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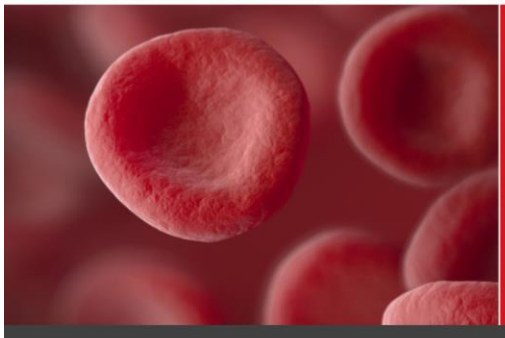
Sample Prep that Matters

Slide Presentation with commentary presented at

COMPANION DIAGNOSTICS FORUM
#CompanionDiagnosticsForum
October 29-30, 2019
Princeton, NJ
www.CompanionDiagnosticsForum.com



Stroma Liquid Biopsy™

Blood-based biomarkers to monitor stromal conditioning in cancer

U.S. Patent Application No. 15/953,260, entitled “Monitoring Dysregulated Serum Complement, Coagulation, and Acute-Phase Inflammation Sub-Proteomes Associated with Cancer," filed April 13, 2018.

Located near Princeton, NJ, we supply research products used in proteomic analysis. Through the evaluation of these products, we have discovered patent pending biomarkers *central to understanding how individuals are uniquely predisposed to cancer, how individuals uniquely adapt to the presence of cancer anywhere in the body, and how individuals uniquely respond to medical intervention.*

BSG

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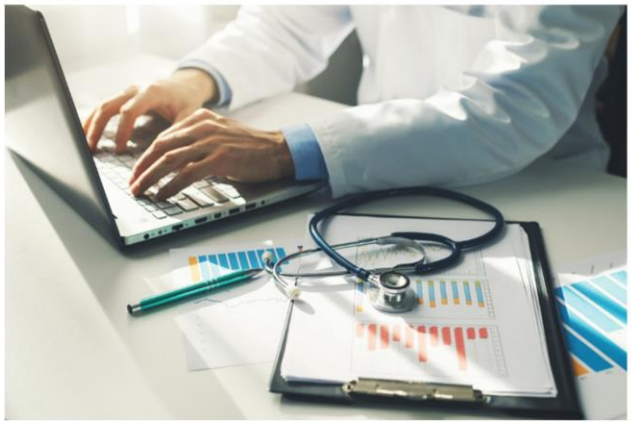
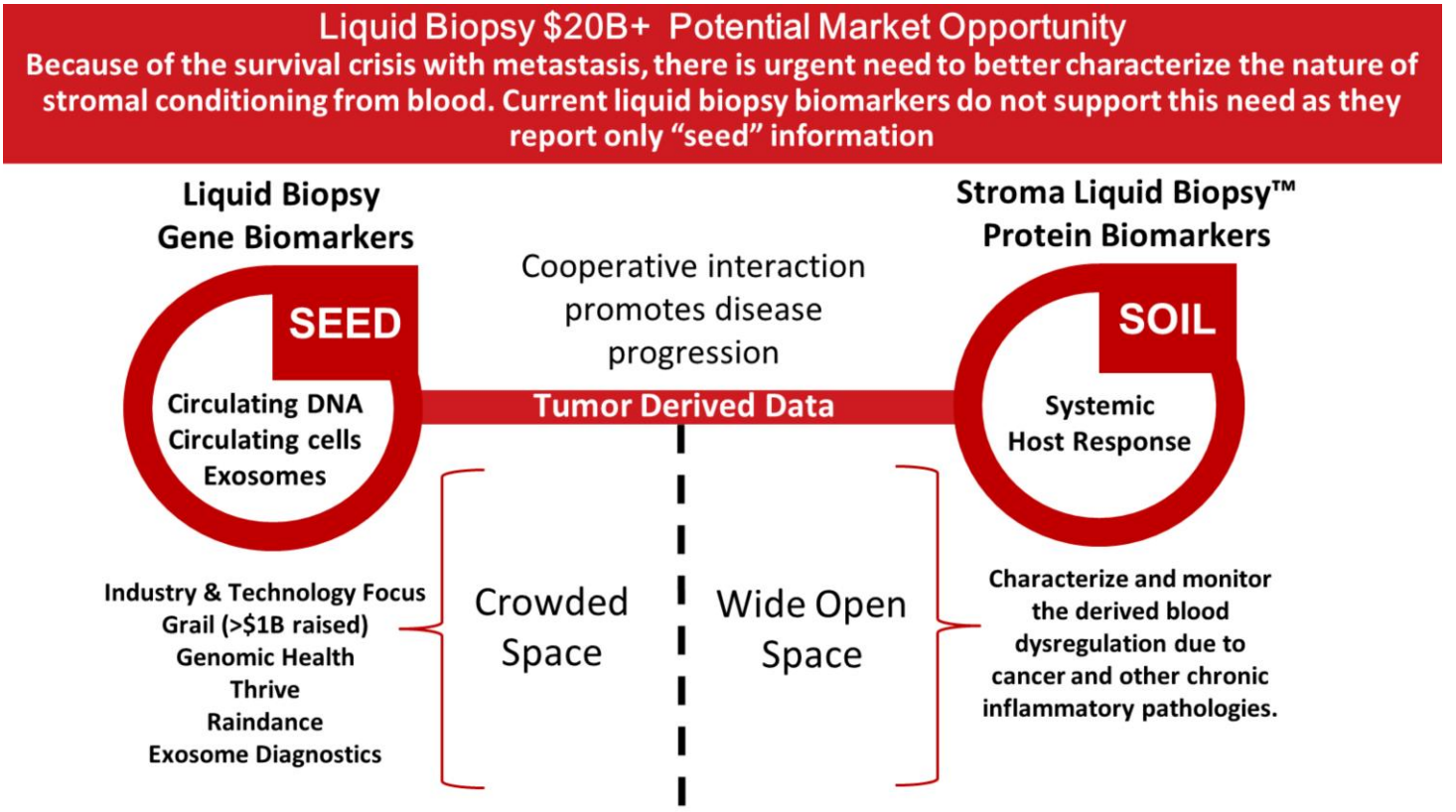
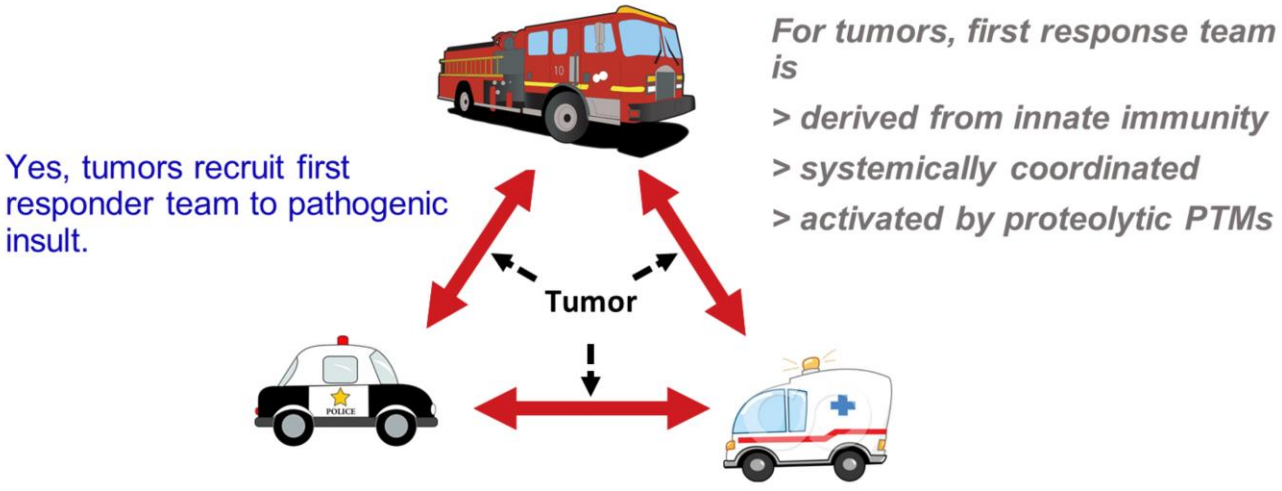




Liquid Biopsy is a term that encompasses methods to monitor one or more biomarkers from non-invasive samples like blood, urine, saliva, etc. Factors such as an aging population, preference for noninvasive procedures, wellness initiatives by governments and health organizations, and rising emphasis on personalized medicine in clinical practice, are driving the growth of the liquid biopsy market. While the field is most advanced with genomic markers, and commercial potential remains enthusiastic, intrinsic challenges remain. Foremost, is how to report mutational heterogeneity which occurs throughout disease progression. In sharp contrast to this, our small panel of protein biomarkers report the systemic host response, to which is remarkably conserved regardless of the primary tissue of origin and stage of disease. Finally we present here a case that stromal conditioning represents an entirely new way to obtain data on metastatic potentiation, and with that, new ways to therapeutically modulate metastasis for overall survival benefits.

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.



The protein level orchestration of this first responder team forms the basis of Stroma Liquid Biopsy™ and our Intellectual Property (IP)

The Immune System contains two branches:

- **Adaptive**
- **Innate**

Innate

- Non-Specific alarm system to pathogens, damaged or stressed cells
- First responders, clinically called acute inflammatory response, dissipates in 1 to 14 days, the common cold serves as model example
- No immunological memory

Cell mediators

Leukocytes (white blood cells)

Neutrophils 50-60%

Innate lymphoid cells

Protein mediators

Complement cascade – crosstalk with Adaptive

Chronic/reoccurring inflammation, driven by mechanisms of innate immunity, is a widely accepted contributor to cancer and many progressive debilitating diseases.

Adaptive

- Lag time, days between exposure and maximal response
- Immunological memory, response dissipates in years not days, and can vary dependent upon the initial insult

Cell mediators

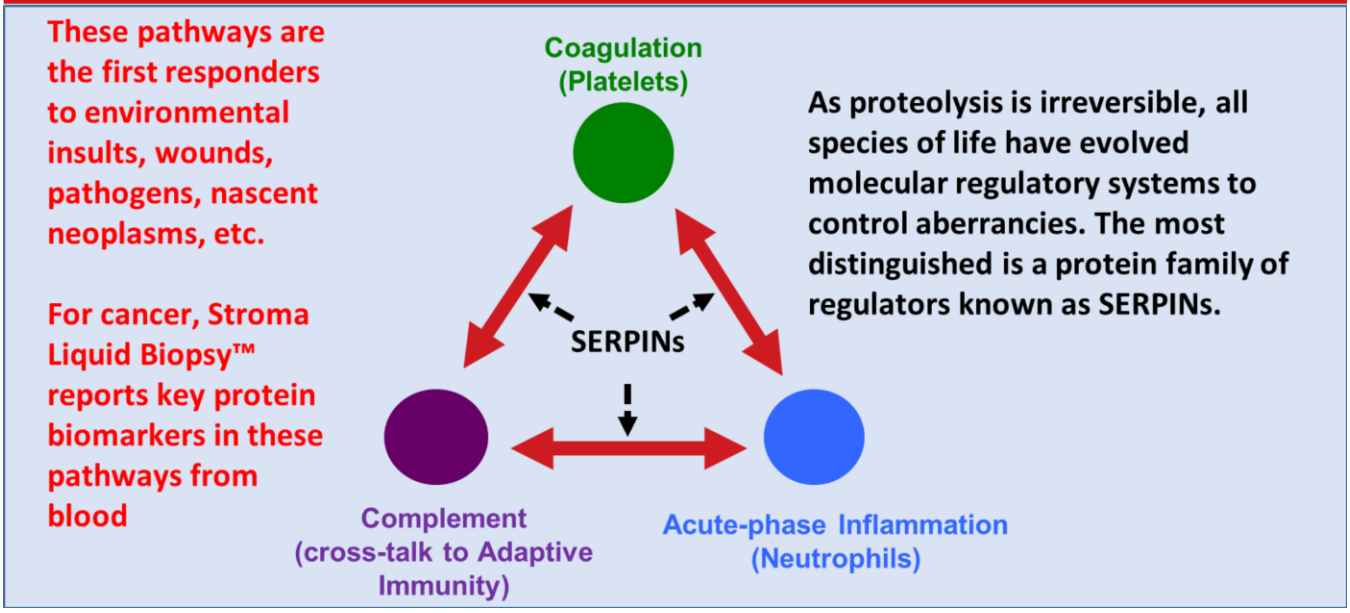
B-cell & T-cell Lymphocytes

Protein mediators

Antigens/Antibodies

Noteworthy is that the Complement cascade provides the conduit for communication between the innate and adaptive branches.

The basis of Stroma Liquid Biopsy™, comes from innate immunity response and the cooperative relationship between cancer cells and a repertoire of recruited, normal host cells - the stroma, promoting an interdependent triangle of dysregulation in cancer



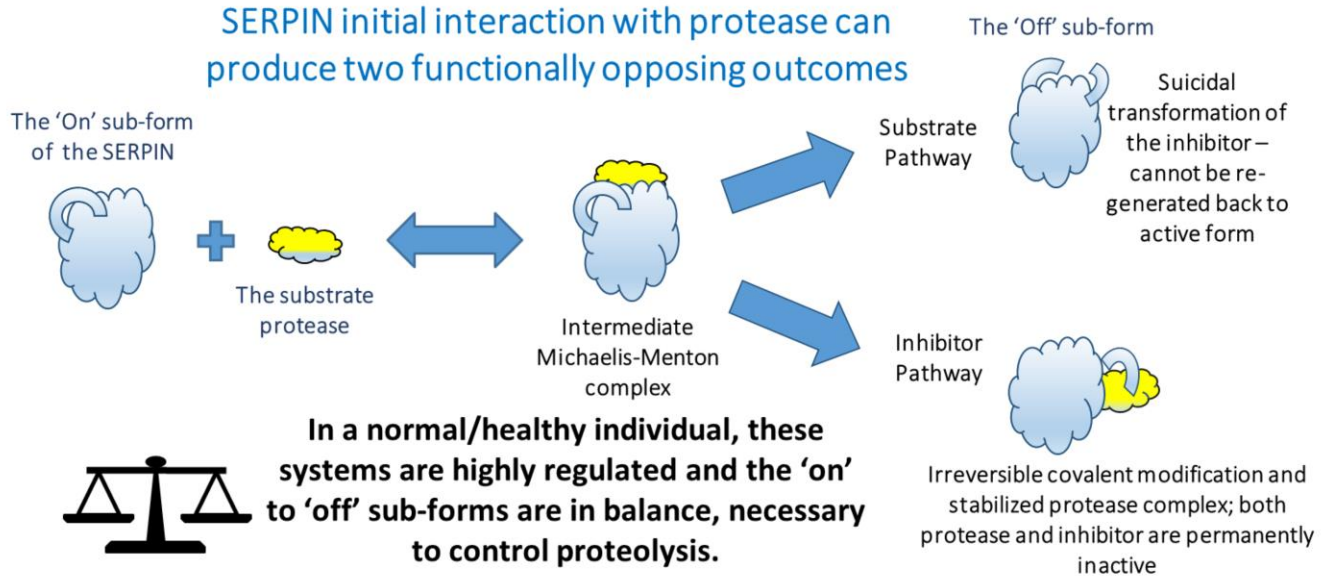
Unlike chemical and biological reactions which are subject to stoichiometric equilibria between the reactants and products, proteolysis is irreversible. Because of this, all organisms have evolved a complex system of regulation whereby multiple factors, both macromolecules and small molecules, control aberrant proteolytic events. In blood, these regulating events are all subject to periodic insults which may perturb this very delicate balance. Once disturbed however, this triangulated network of dysregulation can foster microenvironments suitable for the seeds of neoplastic cells to continue to grow unabated and metastasize. Such opportunistic events occur with localized inflammation, which impacts every step of tumorigenesis, from nascent neoplasms to primary tumor promotion, all the way through to metastatic disease.

Stroma Liquid Biopsy™ biomarkers observed and reported by LC-MS

Protein Conc. Range > 5 log measurable in 1 LC-MS Analysis					
Systemic Pathway	Protein Gene Name	Protein Description	Apprx Serum Conc.	Spectral Intensities cancer relative to normal	Comments
Coagulation	PF4	Platelet Factor 4	10 ng/ml	↑	Severe, released from platelets
Coagulation	PPBP	Pro-platelet basic protein	5 µg/ml	↑	Severe, released from platelets, activates Neutrophils
Coagulation	TIMP1	Tissue inhibitor of metalloproteinases-1	100 ng/ml	↑	Severe
Coagulation	THBS1	Thrombospondin 1	200 ng/ml	↑	Released from platelets, multifunctional with some sequence and functional similarities to Complement regulating protein – Properdin below
Complement	C3	C3	1,500 µg/ml	↓	Complement cascade function & regulation is multi-faceted, Coagulation protein Thrombin activates C3
Complement	C4BPA	Complement Component 4 binding alpha	300 µg/ml	↓	Complement cascade function & regulation is multi-faceted
Complement	PROP	Properdin	25 µg/ml	↓	Released from Neutrophils, some sequence and functional similarities to coagulation protein THBS1
Acute-phase Inflammation	SAA2	Serum Amyloid 2	5 µg/ml	↑	Near limits of detection with current methods
Acute-phase Inflammation	ELANE	Neutrophil Elastase	250 ng/ml	↑	Near limits of detection with current methods
Acute-phase Inflammation	ECM1	Extracellular Matrix Protein 1	800 ng/ml	↑	Released from Platelet dense granules, severe in many chronic inflammatory conditions, might be rule in/out marker based on severity stratification
Acute-phase Inflammation	CMGA	Chromogranin A	-	↑	Only Lymphoma differential from 5 primary tumors tested, below limits of detection for all others
SERPIN Function	SERPINA1	Alpha-1-Antitrypsin	1,500 µg/ml	↓	Inhibits Neutrophil Elastase, and activated Protein C (a regulator of the coagulation cascade)
SERPIN Function	SERPIND1	Heparin Cofactor II	60 µg/ml	↓	Inhibits extravascular Thrombin, Neutrophil Cathepsin G
SERPIN Function	SERPING1	C1 Inhibitor	300 µg/ml	inconclusive	Cross regulates complement and coagulation
SERPIN Function	SERPINA3	Antichymotrypsin	300 µg/ml	inconclusive	Might be Tissue Specific? Complexes with Prostate Specific Antigen

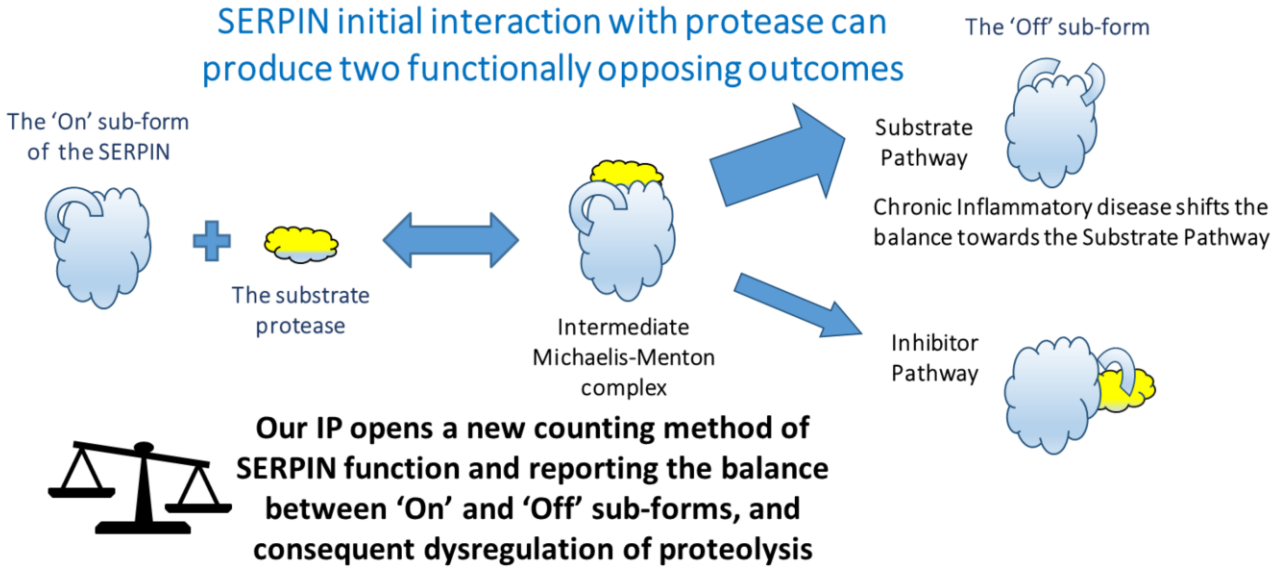
Here are the panel of protein biomarkers for the essential interactions between stroma and proliferating cells that report differentially by LC-MS/MS analysis, into the systemic circulation of cancer patients. This rewiring of the blood circuitry is measurable even at early stages of cancer, for most if not all primary tumors. The two proteins highlighted in yellow here, Alpha-1-Antitypsin (Serpin A1) and Heparin Cofactor II (Serpin D1), are critically important to regulate proteolysis within this network and so deserve special attention. Both proteins are part of a family of protease inhibitors called the SERPINs. Because of their very unique and largely unfamiliar mechanism of action, SERPINs demand a more nuanced approach to monitor and report their function.

Understanding the real balancing act of blood biology is severely compromised by the dogma of one-gene counting as one function. For Serpin level function, this can lead to egregiously misleading functional results.



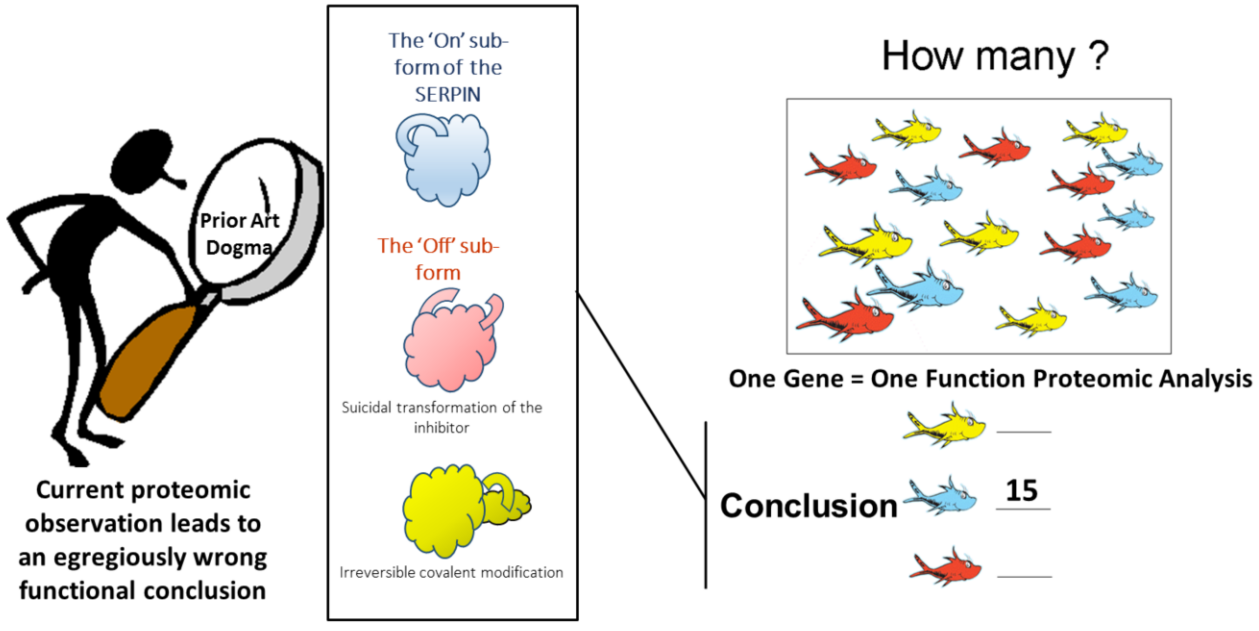
To quantify the function of Serpins, their suicidal mechanism of action demands new proteomic accounting methods than if their functions were simply stoichiometric, as would be the case if they were more mundane Michaelis-Menton type complexes. With Michaelis-Menton complexes, one could infer function by measuring the concentration or the relative abundance of the inhibitor by conventional means, typically by some form of immunoassay. However, because of the bifurcated mechanism of Serpins, the final outcome demands an entirely different analysis.

In chronic inflammatory disease, the Substrate Pathway becomes more pronounced, shifting the balance of Serpin level function to an unbalanced state.



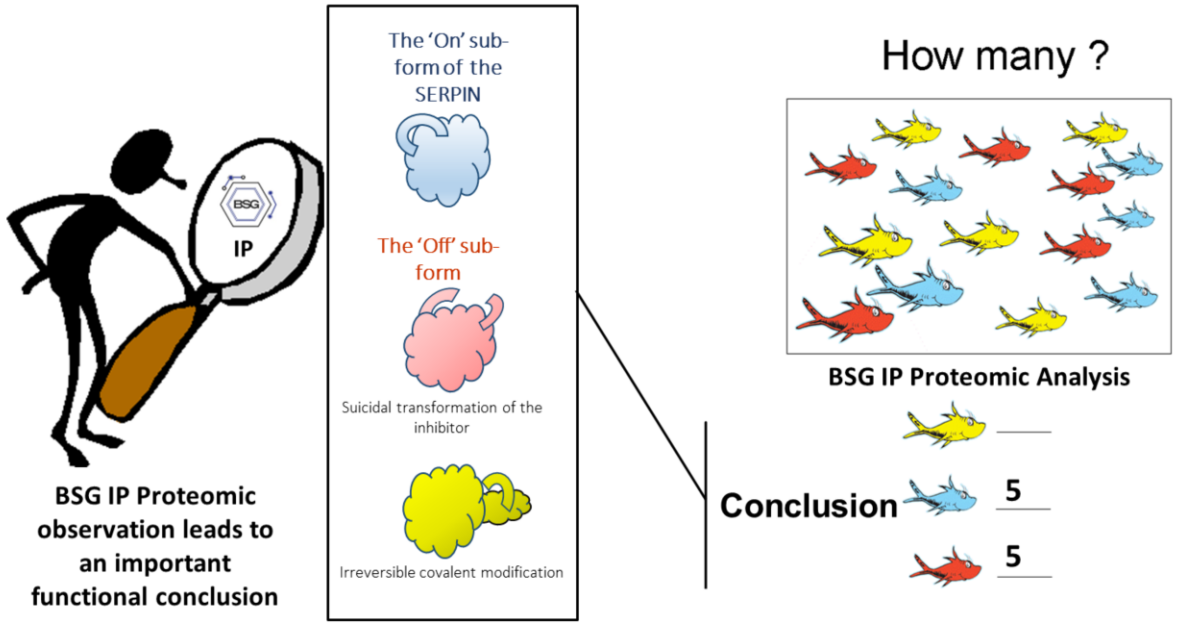
We have developed methods to make this critical distinction by measuring Liquid Chromatography-Mass Spectrometry (LC-MS) traceable peptide features within the decoy loop region of Serpins. These distinct sub-populations now can be observed and measured as:
"On" Serpin sub-populations that report as having inhibitory potential, or
"Off" Serpin sub-populations that report a suicidal transformation with permanent loss of inhibitory capacity.

Three functionally different SERPIN sub-forms circulate in the bloodstream. Current proteomic methods (ELISA) do not differentiate these sub-forms, and count all as being 'On'.



This is in contrast to the more conventional quantitative approaches which only observe the total population of Serpins by antigen presentation (i.e., ELISA). Such measurements not only discount the significance of whether the Serpin is functional or not, but can lead to an egregious misinterpretation of biological consequence. Such is the case in cancer, when Serpins are observed by total populations rather than functional sub-populations. One example of this discrepancy is that in many previous reports on Alpha-1-Antitrypsin (AAT) in cancer sera, AAT is measured in higher amounts in cancer sera relative to normal. Yet we observe just the opposite, that in cancer patients, there a a decline in the abundancy of the 'On' AAT sub-form.

Transformative IP
The 'on' and 'off' sub-form pattern circuitry defines cancer and likely all other chronic inflammatory diseases



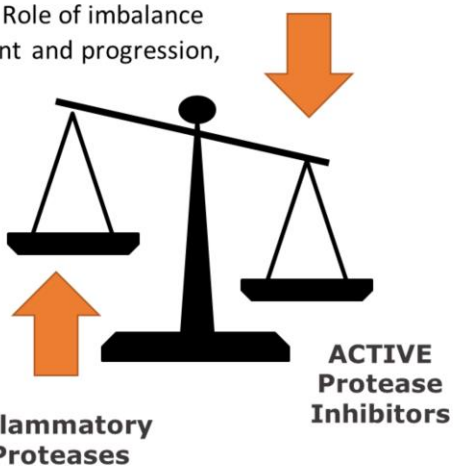
There is profound consequence to this dysregulation; cancer being often described as the wound that does not heal. Serpins account for about 5% of total protein mass in serum, so in a normal and healthy population, there is a sufficient blood reservoir of 'On' Serpins to regulate acute insults. However collectively, because of heredity, lifestyle, environmental exposures, and/or progressive disease, in cancer populations this reservoir becomes depleted. So once depleted, the body can no longer maintain sufficient quantities of functional Serpins to regulate the many inflammatory proteolytic mechanisms that occur as first response to a non-healing wound.

Serpin imbalance with protease activity is well documented in cancer, and used clinically, bound vs. free PSA

"…a deficiency in α1-antitrypsin is associated with increased risk of liver, bladder, gall bladder, lymphoma and lung cancer." From: Role of imbalance between neutrophil elastase and α1-antitrypsin in cancer development and progression, The Lancet Oncology 2004

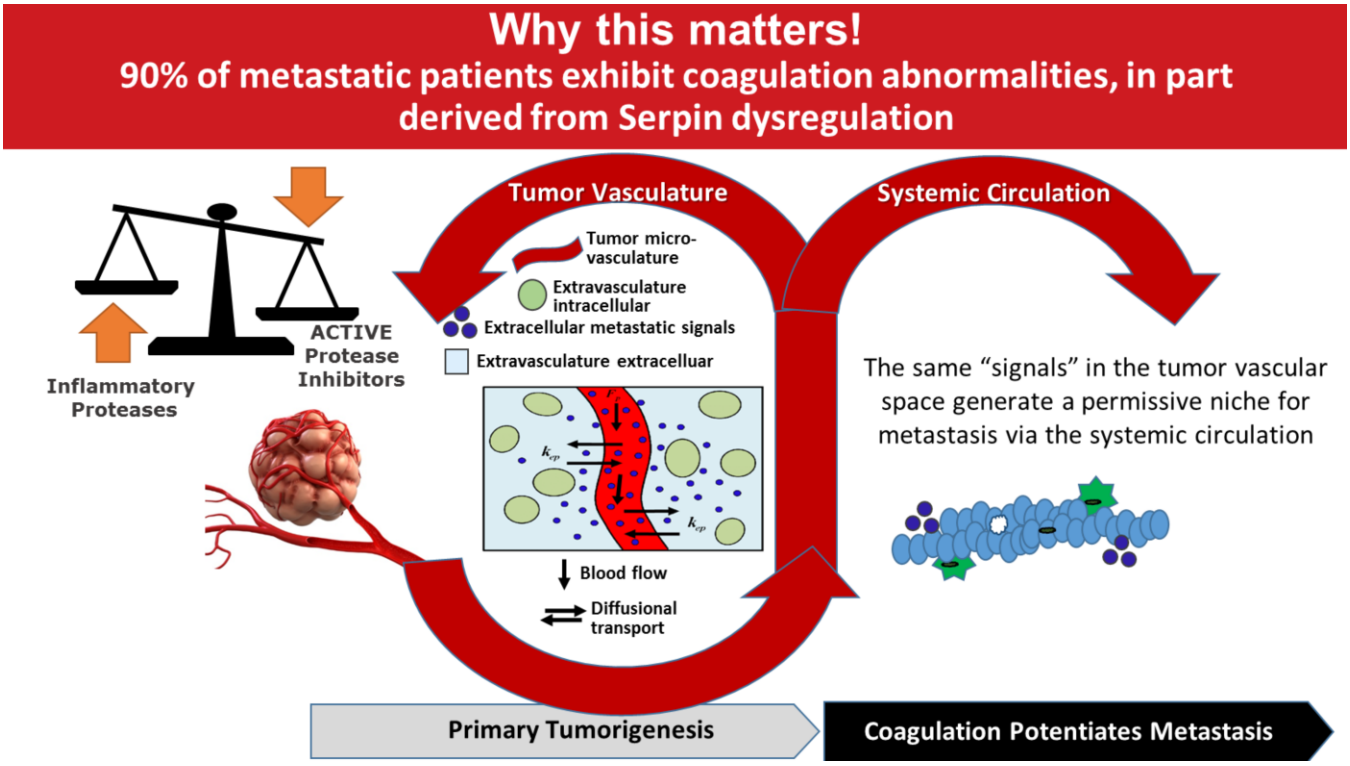
Antigen presentation (i.e., ELISA) has been the basis for counting, so past proteomic analyses counted all SERPINS as one homogeneous population.

Our IP discloses methods to differentiate ACTIVE from INACTIVE SERPIN Proteoforms

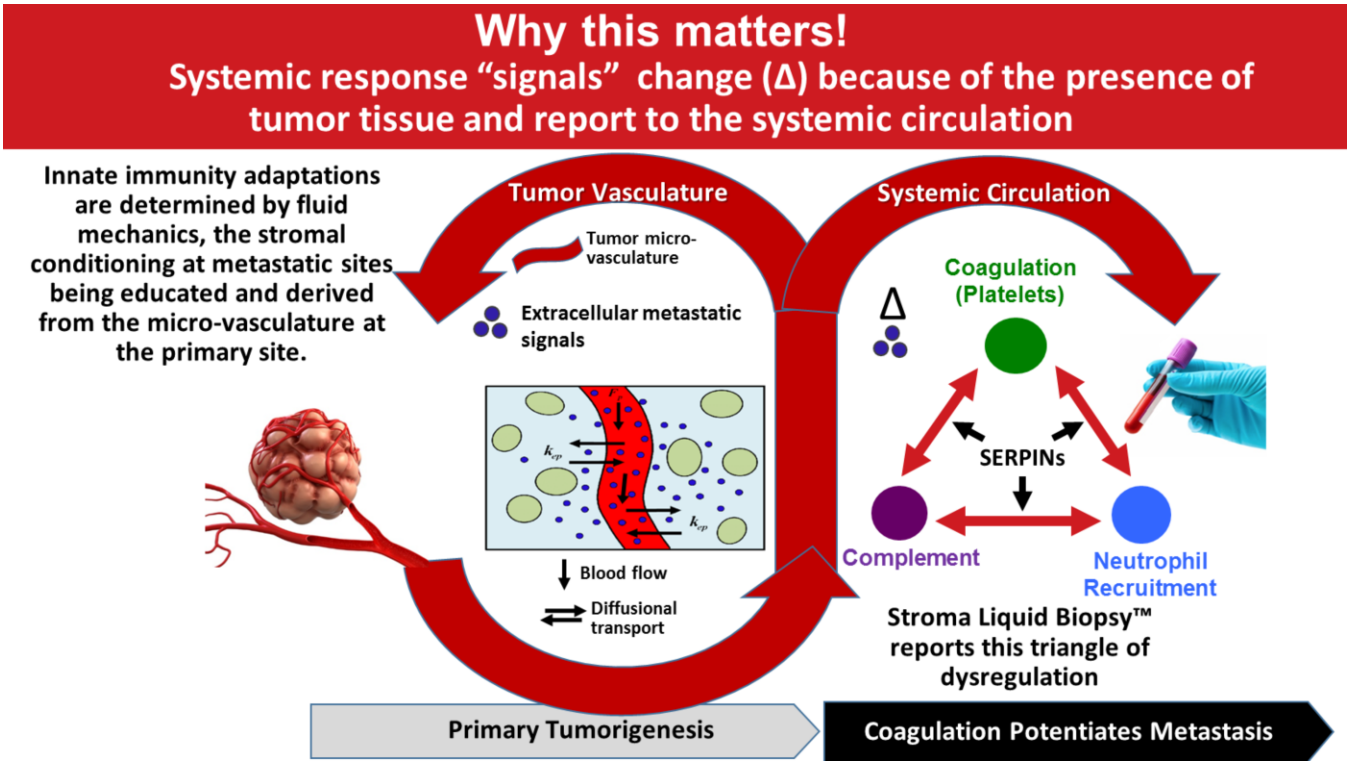


By monitoring these sub-forms, it will be possible to characterize and monitor all chronic inflammatory diseases!

Genetic deficiencies of Serpins as risk factors for cancer highlight there important role as protease regulators. Our data supports that inflammatory proteolysis becomes unregulated due to chronic exhaustion of ACTIVE subforms of SERPIN protease inhibitors! Without a sufficient reservoir of functionally active protease inhibitors, the irreversible cascading proteolytic events supporting coagulation, complement and neutrophil recruitment become progressively malevolent. We now advance this mechanism as a major contributor to metastatic disease. The next slide shows why.



Many reports conclude that there is a strong association of coagulation to metastatic potential. A recent article states "The correlation between Circulating Tumor Cells, hypercoagulability and reduced survival in metastatic breast cancer suggests the coagulation system supports tumour cell metastasis and is, therefore, a potential therapeutic target." From Kirwan, C.C., Descamps, T. & Castle, J. Clin Transl Oncol (2019). https://doi.org/10.1007/s12094-019-02197-6.



Tumors have a more narrow and tortuous vasculature relative to surrounding tissue. However, what happens in the primary tumor does not stay in the primary tumor. The stromal conditioning that supports a permissive niche for metastasis, is derived from the micro-vasculature at the primary site. This re-education is reportable from the systemic circulation with Stroma Liquid Biopsy™ biomarkers. Now with these biomarkers we can generate profiles to more precisely analyze an individualized host inflammatory response to cancer.

May 2019 News Release

Leiden University Medical Center and Biotech Support Group Enter Joint Research Agreement for Stromal Conditioning Biomarkers in Cancer

Transitioning from Tissue to Liquid Biopsy

Objectives are to characterize and quantify Stroma Liquid Biopsy™ biomarkers so as to correlate to the Tumor-Stroma Ratio (TSR) scoring methods developed by Leiden.

Leiden University Medical Center

TSR in epithelial carcinoma

The Tumor-Stroma Ratio (TSR) is a primary tissue based prognostic score. Stroma-high tumors are associated with worse overall and disease-free survival. The prognostic value has been shown for a range of solid epithelial tumors.

The UNITED study is an international multicenter study to validate the Tumor-stroma ratio (TSR) in a prospective study for colon carcinoma. 17 hospitals from 14 countries are participating with 1500 patients, all pStage II and III colon carcinoma.

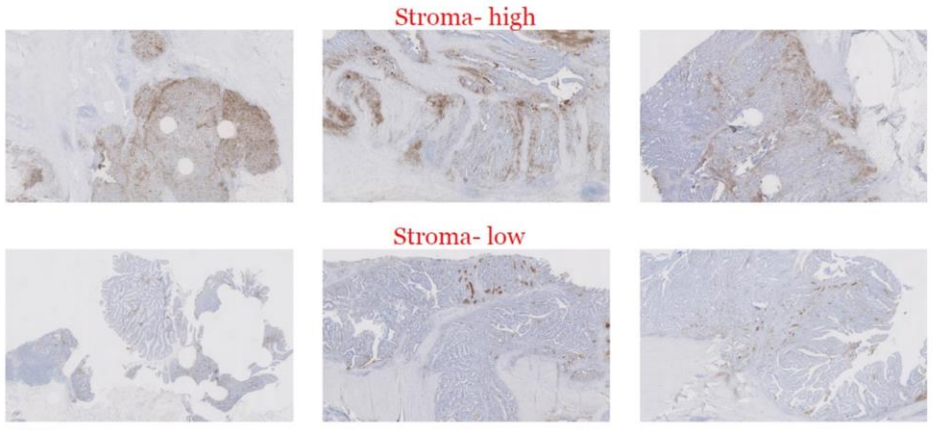
Primary Tissue availability severely limits the clinical utility of TSR. **Stroma Liquid Biopsy™** solves this problem and provides deeper and less subjective clinical characterization.

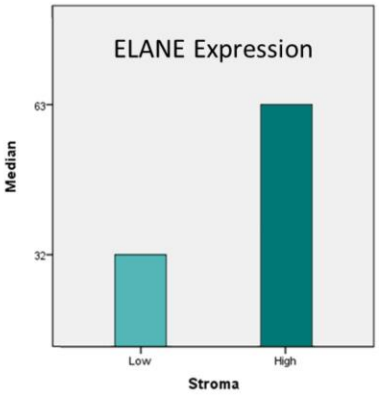
Our academic partners see this as an opportunity to advance cancer management at all levels. Dr. Wilma Mesker (Associate Professor) and Prof. Rob Tollenaar (Surgeon) of the Leiden University Medical Center concur, and state further that, “the tumor-stroma microenvironment is an important prognostic parameter for patients with epithelial cancer types. Patients with a high amount of stromal cells in the primary tumor have a bad prognosis and respond worse to current chemotherapy regimens. Blood derived information about various tumor environmental factors could reduce under and over-treatment of cancer patients with chemotherapy, and offers unique possibilities and insight for monitoring during treatment and personalized therapy”.

Initial Conclusion from Leiden Research Collaboration

ELANE (Neutrophil Elastase) expression is higher in the stroma-high patients (worse prognosis) compared to stroma-low patients

Highest ELANE scores: stroma-high vs stroma-low





Leiden University Medical Center

Early reports are demonstrating that there is a correlation between one of the biomarkers in our Stroma Liquid Biopsy™ panel – Neutrophil Elastase. It is worthwhile to note here that the primary role of Alpha-1-Antitrypsin is to neutralize the protease activity of Neutrophil Elastase.

Heparin Cofactor II (Serpin D1) largely controls the activity of the inflammatory protease thrombin in the extravascular space. So regulation of thrombin activity either by targeting PAR-1 signaling or inhibiting substrate Complement C3 may be of therapeutic benefit.

Ingrid Verhamme, PhD
Leading Serpin investigator

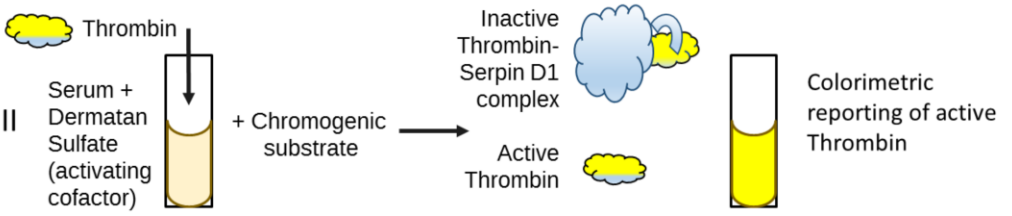
SERPINS2019

Loss of Functional Alpha-1-Antitrypsin and Heparin Cofactor II in Inflammation and Cancer

"Our functional results support a net decrease in inhibitory active Serpin D1, otherwise known as Heparin Cofactor II, in cancer patients. This brings to light a potential new cancer therapeutic strategy, to selectively inhibit Thrombin in the extravascular space, as 60% of Heparin Cofactor II is extra-vascular."

VANDERBILT UNIVERSITY MEDICAL CENTER

Functional Assay for Heparin Cofactor II (Serpin D1)



Using functional enzyme assays, preliminary results correlate to LC-MS/MS results, confirming a significant increase in the cleaved/total ratio in cancer sera, corresponding to a net decrease in inhibitory active serpin, relative to a normal/healthy status. Highlighting the importance of this poster report at SERPINS2019 Conference in Spain, Dr. Verhamme states that "Our hypothesis is that there may be a common host systemic response to many forms of cancer, regardless of primary tumor, stage or development of metastatic disease. This response is proposed to involve interconnected pathways attributable to thrombo-inflammation and innate immunity. All these pathways are activated by proteolysis and regulated by protease inhibition. So it is therefore likely that tumorigenesis can systemically be characterized by chronic exhaustion of inhibitory active serpins, and resulting increased protease activity. Our results support such a net decrease in inhibitory active Serpin D1, otherwise known as Heparin Cofactor II. We believe this is a very important discovery as it brings to light a potential new cancer therapeutic strategy, to selectively inhibit Thrombin in the extravascular space, as 60% of Heparin Cofactor II is extra-vascular."

Therapies reliant on stromal modulation is reportable through Stroma Liquid Biopsy™ Biomarkers

Monitor Efficacy
- Prognostic Indicators
- Alarm bells to attending physician to off-target effects
  - bleeding
  - pulminary

Developing Stroma Liquid Biopsy™ Cancer Biomarker Assays

Therapy Options

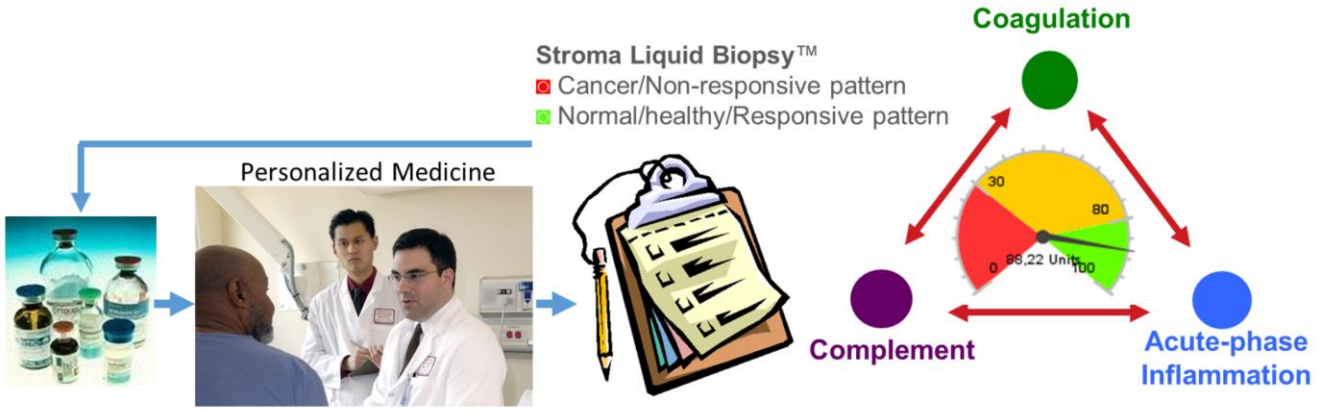
Decide
- Mono
- Combination

Dosing
- how much
- when

Patient Stratification
- Stromal Conditioning
- Hereditary Factors
- Clinical Stage
- Primary Tumor

Current liquid biopsy efforts focus on genomic data which relies on a largely reductionist view that tumors form and progress only through the collection of its immortalized cells. These contributions notwithstanding, it is now overwhelmingly apparent that throughout cancer progression, there are necessary adaptive microenvironments to support metastatic disease. Advancements in proteomics have now made it possible to monitor and measure in blood these adaptive microenvironments.

Biopharma can aspire to move the dial towards a normal/healthy characteristic pattern. Any oncology therapy can now monitor a companion Stroma Liquid Biopsy™ biomarker panel to determine if it is moving the dial to a more normal/healthy state.



Biomarkers that reflect stromal conditioning will be especially useful to follow the disease response for all agents and drug combinations that help to unwind the microenvironments contributing to disease progression. So **Stroma Liquid Biopsy™** biomarkers can be an important way to correlate and represent the density of stroma, its phenotype signature, and - albeit indirectly, the array of host cell populations within the tumor microenvironment at the earliest stages.

IP Presents Many Commercial Avenues to Pursue

Risk Investment Capital

Monitor Risk Factors & Wellness Surveillance throughout lifetime

First thoroughly characterize Systemic Response to Cancer

Follow with other chronic inflammatory pathologies including AD, organ transplant rejection, Rheumatoid Arthritis, Age-related macular degeneration, cardio-vascular, etc.

Stroma Liquid Biopsy™ Cancer Biomarkers

Companion Diagnostics

Personalized Treatment Immuno-oncology

Corporate Partners

New Therapeutic Strategies

Measure Progressive Disease

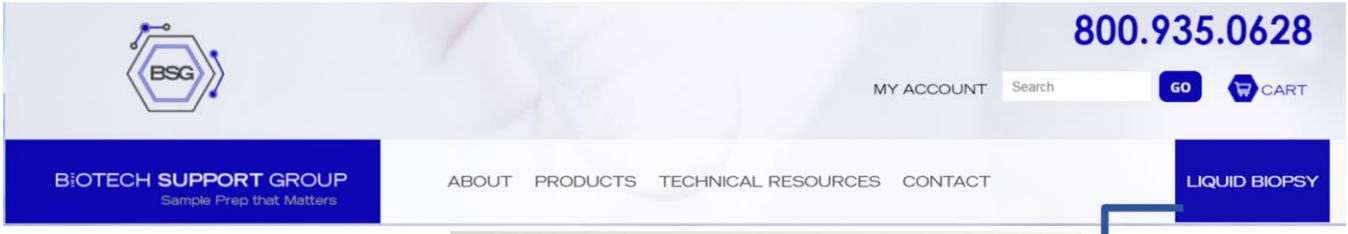
By using Stroma Liquid Biopsy™ biomarkers in blood, our collaborators and partners will gain invaluable information central to understanding how individuals are uniquely predisposed to cancer, how individuals uniquely adapt to the presence of cancer anywhere in the body, and how individuals uniquely respond to medical intervention.

Conclusions
Stroma Liquid Biopsy™

- Stromal mechanisms drive cancer pathogenesis and metastatic dissemination, now can be monitored for early detection of high risk individuals, prognosis and medical intervention decisions, and as companion diagnostics.
- The pleiotropic role of SERPINs, especially α-1-Antitrypsin (SERPINA1) and Heparin Cofactor II (SERPIND1), act as critical nodes in the tumor and systemic microenvironments. The equilibrium of their inherently mobile conformations can be modulated for therapeutic effect.
- Advance that extravascular Thrombin inhibition is not sufficiently regulated in cancer, and specific targeting of extravascular Thrombin, or its proteolytic substrates (C3 for example) is warranted for survival benefits as:

  -- monotherapy to relieve metastatic potential; for maintenance after primary tumor burden reduction, or prophylactic for high risk individuals, or

  -- combination therapy to enhance other modalities, especially for later stages



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