Abstract #16 | Poster

Rituximab-induced liquid biopsy signatures inform on cellular and microenvironmental contexts in B-cell lymphoma patients

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Background: The clinical management of B-cell lymphomas is often hampered by their molecular heterogeneity, systemic nature, and variable clinical course due to continued evolution and adaption. Plasma-derived extracellular vesicles (EVs) are accessible and information-rich clinical materials, that are widely profiled using omics approaches and reflect the states of their cells of origin and their microenvironment, supporting real-time disease characterization and treatment decisions. However, the low signal-to-background ratio of plasma EV proteomics, limits its ability to capture high-resolution physiological states, which we aim to address through targeted pharmacologic perturbations to improve resolution.

Methods: 10 patients with CD20-positive B-cell lymphoma underwent blood sampling before and after rituximab administration. EVs were isolated from plasma using a clinically optimized workflow. Bioinformatic analyses were performed to identify response modes through differential protein expression, pathway enrichment, patient clustering, feature selection, and associations with clinical characteristics.

Results: Changes in EV ultrastructure, concentration, and size as well as raw proteomics data lack the resolution to distinguish acute rituximab-induced responses beyond common innate immune and complement cascade activation, consistent with known rituximab effects. To improve resolution, we applied a paired-centering approach to normalize each patient’s background and represented proteomic changes as vectors in a Principle Coordinate Analysis space, defining response modes between pre- and post-treatment states. Based on angles between vector (i.e., response-mode similarity), 9 patients clustered into 3 groups, with 1 outlier. While these clusters did not correspond to lymphoma subtype, pathological markers, or demographic characteristics, patients within each cluster shared anatomical disease locations: 2 in neck soft tissue, 3 in the central nervous system, and 4 in the trunk. Enrichment analysis of cluster-specific proteins showed significant involvement of receptor-ECM interactions, integrin signaling, and cytoskeleton regulation in all clusters, mediated by different components and abundance patterns, yet consistent with the corresponding anatomical sites.

Summary: *In vivo*-perturbation of CD20-positive B-cell lymphoma with rituximab generates EV proteomic response modes that inform on tumor cell state and microenvironmental interactions.