Longitudinal transcriptional profiling of CTCs in metastatic breast cancer patients receiving CDK4/6 inhibitors to predict response

Corinna Keup[1], Charlotte Gruber[1], Oliver Hoffmann[1], Rainer Kimmig[1] and Sabine Kasimir-Bauer[1]

[1]Department of Gynecology and Obstetrics, University Hospital of Essen, Germany

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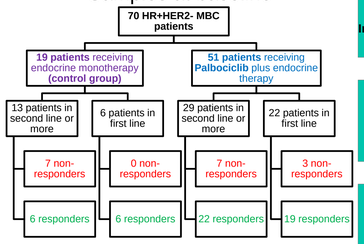
Background

CDK4/6 inhibitors recently became the first choice for treatment of metastatic (M), hormone receptor-positive/HER2-negative (HR+/HER2-) breast cancer (BC) patients (pts). However, predictive markers are missing. Circulating tumor cells (CTCs) represent the heterogeneous disease in real time.

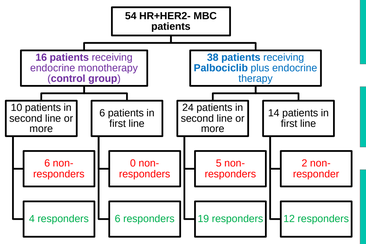
Here, we aim to identify resistance markers to CDK4/6 inhibitors by mRNA profiling of CTCs before therapy start (baseline) and after six months under treatment.

Patients and Methods

Samples at baseline



Samples after six months



Non-Responders: patients with PFS ≤ 6 months
Responders: patients with PFS>6 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

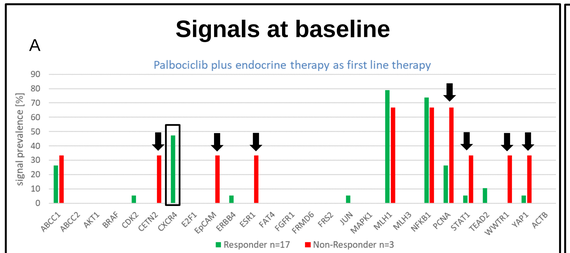
cDNA
by AdnaTest EMT2/StemCell Detect, QIAGEN from the mRNA eluate

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

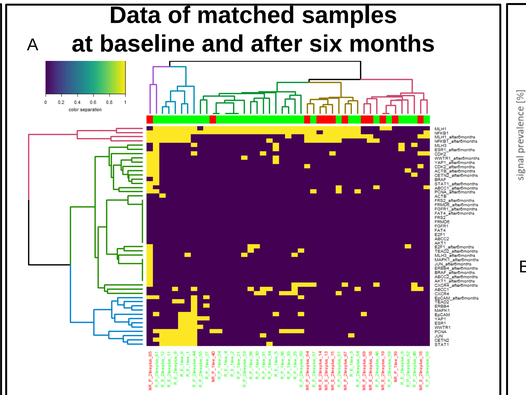
Data evaluation
C_T values >30 excluded; normalization to CD45 and 20 healthy donors -> overexpression yes/no (binary); if one duplicate shows overexpression -> whole sample is evaluated as positive

Updated Results

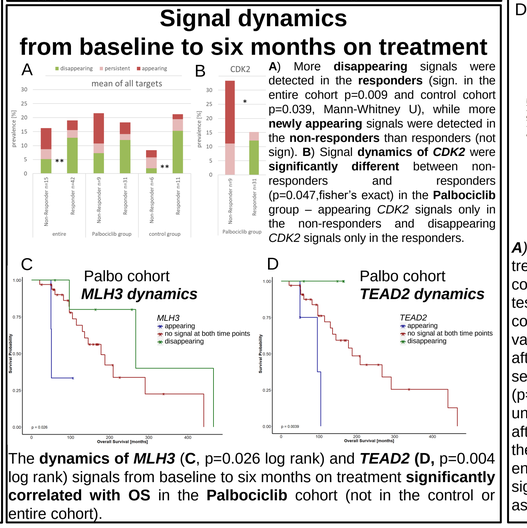


A) CXCR4 signals were detected in half of all responders, but **not in any non-responder** the first line treated Palbociclib plus endocrine therapy group (not significant). In contrast, ***CETN2, EpCAM and ESR1 were only detected in the non-responders****, but not in the responders of this cohort (not significant).* ***PCNA, STAT1, WWTR1*** **and** ***YAP1*** **signals were more frequent in the non-responders** than in the responders (not significant, CAVE: only n=3 non-responders).

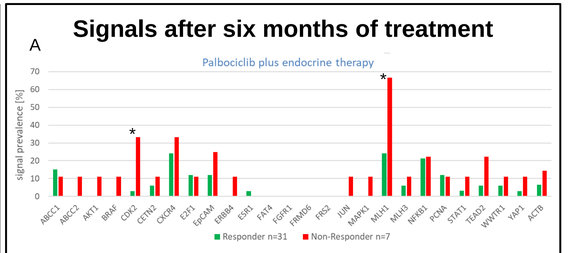
Kaplan-Meier curves showing the significant correlation of ***CXCR4*** **signals with increased PFS in the entire cohort** (**B**, log-rank: p=0.024, univariate cox regression: 0.039) **and Palbociclib plus endocrine treated patients** (**C**, log-rank: p=0.027, univariate cox regression: 0.043). It is to note, that *CXCR4* signals were significantly more often found in first line treated patients and first line treated patients showed a significant longer PFS (data not shown). The ***YAP1*** **signals significantly correlated with decreased PFS** (**D**, log-rank: p=0.018, univariate cox regression: 0.039) **and decreased OS** (**E**, log-rank: p=0.018, univariate cox regression: not sign.) **in the first line Palbociclib plus endocrine treated cohort** (CAVE: only n=2 with *YAP1* signal).



A) Heatmap including hierarchical clustering (Ward's method with Euclidean distance) summarizing the difference in overexpression signals of **responders (green** in upper bar and sample legend below) and **non-responders (red** in upper bar and sample legend below) at baseline and after six months. Dividing the patients into five clusters resulted in the **aggregation of 10/12 non-responders within 2/5 clusters** (yellow and red).



The **dynamics of *MLH3*** (**C**, p=0.026 log rank) and ***TEAD2*** **(D,** p=0.004 log rank) signals from baseline to six months on treatment **significantly correlated with OS** in the **Palbociclib** cohort (not in the control or entire cohort).



A) CDK2 **und** ***MLH1*** **signals after six months of Palbociclib** plus endocrine treatment were **significantly more prevalent in the non-responders** compared to the responders (p=0.026 and p=0.041, tested with fisher's exact test and Mann-Whitney U exact test). 1.CAVE: Although not significant in the control group, these signals might be not Palbociclib specific due to the lower p-value in the entire cohort than in the Palbociclib cohort. 2.CAVE: *MLH1* signals after six months of treatment were significantly more common in the patients in second or more line, compared to the patients receiving first line therapy (p=0.011), leading to the implication that more therapy lines and thus, unspecifically acquired resistance is indicated by *MHL1* signals. ***TEAD2*** signals after six months of treatment were significantly associated with **decreased OS** in the **entire (B), Palbociclib (D) and second or more line Palbociclib (C)** plus endocrine treated groups. **E**) In contrast to the correlation at baseline, ***CXCR4*** signal **after six months treatment with Palbociclib** were significantly associated with **decreased OS**.

Conclusions

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

- ***CXCR4*** **signals at baseline predict longer PFS – however, in the Palbociclib group, these signals after six months of treatment predict shorter OS.**
- ***YAP1*** **signals at baseline might be predictive for shorter PFS and OS in patients receiving first line Palbociclib.**
- **(Newly appearing)** ***CDK2*** **and** ***MLH1*** **signals after six months of Palbociclib treatment might be monitoring markers for therapy failure.**
- ***TEAD2*** **signals after six months and** ***TEAD2*** **signal dynamics might be monitoring markers for worse OS in the Palbociclib group.**

The results have to be validated in larger cohorts.

University Medicine Essen

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contact: corinna.keup@uk-essen.de