Detection of circulating tumor DNA following neoadjuvant chemotherapy and surgery to anticipate early relapse in ER positive and HER2 negative breast cancer: Analysis from the PENELOPE-B trial

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Disclosures

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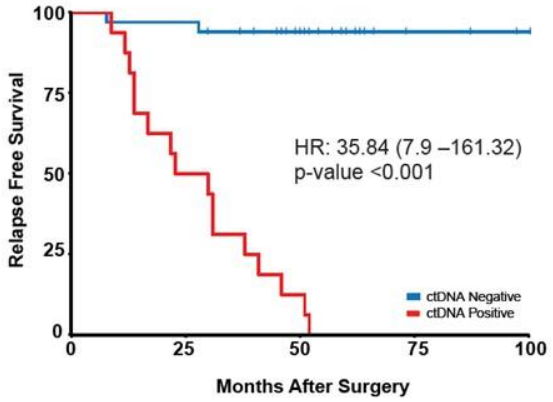
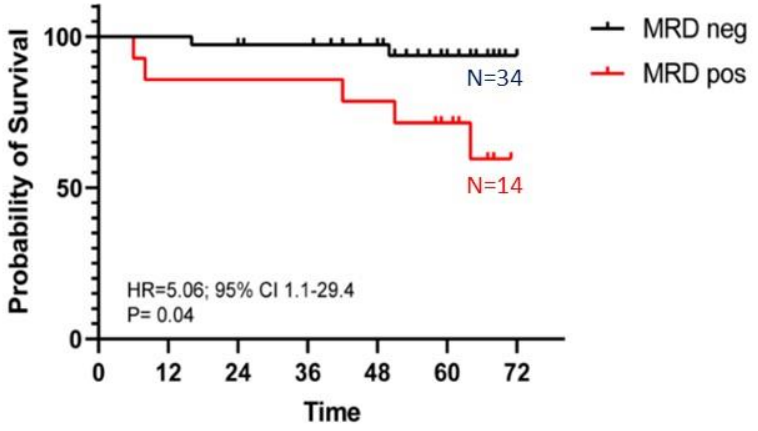
Background – ctDNA in early breast cancer

- Tumors release circulating tumor DNA (ctDNA) into the circulation
- Detection of ctDNA in follow-up 'molecular relapse' anticipates future relapse with high accuracy[1,2]
- Limited data suggest detection of molecular residual disease immediately after surgery, can predict relapse[3]
- Potential use of ctDNA in selection of adjuvant CDK4/6 inhibition is unclear

Molecular relapse monitoring[2]



MRD detection after primary surgery[3]



1. Garcia-Murillas *et al* STM 2015,
2. Coombes *et al* CCR 2019,
3. Garcia-Murillas SABCS 2021

Background - PENELOPE B

N=1250
- HR+/HER2- breast cancer
- no pCR after NACT
- CPS-EG score ≥3 or ≥2 with ypN+

Primary Endpoint: iDFS

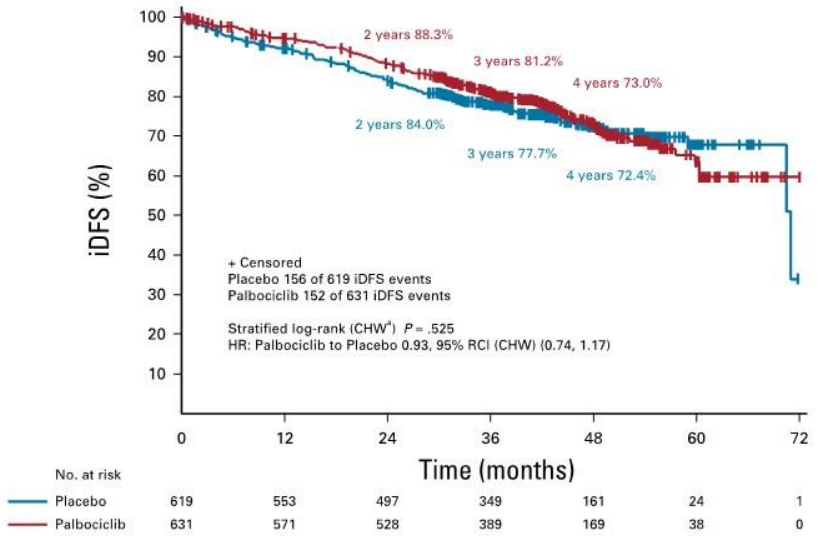
Stratification factors
- Nodal status: ypN 0-1 vs ypN2-3
- Age: ≤50 vs >50 yrs
- Ki-67: >15% vs ≤ 15%
- Region: Asian vs non Asian
- CPS-EG Score: ≥3 vs 2 and ypN+

PENELOPE B

Neoadjuvant Chemotherapy → **Surgery +/- Radiotherapy** → **R 1:1**
- **Palbociclib** 1 year
- **Placebo**

All patients received concomitantly endocrine therapy according to local standards.

Primary endpoint



Samples for ctDNA analysis

Baseline ↓ Cycle 7 ↓ EoT ↓

PenelopeB ET + Palbociclib/Placebo

1. Loibl S et al. J Clin Oncol 2021

Objectives

- To assess the potential of ctDNA analysis to predict future clinical relapse of patients enrolled in the PENELOPE-B trial
- To assess the potential role of sequential ctDNA analysis, ctDNA dynamics, in predicting future clinical relapse
- To assess whether a full analysis of baseline samples is indicated to assess whether palbociclib has benefit in ctDNA positive patients

Methods

- Patients endocrine naive at the time of study entry were selected (129 of 1250)

- Biomaterial was available and ctDNA analysis was performed for 83 patients
  - 78 patients successful ctDNA analysis at baseline
  - ctDNA analysis set was representative of the overall endocrine naive group, with median follow-up of 42.9 months

- 210 plasma samples were collected in Streck tubes and processed

- Association of ctDNA detection with invasive disease-free survival (iDFS) and distant metastasis-free survival was analysed using Cox proportional hazard models.

ctDNA analysis methods

A tumor sample was exome sequenced, and up to 48 tumor variants were tracked in plasma using error-corrected sequencing for ctDNA detection (RaDaR assay).

Step 1

Patient's tumor sample (FFPE) is sent to the NeoGenomics laboratory

Step 2

Patient's tumor DNA is sequenced to determine the tumor's unique mutation profile

Step 3

A personalized RaDaR panel is designed for the patient

Step 4

After initial panel design, ctDNA is tested using blood samples and the patient's custom RaDaR panel

Step 5

Report is generated

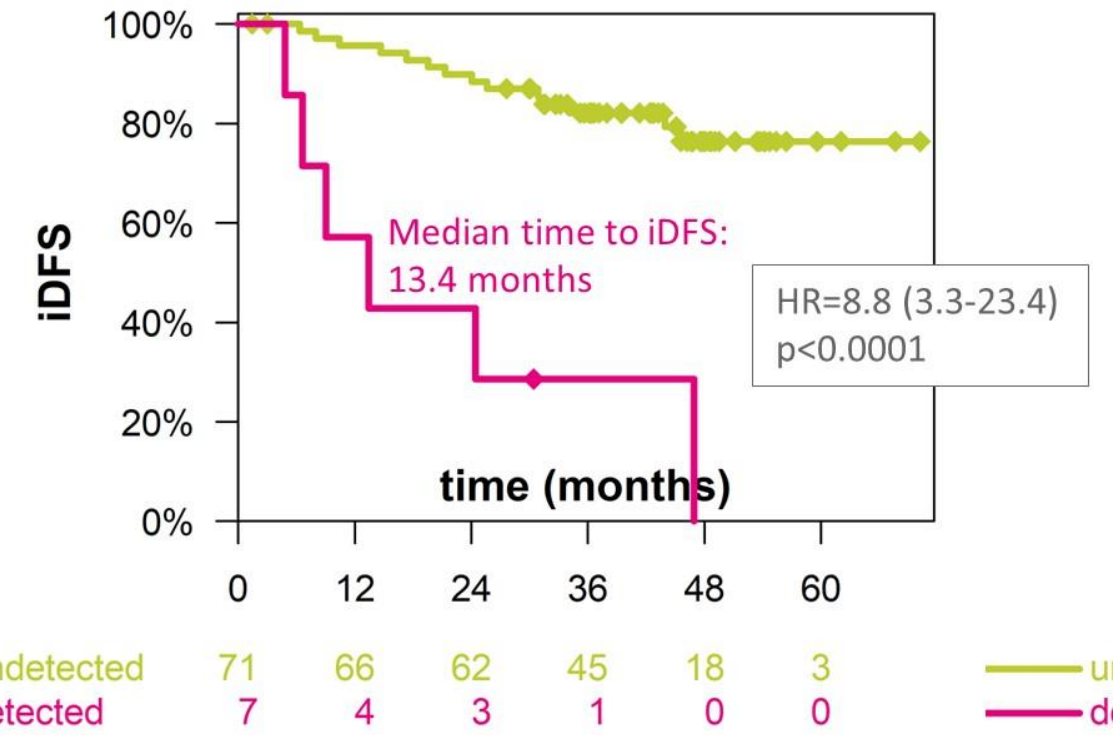
ctDNA detection

- Baseline ctDNA detection 9% (7/78) patients
- Of patients undetected at baseline 5% (3/66) had ctDNA detected in later samples
- Of patients detected at baseline, 29% (2/7) became undetected in later samples

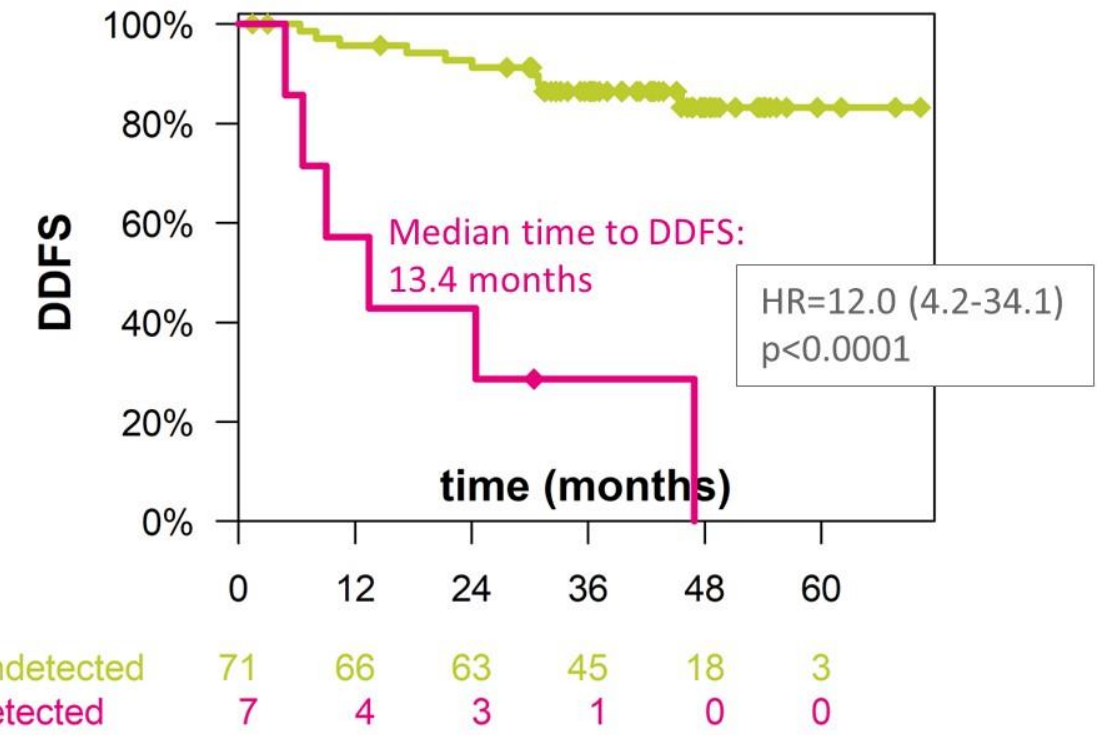
n	baseline	before cycle 7	EoT	Dynamics classification
48	undetected	undetected	undetected	all undetected
8	undetected	undetected		all undetected
7	undetected		undetected	all undetected
5	undetected			Not classified (one sample)
1	detected	undetected	undetected	becoming undetected
1	detected	undetected		becoming undetected
2	undetected	undetected	detected	becoming detected
1	undetected	detected	detected	becoming detected
2	detected		detected	all detected
3	detected	detected	detected	all detected

Results – baseline ctDNA detection

Invasive disease free survival



Distant disease free survival



Results – baseline ctDNA detection

Multivariable analysis:

Invasive disease free survival

variable	comparison	HR	P
ctDNA at baseline	detected vs undetected	6.47 (2.19-19.12)	0.0007
Ki-67	>15% vs ≤15%	1.90 (0.70- 5.14)	0.2054
ypT	ypT3-4 vs ypT0-2	2.03 (0.75- 5.52)	0.1644

Distant disease free survival

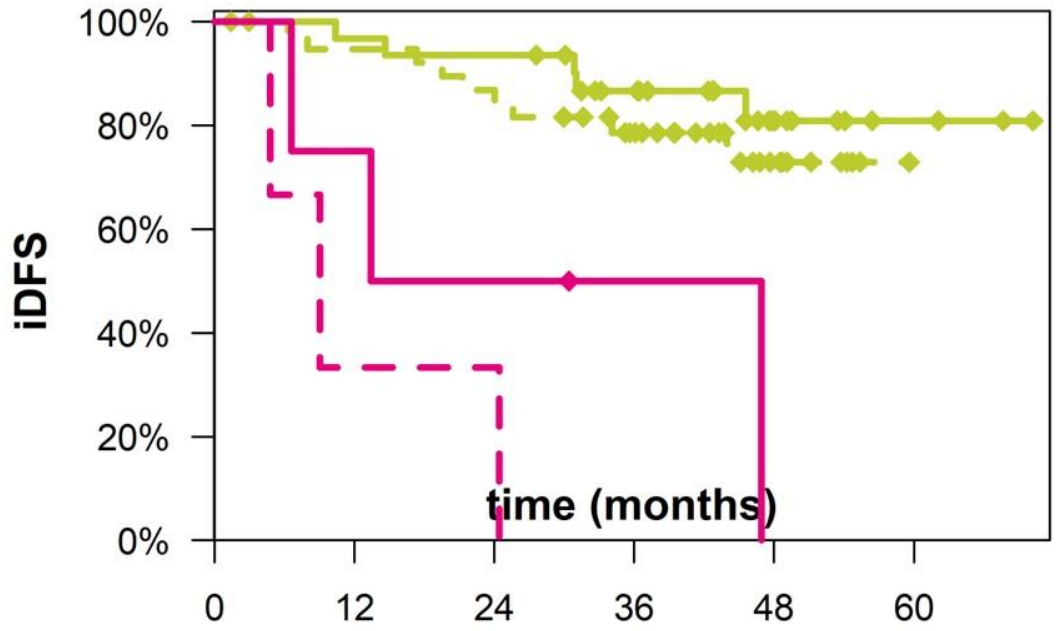
variable	comparison	HR	P
ctDNA at baseline	detected vs undetected	10.93 (3.47-34.48)	<0.0001
Ki-67	>15% vs ≤15%	1.17 (0.37- 3.74)	0.7875
ypT	ypT3-4 vs ypT0-2	1.96 (0.63- 6.11)	0.2434

ctDNA analysis dominated multivariable analysis

Results – baseline ctDNA detection

Invasive disease free survival

Test for interaction: p=0.5456



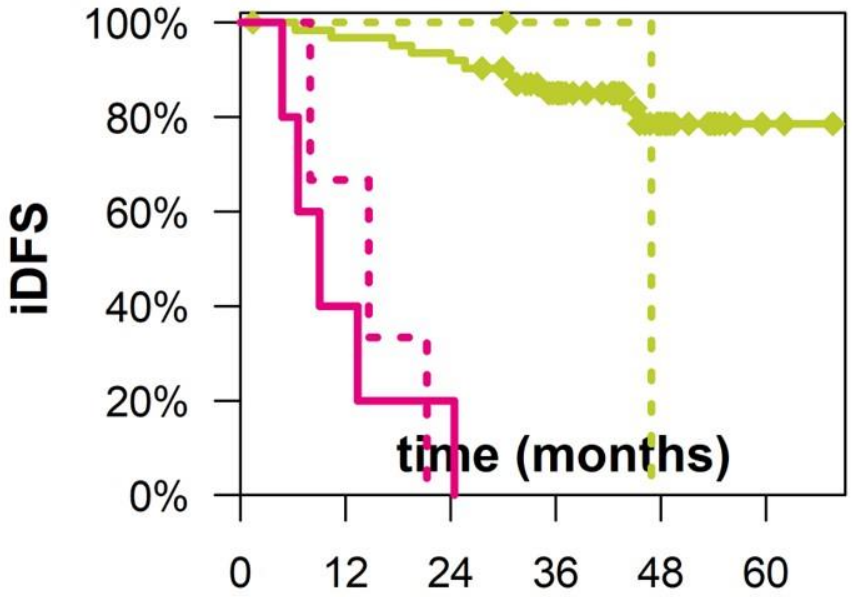
undetected, palbociclib	33	30	29	21	9	3
undetected, placebo	38	36	33	24	9	0
detected, palbociclib	4	3	2	1	0	0
detected, placebo	3	1	1	0	0	0

Split by treatment allocation

Groups too small to draw conclusions

Results – ctDNA dynamics

Invasive disease free survival



	0	12	24	36	48	60
all undetected	63	60	58	43	17	2
becoming undetected	2	2	2	1	0	0
becoming detected	3	2	0	0	0	0
all detected	5	2	1	0	0	0

iDFS by ctDNA dynamic groups

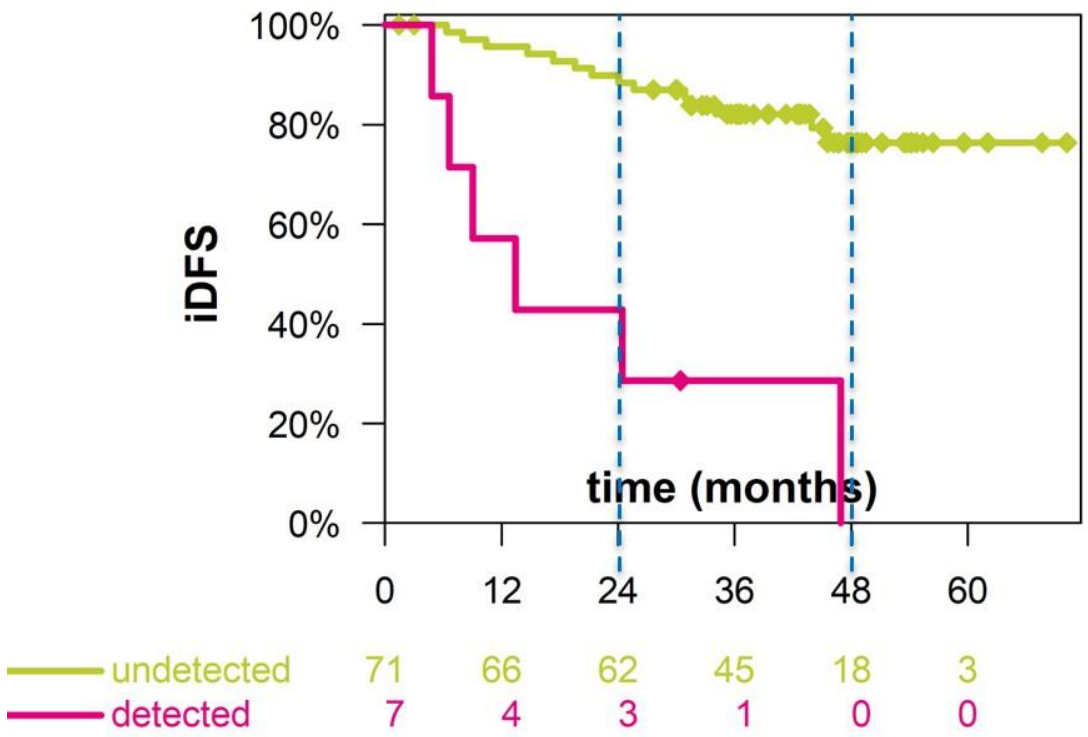
Patients with undetected baseline ctDNA, who become positive during treatment have poor outcome

Both patients who became undetected were on palbociclib

Analysis limited by small groups

Excludes 5 patients with a baseline sample and no subsequent samples

Limitations of baseline ctDNA analysis



'Sensitivity' for relapse analysed in time windows

Higher sensitivity (49%) for relapses within 12 months

Low/Moderate sensitivity for early relapses within 24 months (36% of currently observed)

Low sensitivity for relapses >48 months (relapses not yet observed, but will occur with longer follow-up)

Analysis limited by follow-up

Conclusions

- Detection of ctDNA following neoadjuvant chemotherapy, and surgery, is associated with a very high risk of early relapse suggesting limited efficacy of adjuvant endocrine therapy
  - Studies of clinical imaging and experimental therapy are warranted for these patients

- 'Sensitivity' for future relapse is imperfect, in particular for later relapses, in patients who had prior neoadjuvant chemotherapy and surgery
  - Response to prior neoadjuvant chemotherapy may reduce ctDNA detection rates
  - Sequential testing improves 'sensitivity' for relapse

- Although Signatera ctDNA analysis has been approved by Medicare, use is likely not appropriate in deciding whether to give adjuvant CDK4/6 inhibitor in patients otherwise eligible for treatment, in patients who have had neoadjuvant chemotherapy

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Cooperating partners

Collaborating study groups

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BioBank team at Philipps Universität Marburg

Central Lab team at Inivata A Leader in Liquid Biopsy Subsidiary of NeoGenomics

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GBG

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