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Baseline and pharmacodynamic changes of circulating exosomal microRNAs predict early versus late progression to palbociclib plus endocrine therapy in patients with metastatic breast cancer. A sub–analysis from the PARSIFAL trial

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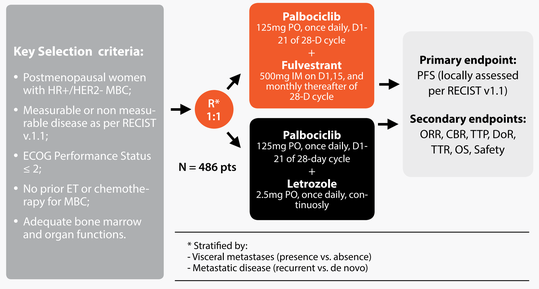
BACKGROUND

- Exosomes are extracellular vesicles from endosomal origin that enable cell-to-cell communication in multiple organisms[1]. Exosomes have been implicated in many cancer hallmark properties, such as immune evasion, metastatic niche formation and drug resistance[2].
- microRNAs (miRNAs) constitute one of the most abundant molecules transported by exosomes[3]. miRNAs play a capital role as modulators of gene expression[4] and their dysregulation has been associated with carcinogenesis in many contexts and tumor types[5]. Exosomal miRNAs are promising means of studying cancer biology and monitoring anticancer therapy in liquid biopsies [6,7].
- Palbociclib in combination with endocrine therapy (ET) is the first– or second–line standard of care for patients (pts) with hormone receptor (HR)–positive (+) / human epidermal growth factor receptor 2 (HER2)–negative (-) metastatic breast cancer (MBC)[8,9]. No clinically validated markers of long–term benefit from palbociclib have been established and the clinically relevant resistance mechanisms to cyclin–dependent kinases 4 and 6 inhibitors remain undefined.
- In the present study, we explored candidate circulating exosomal miRNAs as putative predictors of benefit and/or resistance to palbociclib in combination with ET in pts included in the PARSIFAL trial **(Figure 1)**[10].

Figure 1. PARSIFAL trial design

- PARSIFAL was a randomized, open–label, phase II trial aimed at evaluating the efficacy and safety of palbociclib plus either fulvestrant or letrozole in HR+ / HER2- MBC pts[10].

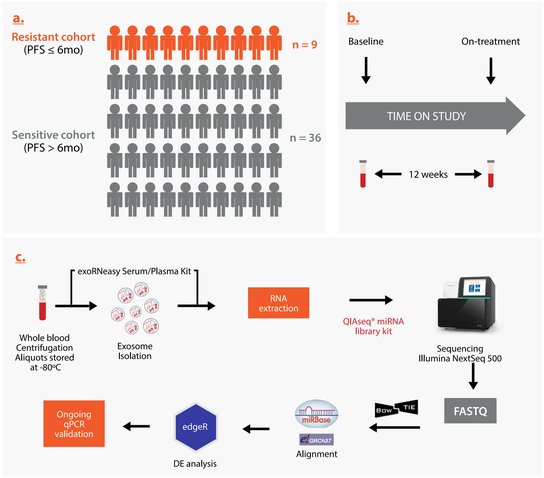


CBR: Clinical benefit rate, D: Day; ECOG: Eastern Cooperative Oncology Group; ET: Endocrine Therapy; HER2-: Human Epidermal Growth Factor Receptor 2-negative; HR+: Hormone receptor-positive; IM: Intramuscular injection; MBC: Metastatic breast cancer; PFS: Progression-free survival; ORR: Overall response rate; OS: Overall survival; PO: Oral administration; RECIST: Response Evaluation Criteria in Solid Tumors; TTP: Time to progression; TTR: time to response.

METHODS

- For the study of exosomal miRNAs, forty–five consenting pts were selected based on primary endocrine resistance according to the ABC–4 criteria[11]. Nine pts who progressed within the first six months (mo) after treatment initiation in the absence of an objective response were considered Resistant and thirty–six pts who progressed more than six mo after starting therapy were classified as Sensitive **(Figure 2a)**. A total of twenty-four (53%) and twenty-one (47%) pts were randomized to fulvestrant and letrozole, respectively.
- Exosomes were isolated from plasma samples collected at study entry (baseline) and after 12 weeks of treatment initiation **(Figure 2b)**. Differences in miRNA expression between Resistant and Sensitive pts as well as miRNA pharmacodynamic changes between baseline and 12–week samples were assessed. Ribonucleic acid (RNA) was isolated using the miRNeasy plasma kit and the library preparation was done using the QIAseq® miRNA library kit according to manufacturer's instructions **(Figure 2c)**. All experiments were conducted at QIAGEN Genomic Services in Germany.

Figure 2. Patient selection and analysis workflow



Differential expression (DE) of miRNA between different conditions was studied using EdgeR statistical software package from Bioconductor (Figure 2c). Estimated p–values for significantly differentially expressed miRNAs were adjusted using Benjamini–Hochberg's False Discovery Rate (FDR). Differences in expression of miRNA with an FDR below 0.05 were considered significant (Figure 3). Validation of the most promising miRNAs by custom quantitative polymerase chain reaction (PCR) is ongoing.

RESULTS

- Sequencing of exosomal RNA and preparation of miRNA libraries were successful for all included samples, with good technical performance.
- On average, 2.8 million Unique Molecular Index–corrected reads were obtained for each sample and the average percentage of mappable reads was 34.5%.
- Overall, expression of miRNAs was higher in Resistant compared with Sensitive pts. Eight miRNAs were called as differently expressed between the two groups.
- Four miRNAs (miR-1246; miR-375; miR-193a-5p; miR-181d-5p) were differently expressed at baseline, three (miR-196a-5p, miR-200a-3p, miR-320d) were differentially expressed at 12 weeks, and expression of one miRNA (miR-141-3p) was consistently higher across both time points in Resistant pts **(Figures 3 and 4).**
- Significant pharmacodynamic changes in miRNA expression were observed both in Resistant and Sensitive pts. Seven miRNAs (miR-224-5p, miR-16-5p, let-7a-5p, miR-381-3p, miR-200c-3p, miR-493-3p, let-7b-5p) in Sensitive pts and three miRNAs (miR-223-3p, miR-126-3p, miR-320b) in Resistant pts were differently expressed between baseline and 12–week samples **(Figures 3 and 4).**

Table 1. Patient characteristics

Characteristic	Resistant (N=9)	Sensitive (N=36)	All patients (N=45)
Age			
Median (range) - yr	60 (49-68)	64 (39-88)	62 (39-88)
<65 yr. - no (%)	8 (88.9)	20 (55.6)	28 (62.2)
≥65 yr. - no (%)	1 (11.1)	16 (44.4)	17 (37.8)
Race - no. (%)			
White	8 (88.9)	34 (94.4)	42 (93.3)
Other	1 (11.1)	2 (5.6)	3 (6.7)
ECOG PS - no. (%)			
0	4 (44.4)	23 (63.9)	27 (60)
1	5 (55.6)	9 (25)	14 (31.1)
2	0 (0)	4 (11.1)	4 (8.9)
Recurrence type - no. (%)			
Newly diagnosed	3 (33.3)	18 (50)	21 (46.7)
Locoregional	0 (0)	1 (2.8)	1 (2.2)
Distant	6 (66.7)	17 (47.2)	23 (51.1)
Disease-free interval - no. (%)	n = 6	n = 18	n = 24
≤12 mo.	0 (0)	0 (0)	0 (0)
>12 mo.	6 (66.7)	18 (50)	24 (53.3)
Disease site - no. (%)			
Visceral	8 (88.9)	14 (38.9)	22 (48.9)
Non visceral	1 (11.1)	22 (61.1)	23 (51.1)
Bone only	1 (11.1)	5 (13.9)	6 (13.3)
No. of disease site - no. (%)			
<3	5 (55.6)	23 (63.9)	28 (62.2)
≥3	4 (44.4)	13 (36.1)	17 (37.8)
Previous therapies - no. (%)			
Chemotherapy			
Neoadjuvant	3 (33.3)	3 (8.3)	6 (13.3)
Adjuvant	4 (44.4)	14 (38.9)	18 (40)
Adjuvant ET			
Tamoxifen	2 (22.2)	12 (33.3)	14 (31.1)
Letrozole	1 (11.1)	3 (8.3)	4 (8.9)
Anastrozole	1 (11.1)	1 (2.8)	2 (4.4)
Goserelin	0 (0)	1 (2.8)	1 (2.2)

ECOG: Eastern Cooperative Oncology Group; ET: Endocrine Therapy; no.: Number; PS: Performance Status; yr.: Years.

Figure 3. Differentially expressed miRNAs in each comparison

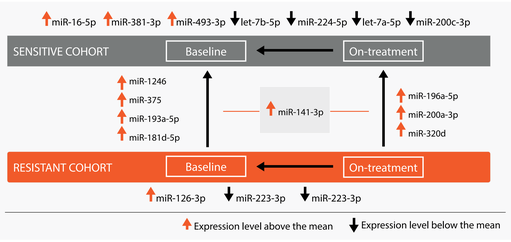
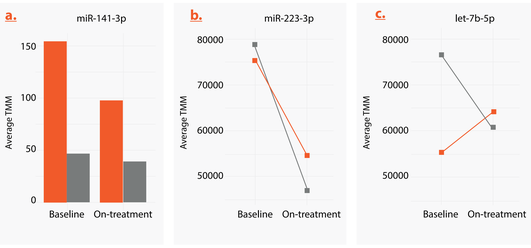


Figure 4. Examples of promising miRNAs (validation on-going)



a. Persistent elevation of miRNA expression levels in Resistant vs. Sensitive pts. b. Decrease of miRNA levels in both Sensitive and Resistant pts. c. Decrease of miRNA levels in Sensitive pts but increase in Resistant pts.
Values expressed as trimmed mean of M-values (TMM). P < 0.05 for all comparisons.

CONCLUSIONS

- Circulating exosomal miRNA profiling is feasible in liquid biopsies from MBC pts.
- Differential expression of selected miRNAs at baseline or their pharmacodynamic modulation may predict benefit from palbociclib combined with ET in pts with HR+ / HER2- MBC.
- Validation of the most promising miRNAs by custom quantitative PCR is warranted.

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Conflicts of interest: https://medsir.org/transfal/

PARSIFAL study Trial Registration: NCT02491983 on July 8, 2015