HELLENIC REPUBLIC
National and Kapodistrian University of Athens

PROTEOMIC ANALYSIS OF BIOMARKERS PREDICTIVE OF CDK4/6 INHIBITOR RESPONSE IN HR+/HER2- BREAST CANCER

Apostolidou K.[1]; Zografos E.[1]; Papatheodoridi AM[1]; Fiste O.[1]; Papadopoulos L.[2]; Filippidou S.[2]; Fokianou A.[2] ; Alevizou R.[2] ; Marinopoulos S.[3] ; Dimitrakakis C.[3] ; Xepapadakis G.[2] ; Samiotaki M.[4] ; Dimopoulos MA[1] , Zagouri F.[1]

1. Department of Clinical Therapeutics, Alexandra Hospital, Medical School, Athens, Greece 11528, 2. Iaso, 2nd Breast Clinic, General Maternity and Gynecology Clinic, Athens, Greece, 3. 1st Department of Obstetrics & Gynecology, “Alexandra” Hospital, Medical School, University of Athens, 115 28 Athens, Greece, 4. Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 166 72 Vari,Greece.

ΕΛΛΗΝΙΚΟ ΣΥΝΕΔΡΙΟ ΚΛΙΝΙΚΗΣ ΟΓΚΟΛΟΓΙΑΣ 31° ΕΣΚΟ

INTRODUCTION

The introduction of CDK4/6 inhibitors has significantly improved treatment outcomes for patients with hormone receptor-positive, HER2-negative (HR+/HER2-) breast cancer.

However, a substantial proportion of patients develop resistance to CDK4/6 inhibitors, which limits their therapeutic efficacy. Despite their widespread clinical use, reliable biomarkers for predicting patient response to CDK4/6 inhibitors are still lacking, emphasizing the need for continued research in this area.

AIM

This study aims to identify potential protein biomarkers predictive of therapeutic response to CDK4/6 inhibitors in HR+/HER2- breast cancer patients.

MATERIALS AND METHODS

This prospective, case-control study included adult women with histologically confirmed HR+/HER2- metastatic breast cancer who initiated ribociclib in combination with endocrine therapy (ET).

Baseline blood samples were collected prior to treatment initiation. Patients who experienced early disease progression were classified as cases, while those without progression were matched in a 1:1 ratio as controls. Serum samples were analyzed using data-independent acquisition liquid chromatography-tandem mass spectrometry (DIA LC-MS/MS) for quantitative proteomic profiling. Gene Ontology (GO) pathway enrichment analysis was performed to elucidate the biological mechanisms underlying the proteomic changes.

RESULTS

- The exploratory DIA LC-MS/MS analysis of 10 samples identified 1,086 proteins, with an average of approximately 700 proteins detected per plasma sample.
- Comparative statistical analysis revealed 107 differentially expressed proteins (DEPs) between cases and controls (p-value<0.05; S0 = 0.1), including 39 upregulated and 67 downregulated proteins.

Table 1. *Demographic and clinical characteristics.*

Variable	Cases subgroup	Controls subgroup
Age at diagnosis		
Mean (SD)	64.20 ± 19.78	63.60 ± 18.73
Median (min, max)	75.00 (40.00 – 84.00)	73.00 (43.00 – 83.00)
Age at ribociclib initiation		
Mean (SD)	65.20 ± 18.59	66.40 ± 15.68
Median (min, max)	75.00 (41.00 – 84.00)	73.00 (44.00 – 83.00)
Smoking status N (%)		
Non-smoker	4 (80%)	4 (80%)
Smoker	1 (20%)	1 (20%)
Menopausal status N (%)		
Post-	4 (80%)	4 (80%)
Pre-	1 (20)	1 (20)
Molecular subtype N (%)		
Luminal A/HER2-	2 (40%)	2 (40%)
Luminal B/HER2-	3 (60%)	3 (60%)
Line of Treatment with Ribociclib N (%)		
1st	5 (100%)	5 (100%)
Metastatic sites N (%)		
Bones	4 (80%)	4 (80%)
Lung	1 (20%)	1 (20%)
Liver	1 (20%)	0 (0%)
Lymph Nodes	1 (20%)	2 (40%)
Cutaneous metastasis	1 (20%)	0 (0%)

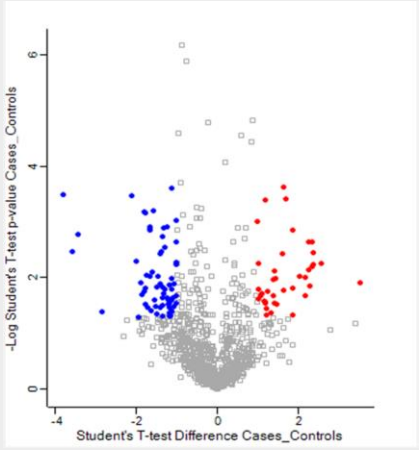

Figure 1. *Scatter plot of differential gene expression in cases (red) vs. controls (blue).*

Table 2. *Selected DEPs between cases and controls.*

Gene name	UniProt Protein name	UniProt Primary accession	- LOG(P)	Difference	Deregulation (cases)
ITGAL	Integrin alpha-L	P20701	3.48	-3.81	↓
KRT85	Keratin, type II cuticular Hb5	P78386	2.46	-3.59	↓
MICU2	Calcium uptake protein 2, mitochondrial	Q8IYU8	2.77	-3.45	↓
CDH23	Cadherin-23	Q9H251	1.39	-2.84	↓
IGLV2-14	Carbonic anhydrase 2	P00918	2.20	2.34	↑
KRT4	Keratin, type II cytoskeletal 4	P19013	2.23	2.36	↑
PRKCE	Protein kinase C epsilon type	Q02156	2.44	2.37	↑
OR51L1	Olfactory receptor 51L1	Q8NGJ5	2.65	2.34	↑
NEK9	Serine/threonine-protein kinase Nek9	Q8TD19	2.25	2.57	↑
FAM110A	Protein FAM110A	Q9BQ89	1.91	3.53	↑

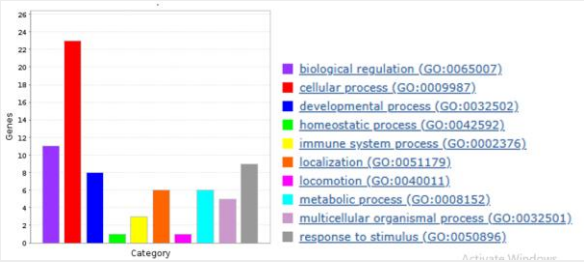

Figure 2. *GO Biological process (upregulated proteins in cases).*

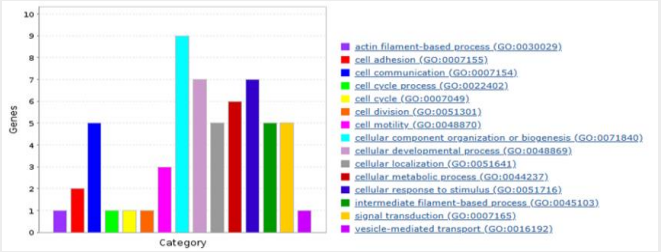

Figure 3. *GO Biological process/cellular process (upregulated proteins in cases).*

CONTACT INFORMATIONS

Kleoniki Apostolidou

Department of Clinical Therapeutics, Alexandra Hospital, Medical School, Athens, Greece

CONCLUSIONS

This study identifies distinct protein expression profiles between cases and controls in the context of treatment response to CDK4/6 inhibitors in HR+/HER2- metastatic breast cancer, suggesting potential biomarkers for predicting treatment efficacy. These findings underscore the importance for further validation of these biomarkers to optimize personalized treatment strategies and improve clinical outcomes in breast cancer therapy.

REFERENCES

- Zhou, F. H. et al. CDK4/6 inhibitor resistance in estrogen receptor positive breast cancer, a 2023 perspective. Front Cell Dev Biol 11, (2023).
- Gomes, I., Abreu, C., Costa, L. & Casimiro, S. The Evolving Pathways of the Efficacy of and Resistance to CDK4/6 Inhibitors in Breast Cancer. Cancers (Basel) 15, 4835 (2023).
- McCartney, A. et al. Mechanisms of Resistance to CDK4/6 Inhibitors: Potential Implications and Biomarkers for Clinical Practice. Front Oncol 9, (2019).
- Lee, J. S., Hackbart, H., Cui, X. & Yuan, Y. CDK4/6 Inhibitor Resistance in Hormone Receptor-Positive Metastatic Breast Cancer: Translational Research, Clinical Trials, and Future Directions. Int J Mol Sci 24, 11791 (2023).