Proteomic profiling of antibody-drug conjugate biomarker panel for rational drug design

With the advancement of linker technology and new payloads, antibody drug conjugates (ADC) approvals have improved in the recent years. Most ADC approvals have occurred in hematological malignancies. For solid tumors, several ADCs have failed clinical trials due to futility or toxicity. While a great deal of attention has been focused on measuring the receptor protein levels mostly by IHC, the payload targets remain generally ignored. Due to the unique mechanism of ADCs, quantitative protein measurement of both the antibody target and markers of sensitivity or resistance to the payload can help in the design of rational drug combinations. We have developed a method to solubilize formalin fixed paraffin embedded (FFPE) tissue and measure the protein concentrations of both receptor and payload targets using quantitative mass spectrometry. We quantitate simultaneously 72 protein biomarkers that are important to oncology. These include multiple antibody target proteins necessary for ADC response, such as receptor tyrosine kinases (EGFR, HER2, HER3, AXL), transporters (FRalpha, NaPi2b), and tumor antigens (Trop2,Mesothelin, DLL3, CD30, GPNMB,CD56, Nectin-4, CD19, CECAM5, PSMA etc.) that are currently approved or in clinical trials. Besides the antibody target, we also measure the payload biomarkers of sensitivity (TOPO1) or resistance (TUBB3).

On exploration of our large quantitative biomarker data from FFPE clinical tissue, we determined that in colorectal cancer, expression of EGFR(83%), HER2(52%), HER3(21.5%), Axl(3.7%), Mesothelin(26.5%), FRalpha(3.7%), and Trop2(60%) showed a wide a dynamic range. These needs to be paired with appropriate payloads (anti-tubulins or anti-TOPO1). Previously we identified that HER2 expression >750amol/µg correlated with HER2 overexpression. Accordingly, 1.4% of CRC patients overexpressed HER2, of which 40% had TOPO1 expression >1350amol/µg (75th percentile) suggesting that these patients may receive benefit from a HER2/TOPO1 ADC. However, the newer generation of ADCs work at low levels of Her2 which expands the potential responders to 52%. If the cut-off of 1350 amol/µg of TOPO1 (75^{th} percentile) is considered, then 27% of CRC is likely to respond to a HER2-TOPO1 ADC (e.g. DS-8201). Similarly, 60% of CRC has Trop2 expression and applying similar cut-off for TOPO1 results in 20% potential responders to HER2-TOPO1 ADC (e.g. Sacituzumab govitecan or DS-1062).

Similarly analysis of our glioblastoma dataset revealed that majority of glioblastoma expresses EGFR(84%) which suggested likely response to anti-EGFR ADC, however, concurrent expression of TUBB3(97%) may indicate resistance to several known payloads, such as taxanes and MMAE. Conjugation with another payload that targets sensitivity marker TOPO1 (68% expression) is a likely option. Proteomic analysis also revealed detectable levels of multiple RTKs (AXL(20%), IGF1R(10%), MET (5%), and HER2 (9%), indicating potential response to RTK inhibitors.

The ability to quantitate protein biomarkers of receptors and payloads using multiplexed mass spectrometry from just two sections of FFPE tissue enable rational drug design based on actual targets of the ADC. Additionally, proteomics enabled patient stratification may result in proper matching of patients for clinical trials.