WHITE SHEET

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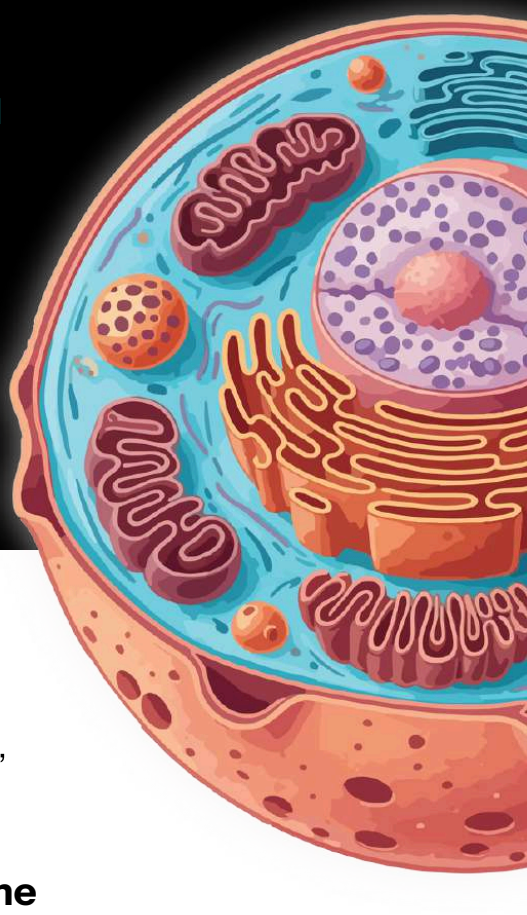
# SignalingSeek™

## Quantifying **functional tumor signaling** pathways



SignalingSeek directly measures phosphorylation-driven signaling activity in human tumors, enabling precise characterization of pathway activation, therapeutic response, and resistance across modern oncology therapies.

### Mapping the Functional Tumor Signaling Proteome

Oncology drug development is increasingly limited not by the availability of targets, but by the **ability to understand whether those targets are functionally active within human tumors.**

Across kinase inhibitors, immunotherapies, antibody-drug conjugates (ADCs), T-cell engagers, and combination strategies, **therapeutic success depends on the activation state of signaling pathways,** not simply the presence of mutations or protein expression.

For signaling biology to be actionable in drug development, several key dimensions must be understood, including:

- **Pathway activation state** (on vs. off)
- **Strength & distribution of signaling output**
- **Feedback and compensatory pathway activation**
- **Network-level pathway interactions**
- **Dynamic response to therapeutic intervention**

*These properties, however, are governed by phosphorylation-driven signaling events that are rarely captured by conventional assays.*

**SignalingSeek addresses this challenge by directly quantifying the phospho-proteome in human tumors using a mass spectrometry-based workflow.**

## Why Signaling Biology is Frequently Mischaracterized

While genomics and transcriptomics are foundational tools in oncology, they offer limited visibility into functional signaling activity. DNA mutations or RNA expression profiling do not reveal function, and **pathway activity can vary significantly across patients with similar genomic profiles.**

| Common Approach | Key Limitation |
|---|---|
| **DNA sequencing** | Identifies mutations but not pathway activation. |
| **RNA expression profiling** | Does not reflect signaling output or protein activation. |
| **Immunohistochemistry (IHC)** | Lacks specificity for phospho-proteins |

This can lead to poor patient stratification, missed biomarkers, and an incomplete understanding of resistance – and can ultimately result in clinical trial failure despite strong preclinical rationale.

## The Power of the SignalingSeek Approach

SignalingSeek addresses these gaps by directly measuring signaling pathway activation in human tumors at the protein level, applying **deep quantitative phosphoproteomics to quantify signaling activity across thousands of phosphorylation sites** for systems-level view of pathway activation.

### Direct Measure of Pathway Activation

Cell signaling is governed by phosphorylation events that regulate protein activation and inhibition, signal propagation through pathways, feedback and cross-talk between networks, and cellular responses to therapeutic intervention.

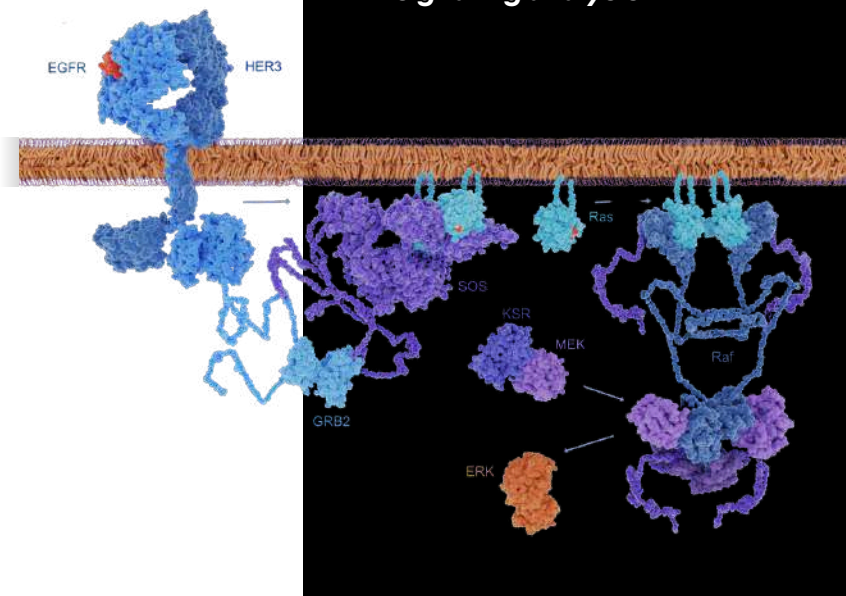
**SignalingSeek directly quantifies these phosphorylation events across critical oncogenic pathways, providing a functional readout of pathway activity rather than inferred potential.**

- **ERK/MAPK** signaling
- **PI3K/AKT/mTOR** signaling
- **JAK/STAT** signaling
- **NF-κB** signaling
- **Cell cycle** and **DNA damage** response pathways

SignalingSeek is **optimized for both fresh-frozen and FFPE human tumor samples,** enabling:

- **Retrospective analysis** of clinical trial cohorts
- **Mechanistic insight** from archived samples
- **Biomarker discovery** without new biopsies

*This unlocks the large repository of FFPE clinical samples for **functional signaling analysis.***

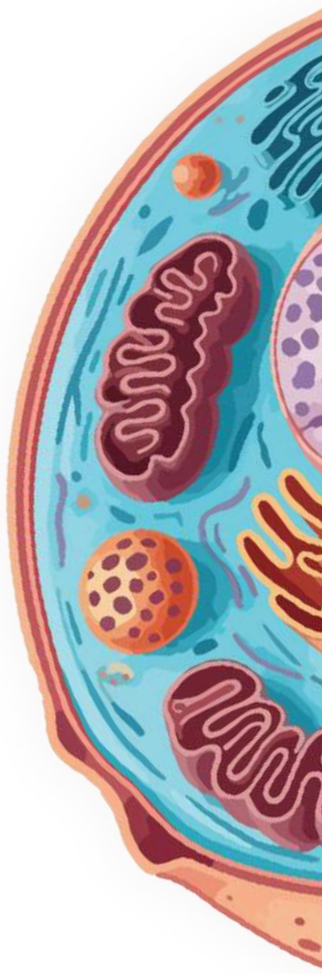


## Network-Level Signaling Insight

Signaling pathways do not operate in isolation. Tumors frequently exhibit parallel pathway activation, feedback loop compensation, and adaptive rewiring in response to therapy.

SignalingSeek **captures these network dynamics** by measuring thousands of phosphorylation events simultaneously, enabling:

- Identification of **dominant and drug-relevant** signaling programs
- Detection of **pathway cross-talk** and network dependencies
- Mapping of **adaptive and resistance-associated** signaling responses



## Pharmacodynamic and Mechanism-of-Action Readouts

Understanding **whether a drug is effectively modulating its intended pathway** is critical in development. SignalingSeek enables direct measurement of:

- **Pathway inhibition or activation** following treatment
- **Feedback activation** and pathway rebound
- Downstream **signaling suppression**
- **On-target** pharmacodynamic effects

# How SignalingSeek Guides Development Decisions

SignalingSeek **generates quantitative signaling data** that informs critical decisions across oncology development.

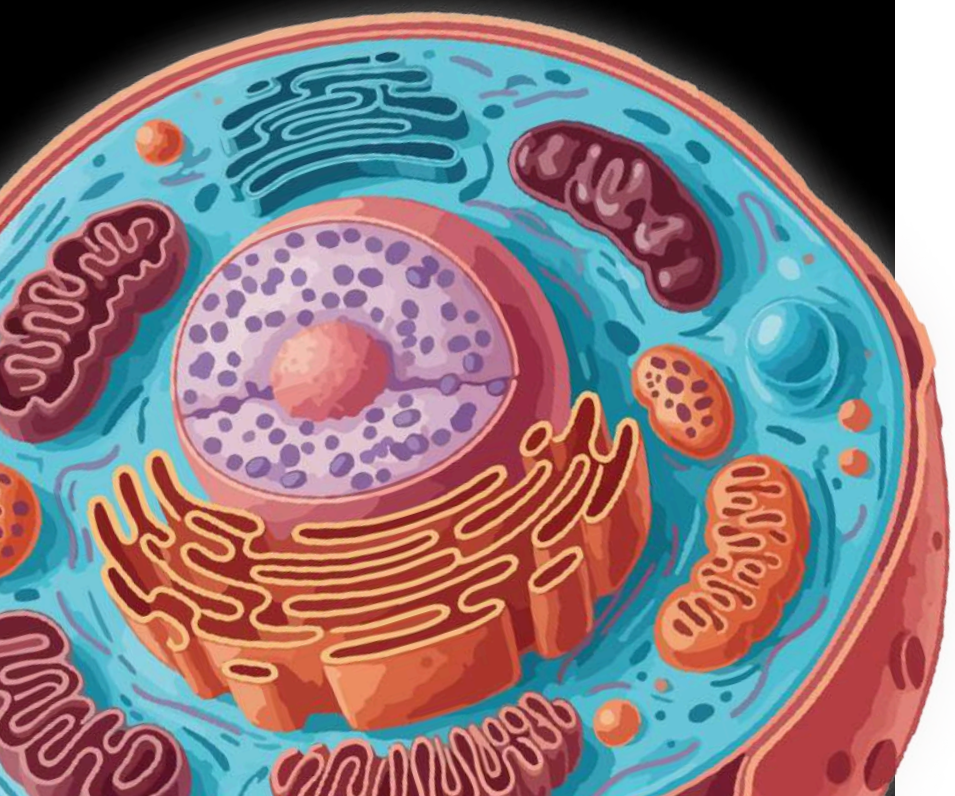
| Development Question | SignalingSeek Insight |
|---|---|
| **Is a certain signaling pathway functionally active in patient tumors?** | Direct measurement of phosphorylation-driven activation |
| **Are patients appropriately selected for therapy?** | Functional stratification based on pathway activity |
| **Why is a therapy failing or losing efficacy?** | Identification of adaptive signaling and resistance pathways |
| **Is the drug engaging its intended target?** | Pharmacodynamic measurement of pathway modulation |
| **Which combinations should be pursued?** | Identification of compensatory signaling networks |

# SignalingSeek:

## A **quantitative solution** to map functional tumor signaling activity

Historically, oncology development has relied on genomic inference to guide signaling biology – yet **signaling is governed by dynamic protein activation,** not static DNA alterations.

SignalingSeek **shifts development from mutation-based inference to functional pathway measurement,** with insights derived from the direct observation of dynamic human tumor biology.



## De-Risking Oncology Drug Development

By measuring pathway activity directly in human tumors, SignalingSeek **enables functionally grounded development strategies** to understand pathway activation, therapeutic response, and resistance, It is particularly valuable in programs where teams are asking:

- Is a drug effectively modulating its intended pathway?
- Why are patients with the same mutation responding differently to the same therapy?
- What mechanisms are driving resistance to targeted therapies?
- Which signaling pathways should be targeted in combination strategies?

**To request a SignalingSeek study, contact discover@sapient.bio.**

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**+ sapient.bio**
**+ discover@sapient.bio**
**+ 858.290.7010**