OXFORD DEBATE

Biopsia liquida: siamo pronti per un uso clinico

Contro

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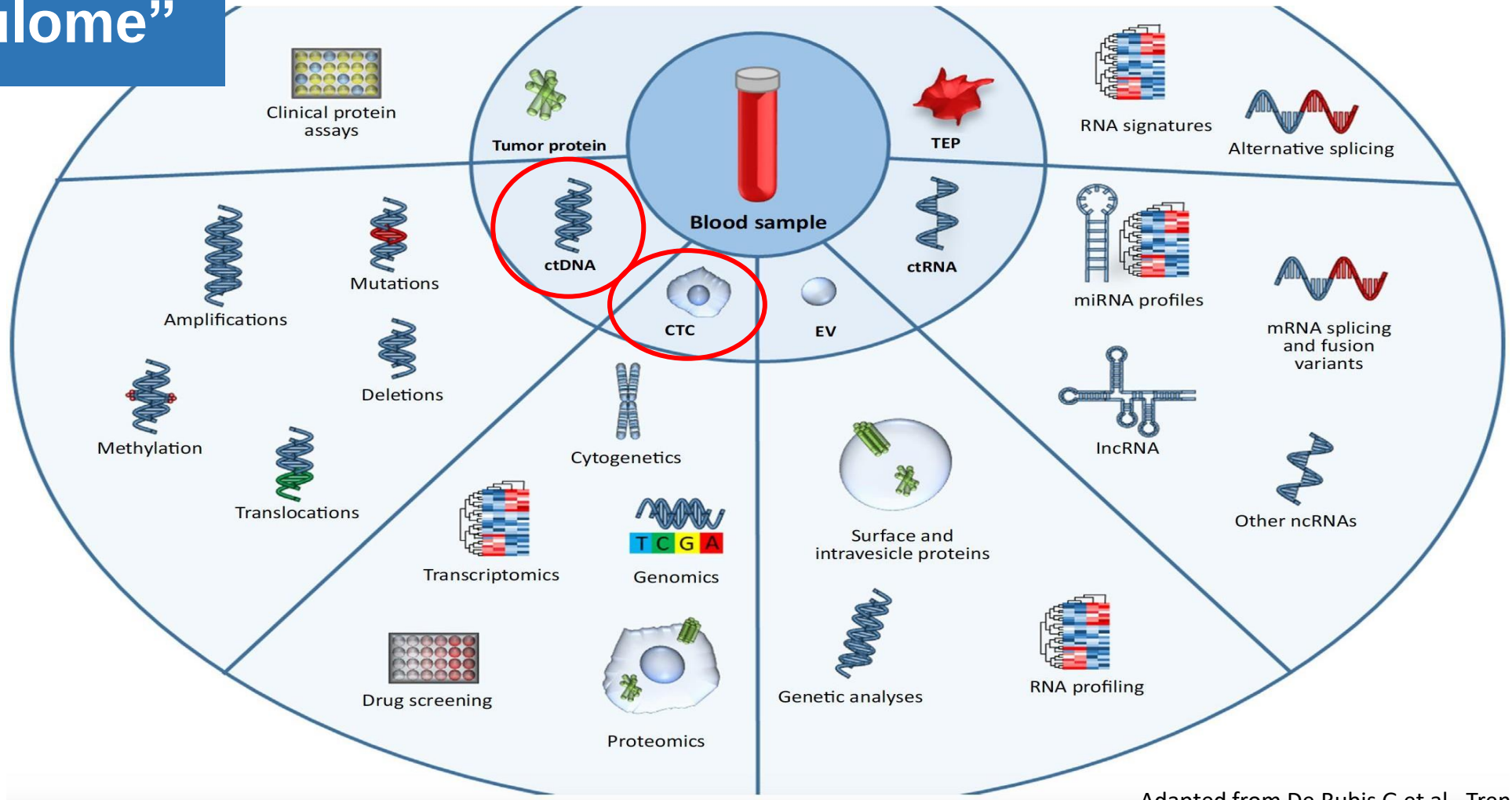
UOSD Ricerca Clinica e Traslazionale in Senologia

IRCCS Istituto Nazionale Tumori

"Fondazione G.Pascale"

Liquid biopsy: beyond ctDNA

Tumor "circulome"



Adapted from De Rubis G et al., Trends Pharmacol Sci. 2019

Cancer Treatment Reviews 73 (2019) 73–83

Contents lists available at ScienceDirect

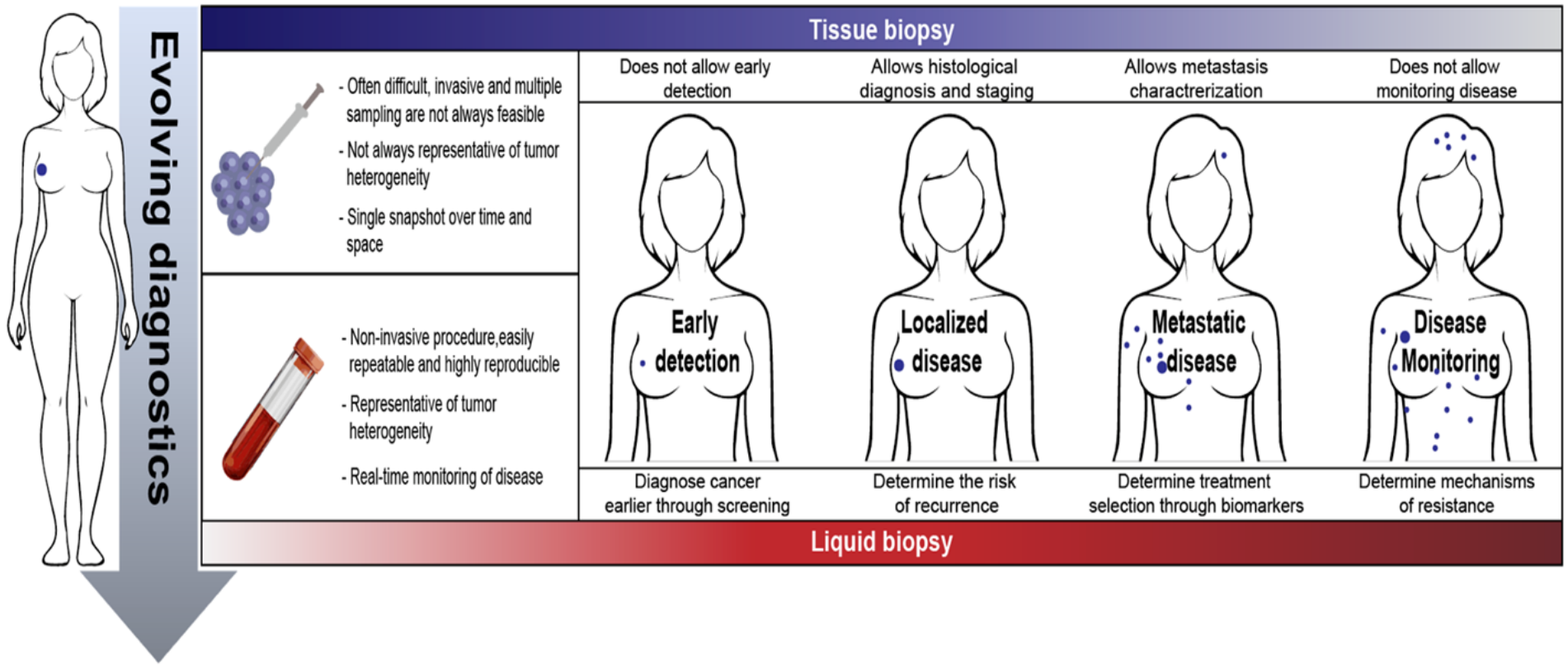
Cancer Treatment Reviews

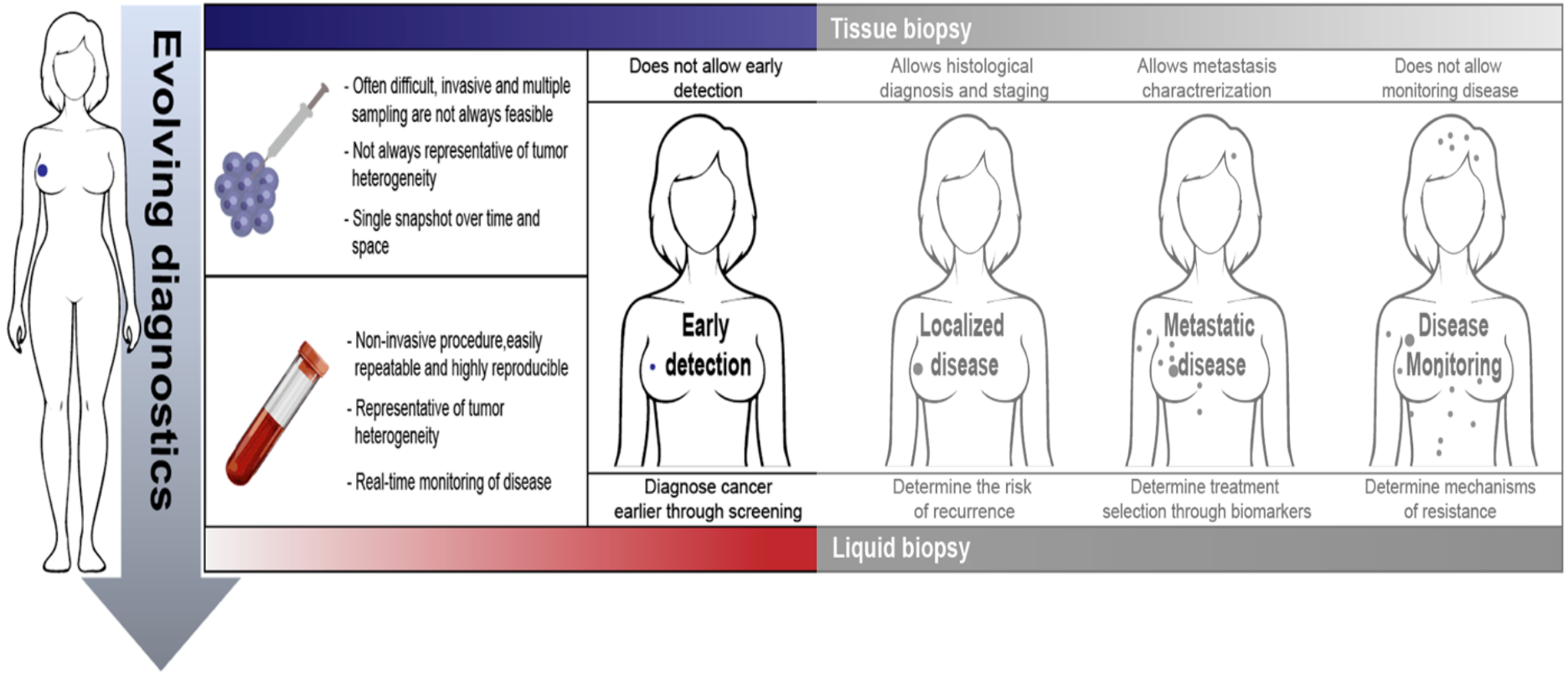
journal homepage: www.elsevier.com/locate/ctrv

Hot Topic

Circulating tumor DNA analysis in breast cancer: Is it ready for prime-time?

Giuseppe Buono[a,1], Lorenzo Gerratana[b,c,1,*], Michela Bulfoni[d], Nicoletta Provinciali[e], Debora Basile[b], Mario Giuliano[a,f], Carla Corvaja[b], Grazia Arpino[a], Lucia Del Mastro[g], Sabino De Placido[a], Michele De Laurentiis[h], Massimo Cristofanilli[c,2], Fabio Puglisi[b,i,2]


Evolving diagnostics
Tissue biopsy
- Often difficult, invasive and multiple sampling are not always feasible
- Not always representative of tumor heterogeneity
- Single snapshot over time and space
Does not allow early detection
Allows histological diagnosis and staging
Allows metastasis charactrerization
Does not allow monitoring disease
Early detection
Localized disease
Metastatic disease
Disease Monitoring
- Non-invasive procedure,easily repeatable and highly reproducible
- Representative of tumor heterogeneity
- Real-time monitoring of disease
Diagnose cancer earlier through screening
Determine the risk of recurrence
Determine treatment selection through biomarkers
Determine mechanisms of resistance
Liquid biopsy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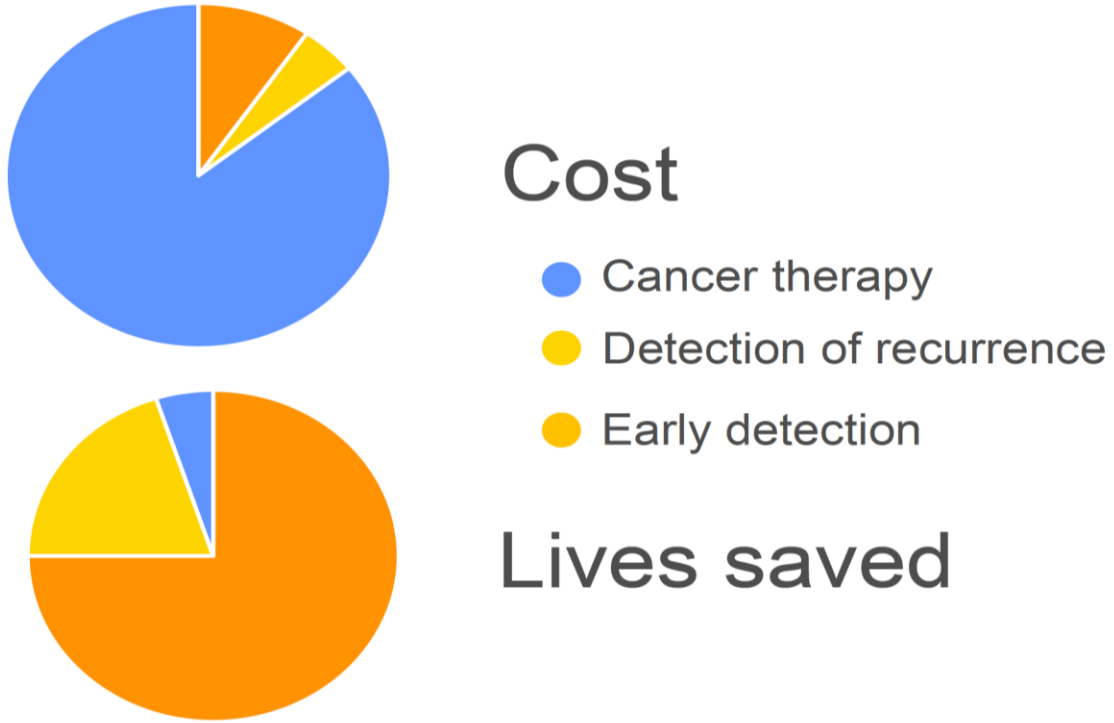
Early detection save lives and is cost-effective

Early detection can save 4-6 million lives per year

RELATIVE 5 YEAR CANCER SURVIVAL RATES		
Cancer Type	**Late Detection**	**Early Detection**
Breast	27%	99%
Colorectal	14%	90%
Lung	5%	56%
Ovary	29%	92%
Stomach	5%	68%

Economic savings of early detection could be $100-1,000 Bn



Mariotto et al, 2011; Bradely et al, 2008; Yabroff et al, 2011; Blumen et al, 2016; WHO, 2014. G. Meijer et al., unpublished.

Criteria for successful Screening Test

- Very Safe
- Acceptable to subjects and providers
- Simple and inexpensive
- Cost-effective
- Reliable (repeatability, reducibility, precision)
- Valid (sensitivity – specificity)
- Exit strategy (facilities for diagnosis an appropriate treatments should be available for positive subjects)

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What should be the maximum-price of LB to be cost-effective?

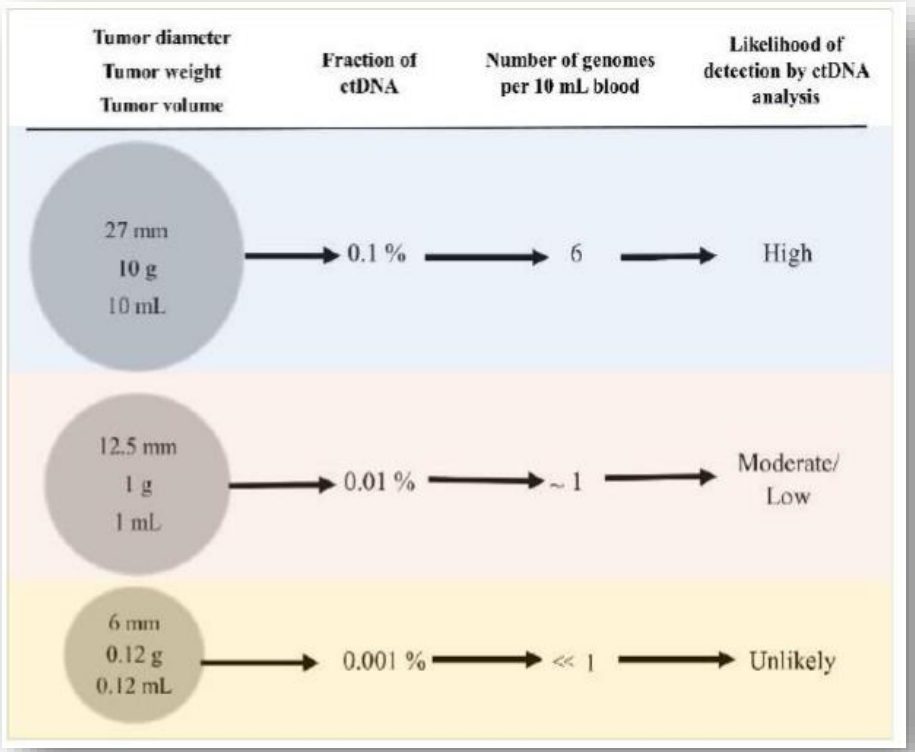
Model Inputs		Outcomes per 1000 Women Screened over Their Lifetime					
Combined Sensitivity [a], %	Specificity, %	Mortality Reduction %	False Positives	Overdiagnoses	QALYs-Gained	Total Costs, USD 1000	Maximum Price, USD
			Digital mammography (comparator)				
74	88	25.1	913.8	18.1	40	7022	-
			DCIS detection				
70	100	22.4	0	17.7	43	6786	195
71	96	23.2	406.3	17.8	41	6888	171
71	100	23.2	0	17.8	44	6796	202
73	96	24.2	406.2	18.0	42	6899	179
73	100	24.2	0	18.0	45	6807	210
74	96	25.1	406.1	18.1	44	6911	186
74	100	25.1	0	18.1	47	6819	217
76	88	26.0	913.6	18.2	42	7035	156
76	96	26.0	406.0	18.2	45	6924	193
76	100	26.0	0	18.2	48	6832	224
78	88	27.1	913.4	18.4	43	7050	164
78	96	27.1	405.9	18.4	47	6939	201
78	100	27.1	0	18.4	50	6847	232
79	88	28.3	913.2	18.5	45	7068	173
79	96	28.3	405.8	18.5	49	6957	210
79	100	28.3	0	18.5	52	6868	241
			No DCIS detection				
67	100	23.1	0	1.9	41	6951	161
69	100	23.7	0	2.1	42	6964	169
71	100	24.8	0	2.2	44	6979	177
~~73~~	~~96~~	~~26.1~~	~~409.2~~	~~2.4~~	~~43~~	~~7090~~	~~156~~
73	100	26.1	0	2.4	46	6998	187
			Perfect test, DCIS detection				
~~90~~	~~88~~	~~38.4~~	~~910~~	~~20.5~~	~~59~~	~~7273~~	~~235~~
~~90~~	~~100~~	~~38.4~~	~~0~~	~~20.5~~	~~65~~	~~7071~~	~~303~~
			Perfect test, no DCIS detection				
87	88	36.6	918	4.5	53	7413	183
~~87~~	~~100~~	~~36.6~~	~~0~~	~~4.5~~	~~60~~	~~7210~~	~~253~~

Van der Poort E. Cancers 2022

Mutant allele fraction (MAF) is very low for small tumours

Tumor Diameter, mm	Tumor Weight, mg	Tumor Volumen mL (cm^3)	Number of Cancer Cells	Percentage Fraction of Mutant ctDNA	Number of Cancer Genomes per 10 mL of Blood	Chance of Progression [c]	Mammographic Screen Sensitivity [d]
27	10,000	10 [a]	10,000,000,000	1:1000	6	-	-
12.5	1000	1 [b]	1,000,000,000	1:10,000	0.6	-	-
10	500	0.5	500,000,000	1:20,000	0.3	50%	91%
8	250	0.25	250,000,000	1:40,000	0.15	25%	-
6	125	0.12	125,000,000	1:80,000	<0.1	-	-
5	62	0.06	62,000,000	1:160,000	<0.1	6%	26%
4	31	0.03	32,000,000	1:320,000	<0.1	-	-
3	16	0.015	16,000,000	1:640,000	<0.1	-	-
2.4	8	0.007	8,000,000	1:1,300,000	<0.1	-	-
2	4	0.0035	4,000,000	1:2,600,000	<0.1	-	-
1.5	2	0.0017	2,000,000	1:5,200,000	<0.1	-	-
1.1	1	0.0008	1,000,000	1:10,000,000	<0.1	0.05%	-

ctDNA: circulating tumor DNA. [a] As reported by Abbosh et al. [36]. [b] As reported by Del Monte [37]. [c] As reported by Narod and others [38,39]. [d] As reported by Wedon-Fekjaer et al. [39]. Adapted from ref. [34].



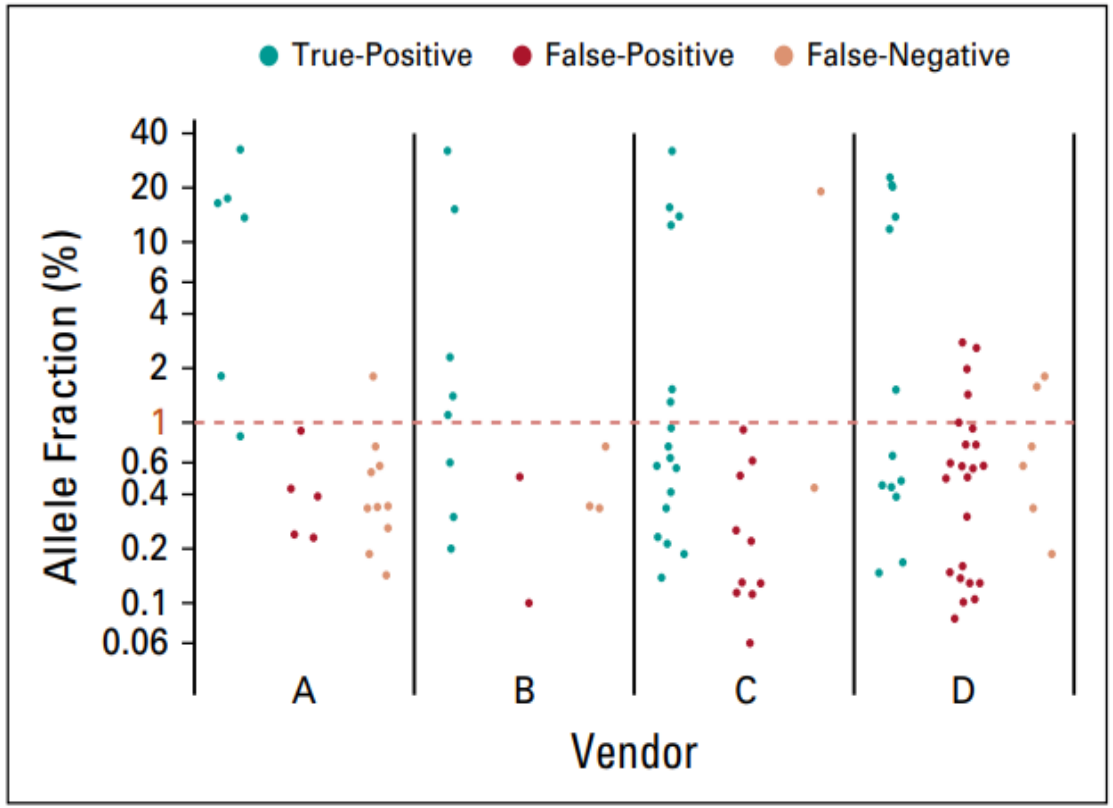
Pons-Belda O. Diagnostics 2021

The importance of test reproducibility

- 24 matched, tumor-normal pairs with matched plasma from lung, breast, ovary, and prostate cancers
- 4 NGS gene panels assays evaluated
- Substantial variability among the ctDNA assays, with a range of sensitivity (38-89%) and positive predictive value (36-80%), particularly in the detection of allele frequency variants <1%
- Most NGS assay discordance is a result of technical variations

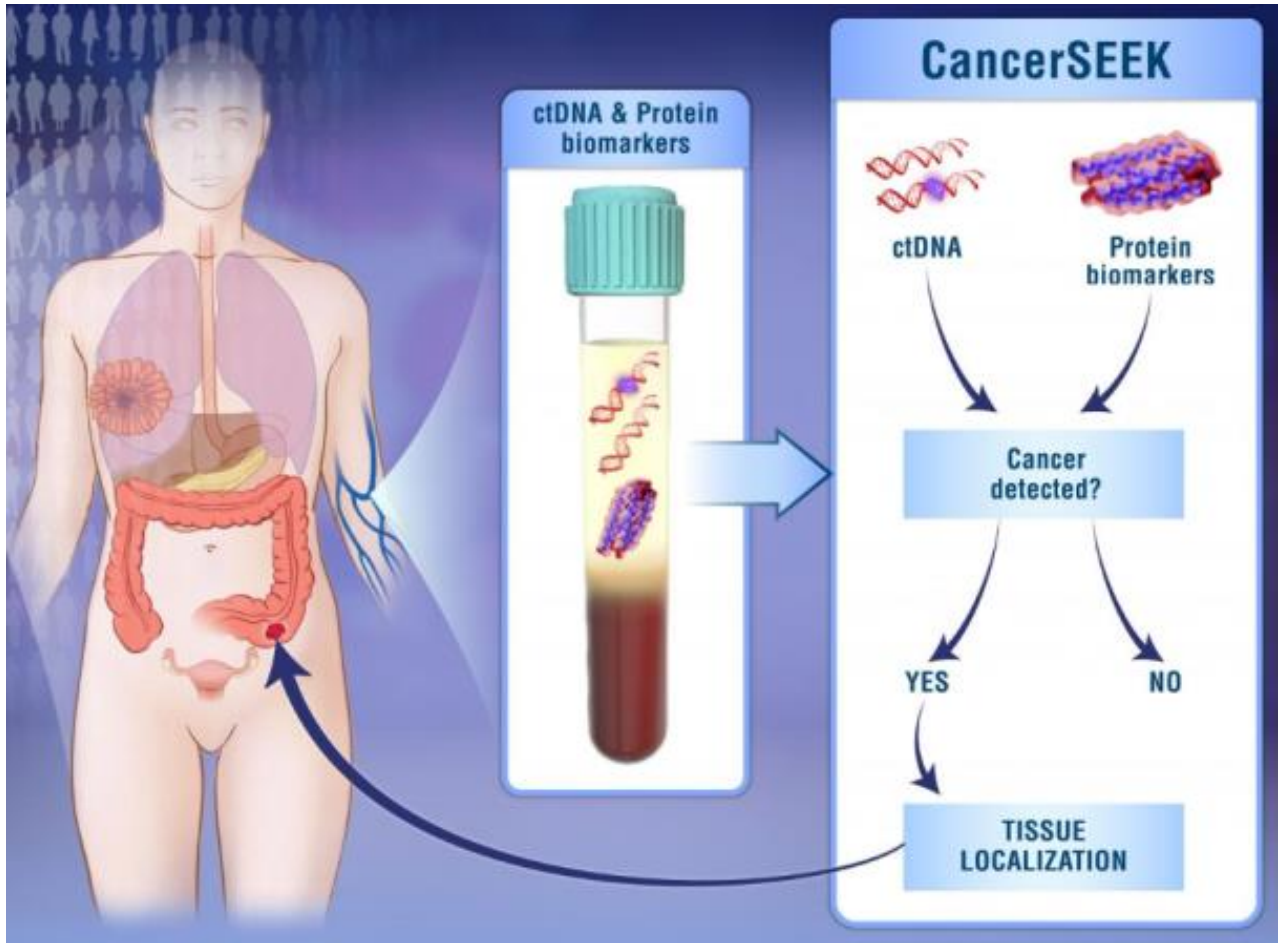
TABLE 2. Sensitivity and Positive Predictive Value of All Variants

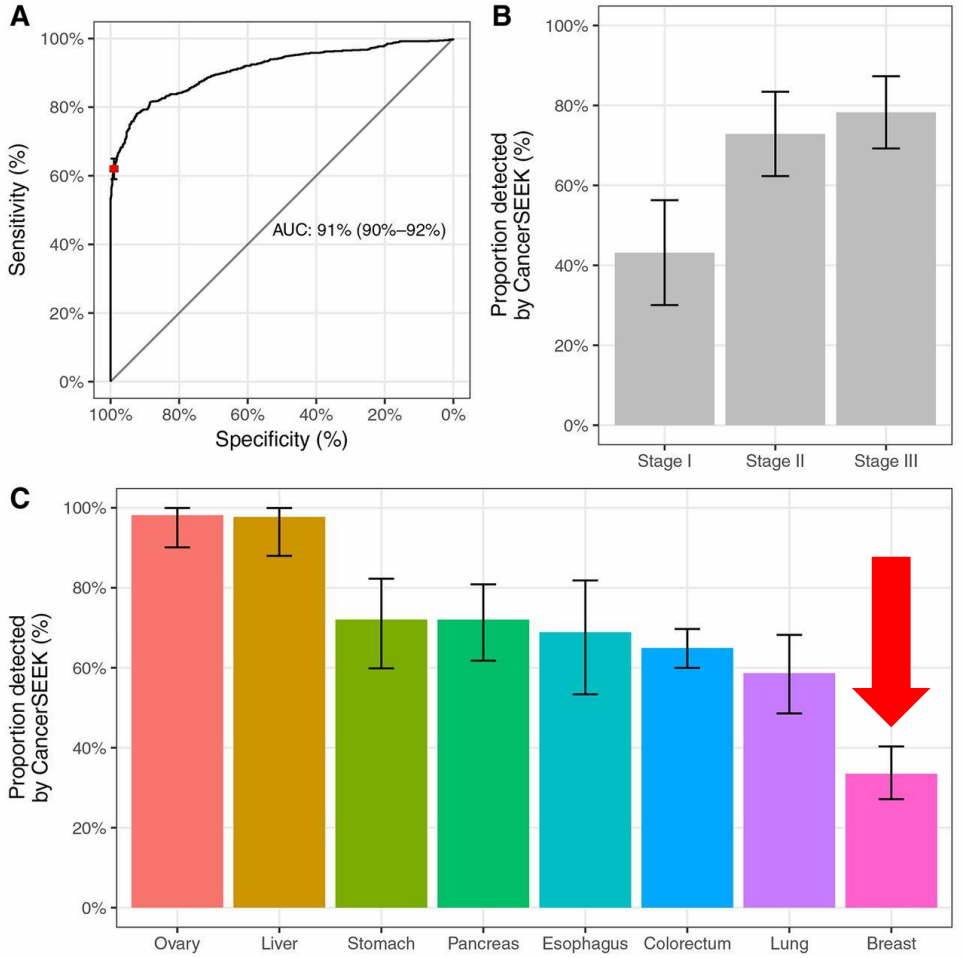
Vendor	TP	FP	FN	Sensitivity* (%)	PPV† (%)
A	6	5	10	38	55
B	8	2	3	73	80
C	17	10	2	89	63
D	13	23	6	68	36



Stetson D. JCO Precis Oncol 2019

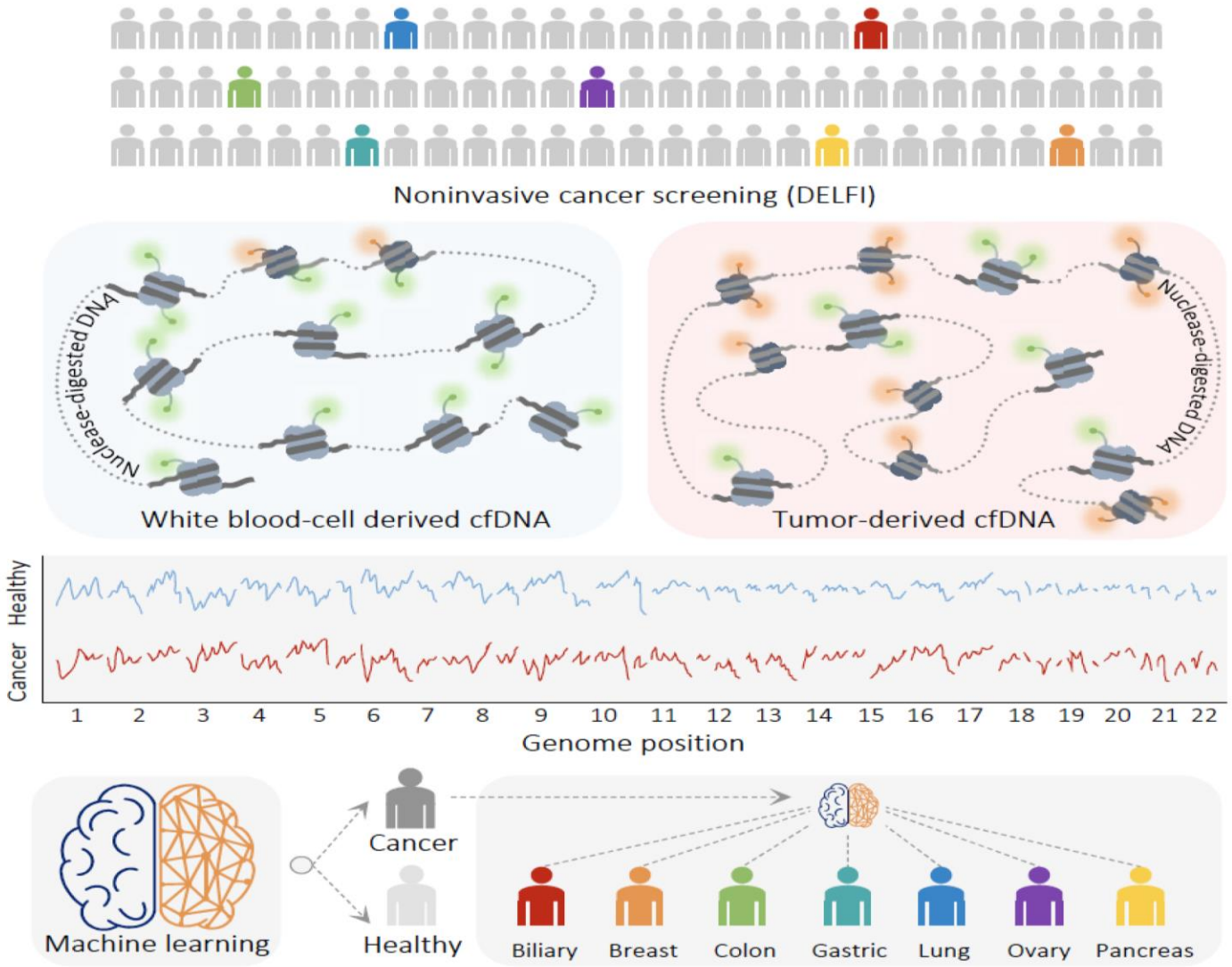
CancerSEEK: low sensibility for BC





33%

DNA evaluation of fragments for early detection (DELFI)



Cristiano, Leal, Phallen, Fiksel, Scharpf et al., *Nature*, 2019

DNA evaluation of fragments for early detection (DELFI)

Cancer type	Individuals analyzed	Sensitivity	
		95% specificity	98% specificity
Lung	12	100%	100%
Ovarian	28	89%	89%
Bile duct	26	88%	81%
Gastric	27	81%	81%
Colorectal	27	81%	80%
Pancreatic	34	71%	65%
Breast	54	70%	57%
Healthy	215	<5%	<2%

Cristiano, Leal, Phallen, Fiksel, Scharpf et al., *Nature*, 2019

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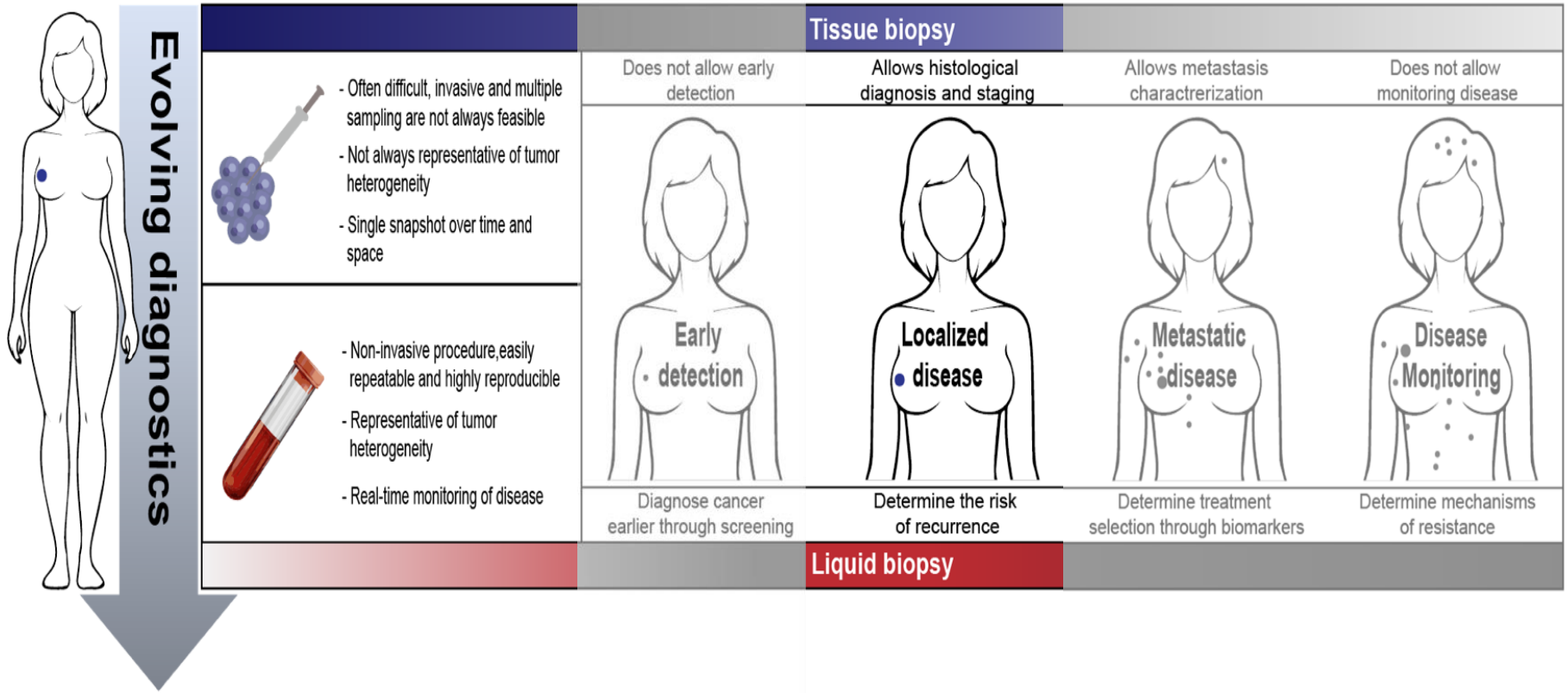
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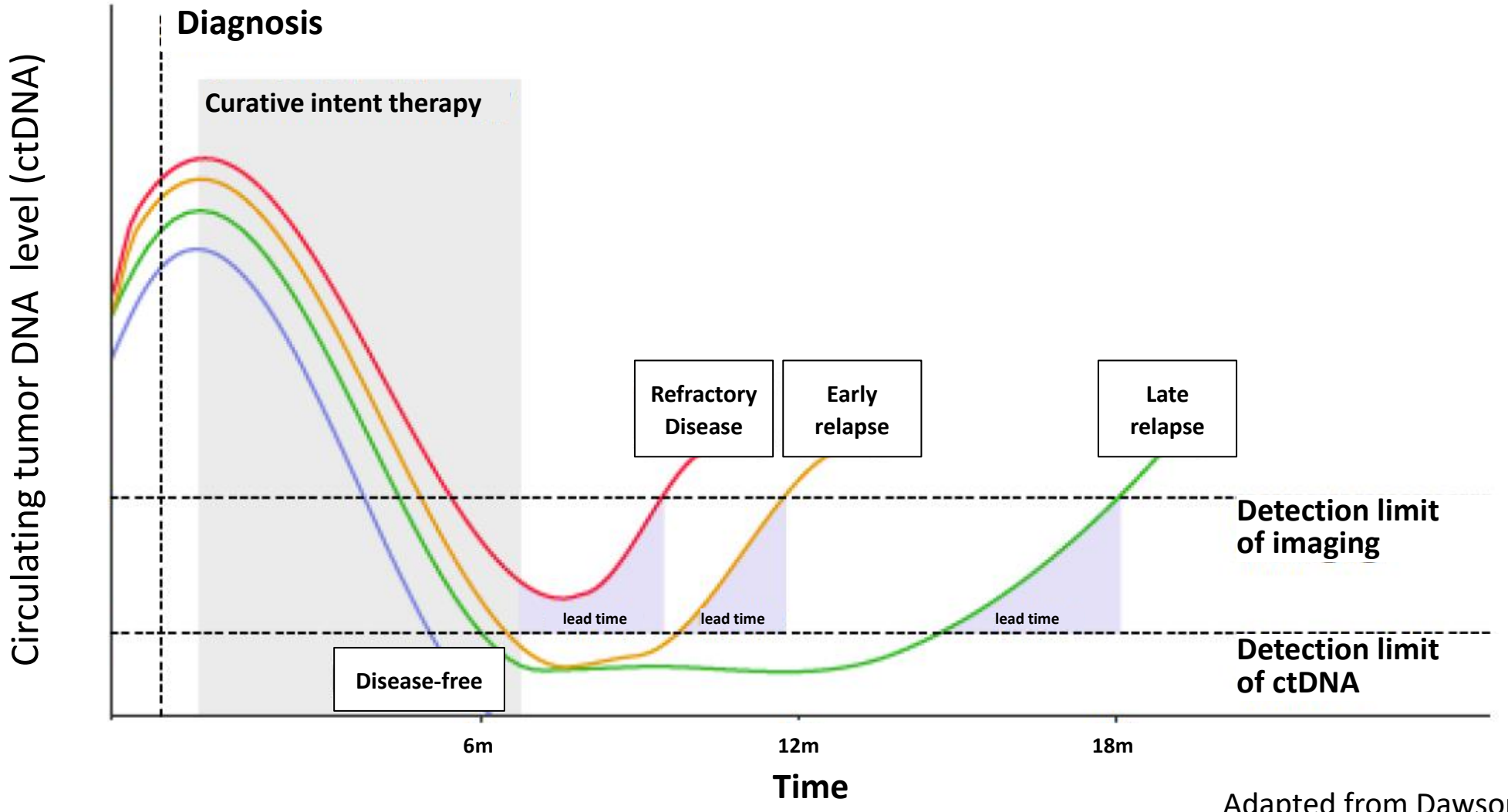
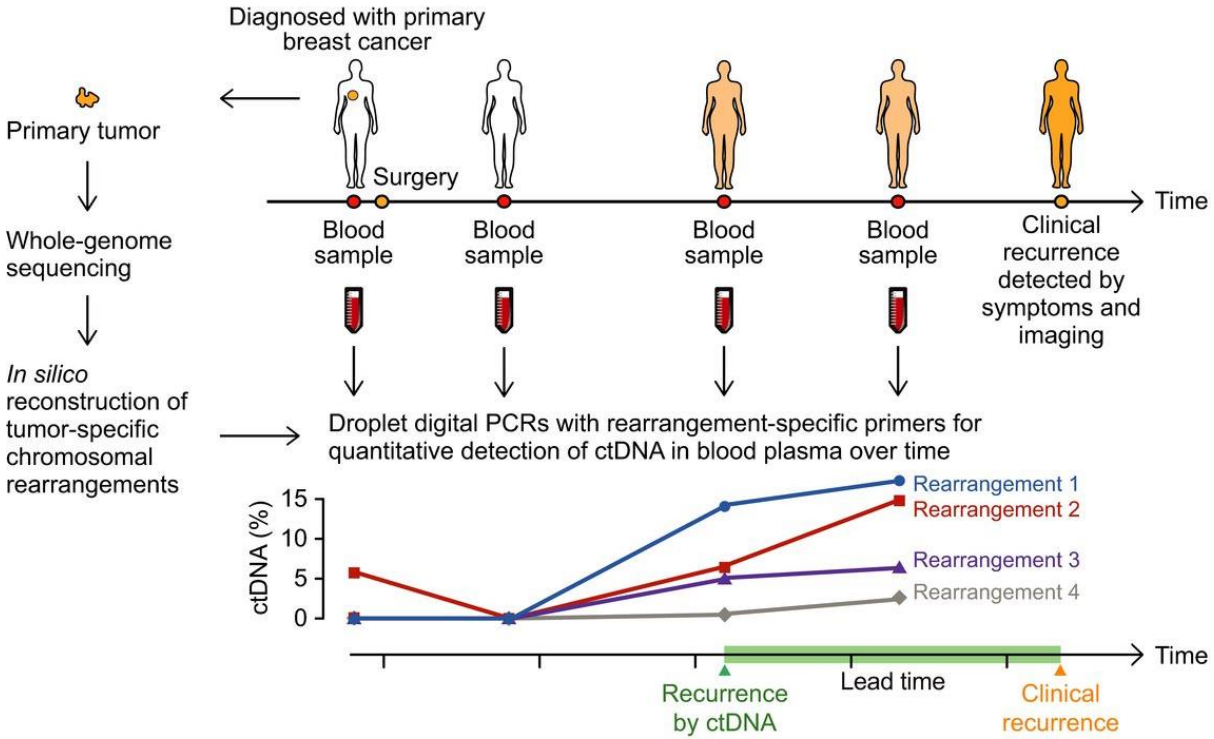
Detecting Minimal Residual Disease (MRD)



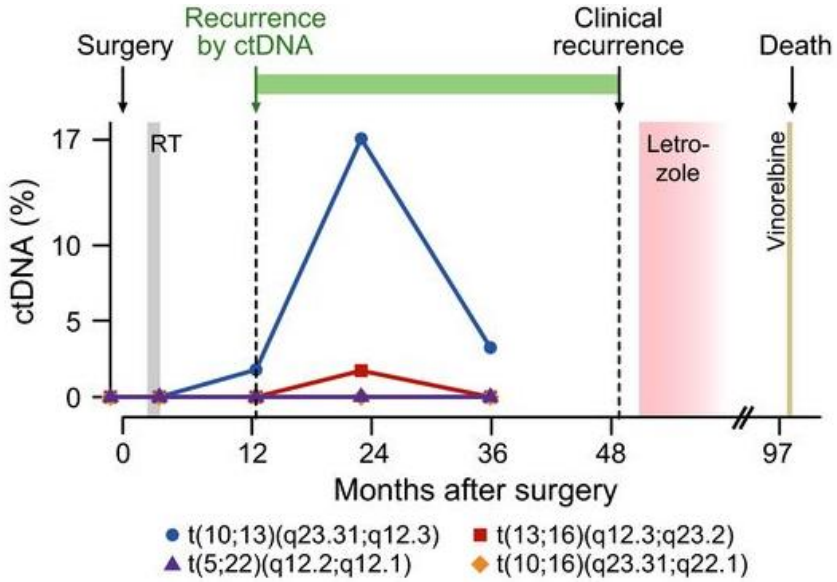
Adapted from Dawson SJ, SABCS 2017

Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease

Eleonor Olsson, Christof Winter, Anthony George, Yilun Chen, Jillian Howlin, Man-Hung Eric Tang, Malin Dahlgren, Ralph Schulz, Dorthe Grabau, Danielle van Westen, Mårten Fernö, Christian Ingvar, Carsten Rose, Pär-Ola Bendahl, Lisa Rydén, Åke Borg, Sofia K Gruvberger-Saal, Helena Jernström & Lao H Saal

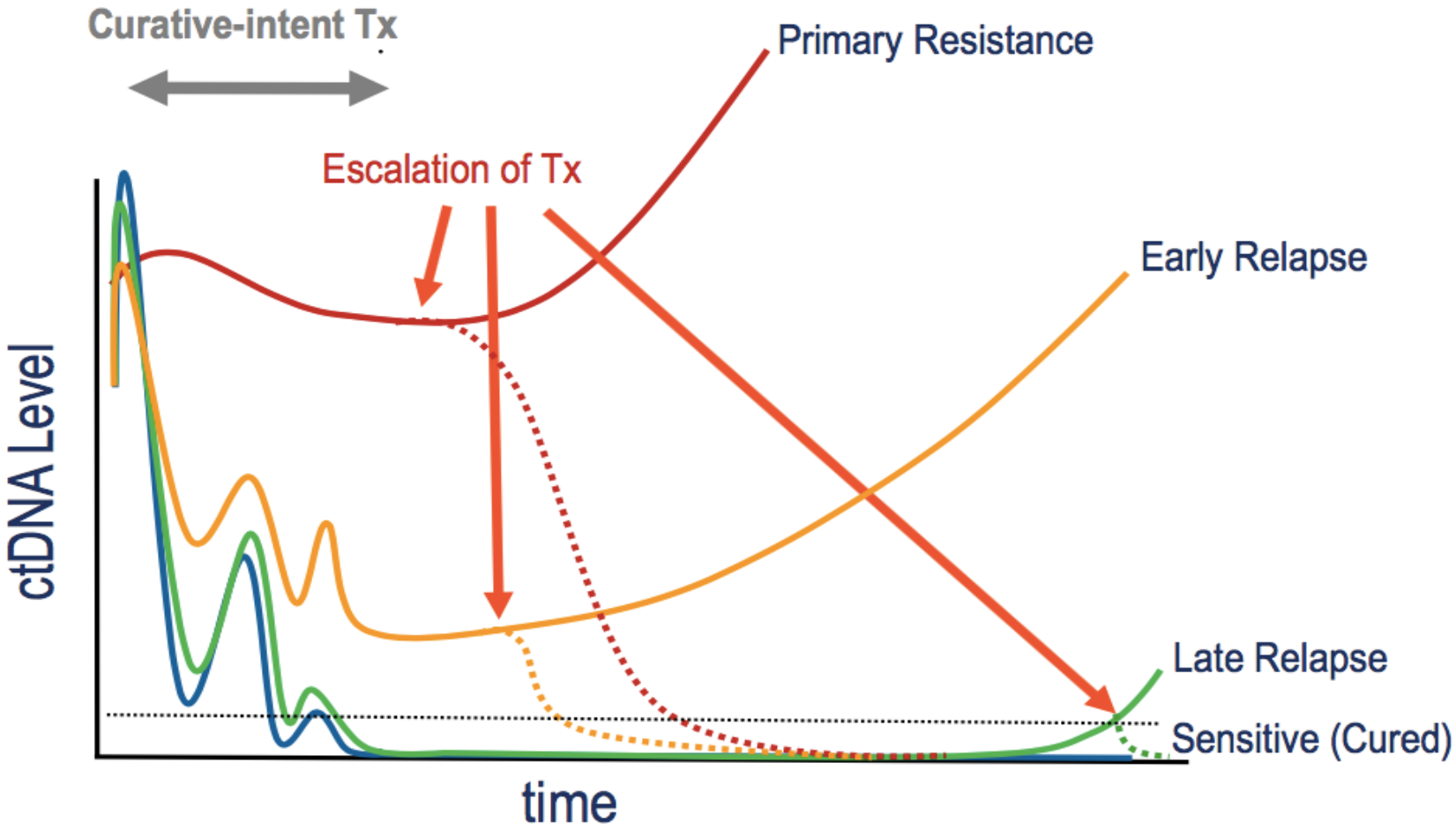


Patient EM5



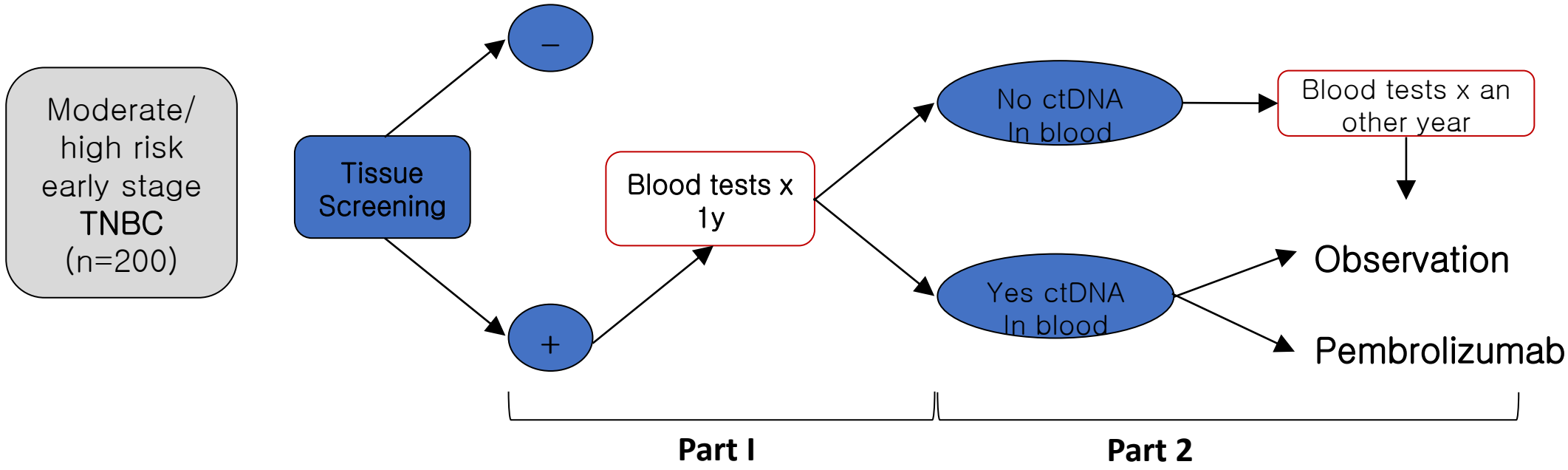
Olsson et al. EMBO Mol Med 2015

Detecting MRD: principles of clinical utility



Are we ready to a clinical use of MRD?

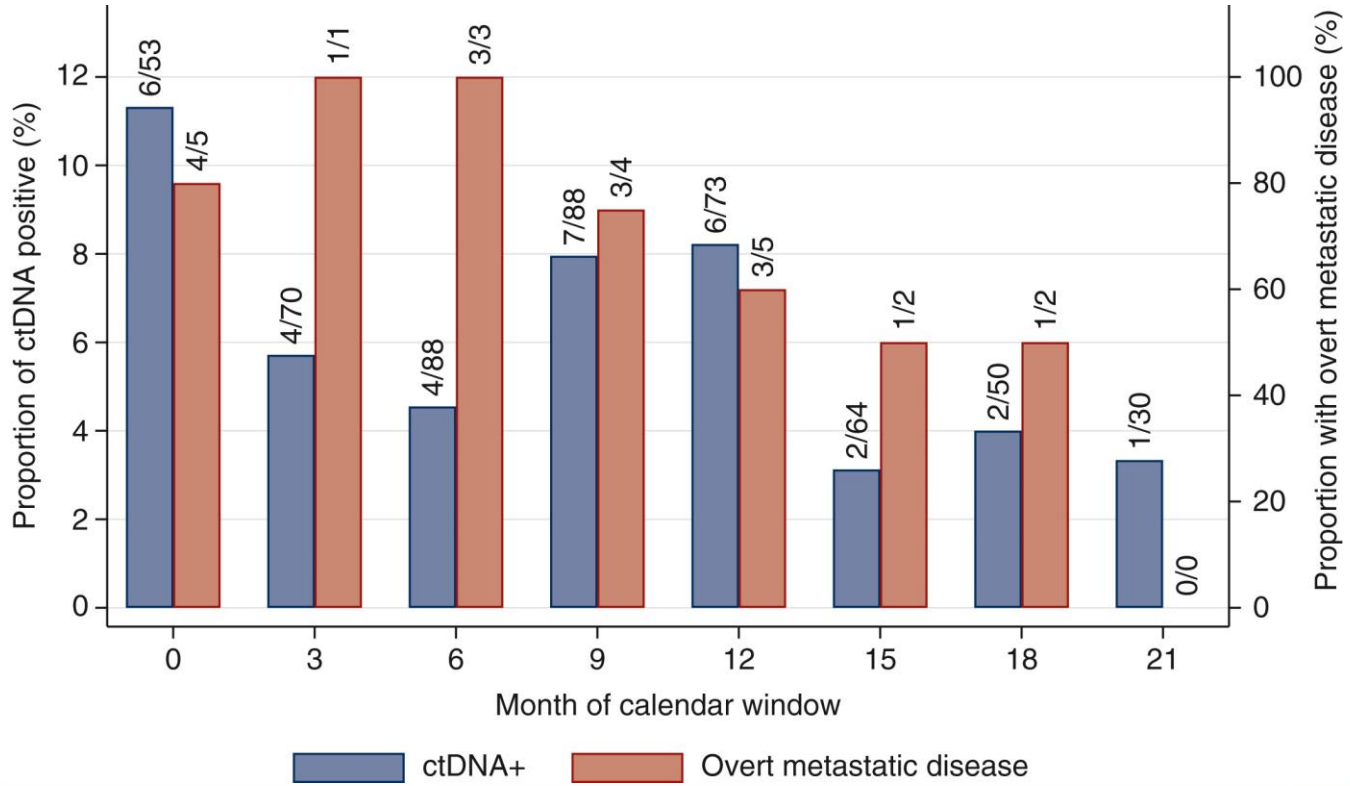
c-TRACK TN: A randomized trial utilizing ctDNA mutation tracking to detect minimal residual disease and trigger intervention in patients with moderate and high risk early stage triple negative breast cancer.



Turner N. et al. Annals of Oncol 2022.

Are we ready to a clinical use of MRD? C-TRACK TN

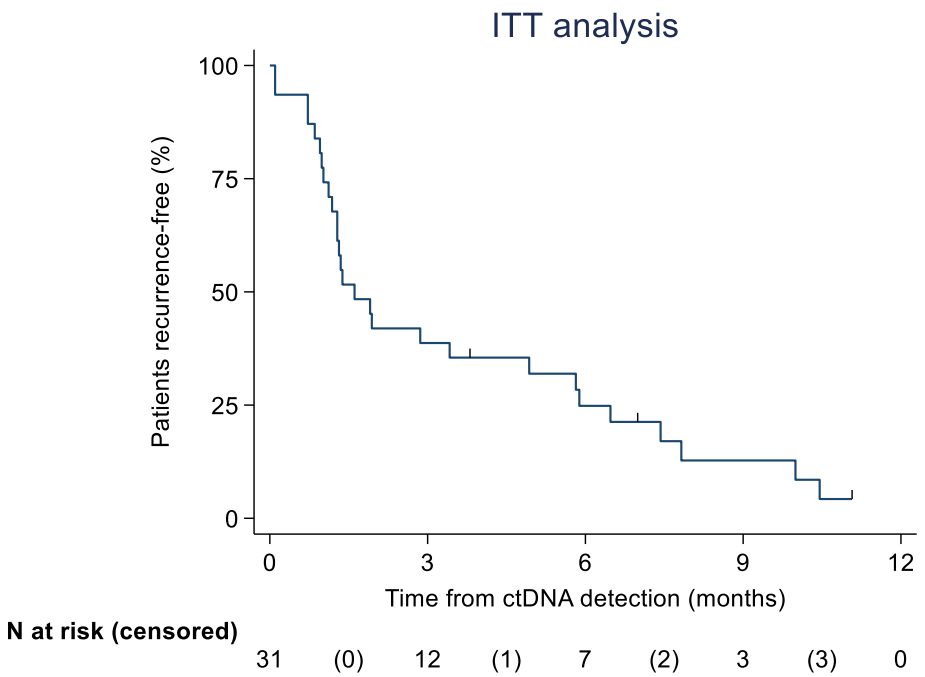
- Of the patients allocated to intervention, 71.9% (23/32, 95% CI 53.3% to 86.3%) had metastatic disease on staging at the time of ctDNA detection

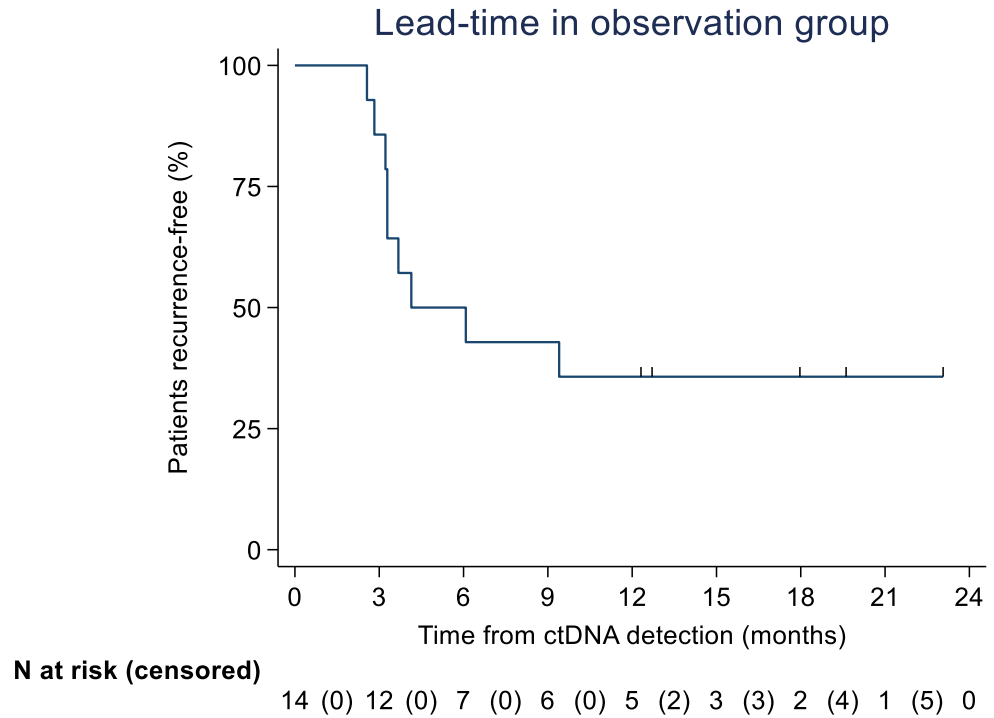


Turner N. et al. Annals of Oncol 2022.

Are we ready to a clinical use of MRD? C-TRACK TN

- Median lead time between ctDNA detection and disease recurrence in the intervention group was 1.6 months (95% CI 1.2-4.9 months) *versus* 4.1 months (95% CI 3.2 months-not defined) in observation arm



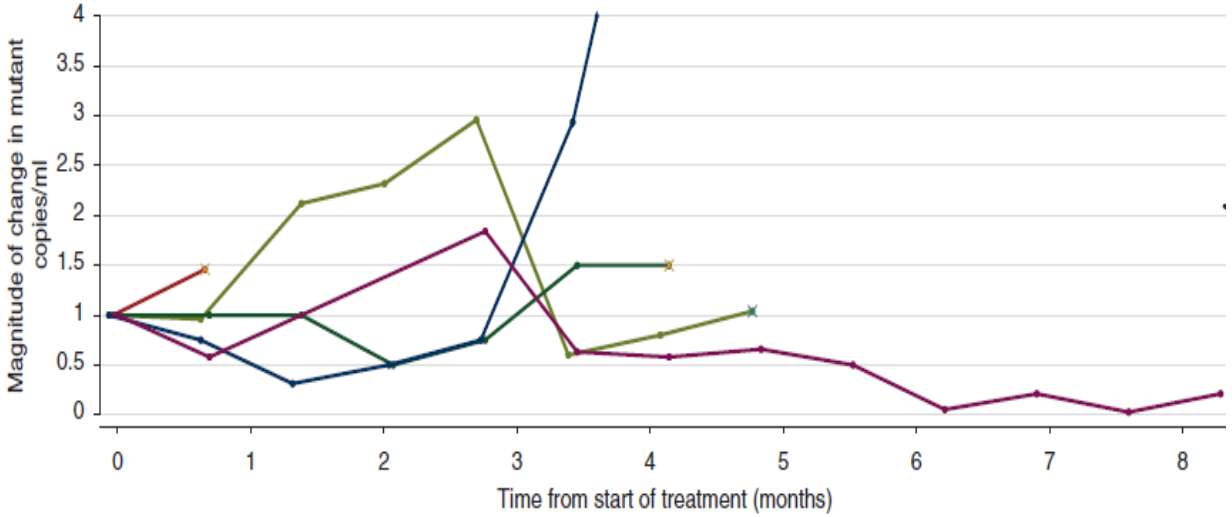


Turner N. et al. Annals of Oncol 2022.

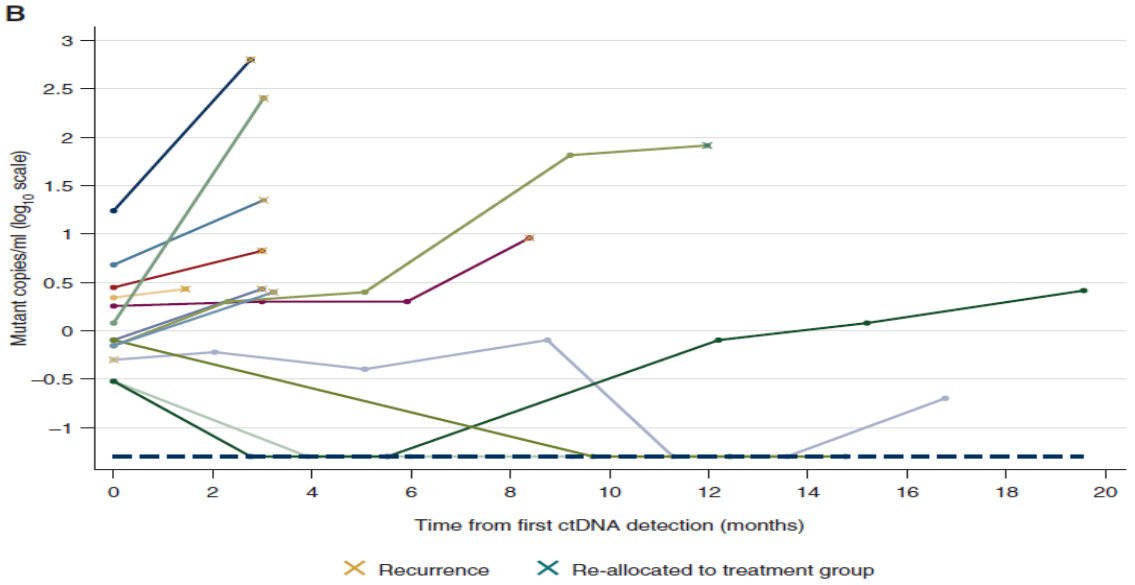
Are we ready to a clinical use of MRD? C-TRACK TN

- 9 pts allocated to pembrolizumab; 4 declined.
- 5 pts commenced pembrolizumab → none (0/5) achieved ctDNA clearance at 6 months, and all subsequently relapsed
- ctDNA clearance after 6 months occurred in 21.4% (3/14, 95% CI 4.7% to 50.8%) of pts in the observation group, and 2 pts have never relapsed (false positive result?)

Pembro arm



Observation arm



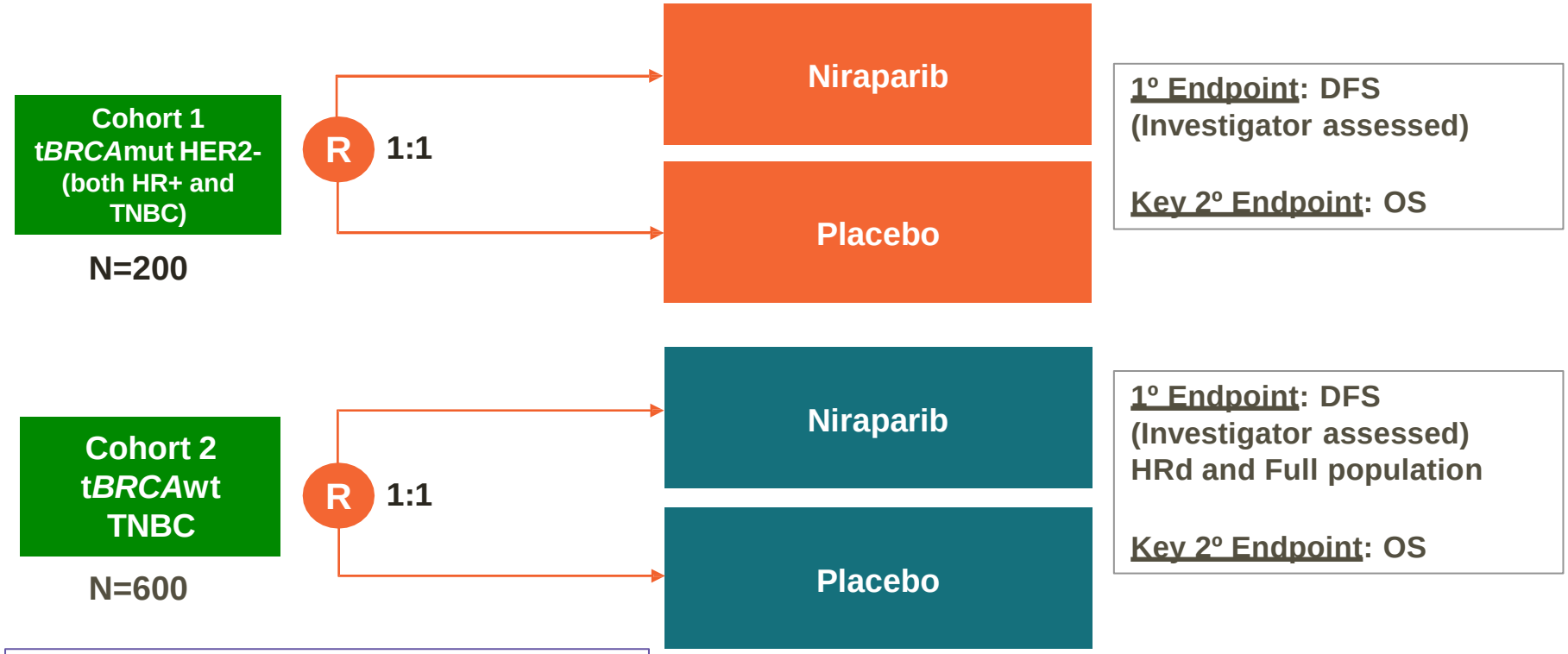
Turner N. et al. Annals of Oncol 2022.

Are we ready to a clinical use of MRD? ZEST trial

Ph 3 trial of the Treatment of *BRCA*mut HER2-Negative or *BRCA*wt Triple Negative Breast Cancer Patients who have Detectable Circulating Tumor DNA Levels after Definitive Therapy; n = 800

Key Eligibility Criteria

- Stage I-III breast cancer with surgical resection of the primary tumor that is confirmed to be either:
  1) t*BRCA*mut HER2-negative, or
  2) t*BRCA*wt TNBC
- ctDNA detected by Signatera (central testing)
- Prior adjuvant therapy allowed
- No sign of radiographic disease recurrence
- Prior exposure to checkpoint inhibitor allowed
- Patients who had neoadj. chemotherapy and tumor showed no response are excluded



Stratification factors

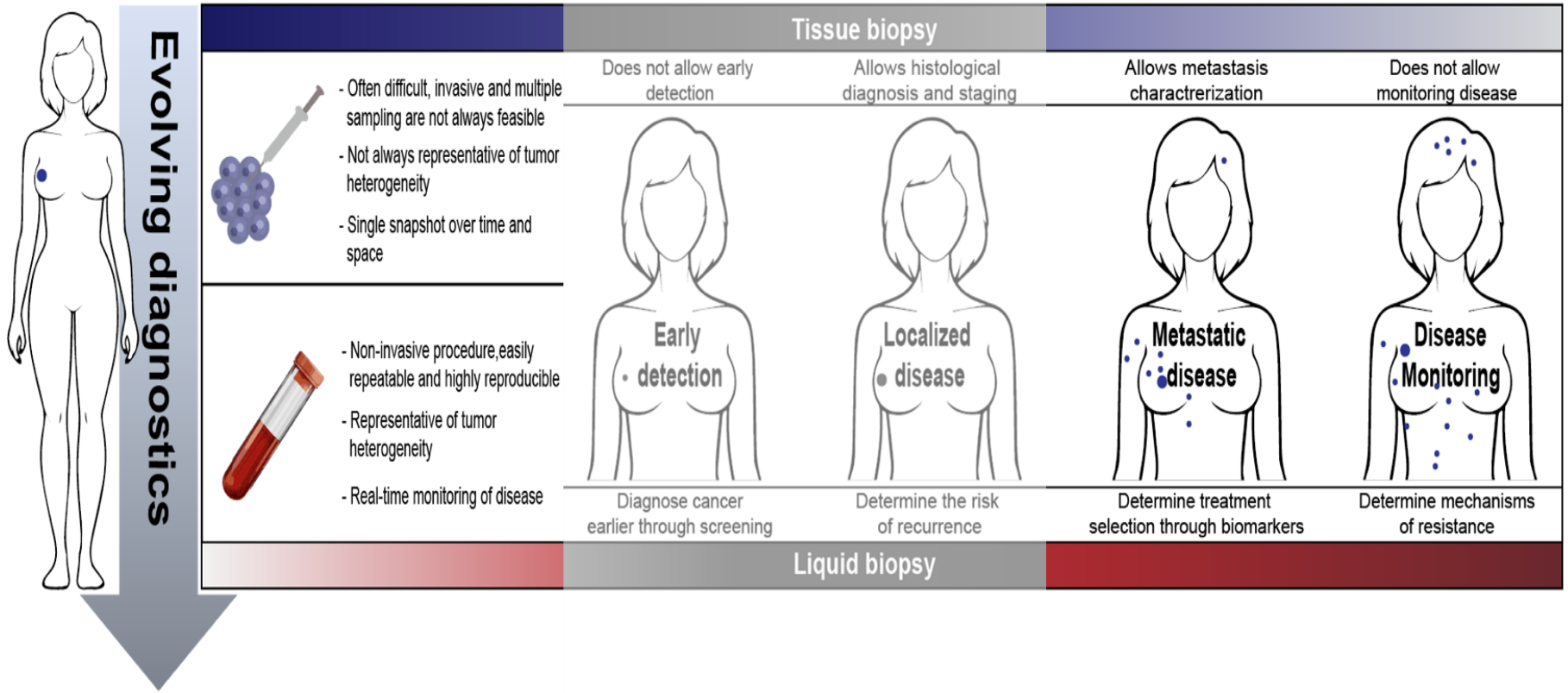
- Time after last intervention (<6 vs ≥6 mo)
- **Prognostic** Stage (I/II vs III)
- Cohort 1 only: HR status (pos or neg)
- Cohort 2 only: Prior use of adj capecitabine

- HR+ patients will receive background SOC hormonal therapy (tamoxifen, anastrozole, letrozole or exemestane by investigator choice)
- TNBC: Concurrent adjuvant pembrolizumab is allowed in both cohorts

Are we ready to a clinical use of MRD? ZEST trial

- 233 pts pre-screened in Italy, 7 ctDNA positive, 5 screening failure, 1 randomized
- Our experience (up-to-now): 54 pts pre-screening, 4 ctDNA positive → 3/4 (75%) metastatic at disease staging ; 1/4 (25%) randomized
- <u>Considerations from C-TRACK TN and initial experience with ZEST:</u>

- In prospective randomized trials ctDNA detection often correspond to metastatic disease (if staged with CT and/or PET scan)
- The lead-time is significantly inferior that previously reported
- High number of pts to be screened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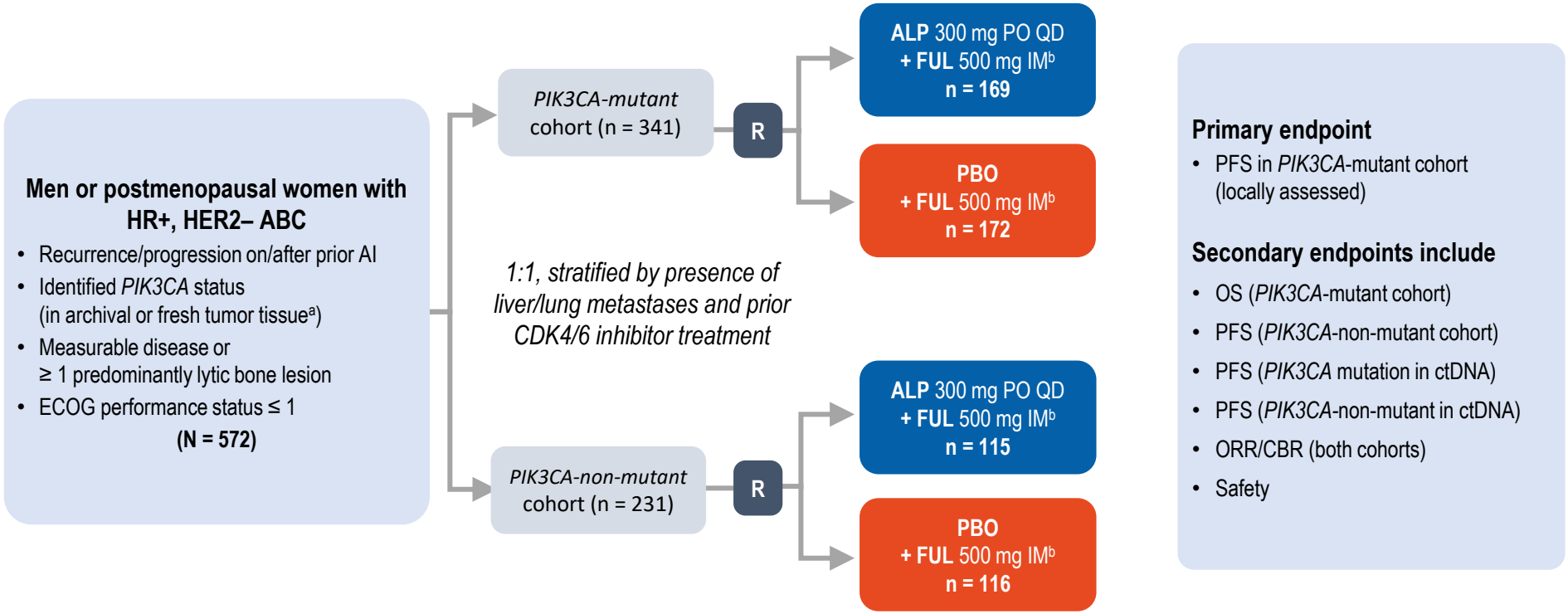
Liquid biopsy

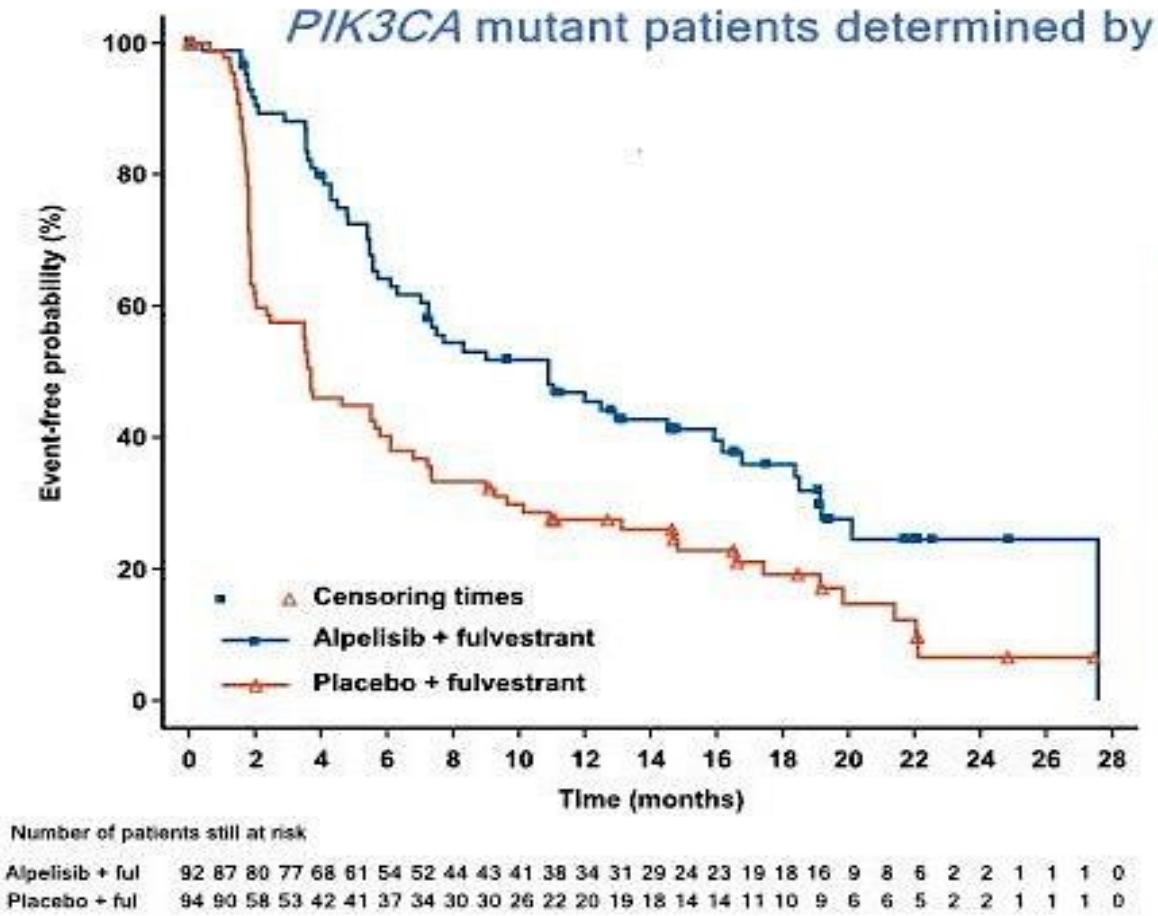
SOLAR-1: A Phase III Randomized, Double-Blind, Placebo-Controlled Trial (NCT02437318)[1]



Tissue biopsy samples collected by investigator sites and sent to a single central laboratory for *PIK3CA* testing

Juric et al. SABCS, 2018

Only about half PIK3CA-mut pts on tissue are also ctDNA+: are we risking to lose some pts potentially candidate to alpelisib if we only evaluate ctDNA?



	ALP + FUL		PBO + FUL		
	Event n/N (%)	Media n PFS	Event n/N (%)	Media n PFS	H R
Patients with *PIK3CA* mutation: tissue	103/169 (60.9)	11.0	129/172 (75.0)	5.7	0.65
Patients with *PIK3CA* mutation: plasma	57/92 (62.0)	10.9	75/94 (79.8)	3.7	0.55
Patients without *PIK3CA* mutation: tissue	49/115 (42.6)	7.4	57/116 (49.1)	5.6	0.85
Patients without *PIK3CA* mutation: plasma	92/181 (50.8)	8.8	103/182 (56.6)	7.3	0.80

- 341 *PIK3CA* mutations by tissue, 322 had *PIK3CA* results by ctDNA of whom 178 (55.3%) had a *PIK3CA* mutation by ctDNA
- Tissue: >90% archive
- *PIK3CA* mut is quite stable, loss is not so frequent

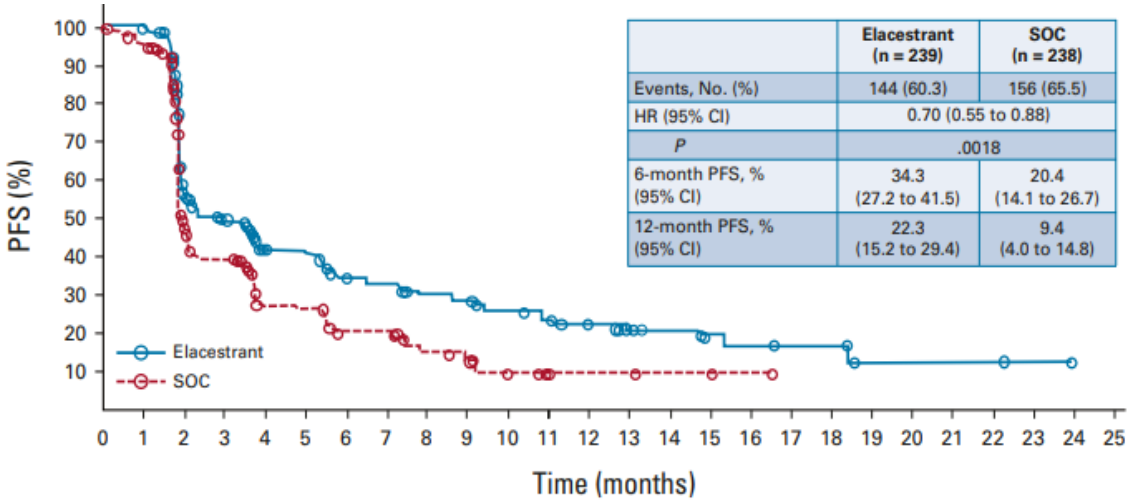
→false negatives?

→results in concordant and discordant?

Juric D, SABCS 2018

EMERALD: Phase III randomized trial of elacestrant *vs* standard endocrine therapy

PFS in All patients

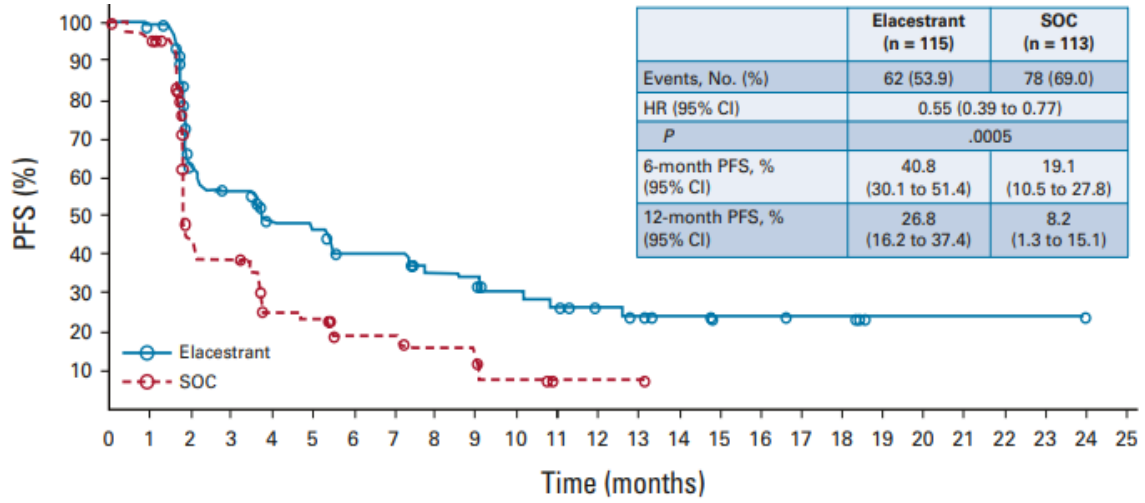


	Elacestrant (n = 239)	SOC (n = 238)
Events, No. (%)	144 (60.3)	156 (65.5)
HR (95% CI)	0.70 (0.55 to 0.88)	
P	.0018	
6-month PFS, % (95% CI)	34.3 (27.2 to 41.5)	20.4 (14.1 to 26.7)
12-month PFS, % (95% CI)	22.3 (15.2 to 29.4)	9.4 (4.0 to 14.8)

No. at risk:

Elacestrant	239	223	106	89	60	57	42	40	34	33	27	24	19	13	11	8	7	6	6	2	2	2	2	1	0
SOC	238	206	84	68	39	38	25	25	16	15	7	4	3	3	2	2	1	0							

PFS in ESR1mut patients



	Elacestrant (n = 115)	SOC (n = 113)
Events, No. (%)	62 (53.9)	78 (69.0)
HR (95% CI)	0.55 (0.39 to 0.77)	
P	.0005	
6-month PFS, % (95% CI)	40.8 (30.1 to 51.4)	19.1 (10.5 to 27.8)
12-month PFS, % (95% CI)	26.8 (16.2 to 37.4)	8.2 (1.3 to 15.1)

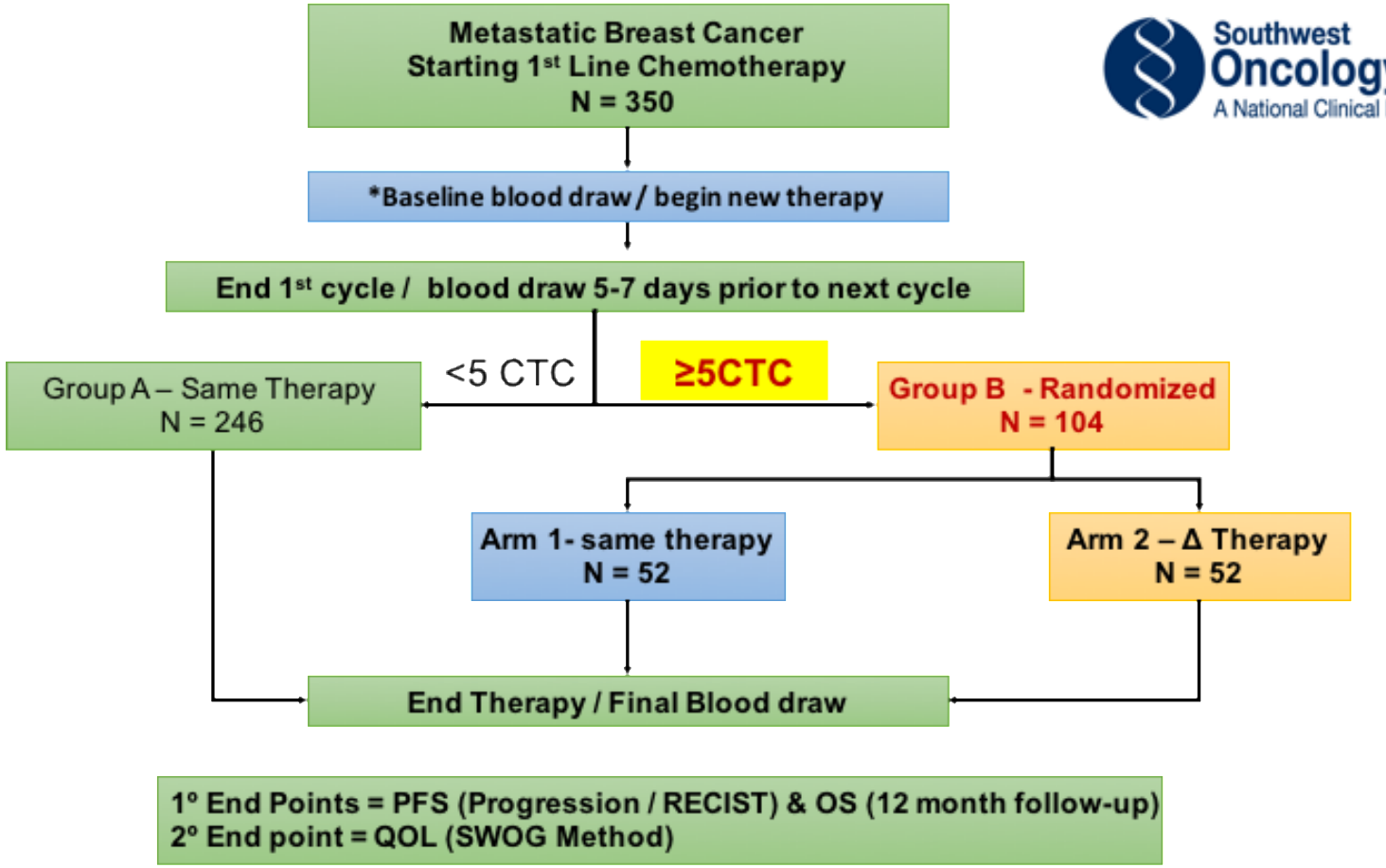
No. at risk:

Elacestrant	115	105	54	46	35	33	26	26	21	20	16	14	11	9	7	5	5	4	4	1	1	1	1	1	0
SOC	113	99	39	34	19	18	12	12	9	9	4	1	1	1	0										

CO-PRIMARY endpoints were PFS in all pts and in ESR1mut pts. Formally, the trial is positive for both the co-primary endpoints. Do we really need ESR1mut to candidate pts to elacestrant? (FDA)
Are we ready to deny an ORAL potentially valid alternative to fulvestrant in ESR1wt pts? Waiting for EMA

Bidard F-C et al. J Clin Oncol 2022;40:3246–56

Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500



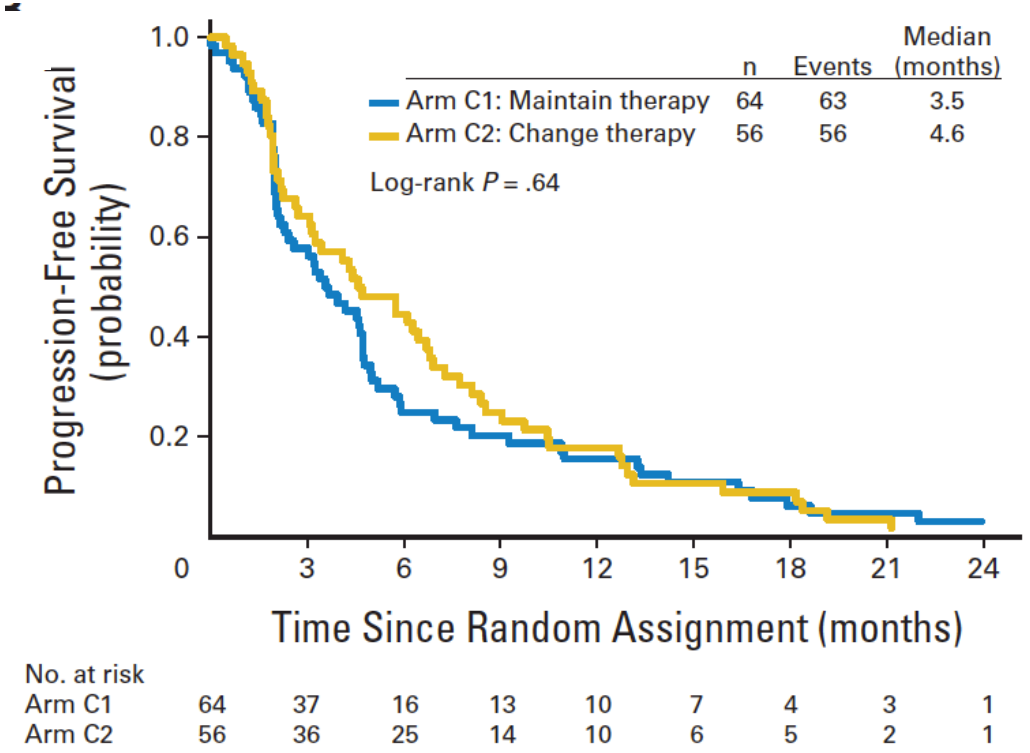
Southwest Oncology Group
A National Clinical Research Group

Smerage JB et al. JCO 2014

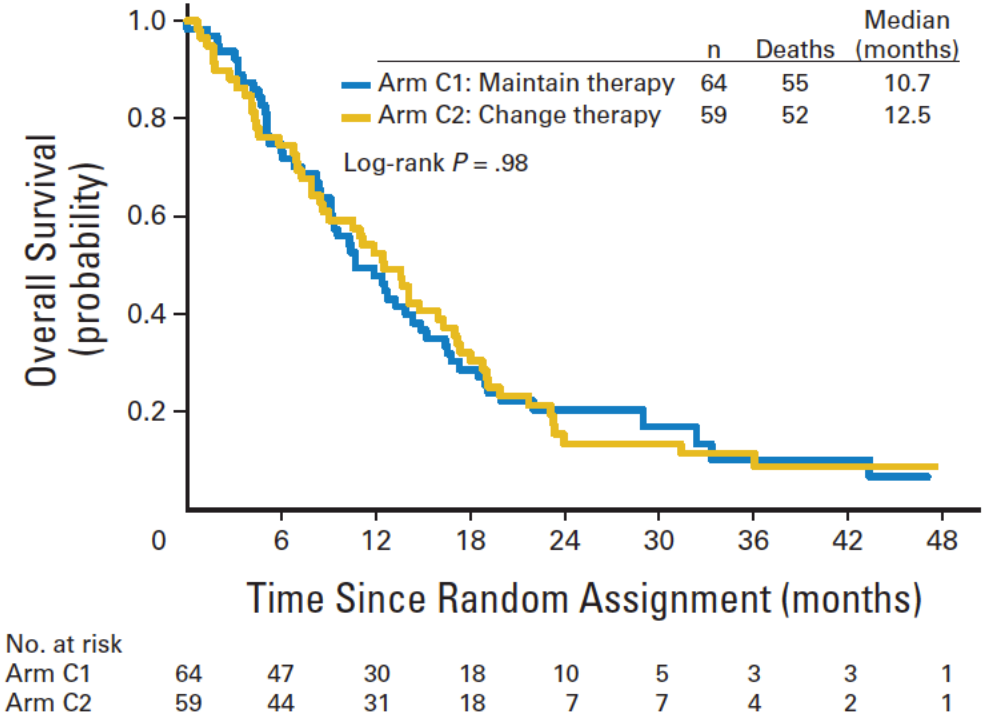
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Circulating Tumor Cells and Response to Chemotherapy in Metastatic Breast Cancer: SWOG S0500

Progression-free Survival

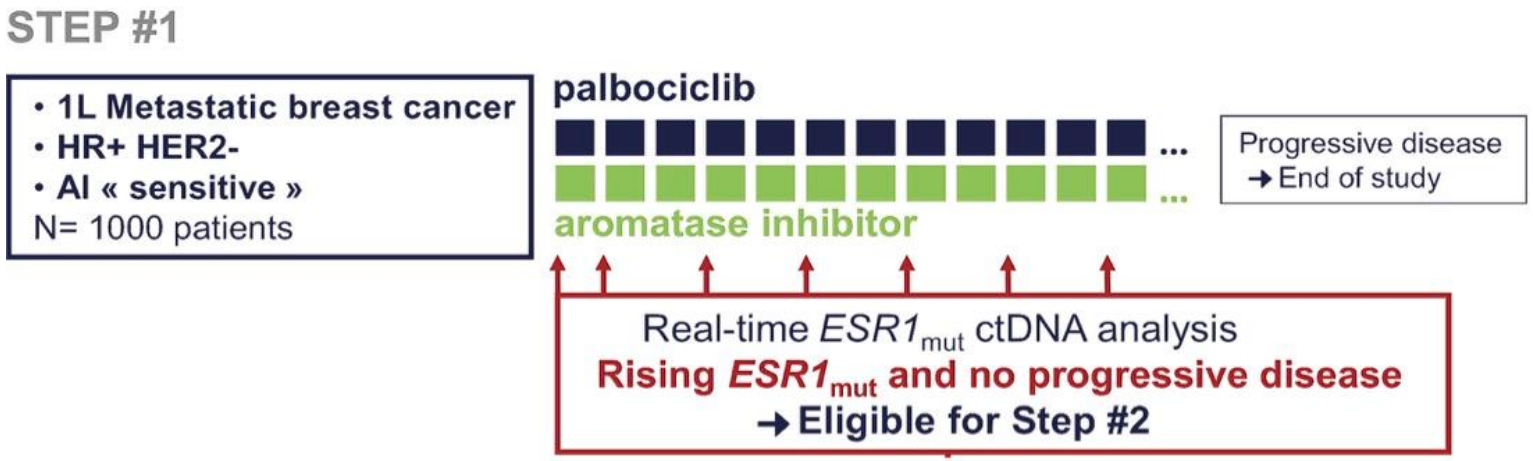


Overall Survival



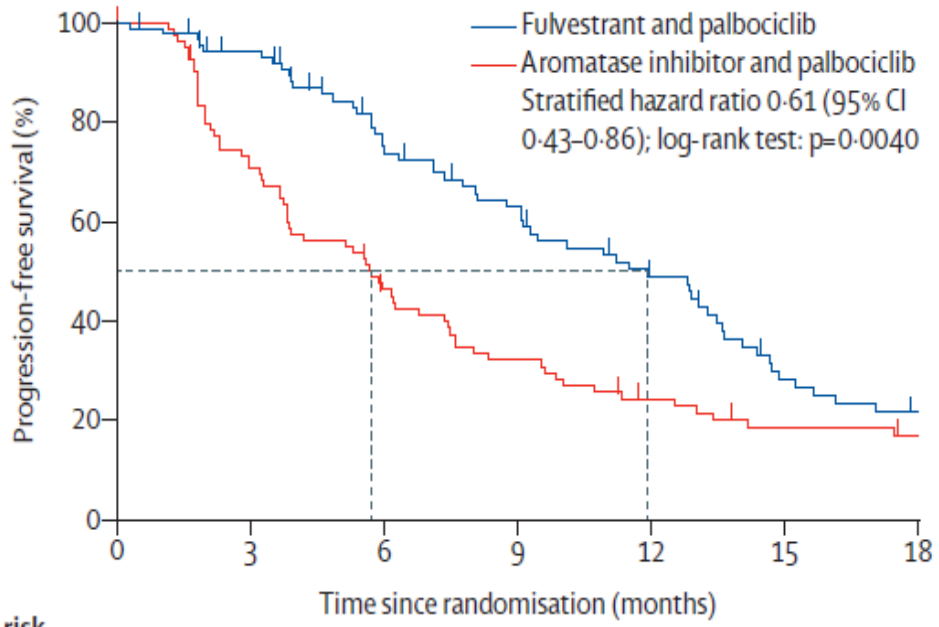
Smerage JB et al. JCO 2014

Testing clinical utility of real-time ESR1 mut. detection: PADA-1 Trial



Bidard et al, SABCS 2018

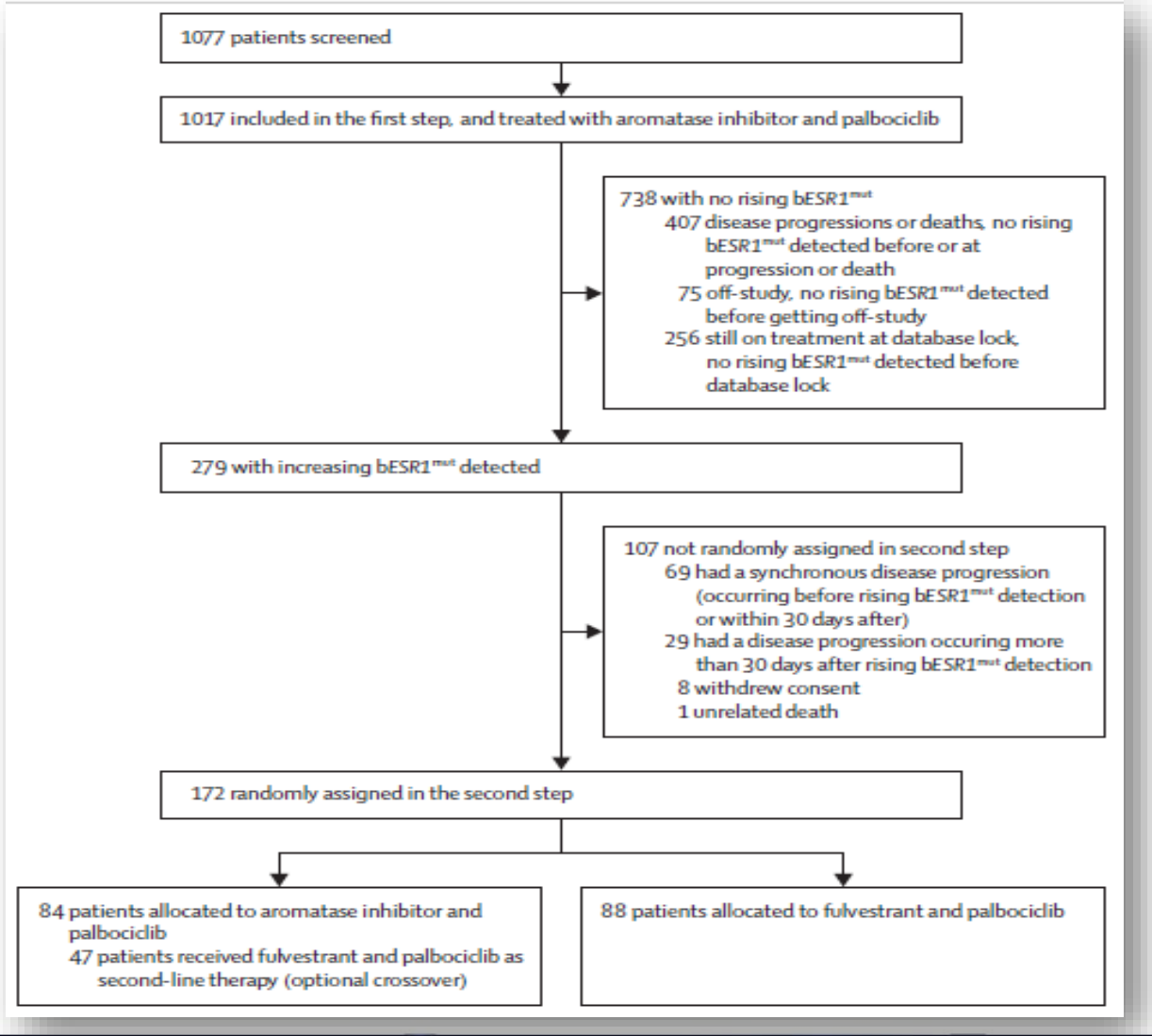
Testing clinical utility of real-time ESR1 mut. detection: PADA-1 Trial



Number at risk (number censored)							
Fulvestrant and palbociclib	88 (0)	78 (5)	57 (11)	46 (13)	32 (17)	17 (19)	12 (20)
Aromatase inhibitor and palbociclib	84 (1)	58 (2)	36 (4)	25 (4)	17 (6)	12 (7)	10 (8)

Bidard F-C et al. Lancet Oncol 2022;23:1367–77.

Testing clinical utility of real-time ESR1 mut. detection: PADA-1 Trial



Some considerations

- For 107/279 (38%) ESR1 monitoring strategy failed
- Is a PFS advantage enough to consider an anticipated line? Or shoud we wait for OS data? Other endpoints to be considered?
- In all randomly assigned pts (n=172), the median time to strategy failure was 11.9 months (9.1–13.6) in the fulvestrant and palbo group and 10.6 months (8.0–13.4) in the AI and palbo group (HR 1.02, 95% CI 0.71–1.45; log-rank test p=0.90)
- The median CT-free survival was 14.6 months (11.8–17.0) in the fulvestrant and palbo group and 13.1 months (10.8–17.6) in the AI and palbociclib group (HR 0.91, 0.62–1.33; log-rank test p=0.60)
- Possibility to switch to elacestrant at progression

Bidard F-C et al. Lancet Oncol 2022;23:1367–77.

Does liquid biopsy have a clinically utility NOW? **NO!**