BIOTECH SUPPORT GROUP
Sample Prep that Matters

AlbuVoid™ Application Report

Poster reprint from US HUPO 2018 Conference,
Minneapolis, MN, USA March 12-14, 2018

Stroma Liquid Biopsy – Pan-Cancer Dysregulation of the Serum Proteome

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Abstract

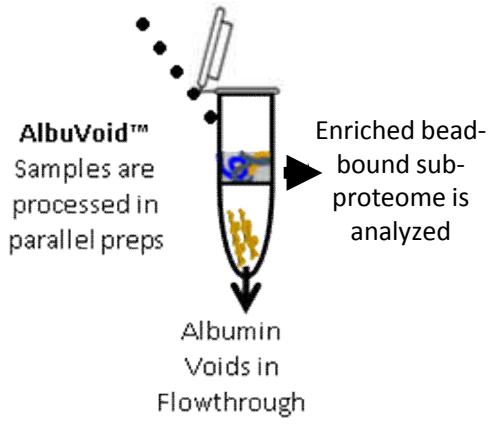
We hypothesized that because of tumor vasculature, changes in the serum proteome might result from the host cell response within the tumor-associated stromal microenvironments, and can thus be monitored by blood tests. We now report on protein measurements contributing to a Stroma Liquid Biopsy™, a pan-cancer proteome profile of dysregulation and consider its differential utility from previous liquid biopsy approaches. The special significance of this profile is that serum proteome changes were categorical and primarily contained within three host systemic response pathways: acute-phase inflammation, coagulation, and the complement cascade. Furthermore, this study signifies the importance of these pathways intercommunicating in the vast circuitry of cascading proteolytic events, the predominant mechanism for controlling acute insults in the bloodstream. Because proteolysis is irreversible and therefore highly regulated, the pathways in our model cannot be viewed as separate independent cascades, but rather as one interdependent system with extensive cross-talk, mutually fine-tuning their functional status.

Introduction:
Is there a common systemic response to cancer?

Unique workflow supported a new window of observation, uncovering a common dysregulated pattern of proteins, across different primary tumors which can be monitored and quantified by LC-MS.

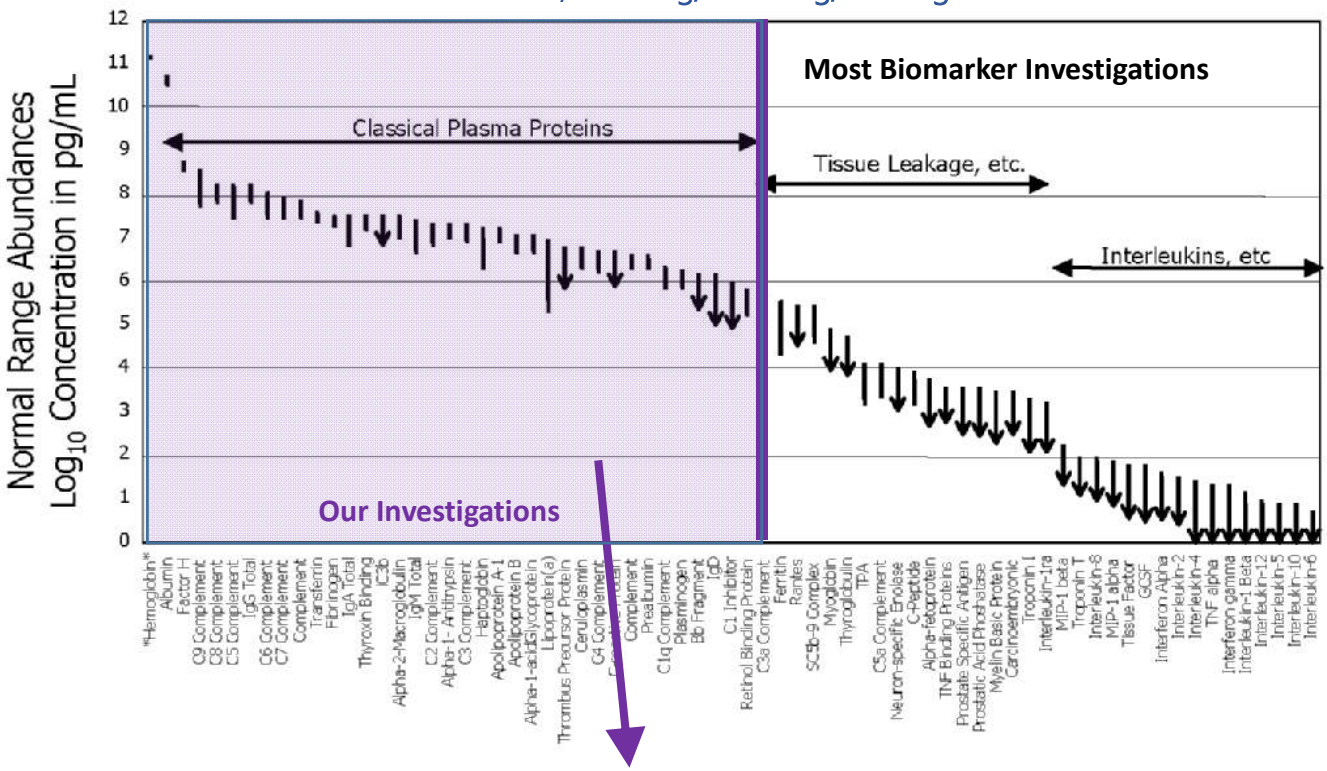
Workflow Considered:

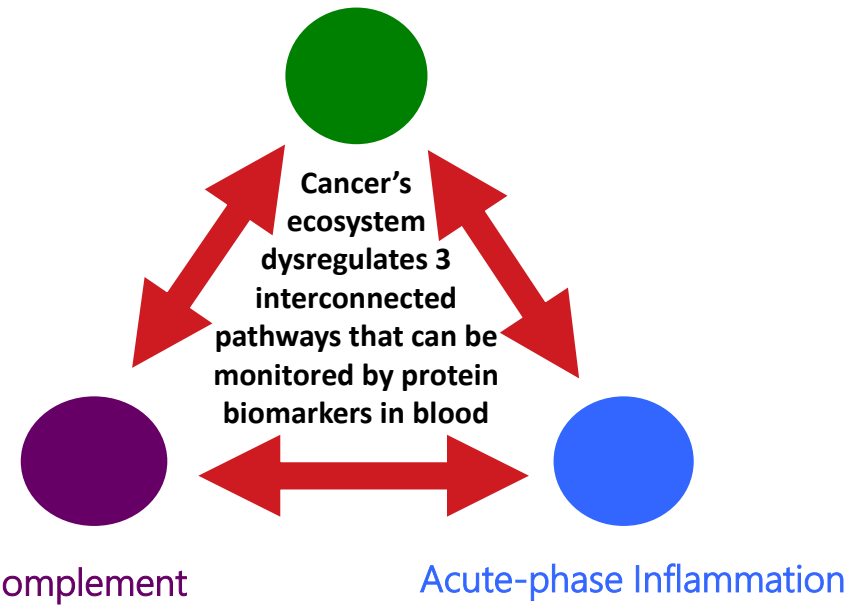
Albumin Depletion Using AlbuVoid™ bead separation
On-Bead & Eluate Digestion
Single 3 hour LC-MS acquisition
TMT labels or targeted label-free
>200 Proteins observed
Primary tumors: Breast, Lung, Pancreas, Lymphoma, Ovary



Because of tumor vasculature, quantitative changes in the serum proteome results from the normal cell host response within the tumor stroma, and can thus be monitored by blood tests.

Workflow only considered proteins in the classical concentrations, the >ng/ml to mg/ml range





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We set out to answer whether there is a common blood response to most if not all cancers, regardless of primary tumor, stage, or metastatic disease.

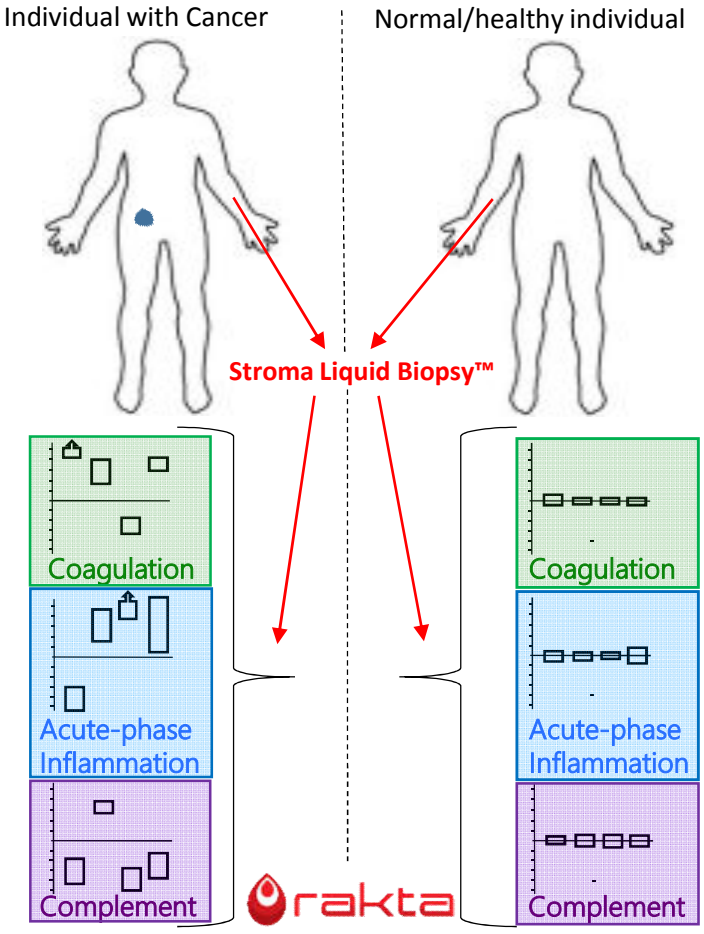
Yes, the common response is reflected in biomarkers for:

- Coagulation
- Acute-phase Inflammation
- Complement Cascade

All of these pathways are

> systemically interconnected

> regulated through proteolytic PTMs



The localized microenvironments of tumors cooperate to subvert normal homeostatic mechanisms in blood, consistent systemically with the presence of cancer

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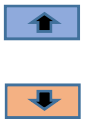
So we now present a way to report the systemic dysregulation using protein biomarkers

Stroma Liquid Biopsy™
Model of Proteome Dysregulation in Cancer

Protein Conc. Range > 5 log measurable in 1 LC-MS Analysis		Normal/Healthy Females, age 40-60				Cancer Females, age 40-60				
Systemic Pathway	**Rakta Protein Code**	**N1**	**N2**	**N3**	**N4**	**Breast Stg 1**	**Lung Stg 2**	**N-Hod Lymph**	**Panc Stg 2b**	**Ovary**
Coagulation	CA	Nd	0.3	0.4	Nd	0.8	3.2	4.0	1.0	2.5
Coagulation	PPBP	5	3	2	3	107	201	26	80	15
Coagulation	CC	Nd	Nd	Nd	Nd	39	87	11	22	11
Coagulation	THBS1	0.1	Nd	Nd	Nd	7	11	2	4	1
Coagulation	CE	1.0	1.4	0.9	1.5	0.6	0.5	0.3	0.2	0.2
Complement	TA	1.8	1.3	1.6	1.3	1.2	0.5	1.1	0.5	0.5
Complement	TB	3.1	1.2	1.5	2.6	0.8	3.2	0.6	0.8	0.2
Complement	TC	2.5	1.6	1.3	2.3	1.2	1.5	0.6	0.8	0.4
Acute-phase Inflammation	AAT Ratio	1.8	0.9	1.3	3.2	46	22	7	21	31
Acute-phase Inflammation	AB	0.6	1.4	1.7	2.3	1.8	11.3	1.7	8.7	10.1
Acute-phase Inflammation	SAA2	0.5	0.5	Nd	0.4	0.8	15.2	4.8	4.9	1.0
Acute-phase Inflammation	AD	3.3	3.3	4.2	0.9	5.4	7.8	41.1	6.0	6.2
Other	OA	Nd	0.8	Nd	0.8	3.0	6.8	4.8	3.5	5.1
Other	OB	4.7	5.3	1.6	2.6	0.3	0.6	0.6	1.3	0.5
Other Tissue Specific	SA	Nd	Nd	Nd	Nd	Nd	Nd	16	Nd	Nd

LC-MS Signal intensities



Special Case

Note – We have found that several of these biomarkers are also severely dysregulated in other chronic pathologies, diabetes, obesity, etc, and so additional specialized biomarkers from this panel are under investigation to improve the cancer specificity of the panel. We hope to report on that in the future.

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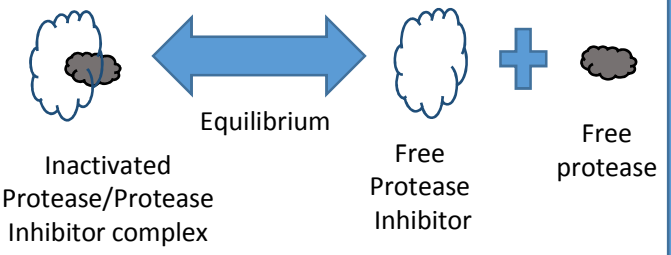
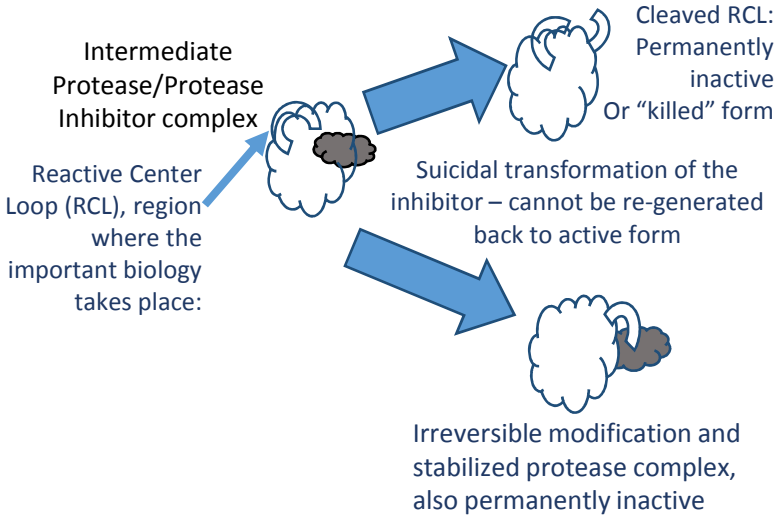
Special Case – AAT (Alpha-1-Antitrypsin)

SERPIN protease inhibitors must be accounted for differently than binary binders

Binary binding event protease inhibition, is regulated by relative concentrations of the reactants – protease and protease inhibitor (antiprotease)



SERPIN initial interaction can produce two substantially opposing outcomes



We have developed LC-MS methods to report and quantify ACTIVE from INACTIVE SERPIN sub-populations

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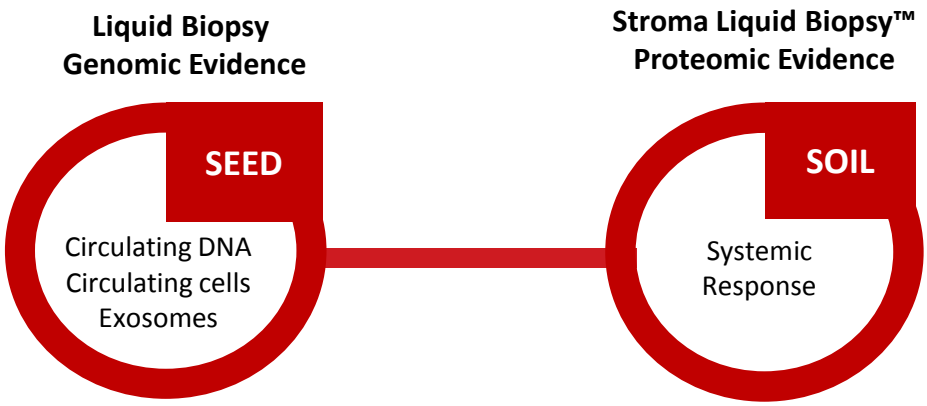
Conclusion

Discovery of protein biomarkers that can detect cancer early and personalize a treatment process has become an important research area in the proteomics field. For this, many proteomic approaches are being implemented in cancer research. Most biomarker investigations focus on very low abundance (pg/ml range) proteins shed from cancer cells, at the very limits of quantitative LC-MS analysis. To discover, characterize and monitor these 'needle in the haystack' biomarkers remains an industry wide challenge. By contrast, our investigations focused on the serum proteome mid-abundance range often not considered in biomarker investigations. Yet, an important advantage of the use of biomarkers within this window is that they are all highly observable with serum concentrations in the [µg-mg]/ml range. Furthermore, this range has been previously determined to be quantitative by LC-MS/MS with precision comparable to current clinical immunoassays.

Cooperative relationship between cancer cells and a repertoire of recruited, normal host cells - the stroma, fosters an interconnected pathway triangle of dysregulation.

All the SEED evidence miss an essential element of cancer pathogenesis, the tumor-associated microenvironment – the SOIL.



Future Directions

- Biopharma can aspire to move the dial towards a normal/healthy characteristic pattern. Any oncology therapy can monitor a companion Stroma Liquid Biopsy™ biomarker panel, suitable to the therapy
- Under physician guidance, Stroma Liquid Biopsy is a realistic goal, forming an early indicator for cancer possibly before clinical evidence
- A Proteogenomic Model now can consider the transient nature of SERPINs as Risk Factors
- Other chronic pathologies promote patterns involving same 3 interconnected pathways, ie, cardiovascular, Alzheimers, etc.
- We welcome opportunities to partner and collaborate with research organizations to explore how Stroma Liquid Biopsy can help in the management and treatment of cancer.

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