910 Circulating tumor DNA and disease recurrence in early stage breast cancer: From a case-control study to a prospective longitudinal trial

S. Di Cosimo[1], V. Appierto[1], E. Ortolan[1], F. Dell'Angelo[1], M. Silvestri[1], G. Bianchi[2], S. Folli[3], L. De Cecco[1], G. Pruneri[4], M.G. Daidone[1]

[1]*Department of Applied Research and Technological Development, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy,* [2]*Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy,* [3]*Breast Cancer Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy,* [4]*Department of Pathology, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy*

Background: Biomarker/s able to detect minimal residual disease (MRD) after breast cancer (BC) surgery are needed to aid optimal systemic therapy. We aimed at investigating the feasibility of using circulating tumor DNA (ctDNA) to early detect MRD in plasma samples collected during the follow-up of patients (pts) with early BC.

Methods: Forty BC pts undergoing surgery with curative intent and regular follow-up for a minimum of 13 years were included in a 1:3 case-control study. Preliminary experiments demonstrated that heparinase I digestion does not affect the quality of DNA extracted from heparin-collected blood and that pre-amplification overcomes limitations due to small (<0.5 ml) plasma aliquots. Mutational analysis of archival BC tissues was performed by Ion AmpliSeq^{TM} targeted sequencing and identified Single Nucleotide Variations (SNV) were validated and tracked in plasma by digital Polymerase Chain Reaction.

Results: One or more circulating SNVs were identified in 27/40 cases prior to surgery. During follow-up (range 80-200 months), 6 pts relapsed locally, 4 at distant sites, and 17 remained disease-free (DF). ctDNA was undetectable in 16/17 DF cases, whilst it was detectable in 9/10 recurrent pts, and anticipated overt metastases/loco-regional

recurrences with a median lead time of 20 (range 10-47) months. Based on these results, we started a prospective study on ctDNA tracking in triple negative (TN) BC pts. At the time of writing, 67 pts with early stage TNBC have been enrolled in a longitudinal trial and followed up (range 10-74 months). Before surgery, ctDNA was detectable in 64% of cases and its clearance was associated with DF status. In additional 12 TNBC pts, ctDNA was analyzed prior and during neoadiuvant chemotherapy (NAC): ctDNA was detectable in 81% of the cases at baseline, and its dynamics during and after NAC reflected tumor response, and anticipated overt metastases with a lead time up to 13 months.

Conclusions: Post-surgical ctDNA can anticipate the diagnosis of new disease manifestations, including loco-regional recurrences, which are amenable of treatment with a curative intent. Additional results on the longitudinal trial currently ongoing in TNBC pts will be presented at the meeting.

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