APPLICATION NOTE

SAPIENT

A Protein Liquid Biopsy for Oncology

A liquid biopsy platform that **defines a tumor's protein variants in tissue and detects them in blood by high-sensitivity mass spectrometry,** complementing ctDNA, methylation, and CTC analysis.

Liquid biopsy has changed how cancer is detected and monitored, yet its reach is still bounded by what circulating nucleic acids and rare cells can report. Circulating tumor DNA (ctDNA) sits at vanishingly low levels in early disease, clears quickly from blood, and is diluted by cell-free DNA from healthy tissue. Circulating tumor cells (CTC) are rarer still and highly heterogeneous, making them hard to characterize versus simply count. More fundamentally, these are all nucleic acid readouts, reporting genotype or cell state and not the tumor's functional output. Proteins are the layer where active biology, druggable surface targets, and treatment response biomarkers actually live. The challenge is that the majority of protein-based liquid biopsies used today are not truly tumor-specific. Conventional protein markers like CEA, CA-125, and PSA measure normal proteins that rise nonspecifically across many conditions, falling short of the specificity oncologists now expect from targeted assays.

Sapient fills this gap with a protein liquid biopsy platform that first **profiles a patient's tumor to define the protein variants that the cancer alone produces – including its sequence isoforms, aberrant glycoforms, and cleaved surface-protein fragments – then uses nanoparticle enrichment with high-sensitivity targeted mass spectrometry to detect those variants in blood.** The result is a protein readout that is tumor-specific by design and complements the genomic and cellular assays already in use.

Why a Protein Layer Has Been Missing

Proteins are harder to read from blood than DNA. They cannot be amplified, span an wide dynamic range, and because most proteins are made by healthy tissue as well as tumor tissue, total abundance rarely separates cancer from background. What marks a tumor is usually not how much of a protein is present, but its form.

Cancers routinely make proteins that diverge from what their own genome and transcriptome predict, through alternative splicing, RNA editing, and non-canonical translation – producing sequence isoforms absent from normal tissue. They add aberrant modifications, most notably

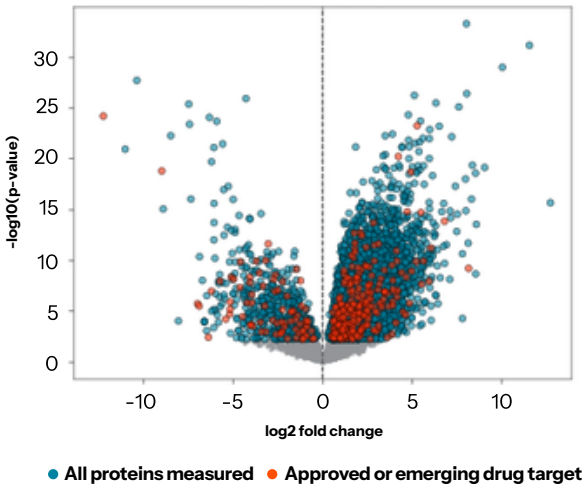
altered glycosylation, and shed cleaved fragments, including the cut ectodomains of cell surface proteins, into the circulation. Each is a biological feature that the tumor creates and that healthy tissue does not. That is precisely why abundance-based protein markers are often nonspecific.

The Sapient Approach

Defining the Tumor's Own Protein Variants

Sapient begins in the tumor itself. Using our Tumor Protein Mapping platform, including SurfaceSeek™ to identify druggable cell surface targets, Sapient measures 10,000 to 12,000 proteins in tumor tissue, from fresh-frozen or archived FFPE blocks, along with the modifications, glycoforms, and isoforms they carry.

Comparing tumor against matched normal tissue isolates the sequence, PTMs, and cleavage variants specific to the cancer, including the shed ectodomains of the surface proteins that antibody-drug conjugates (ADCs), T-cell engagers, and radioligand therapies target. Because these variants are **measured directly in human tumor rather than inferred from sequence, they form a set of candidate circulating markers that is tumor-specific from the start.**

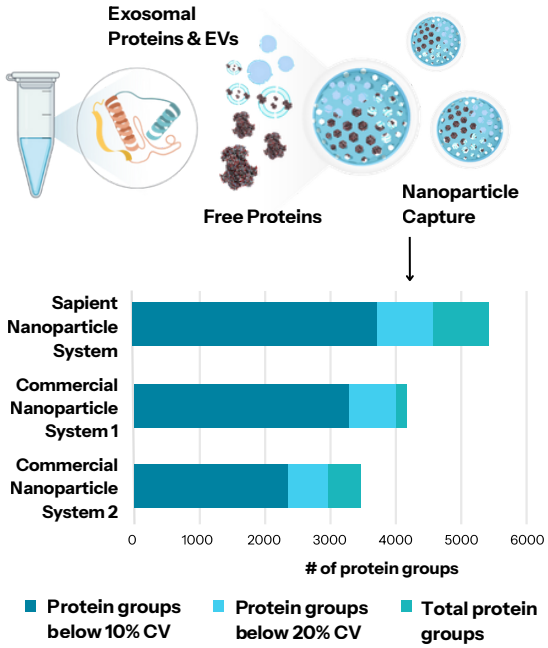


Proteins measured by Sapient's tumor proteomics method that are significantly changed in tumor samples.

Finding Those Variants in Blood

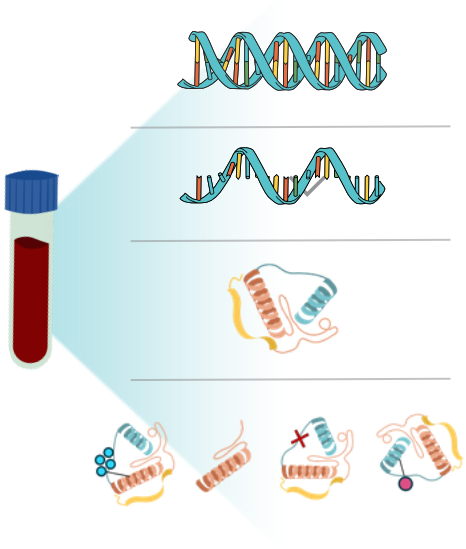
Each variant becomes the target of a high-sensitivity targeted mass spectrometry assay, built to the exact peptide, glycopeptide, or cut site that marks the tumor form. These assays are **antibody-free, quantitative with low pg/ml sensitivity, and because they look for a sequence or modification that only the tumor makes, carry very little normal background.**

To reach the low concentrations at which tumor proteins circulate, Sapient enriches plasma with an engineered nanoparticle system that pulls low-abundance proteins and shed fragments out of the high dynamic range background, deepening the measurable plasma proteome to roughly 5,400 proteins. The same assay runs on plasma directly or on circulating tumor cell-enriched fractions.



Protein groups quantified by CV using Sapient's nanoparticle system.

How the Protein Layer Complements DNA, RNA, and Epigenetic Markers



Each layer of a liquid biopsy answers a different question. ctDNA reports which mutations are present, read from cells that have already died. RNA and epigenetic marks report what a cell is transcribing and its tissue of origin and state. Proteins report what the tumor is making right now: the functional output of the cell. The protein layer is also the only one that carries the surface antigens therapies are built against. **A tumor-specific protein variant is therefore orthogonal to the nucleic acid layers, not a substitute for them.** It confirms that viable disease is present and active, and it reads targets and pathway activity that DNA and RNA can only predict.

Across the Liquid Biopsy Use Cases

In **early detection,** identifying a variant marker that only the tumor produces brings the specificity that abundance markers like CEA and CA-125 lack, and because it comes from viable cells, it can surface even when DNA shedding is minimal.

In **minimal residual disease and recurrence monitoring,** the task is to catch a vanishing signal. A tumor-specific protein marker carries almost no normal background and does not depend on ctDNA shedding, so it reports active disease rather than residual DNA and adds confidence when run alongside a genomic MRD test.

For **therapy monitoring,** quantitative assays can be used to track a circulating variant over time as a measure of tumor burden and response, while shed ectodomains of surface targets give a direct pharmacodynamic read on the antigen a therapy engages.

For **patient selection,** those same shed fragments show whether an ADC, T-cell engager, or radioligand target is expressed, providing a blood-based read on eligibility that today usually requires a fresh tissue biopsy.

Resistance tracking is where the protein layer is most informative. As a tumor escapes a targeted or surface-directed therapy, its protein output shifts, whether by losing the target antigen, changing its glycosylation or cleavage, or switching on a bypass pathway. Tracking resistance and bypass variants in circulation can flag escape as it emerges, often before it appears on imaging, and can point to the mechanism, not just the fact, of progression.

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A Protein Liquid Biopsy Grounded in DynamiQ™ Insights

Candidate circulating variants can be weighed against **DynamiQ™, Sapient's molecular-clinical database of more than 67,000 plasma samples from over 13,000 individuals,** profiled on the same platform and linked to clinical outcomes across more than 60 diseases, and with a median follow-up of well over a decade.

Because the tumor and plasma measurements are made the same way, a variant found in tissue can be checked directly against real patient plasma to see which markers genuinely track cancer. Two further reference sets sharpen the call.

Sapient's **Normal Human Tissue Proteomics Atlas** measures the same proteins across the major human tissues on the same platform, so a candidate can be screened against normal organs and dropped if it is not tumor-restricted. This, paired with Sapient's growing **Human Tumor Proteomics Atlas** – holding many tumor-specific variants already characterized across cancer types – means most programs start from known candidates rather than a blank slate.

Through this analysis, a **variant earns an assay when it clears three bars: present in the tumor, absent from normal tissue, and detectable in blood.**

One Draw, Multiple Omics Layers

It is important to note that the same plasma sample in which protein-level tumor biology can be extracted also carries the tumor's metabolic and lipid signature, which Sapient's metabolomic platform can read out as a further orthogonal layer. **A single blood draw can be measured across proteins, metabolites, and lipids alongside the genomic and cellular assays a program already runs.**

Put Our Protein Liquid Biopsy **to the Test**

To start a **discovery collaboration or a translational liquid biopsy program,** contact **discover@sapient.bio**.

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