ABSTRACT #: 284587

Expression of antibody-drug conjugates (ADC) biomarkers in colorectal cancer.

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BACKGROUND

- Antibody-Drug Conjugates (ADC) represent a powerful class of cancer therapeutics - directing extremely toxic small molecules specifically to tumor cells.
- Multiple ADCs are in clinical trials for colorectal cancer (CRC) and the optimal strategy for selecting patients who may benefit from the treatment is evolving.
- Due to the unique mechanism of ADCs, patient selection should involve screening not only for the presence of the antibody target, but also screening for the presence of any markers of resistance or response to the payload.
- We have built a multiplexed ADC biomarker panel in FFPE tumor tissue that simultaneously quantifies the protein levels of the antibody targets and also the payload markers.
- A survey of patient tumors has identified protein biomarkers with variable expression patterns that may affect treatment response.

METHODS

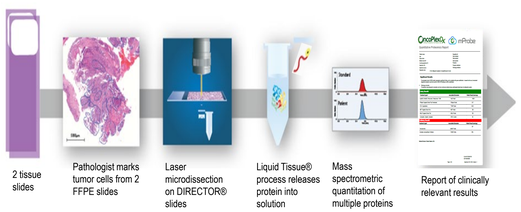

Figure 1. FFPE tumor tissues from 355 clinical CRC patients were microdissected and solubilized for mass spectrometry-based targeted proteomic analysis.

Multiplexed ADC panel

Antibody Targets:
EGFR, HER2, HER3, AXL, Mesothelin, FR-alpha, Trop2

Payload biomarkers:
TOPO1 and tubulin-beta3

RESULTS

Expression Distribution of HER2 ADC & Payload Targets in CRC

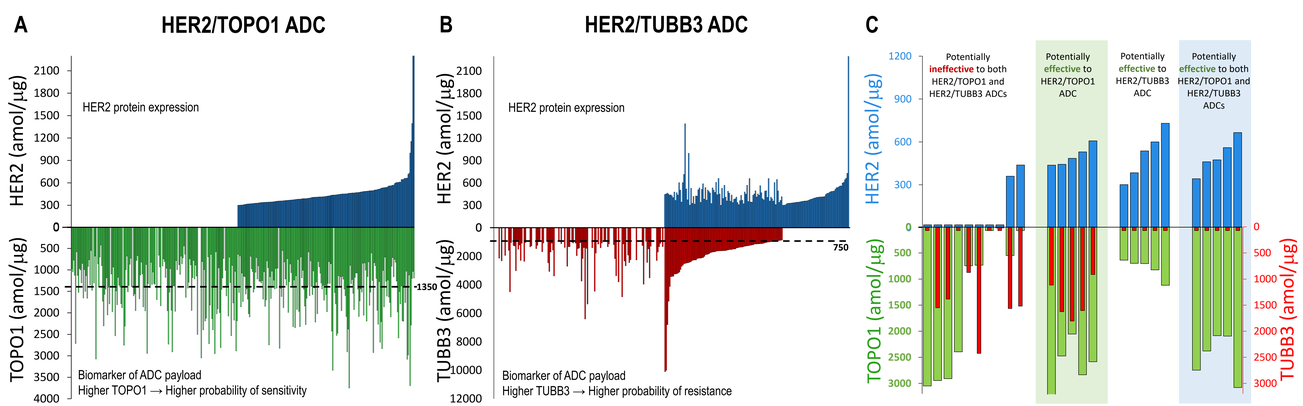

Figure 2. Expression distribution of targets of HER2 ADCs. **(A)** HER2 expression and TOPO1 (payload response biomarker) expression in CRC patients (n=355). The reference cutoff 1350 amol/ug (75th percentile) is indicated by dashed lines. **(B)** HER2 expression and TUBB3 (payload resistance biomarker) expression in CRC (n=298). The reference cutoff 750 amol/µg is indicated by dashed line. **(C)** Combined targeted proteomic analysis of HER2, TOPO1 and TUBB3 protein expression. A multiplex protein expression panel can reveal mechanisms of response and resistance independent of antibody-target expression levels (HER2).

Quantitation of Antibody Targets in CRC

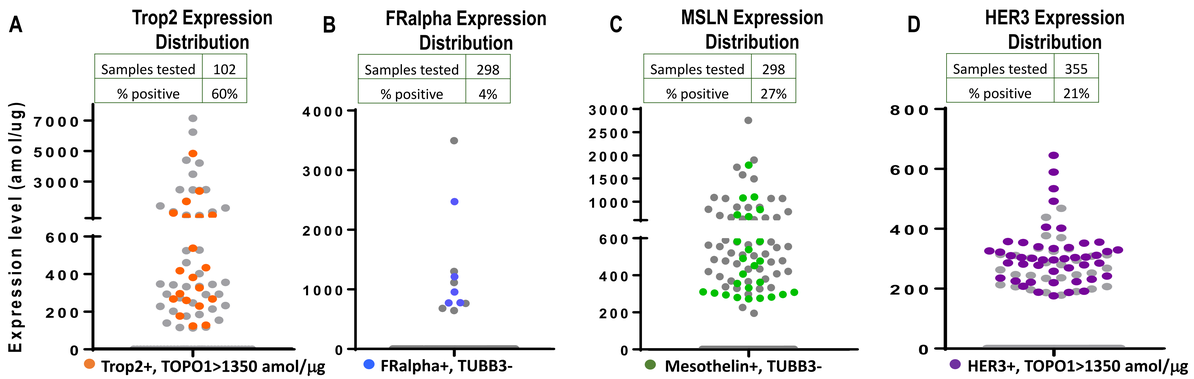

Figure 3. Expression distribution of receptor targets of ADCs. (A) Trop2 expression distribution (Grey dots). Trop2 positive CRC with TOPO1 expression >1350amol/µg are indicated by orange dots. (B) FRalpha expression distribution (Grey dots). Blue dots represent FRalpha positive CRC with TUBB3 expression below the clinical cutoff. (C) MSLN expression distribution (Grey dots). Green dots represent MSLN positive CRC with TUBB3 expression below the clinical cutoff. (D) HER3 expression distribution (Grey dots). HER3 positive CRC with TOPO1 expression >1350amol/µg are indicated by purple dots.

Expression Distribution of Potential ADC Biomarkers in CRC

Biomarkers	N of CRC tested	Prevalence (potential response rate)
HER2(+), TOPO1(>1350)	355	27%
HER2(+), TUBB3(-)	298	18.8%
HER3(+), TOPO1(>1350)	355	12.7%
HER3(+), TUBB3(-)	298	10.1%
EGFR(+), TUBB3(-)	296	32.1%
Axl(+), TUBB3(-)	298	0.3%
Mesothelin(+), TUBB3(-)	298	8.4%
FRalpha(+), TUBB3(-)	298	1.7%
Trop2(+), TOPO1(>1350)	102	19.6%

(+) indicates expression ≥LOQ; (-) indicates expression <LOQ

Table 1. Expression Distribution of antibody targets and markers of resistance or response to the payloads for multiple approved and investigational ADC therapies in CRC.

CONCLUSIONS

- Quantitative proteomics using multiplexed mass spectrometry quantified 72 protein biomarkers from 2 sections of FFPE tissues.
- HER2 protein was found overexpressed[1,2,3] in 1.4% of CRC by mass spectrometry.
- Targeted proteomics detected varying expression levels of antibody-targets: EGFR(83%), HER2(52%), HER3(21.5%), Axl(3.7%), Mesothelin(26.5%), FRalpha(3.7%), and Trop2(59.8%) in CRC
- The multiplexed mass spectrometry assay simultaneously quantified protein markers for several known payloads. While 75th percentile was used to defineTOPO1 overexpressors, lower percentile cut-off could be used for ADC.
- Protein biomarkers expression levels quantified by mass spectrometry are continuous variables, amenable to powerful statistical tools for clinical cut-offs values.

REFERENCE

[1] *Ann Oncol*. 2017; 28(1):110-115. [2] *Mol Oncol*. 2016; 10(1):138-147. [3] *Gastric Cancer*. 2016; 19(4):1066-1079.