Poster board # **13A**

Proteomic analysis of therapeutic biomarkers to guide treatment of patients with bone metastases

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BACKGROUND

- Personalized medicine has revolutionized cancer treatment, but treatment-related biomarkers can be challenging to assess in biopsies of metastatic bone lesions or bone cancer.
- Bone-containing biopsy tissues are routinely softened with rapid, strong-acid decalcification agents prior to sectioning; this can destroy cellular morphology, reduce protein immunoreactivity, and degrade the quality of DNA/RNA.
- We assessed the effect of acid decalcification on quantities of protein biomarkers expressed by archival tumor specimens from patients with various cancers.
- We quantified clinically relevant protein targets in decalcified bone biopsies from patients with various cancers using selected reaction monitoring (SRM).
- KRAS oncogenic mutation is an indicator that a tumor will not respond to EGFR-targeted therapy. We attempted to detect KRAS mutation at the protein level using data-independent acquisition (DIA).

METHODS

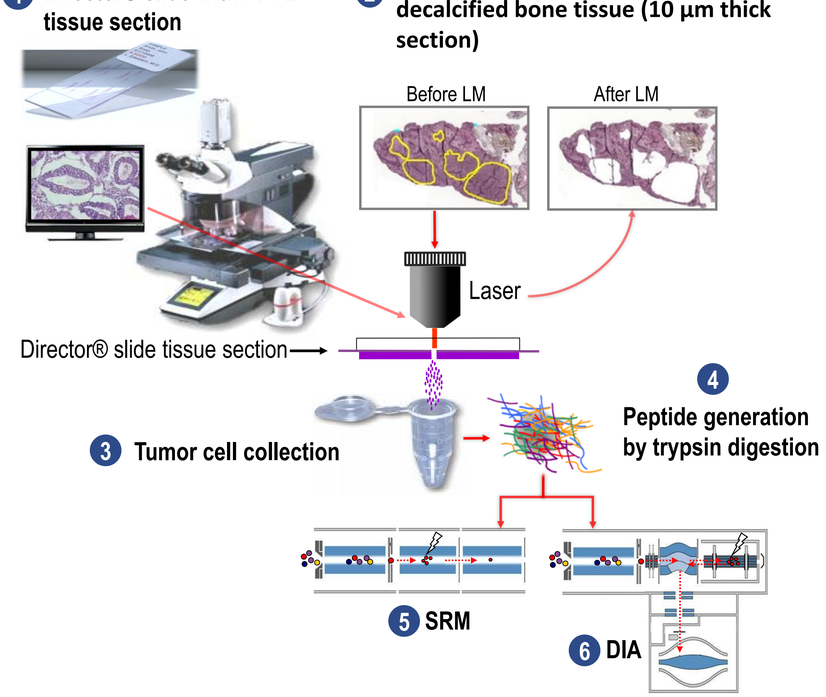

Figure 1. Liquid Tissue™ preparation followed by LC-SRM was used to quantify 27 biomarkers. LC-DIA was used to detect KRAS mutation.

RESULTS

Proteomics unaffected by decalcification conditions

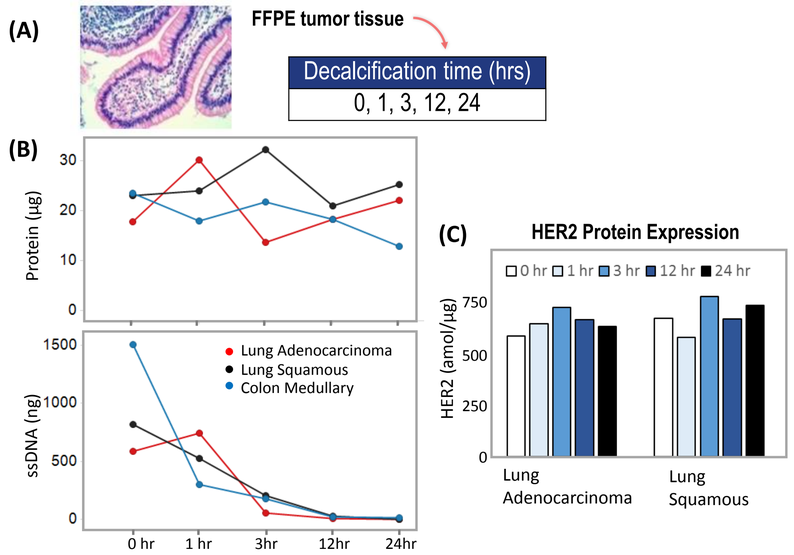

Figure 2. (A) Prior to LM, tissue samples from 3 tumors were processed with Decal-Stat™ solution for 0, 1, 3, 12, and 24 hours **(B)** Total protein remained fairly stable with increasing decalcification time, in contrast to DNA. **(C)** Tumor expression of HER2 protein showed no relationship with decalcification time.

A clinically actionable oncogenic mutation was detected

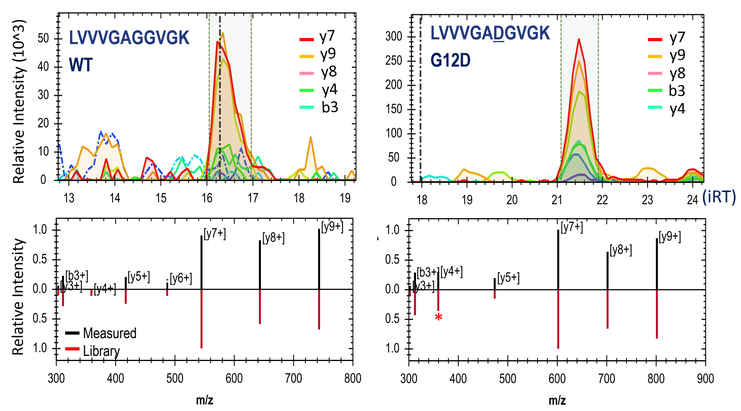

Figure 3. Detection of mutant KRAS (G12D). DIA analysis surveying m/z [470-560] with 2Th window detected both wild type and mutant peptides in a bone biopsy.

SRM detected 18 of 27 treatment-related biomarkers tested in bony biopsies

Table 1. Proteins quantitated in biopsies of bone metastases or bone cancer (N=23)

Protein	Cutoff	Agent	Breast cancer							Lung cancer					Gastric				Sarcomas				Other		
			1	2	3	4	5	6	7	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	3
hENT1	100	gemcitabine																							
TOP1	1340	irinotecan																							
EGFR	1500	targeted																							
TOP2A	1570	antracycline																							
HER2	750	targeted																							
RRM1	700	gemcitabine																							
ERCC1	75	platinum																							
TUBB3	850	taxane																							
KRAS	1650	targeted																							
HER3	175	targeted																							
MET	400	targeted																							
MGMT	200	temozolomide																							
AR	100	targeted																							
AXL	100	targeted																							
MSLN	500	targeted																							
FGFR1-4	200	targeted																							
FRalpha	1300	pemetrexed																							
IGF1R	150	targeted																							

Likely respond (> cutoff) Non actionable (< cutoff)
Likely resistance (> cutoff) Not detected (< LOQ)

The 23 bone biopsy samples expressed 18 of the 30 protein targets tested. Half of samples expressed one or more protein markers of response to targeted therapy, and the vast majority expressed markers of response or resistance to conventional chemotherapies.

CONCLUSIONS

- A commonly used decalcifying solution had no discernable effects on mass spectrometric quantification of biomarker proteins in archived tumor samples.
- In decalcified bone biopsy specimens from cancer patients, an SRM assay quantitated 18 therapeutically relevant protein biomarkers that may inform selection of personalized cancer treatments.
- Mutant KRAS was detected at the protein level by DIA in a bone biopsy.
- Targeted proteomics can be used to rescue decalcified bone samples that are not evaluable by genomics due to insufficient quality or quantity of sequenceable DNA.