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Liquid biopsy as promising source of plasma extracellular vesicle biomarkers of response to Cabozantinib (CABO) plus Durvalumab (DURVA) in advanced urothelial carcinoma (UC) or non-UC variant histologies (VH) patients after platinum chemotherapy (the Phase 2 ARCADIA trial)

Veronica Huber[1], A. Mereu[1], L. Lalli[1], J. Salsetta[1], C. Banfi[2], P. Squarcina[1], F. Sgambelluri[1], R. Mortarini[1], M. Stellato[1], D. Raggi[3], V. Guadalupi[1], M. Claps[1], W. Ferrari Bravo[1], G. Calareso[1], A. Alessi[1], A. Anichini[1], G. Procopio[1], A. Necchi[3], L. Rivoltini[1], P. Giannatempo[1]

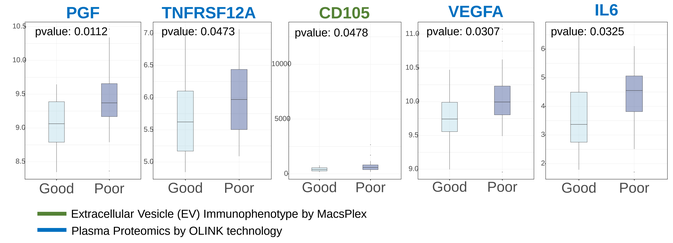
[1]Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; [2]Monzino Cardiologic Center IRCCS, Milan, Italy; [3]IRCCS Ospedale San Raffaele, Milan, Italy

BACKGROUND. As components of the liquid biopsy, Extracellular Vesicles (EVs) have gained major interest as biomarkers of diagnosis, prognosis and prediction of response/resistance to cancer therapies. Here we investigated if plasma EV immune profile, size and concentration in concert with plasma proteomics might discriminate good from poor responding patients affected by advanced urothelial carcinoma (UC) or non-UC variant histologies (VH) undergoing cabozantinib (CABO) plus Durvalumab (DURVA) combination therapy after platinum chemotherapy (NCT03824691).

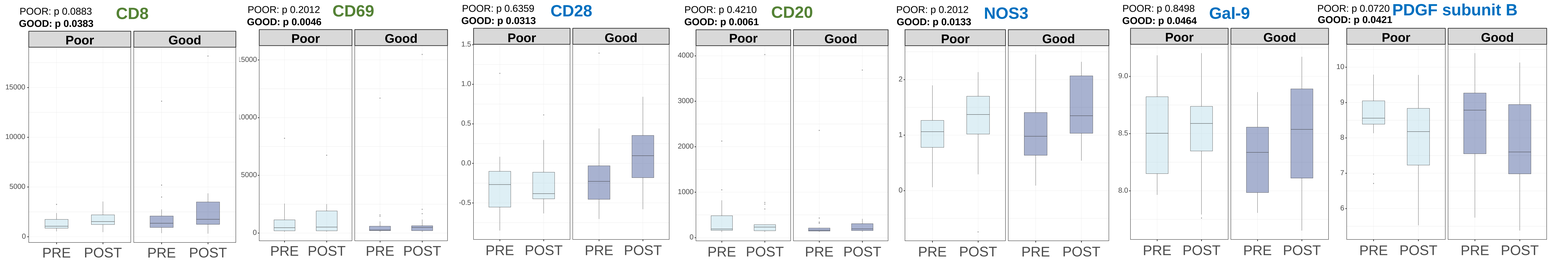
METHODS. We evaluated 40 patients for their plasma EV profile at baseline and first reassessment after 2-4 months of therapy. Baseline samples of 50 patients (40 plus additional 10 patients) were evaluated for their predictive potential. EVs were profiled using modified MACSplex technology (Miltenyi Biotec) coupled with flow cytometry and nanoparticle tracking analysis (NTA). Whole plasma was searched for indicators of good/poor response by proteomics (92 analytes, Immune-Oncology panel, Olink). Differences were considered as statistically significant when $p<0.05$, achieved by Wilcoxon (paired) and Mann-Whitney (unpaired) test.

RESULTS. Preliminary analysis of the single EV and proteomic markers measured in baseline samples evidenced an association with response of PGF, TNFRSF12A, VEGFA, CD105 and IL6, significantly enriched in poor responders with respect to good responders (**A**). Upon inclusion in the analysis of the on-therapy time point we were able to detect a significant increase of NOS3 and immune markers CD8, CD69, CD28, CD20, Gal-9 and a decrease of PDGF in good responders (n=21, **B**). Poor responders (n=19, **C**) displayed a significant decrease of IL12 and CD83, while VEGFA and KLRD1 increased. Finally, CABO plus DURVA induced a remarkable increase of the immune markers PD-L1, PDCD1 and LAG3 together with HO-1 and a decrease of VEGFR-2 and LAP TGF-beta-1, which was detectable in the whole case set (**D**). Further analysis of the markers in VH patients and NTA for EV quantification and sizing are currently ongoing.

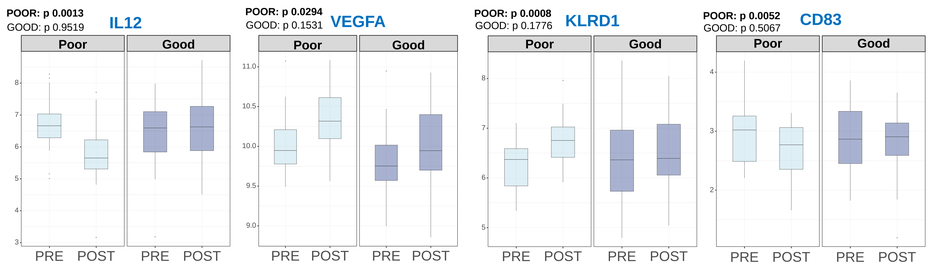
A BASELINE. Significantly different factors are tumor- and angiogenesis-related and are increased in samples of poor responders



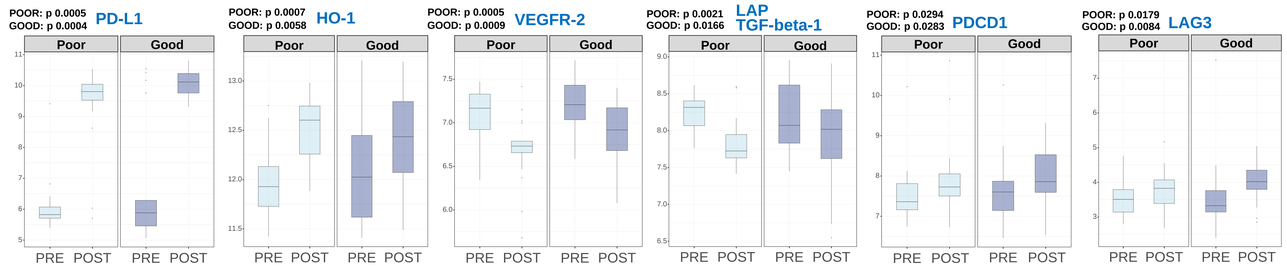
B PRE – FIRST REASSESSMENT. Significantly different factors associated with good response



C PRE – FIRST REASSESSMENT. Significantly different factors associated with poor response



D PRE – FIRST REASSESSMENT. Significantly different factors modulated by therapy in samples from all patients



Conclusion: Our preliminary results suggest that the early dynamics of EVs and proteins in plasma may inform on the clinical outcome to DURVA plus CABO. The significant increase of EVs expressing immune markers together with the decrease of tumor-associated markers measured at first reassessment in responding patients may derive from the synergic activation of the immune system and anti-tumor activity induced by therapy. The comprehensive analysis of EV profiles, size and concentration together with plasma proteomics could give rise to predictive/prognostic biomarkers of response in this clinical setting.

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For inquiries, please contact: *veronica.huber@istitutotumori.mi.it*

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