**Presentation title:** Exploring the vulnerabilities of EGFR-dependent breast cancer cells with single cell lineage-tracing

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**Abstract (250-300 words):**

Triple negative breast cancer (TNBC) is characterized by the absence of known targetable biomarkers which still results in no clear therapeutic intervention. However, about 75% of TNBC patients are associated with overexpression or amplification of Epidermal Growth Factor Receptor (EGFR). Despite so, conversely to other cancer types like lung or colon ones where EGFR inhibitors have been proved effective, therapies targeting EGFR have yet variable and unpredictable responses in breast cancer (BC). Thus, the poor mechanistic understanding of the dependencies of sensitive breast tumors and the lack of predictors have hampered the translation of EGFR inhibitors into the BC precision medicine paradigm. Here we coupled single-cell transcriptomic and lineage tracing to comprehensively characterize drug-tolerant states of an anti-EGFR responsive MDA-MB-468 TNBC cell line, in response to incremental concentration of Afatinib, a tyrosine kinase inhibitor that irreversibly blocks EGFR. Deep single-cell RNA sequencing of 5088 cells over 5 timepoints from MDA-MB-468 cells, identified a small pre-existing subpopulation of cells composed by 192 lineages (out of the initial 2,336 lineages) displaying distinct biological features, where elevated Insulin-Like Growth Factor Binding Protein 2 (IGFBP2) expression was significantly enriched. Cell viability assays in IGFBP2 overexpressed or depleted MDA-MB-468 cells confirmed the involvement of IGFBP2 in modulating the response to Afatinib. Also, functional studies demonstrate that the restoration or the knockdown of IGFBP2 in TNBC cells further supported its role as a putative resistant biomarker. Our findings provide new understanding of EGFR-dependent hierarchy in TNBC and could be useful for novel strategies of patient stratification and therapeutic intervention