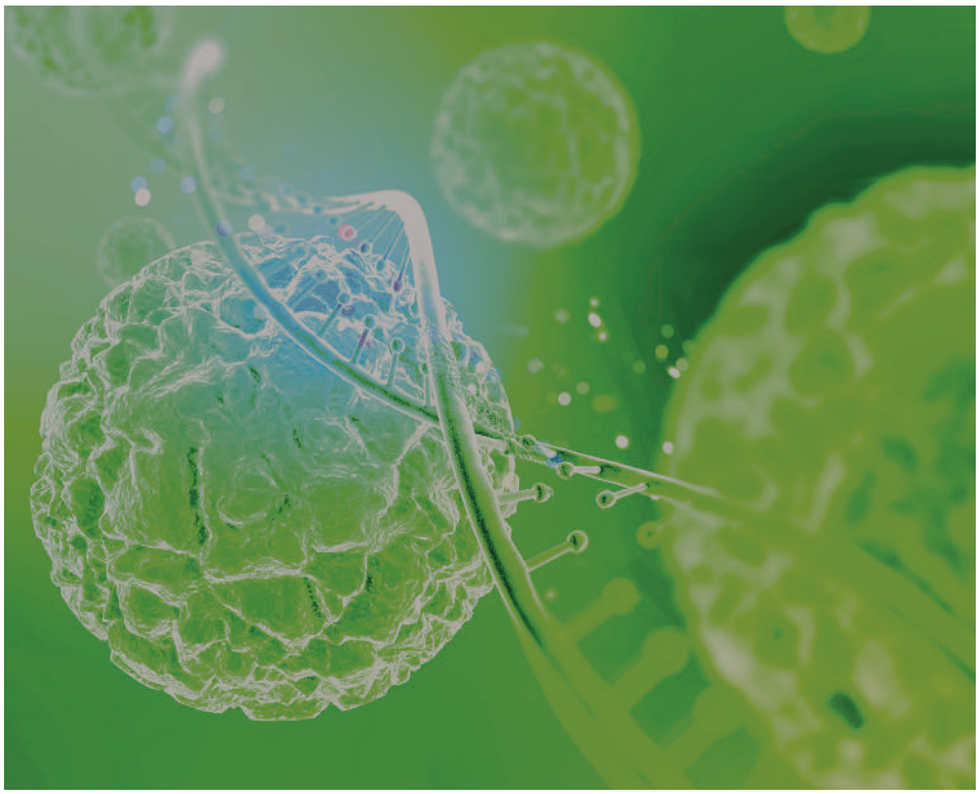


THE HUNT FOR KINASE INHIBITORS

NOVEL ASSAYS AND PROFILING TECHNIQUES are revealing new options for discovery of kinase inhibitors, a drug development stalwart.

Few, if any, classes of enzymes play a greater role in cell signaling than kinases. The human genome encodes for 518 different kinases, which add phosphate groups to substrates. Researchers suspect kinases phosphorylate up to one-third of all proteins in the human proteome.

To biologists, kinases are at the heart of many biological processes, making them compelling research subjects. To drug developers, the number of kinases and their central role in cell signaling makes them attractive targets for modulation.

Since the first small molecular kinase inhibitor Gleevec was approved in 2001 for treatment of chronic myeloid leukemia, kinases proved to be an extremely beneficial target class in cancer. To date, 49 drugs based on small molecule kinase inhibitors have been approved around the world, with 39 inhibitors approved in the last decade alone. More than 80% of focus on cancer, with the primary target being receptor tyrosine kinases.

While other therapeutic targets exist, expanding the universe of kinase inhibitors beyond cancer has presented challenges, including validating kinase targets, overcoming drug resistance, and kinase target selectivity. In recent years, however, several new assays and techniques have emerged that will allow drug developers to identify more selective kinase inhibitors capable of targeting a greater range of human diseases.

Begin with Biochemistry

Developing kinase inhibitors is not a simple process. Researchers first need to identify the kinase(s) to inhibit, a decision based on an understanding of kinase biology, cell signaling and disease. Then a suitable kinase inhibitor molecule, which shows selectivity for that specific kinase(s), must be identified. Assays help validate, or invalidate, those decisions.

"It's the biochemical and biophysical assays that provide important details on a potential kinase inhibitor's potency, selectivity, and mechanism of action," explains Haiching Ma, Chief Science Officer at Reaction Biology, a company that specializes in kinase assays. The better the biochemical assays can evaluate these characteristics in vitro, the better chance the candidate kinase inhibitor will work in vivo.

Biochemical assays are broadly divided into two categories: activity and binding assays. Among activity assays, the radiometric assay remains the gold standard. In the assay, the kinase inhibitor of interest is incubated with a target kinase, substrate, cofactors and radiolabeled ATP. During the kinase reaction, the radiolabeled phosphate is transferred onto the substrate, which is quantified to determine a kinase inhibitor's activity. Recently, in an effort to enable larger numbers of kinase inhibitors to be profiled for activity, Reaction Biology created the HotSpot℠, a high-throughput, miniaturized version of the radiometric assay platform.

Move into cellular assays

Biochemical assays help solve some of the mystery behind an inhibitor's activity and specificity, but they explain little about what a specific kinase inhibitor binds within a cell. That information is very important in determining whether a candidate inhibitor should be developed further.

"Cell assays have an advantage over biochemical assays as they represent a

more physiological situation," says Jan Ehlert, Head of Cellular Drug Discovery at ProQinase, a kinase focused CRO based in Germany that was recently acquired by Reaction Biology to form a global market leader providing the most comprehensive kinase drug development services.

To detect whether an inhibitor binds a target in living cells (target engagement), Reaction Biology has adopted the NanoBRET™ technology in collaboration with the technology developer Promega, a Wisconsin-based company that is the market leader in providing innovative biological technologies and reagents.

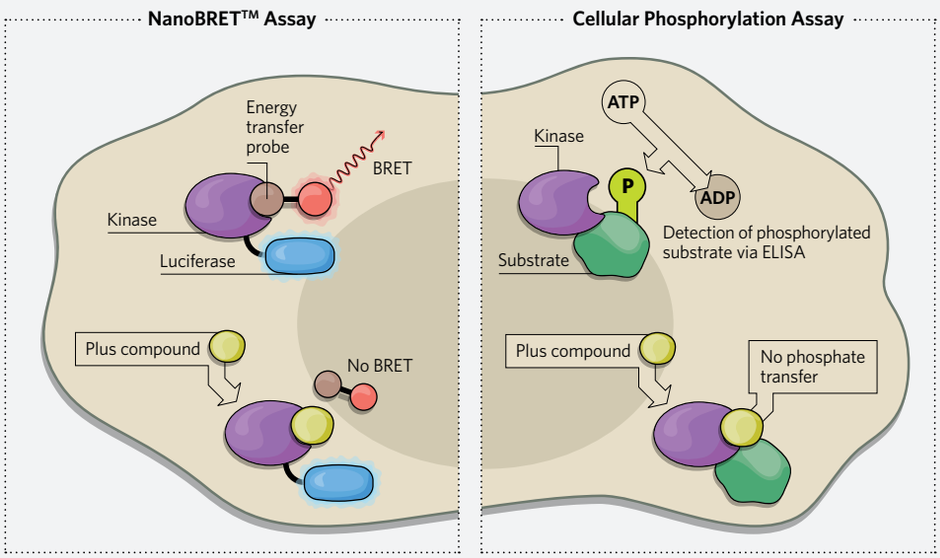
NanoBRET™ is a probe displacement assay that relies on bioluminescence resonance energy transfer (BRET) between the luminescent kinase and a fluorescent probe. In 2018, researchers from Promega and the Structural Genomics Consortium reported on how NanoBRET™ could be adapted to hunt for kinase inhibitors.[1] Using the approach, the authors were able to profile clinically-relevant kinase inhibitors against 178 different kinases, of which 115 targets are available for profiling at Reaction Biology.

"NanoBRET™ not only shows direct interactions between inhibitor and kinase target in living cells, it can also give information on how long the target is bound by the probe," adds Ma, who says information on target engagement helps in understanding the kinase inhibitor's binding kinetics and selectivity.

"BIOCHEMICAL DATA PREDICTS VERY LITTLE ABOUT EFFECTIVENESS IN VIVO AND VICE VERSA."

CELLULAR ASSAYS TO PROFILE KINASE INHIBITORS

For researchers profiling kinase inhibitors, assays with intact cells permit a view into physiology that biochemical ones do not. Those assays account for factors such as ATP concentration, pH, and cell membrane permeability, which have significant bearing on results.



Assays measuring the effect of an inhibitor on the activity of a target kinase have also been well received in the search for therapeutics. Reaction Biology and ProQinase have established 53 assays to directly measure the potency of a kinase inhibitor to prevent the phosphorylation of a physiologic substrate by the kinase in intact cells. This list includes a number of assays to discover novel drugs against EGF receptor mutants that are important for patients bearing drug resistant tumors.

"Because the cellular binding and activity assay provide different but important knowledge about the kinase inhibitor, it is wise for drug hunters to perform both assays."

Embrace In Vivo Testing

"In vivo assays address other questions about kinase inhibitors, such as bioavailability, stability, pharmacokinetics and efficacy," Ehlert says. These properties are usually out-of-bounds for typical biochemical and cellular assays, since cells can have different expression patterns in vivo and in vitro.

A specialized in vivo technique, the In Vivo Hollow Fiber Model, can be used to cross the divide between in vitro cellular assays and traditional pharmacology testing in mice. The In Vivo Hollow Fiber Model was invented by researchers of the National Cancer Institute in 1995. Investigators place cells into inert hollow fibers and implant them in a mouse. The fibers hold the cells in place while still allowing kinase inhibitors to enter.

Unlike traditional testing in mouse models, using the In Vivo Hollow Fiber Model researchers can test three or more different cell lines simultaneously in a single animal. By testing multiple cell lines together in vivo, costs are reduced, throughput is increased, and data needed to decide on how best to advance a potential kinase inhibitor drug can be obtained more quickly.

In the end, biochemical, cellular and in vivo assays are all necessary when hunting for a new kinase inhibitor. "Pure biochemical data predicts very little about effectiveness in vivo and vice versa," Ehlert says. It's the correlative proof accumulated during the totality of the testing and discovery process that makes the difference. As new targets for kinase inhibitors emerge, that correlative proof will become even more important. ■

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