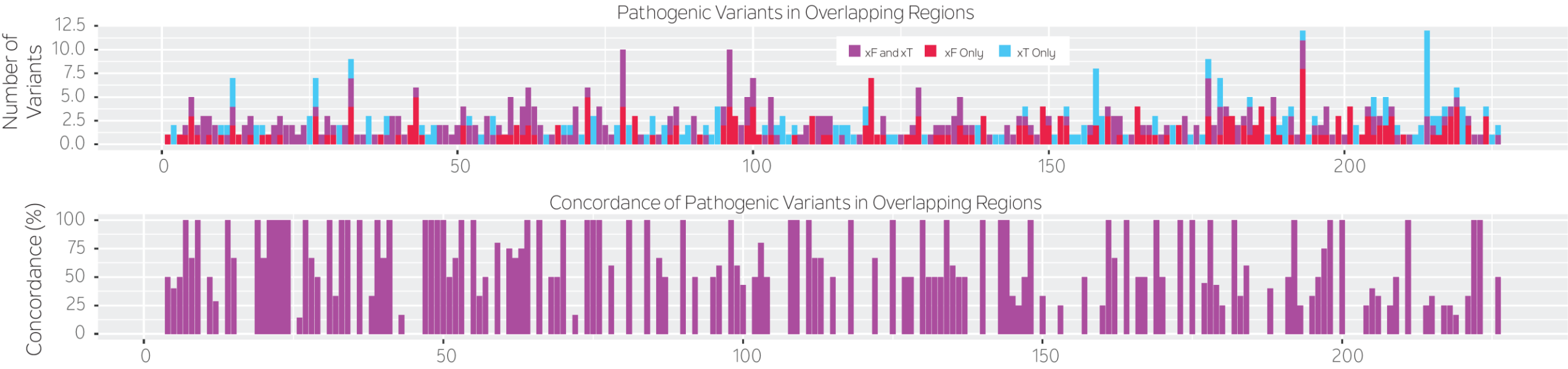


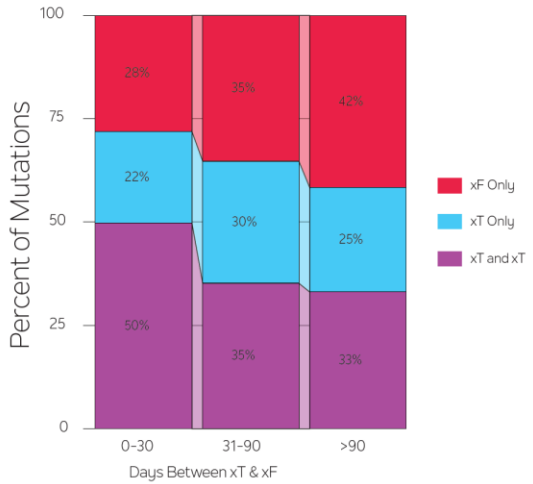
Aim= to evaluate concordance between tissue and cfDNA in paired metastatic breast cancer biopsies

Table 1. Clinical Characteristics of Cohort

		Count (n=226)	Percent
Age at xT test	Median (sd)	57 (11.74)	
Sex	Female	224	99.12%
	Male	2	0.88%
Self-reported race	White	113	50.00%
	Black or African American	29	12.83%
	Asian	8	3.54%
	American Indian or Alaska	1	0.44%
	Other Race	7	3.09%
	Not Reported	68	30.09%
Stage	Stage 4	226	100.00%
Sample type	Primary breast	59	25.66%
	Metastatic lesion	167	74.34%
Subtype	HR+, HER2-	95	42.03%
	TNBC	29	12.83%
	Other*	102	45.13%
Days between xT and xF tests	> 90 days	156	55.32%
	31 – 90 days	35	12.41%
	0 – 30 days	91	32.27%

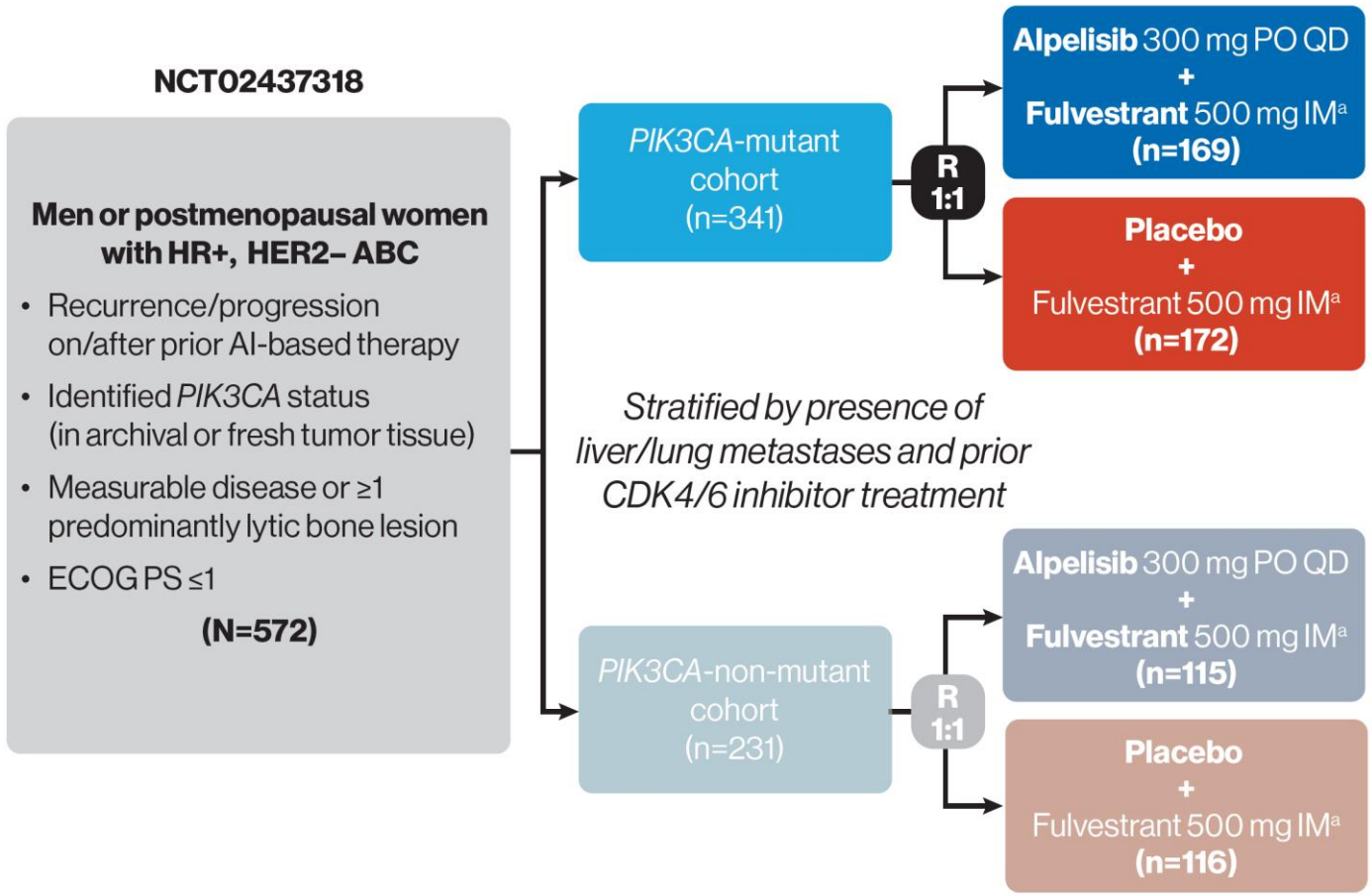
*Other subtypes included those with less than 15 samples, such as HR+/HER2+and HR-/HER2+, and samples in which receptor status was unclear or conflicting.





- **Limited concordance** between tissue and liquid biopsy, with mutations detected only in tissue or in liquid sample (sensitivity assay & intra-patient heterogeneity).
- **Concordance is dependent of time interval between tissue and liquid biopsy**: 50% concordant when tests were performed within 90 days of each other, less if >90 days.
- Authors suggest **concurrent tissue and liquid biopsy genomic testing** at time of diagnosis to provide the most comprehensive genomic characterization.

Ciruelos (PD2-06): PIK3CA testing PCR vs NGS, plasma vs tissue in SOLAR-1 trial

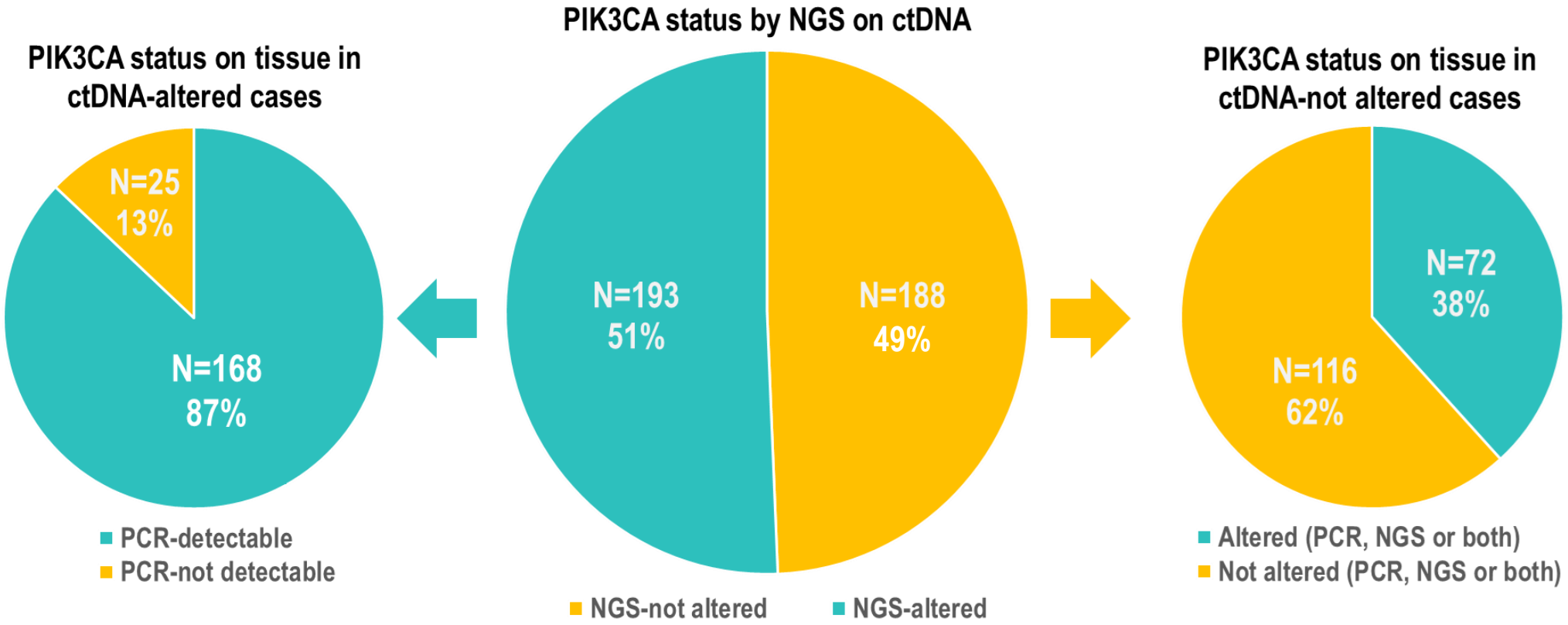


- **Initial *PIK3CA* mutation testing**= 11 specific mutations in exons 7, 9, and 20 by PCR in archived or fresh tumor tissue.

- **PFS in *PIK3CA* mutated population**: hazard ratio [HR] 0.65; 95% confidence interval [CI], 0.50-0.85; 1-sided *P*=0.00065 (primary endpoint)

- the full exonic region of *PIK3CA* gene was sequenced in tumor tissue (FoundationOne® CDx 324-gene panel) and plasma ctDNA (FoundationOne® Liquid CDx 311-gene panel).

➔ report about concordance



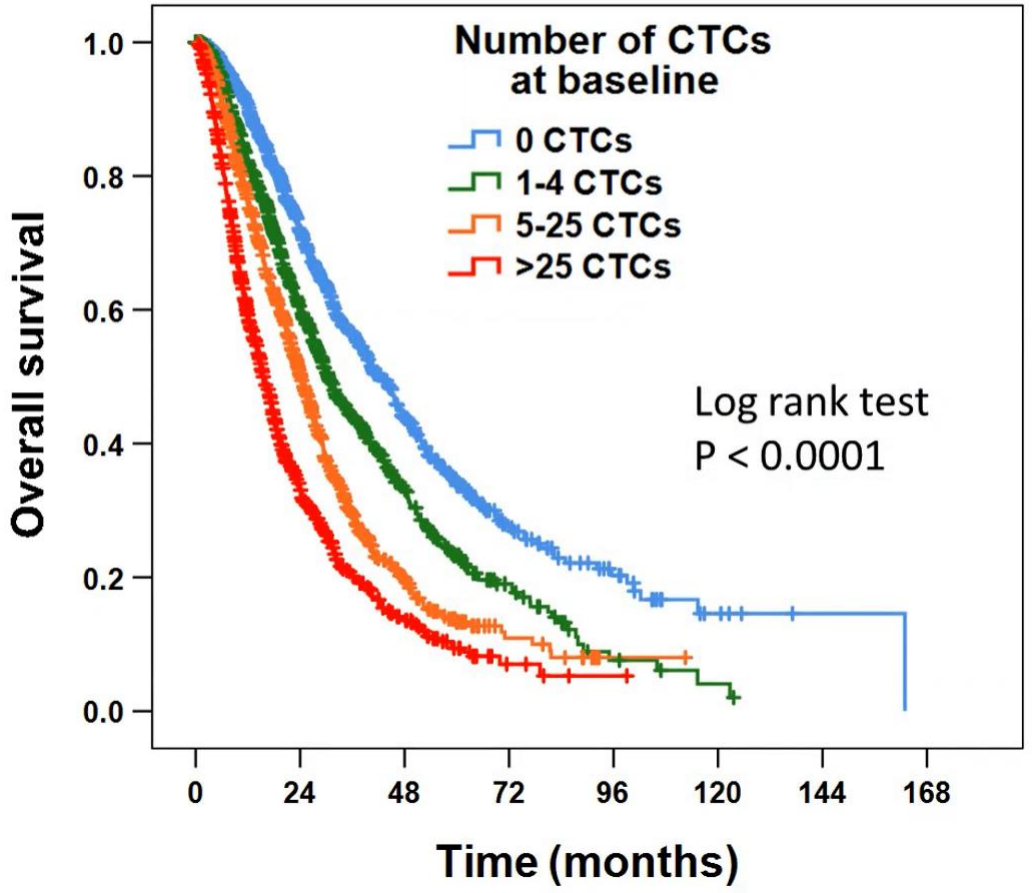
ctDNA alterations by NGS not detected by tissue PCR (yellow): likely due to rare additional mutations covered by NGS.

alterations detected in tissue but not in plasma (green): likely due to heterogeneity of the disease, reduced tumor shedding, and sensitivity of the assays.

Ccl: When no *PIK3CA* alteration is detected by ctDNA liquid biopsy, a tissue test should be performed to confirm the *PIK3CA* status of the tumor.

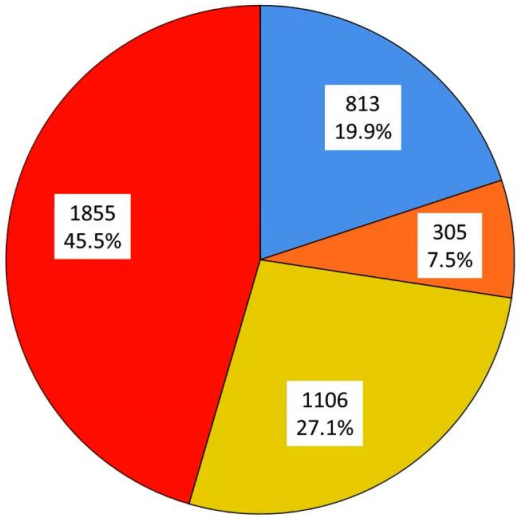
Studying, monitoring and targeting disease progression

Janni (GS4-08): CTCs as monitoring tool in patients with advanced BC



- CTCs have been shown to be prognostic in metastatic BC.
- Here, **pooled analysis of CTC enumeration in 4,079 patients**

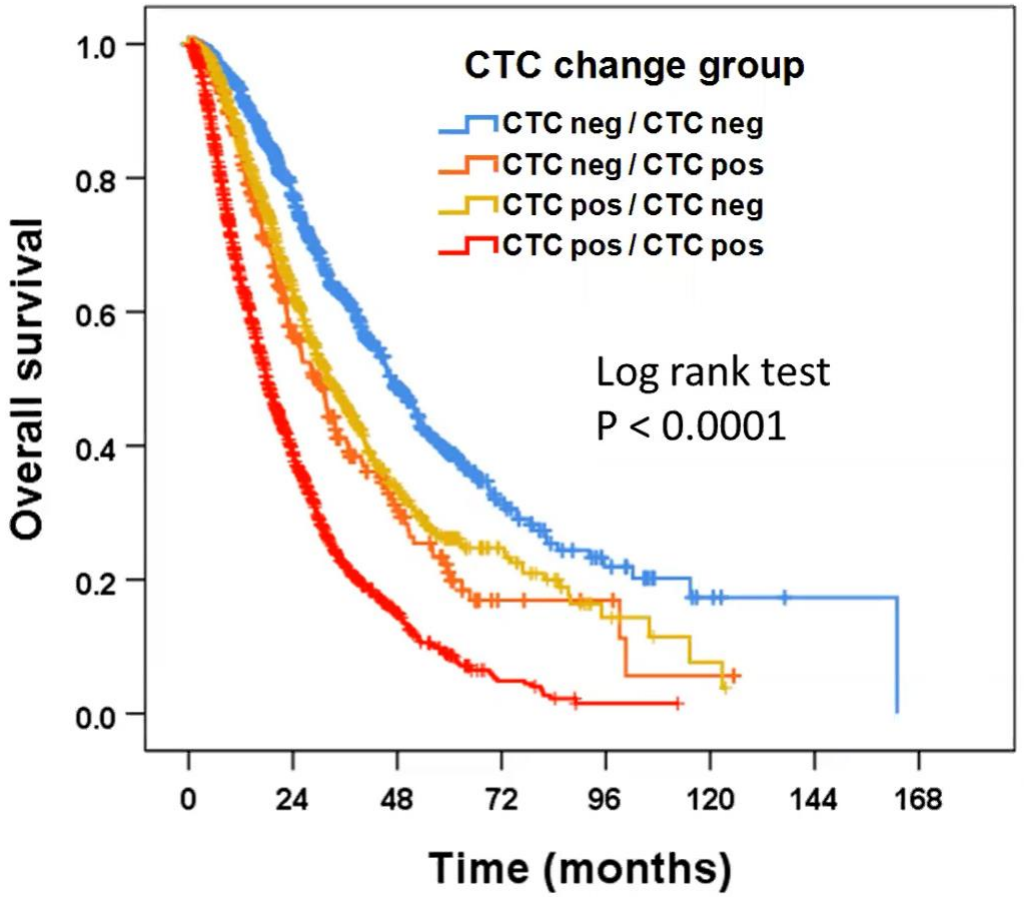
Change in CTC status from baseline to first follow up



CTC change group
(cutoff for CTC positivity: ≥ 1 CTC)

- CTC neg / CTC neg
- CTC neg / CTC pos
- CTC pos / CTC neg
- CTC pos / CTC pos

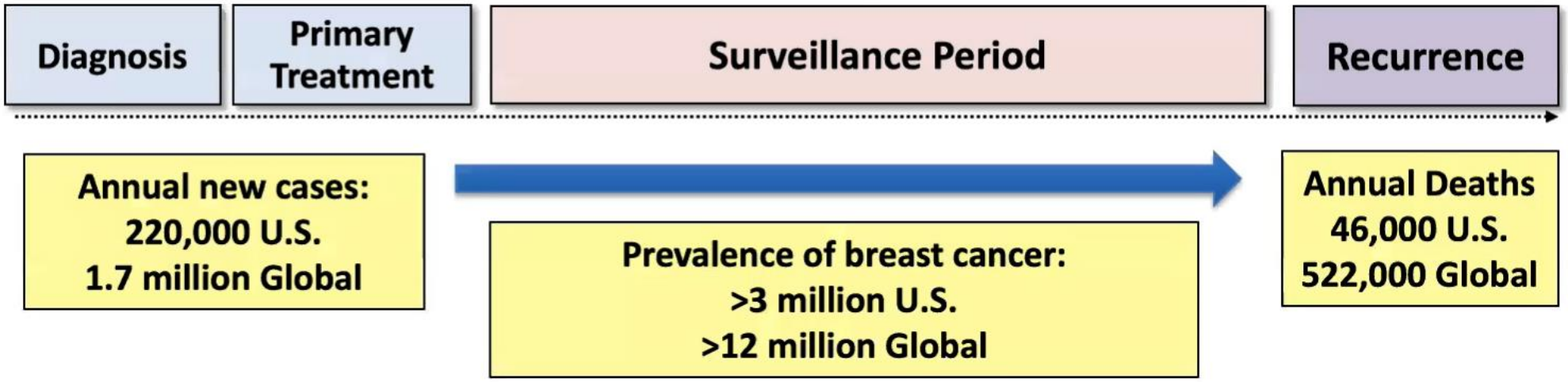
Time from baseline CTC assessment to first follow up CTC assessment:
Median: 29 days
Interquartile range: 26 – 54 days



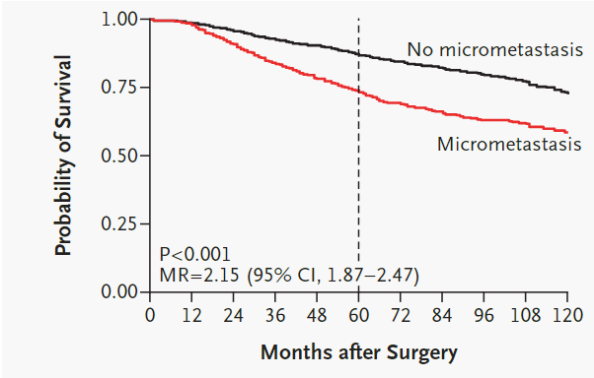
Ccl: 1) clinical validation of CTC monitoring; **2)** after a median of 29 days, **follow-up CTC enumeration predicts OS, 3)** early treatment monitoring seems to work in **all BC subtypes.**

Chodosh (ES3-03): Identifying, characterizing and targeting dormant cancer cells

~1/3 of the patients!



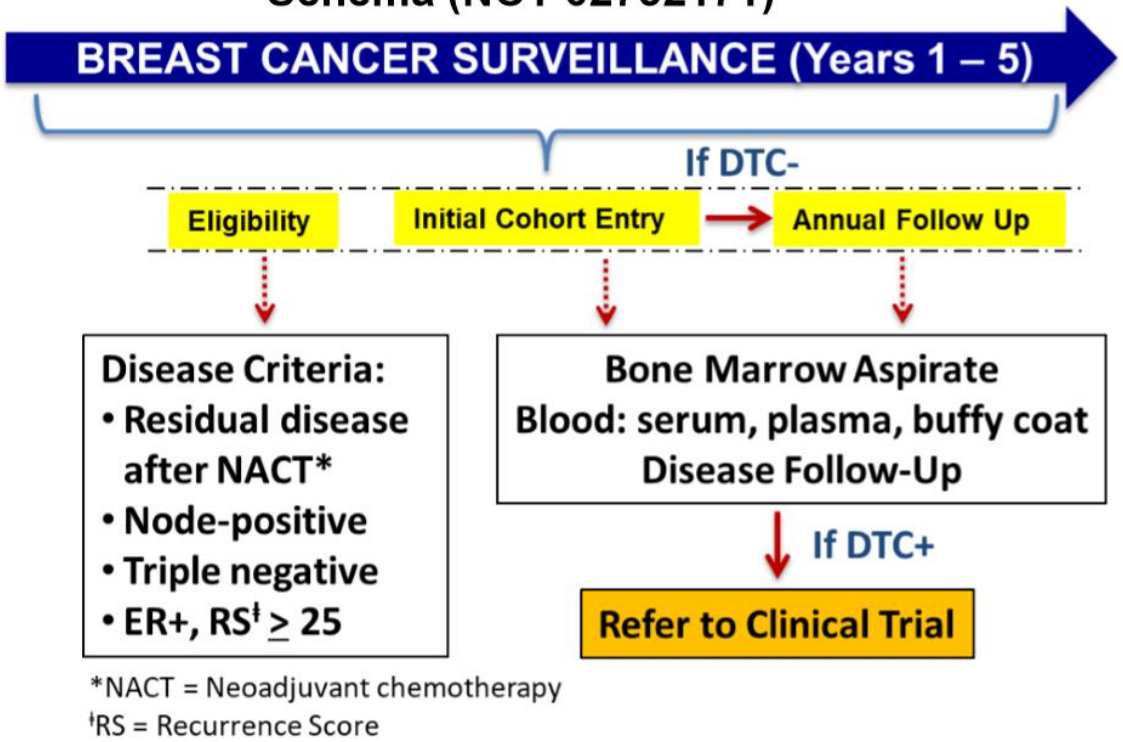
Focus on **disseminated tumor cells (DTCs)** present in the bone marrow (in **~30%** of early BC pts) to detect & treat dormant disease (➔ reduce recurrences)



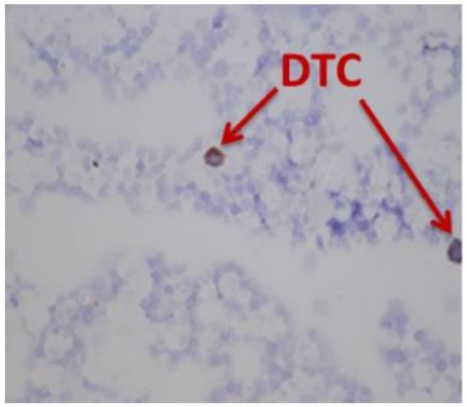
Braun *et al.* NEJM 2005

Penn Medicine

Figure 1: PENN-SURMOUNT Screening Protocol Schema (NCT 02732171)



*NACT = Neoadjuvant chemotherapy
†RS = Recurrence Score



DTC-IHC Assay

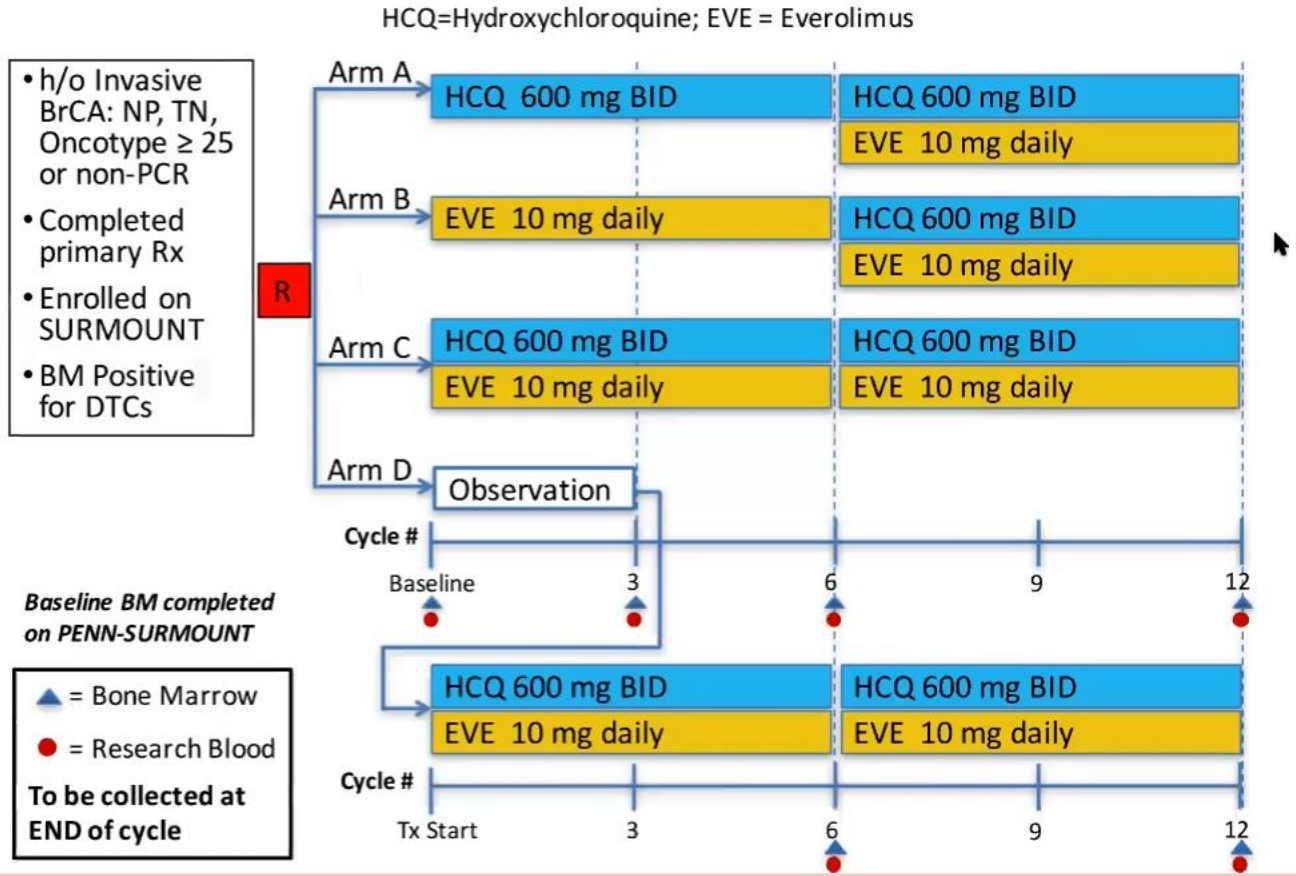
- Performed in CLIA lab
- Positive result defined as ≥ 1 pancytokeratin-DAB positive cell
- Cytospin slides are independently reviewed by two pathologists with adjudication

- **151 pts** enrolled
- All screened @baseline & 46 pts returned for at least 1 annual rescreen
- Cumulative **DTC positivity rate 32.5%** (49/151)
- **98% of DTC+ pts have enrolled on the CLEVER trial**

More info in PD9-11

Penn Medicine

Clever trial: A Phase II Pilot Trial of HydroxyChLoroquine, EVErolimus or the Combination for Prevention of Recurrent Breast Cancer (PI= A. De Michele, NCT03032406)

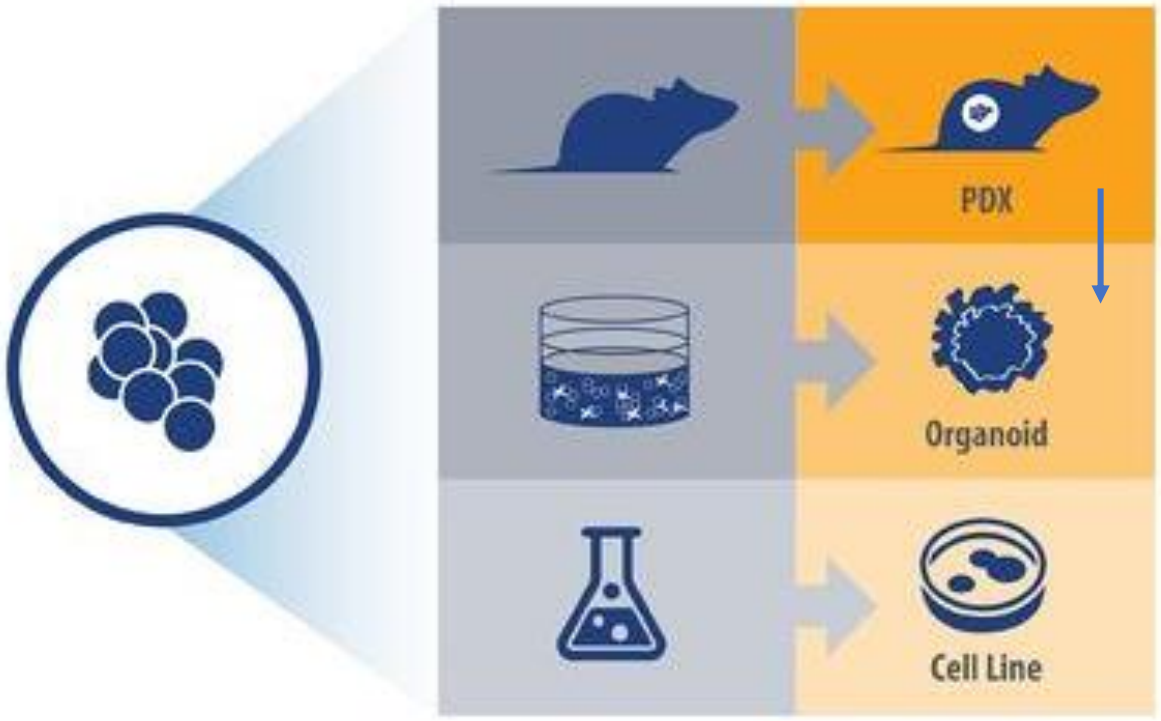


- **60 pts** to be enrolled
- This and other **similar larger trials** (PALAVY and ABBY) will or not demonstrate potential clinical utility of intervention in surveillance period.

Ccl: 1) experimental studies identified vulnerabilities of DTCs that are now being tested in **clinical trials**; **2) intervention is feasible in observation period**, results of the trials will tell us whether the strategy should be expanded.

Models (PDTX & PDTOs)

PDTX: Patient-derived tumor xenograft
PDO: Patient-derived organoid
PDXO: PDX-derived organoid



Advantages PDTX:

- Work with fragments of tumors instead of isolated cells
- Reflect heterogeneity of tumour.
- 3D architechture

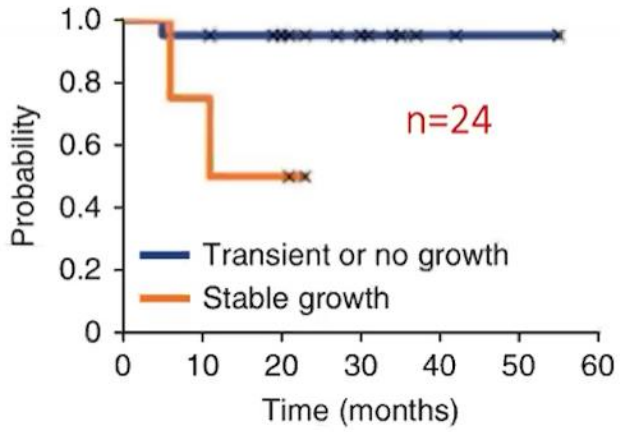
Limitations PDTX:

- Grown in mice lacking fct immune system
- Time
- Cost!
- Failures to ‘take’

Illustration from Crownbio.com, see excellent discussion by S. Goel (SPD7-01)

Vaklavas & Boughey (PD7-02): PDTX 'take' to predict the prognosis of patients

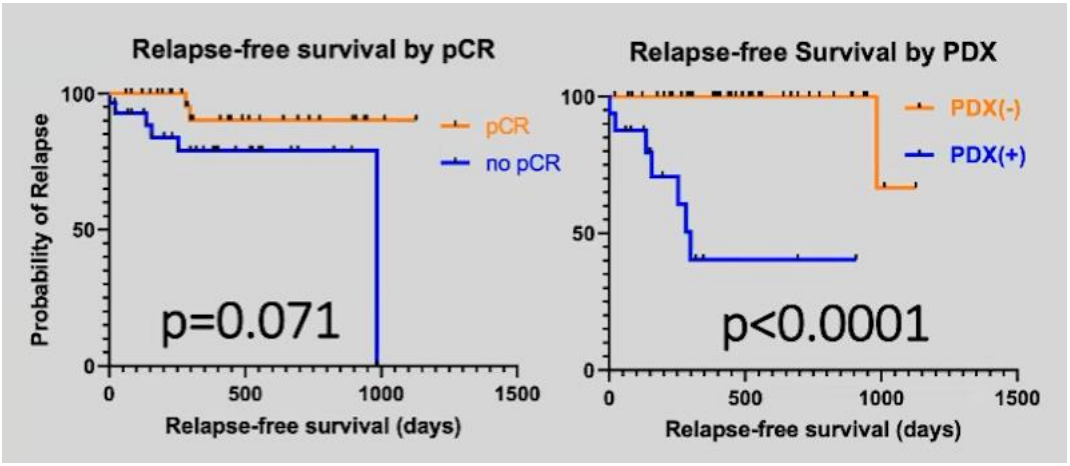
Survival of the fittest:
TNBC>HER2>ER+ BC
High grade tumors
Metastatic tissue



DeRose, Nat Med 2011

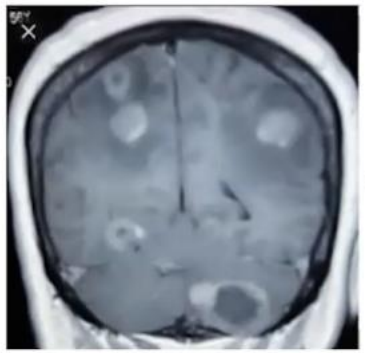
Post-NAC Ki-67*
≤ 14% : 0% take rate
> 14% : 67% take rate
p=0.028

Post-NAC take rate and recurrence
PDX take: 50% recurrence (6 patients)
PDX no take: 19.6% recurrence (28 patients)
p=0.1092

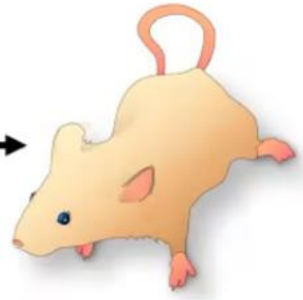


Ccl: 1) confirmation of the seminal study of De Rose Nat Med 2011; **2) still a long way to go** to see whether it could be used to refine prognosis…

Rosenbluth (PD7-01 & PD7-04): PDOs to investigate drug response/resistance mechanisms

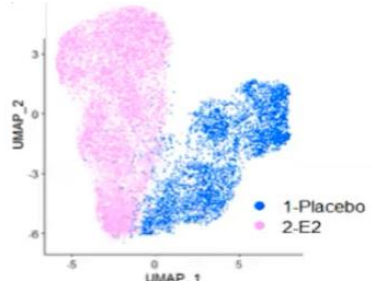


Brain metastasis
ER+ breast cancer
AI-resistant (*ESR1* WT)



PDX (mammary gland)
Tumour growth studies
Single-cell RNA-seq

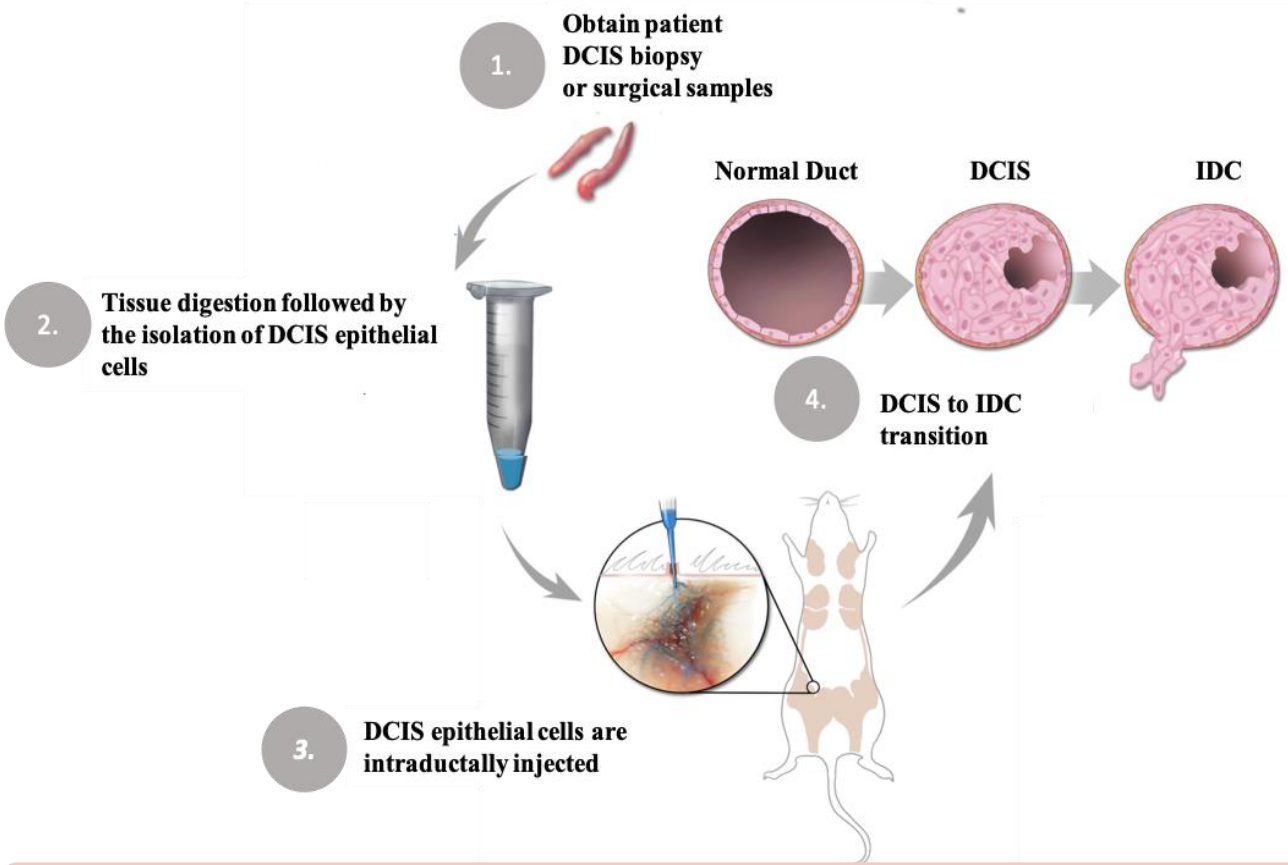
Organoids
Drug treatment studies



Single-cell RNA sequencing before and after treatment

Ccl: Excellent (but very costly) setting to investigate drug response/resistance mechanisms

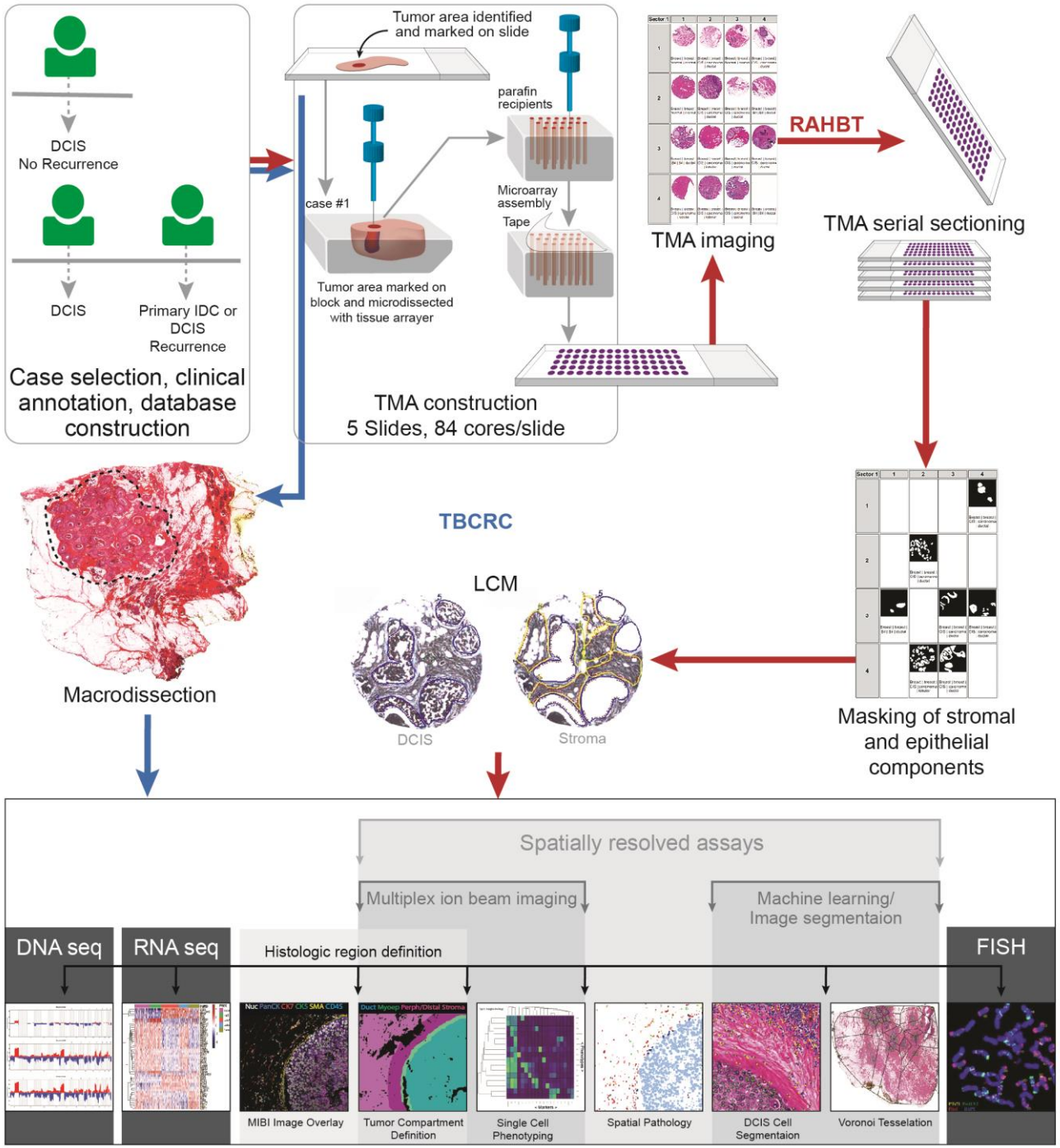
Behbod (PD5-09): PDTX models for DCIS using the MIND technique



- **Histologic features are conserved.**
- **Biomarker expression is conserved,** including ER, PR, HER2, p53 and Ki67.
- **Similar genomic profile**.
- After 9 months, **50% DCIS evolved to invasive BC**.

Ccl: MIND models are valuable resources for the experimental analysis of DCIS progression

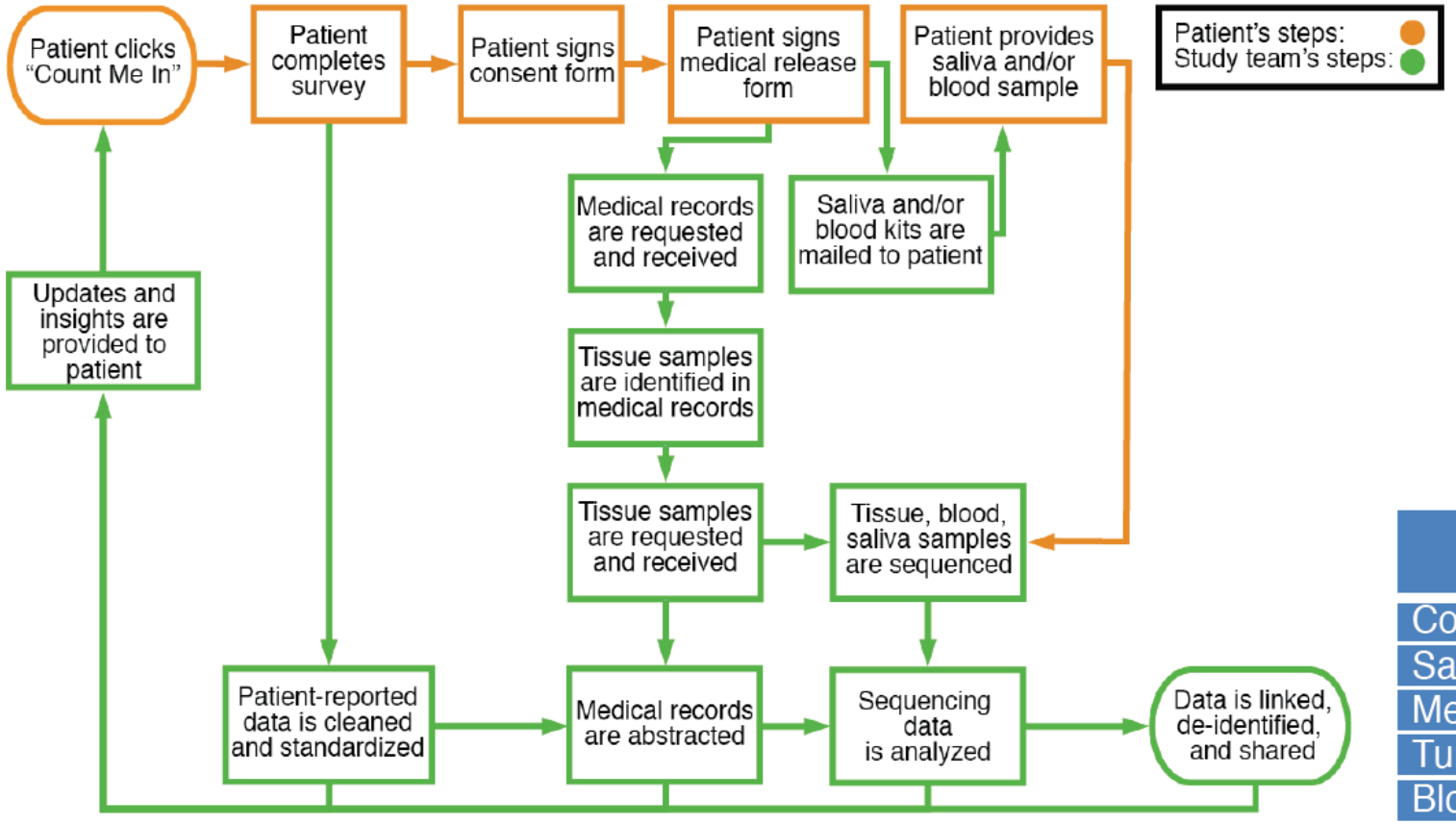
Ressources to advance breast cancer research



Strand (PD5-8): pre-cancer atlas

- Two independent patient cohorts of DCIS (1981 to 2014), cases matched to controls.
- Integrated **multi-omic analysis of DCIS and associated tumor microenvironment** to generate a spatially resolved atlas of breast precancers
- The most enriched pathways for genes upregulated in IBC vs. DCIS epithelium were related to **extracellular matrix and immune system regulation**
- Specific immune cell types are associated with differences in case and control status including CD8 T cells, macrophages, and NK T cells

Wagle (OT-18-01): The Metastatic Breast Cancer Project (mbcproject.org)



	Requested	Received	Yield (Pct)
Consent Form	5418	3386	**63%**
Saliva Sample	3002	2158	**72%**
Med Records	2272	1475	**65%**
Tumor Tissue	873	626	**73%**
Blood (cfDNA)	1471	1124	**76%**

Ccl: 1) Patient-driven initiative that started in 2015; **2) shared omics and clinical data** with the research community (cbioportal.org).

VIRTUAL MEETING
Saturday January 30, 2021

Thank you for your attention!

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KU LEUVEN

LTCR
Laboratory for Translational Breast Cancer Research