Longitudinal transcriptional profiling of CTCs in metastatic breast cancer patients receiving the CDK4/6 inhibitor Palbociclib to predict therapy response.

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Purpose of the Study

CDK4/6 inhibitors represent a new treatment option for metastatic (M), hormone receptor-positive/HER2-negative (HR+/HER2-) breast cancer (BC) patients. Here, we aim to identify urgently needed biomarkers of resistance to Palbociclib by conducting transcriptional profiling of circulating tumor cells (CTCs) before therapy initiation (baseline) and after six months under treatment.

Patients and methods

Samples at baseline

Samples after six months

Transcripts of interest

ABCC1	ABCC2	AKT1	BRAF	CDK2	CETN2	CXCR4	E2F1	EpCAM
ERBB4	ESR1	FAT4	FGFR1	FRMD6	FRS2	JUN	MAPK1
MLH1	MLH3	NFKB1	PCNA	STAT1	TEAD2	WWTR1	YAP1

Blood
In EDTA tube (4°C max. 4h) or ACDA tube (4°C max 24h)

CTC
by AdnaTest EMT2/StemCell Select, QIAGEN (targeting EpCAM, EGFR and HER2) from 2x5ml blood

mRNA
by AdnaTest EMT2/StemCell Detect, QIAGEN from the CTC lysate

cDNA
by AdnaTest EMT2/StemCell Detect, QIAGEN from the mRNA eluate

Preamplified cDNA
by UCP Multiplex PCR protocol, QIAGEN with 18 PCR cycles

mRNA profiling
by singleplex qPCR (QuantiNova LNA Probe PCR assays, QIAGEN); with 1:10 diluted preamplified cDNA; targeting 25 different transcripts of interest

Data evaluation
C_T values >30 excluded; normalization to CD45 and 20 healthy donors -> overexpression yes/no (binary); if one duplicate shows overexpression -> whole sample is evaluated as positive
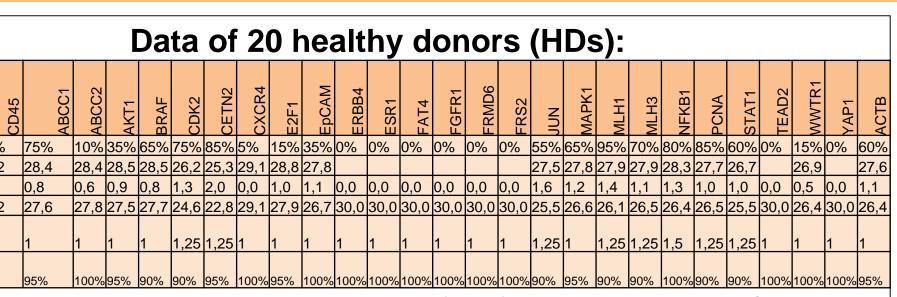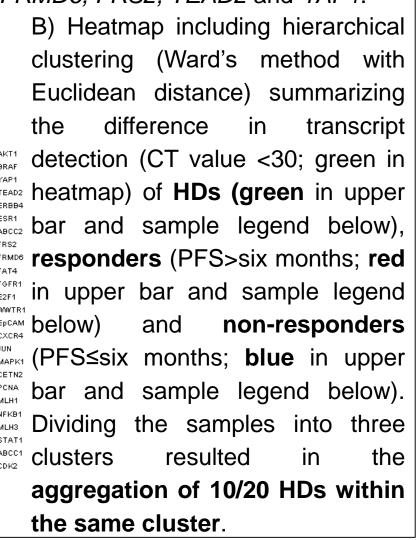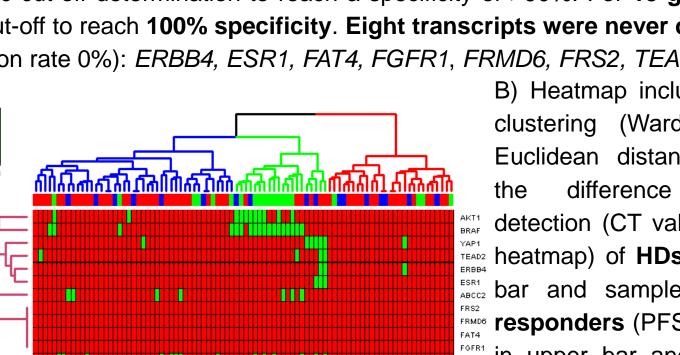
Updated Results

Data of 20 healthy donors (HDs):

A) The detection rate shows the percentage of HDs (n=20) showing at least one C_T value<30 in one duplicate. We subtracted multiples of the standard deviation (SD) from the mean for target-specific cut-off determination to reach a specificity of >90%. For **13 genes of interest**, we set the cut-off to reach **100% specificity**. **Eight transcripts were never detected in all 20 HDs** (detection rate 0%): *ERBB4, ESR1, FAT4, FGFR1, FRMD6, FRS2, TEAD2* and *YAP1*.
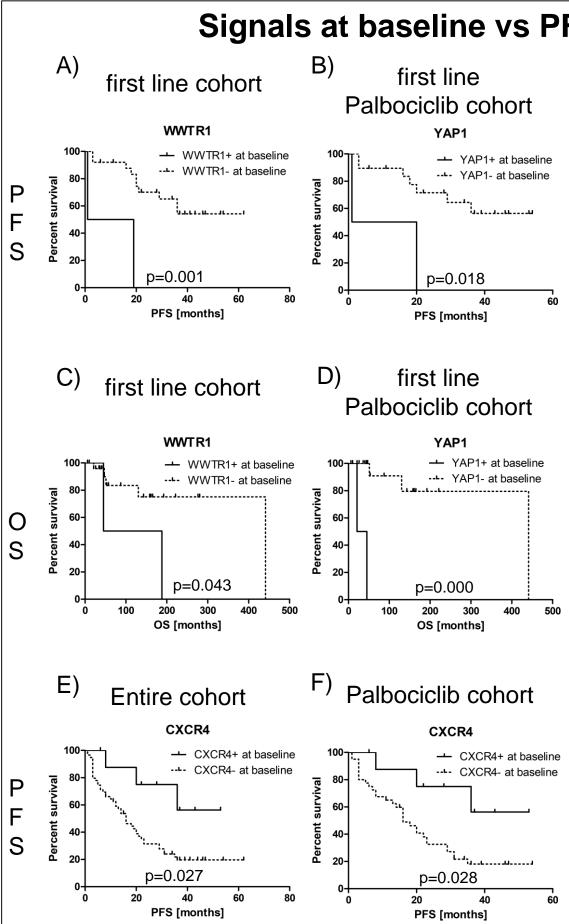
B) Heatmap including hierarchical clustering (Ward's method with Euclidean distance) summarizing the difference in transcript detection (CT value <30; green in heatmap) of **HDs (green in upper bar and sample legend below)**, **responders** (PFS>six months; **red** in upper bar and sample legend below) and **non-responders** (PFS≤six months; **blue** in upper bar and sample legend below). Dividing the samples into three clusters resulted in the **aggregation of 10/20 HDs within the same cluster**.

Signals at baseline vs Response:

Higher prevalence of *EpCAM, PCNA, STAT1* and *YAP1* signals (arrows) at baseline in **non-responders** compared to responders in the cohort consisting of only patients treated with Palbociclib (not significant). *CXCR4* signals (in box) were **only detected in responders** in this cohort (not significant).

Signals at baseline vs PFS and OS:

WWTR1 signals (n=2) in the **first line** treated cohort significantly correlated with **reduced PFS** (A) and **OS** (C).

YAP1 signals (n=2) significantly correlated with **decreased PFS** (B) and **OS** (D) in **the first line Palbociclib** treated cohort and significantly correlated with decreased PFS also in the entire first line cohort (latter not shown).

CXCR4 signals significantly correlated with **increased PFS** in the **entire** cohort (E) and in the **Palbociclib** treated cohort (F). This effect, however, might be due to the fact, that CXCR4 signals were **significantly more common in the first line** treated patients (compared to the second or more line treated patients; data not shown), who might have an increased PFS anyway.

Statistical analysis via log-rank (Mantel-Cox) test.

Signals after six months vs Response:

CDK2 signals after six months were significantly more common in **non-responders** compared to responders in the cohort consisting of only patients treated with Palbociclib (fisher's exact test two-tailed). In addition to *CDK2, ABCC2, AKT1, BRAF, ERBB4, MAPK1, STAT1* and *TEAD2* signals after six months were **only detected in non-responders** of this cohort.

Signals after six months vs OS:

TEAD2 (A) and *CDK2* (B) signals **after six months** significantly (log-rank test) correlated with **worse OS** in the Palbociclib treated group, but not in the control group (not shown) or entire cohort (not shown).

Signals dynamics from baseline to after six months vs Response

Interestingly, the percentage of **disappearing signals** was greater in **responders** than in non-responders and the percentage of **newly appearing signals** (not detected at baseline, but existing after six months) was higher in the **non-responders** compared to the responders.

Signals dynamics vs OS

The **dynamics** of *TEAD2* (A) and *MLH3* (B) signals from baseline to six months after therapy initiation significantly (log-rank test) correlated with OS in the Palbociclib treated cohort (not in the control or entire cohort).

Conclusion

Preliminary results of transcriptional profiling of CTCs that represent a real-time snapshot of the disease indicate that

➤ *WWTR1* and *YAP1* signals at baseline might be predictive markers that do not favor Palbociclib treatment.

➤ *CXCR4* signals at baseline might indicate favorable PFS

➤ *CDK2* signals in CTCs after six months or the transcriptional dynamics of *TEAD2* and *MLH3* from baseline to six months under Palbociclib might be suitable as monitoring markers.

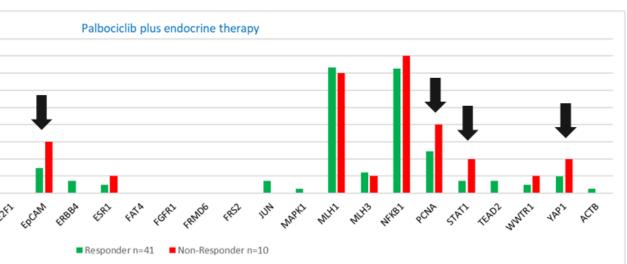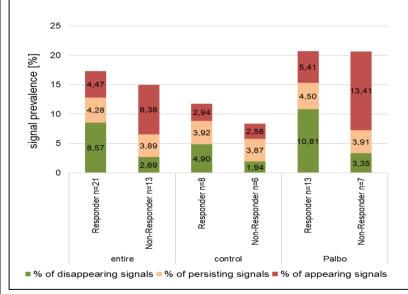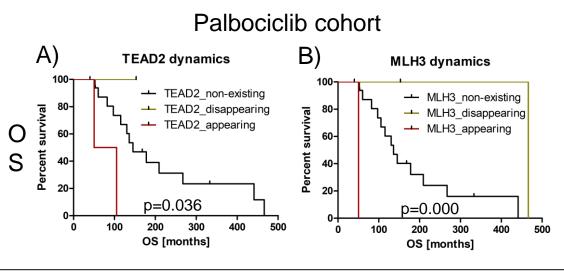**The results have to be validated in larger cohorts.**

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