nature

KINASES: THE SECOND WAVE

In the past 15 years, kinases have become one of the MOST POPULAR SMALL-MOLECULE TARGETS. Now kinase inhibitors are finding application beyond oncology.

Within the human body, few enzymes are more instrumental to basic biological function than protein kinases. The family of 518 enzymes, plus a large number of mutants, catalyzes the transfer of the γ-phosphate group of ATP on to a target substrate, modifying activity. Phosphorylation is one of the most potent and pervasive regulatory mechanisms. Within cells, kinases are mediators of signal transduction and critical processes, including metabolism, transcription, differentiation and apoptosis. Kinases also play critical roles in intercellular communication, and the maintenance of nervous and immune systems.

Because of their ubiquity, kinases are popular discovery targets and kinase inhibitors (KI) have become one of the most successful classes of drug in the past 20 years. Imatinib, the first breakout KI, transformed chronic myelogenous leukemia and gastrointestinal tumors from fatal diseases into manageable conditions. The FDA had approved 38 small molecule protein kinase inhibitors and 3 mTOR kinase inhibitors by the end of 2017, and up to 150 more KIs are in clinical trials.

Yet even after two decades of intense discovery, the therapeutic benefits of KIs are far from exhausted. The next 20 years could see as much, if not more, research into kinases and KIs, ushering in new-generation treatments for cancer and other diseases.

New Indications

Of the 40-plus FDA-approved KIs, only two have been approved for use outside cancer. That trend is set to change as autoimmune diseases come under increasing research scrutiny. As scientists unravel the pathways that regulate inflammatory response, the possibility grows of using KIs to mediate those pathways. Research into KIs for the treatment of rheumatoid arthritis, psoriasis, inflammatory bowel disease and myeloproliferative disorder is ongoing, with particular focus on the Janus family of kinases (JAKs) and the Spleen Tyrosine Kinase (Syk). The EMA approved its first two JAK inhibitors for treatment of inflammatory diseases in 2017, and several more are in trials in the Europe and the U.S.

BEYOND ONCOLOGY, KINASES ARE A KEY TARGET CLASS FOR NEURODEGENERATIVE DISEASES LIKE PARKINSON'S, ALZHEIMER'S AND HUNTINGTON'S DISEASES.

Neurodegenerative diseases, particularly Alzheimer's (AD) and Parkinson's (PD) diseases, are also subjects of intense research. The lack of new AD and PD drug candidates has intensified the search for drugs that can be repurposed or repositioned. KIs are a promising category, particularly those that inhibit the kinases LRRK2 for PD and p38 MAPK and DYRK1A for AD. In early 2017, researchers at the Georgetown Hospital announced two trials to test the impact of Nilotinib, an approved cancer drug, on AD and PD.

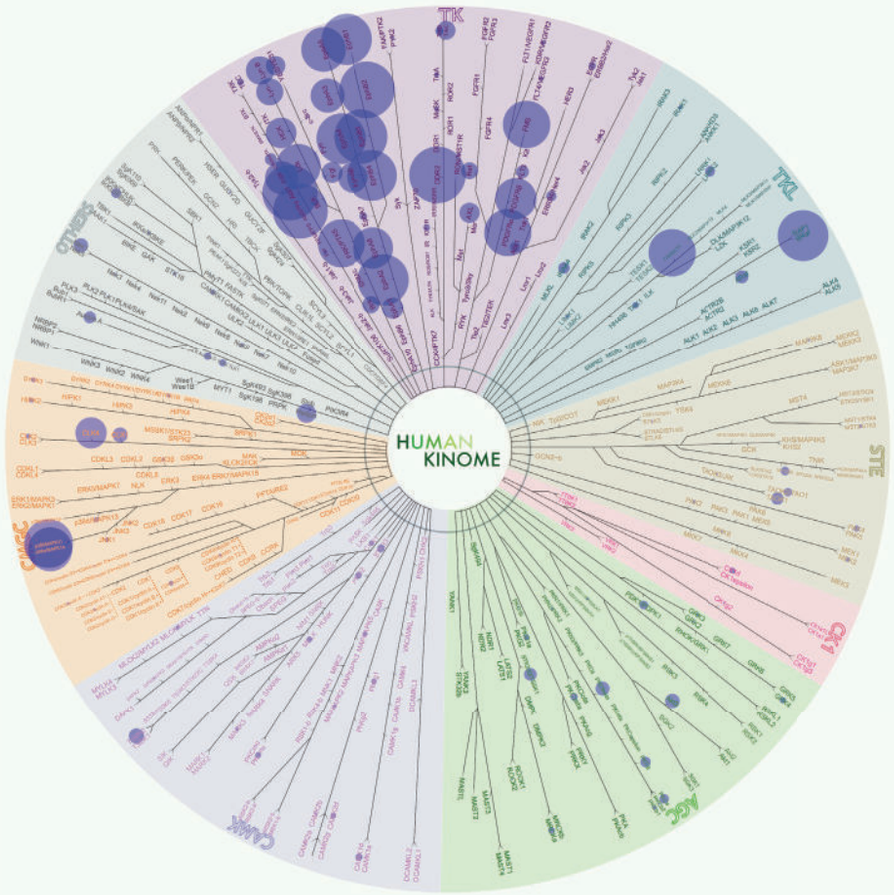
New Kinase Biology

KIs still hold immense potential for the treatment of cancer. Epigenetics is a particularly exciting frontier. Epigenetic changes modify gene expression, impacting the underlying DNA. Phosphorylation is one of the most common epigenetic modifications and has been shown to prompt structural changes in histones, which, in turn, encourage or discourage a gene's translation. Kinases catalyze those modifications. As such, KIs could one day be used to strategically turn genes on or off.

Kinases also have indirect epigenetic impacts. Bromodomains are a family of non-kinase proteins that recognize acetylation marks in histone tails and recruit transcriptional machinery to promote gene expression. The BET family, as it is known, is closely linked to cancer, directly regulating the expression of certain cancer-related genes, such as c-MYC. Recent studies have shown that KIs can serve as BET inhibitors. For example, the PLK1 inhibitor BI2536 potently suppressed MYC expression in the multiple myeloma cell line.

Other non-kinase targets might hold therapeutic potential as well. For example, a number of studies have shown that KIs can interfere with the formation of microtubules, hollow tubes of α- and β-tubulin that form a cell's cytoskeleton. KIs bind directly to tubulin and disrupt polymerization, killing rapidly proliferating cells.

In addition to epigenetics and non-kinase targets, researchers are investigating the potential of poly-kinase or poly-pharmacological combinations. By combining first, second, third and, soon, fourth-generation KIs, or by pairing KIs with other cancer drugs, clinicians could manage tumors as they mutate and become resistant over time. Few cancers better exemplify the challenge of conferred resistance than non-small cell lung cancer (NSCLC). Epidermal growth factor tyrosine kinase inhibitors (EGFR TKIs) are extraordinarily effective in the treatment of NSCLC, but their



The human kinome describes 518 protein kinase genes. Some kinases are highly specific while others vary in function depending on their partner proteins. Reaction Biology Corporation's Kinome Mapper (KM) visualizes the selectivity and potency of KIs against native kinases and kinase complexes collected by RBC. The KM diagram at right illustrates the selectivity of Nilotinib against the 300 kinases profiled. The drug inhibits a number of Tyrosine Kinases (TK) but also kinases in other families. For researchers in the early stages of drug discovery, determining the on- and off-target effects of KIs can focus drug repurposing and ultimately FDA-approvals.

Anastassiadis, T. et al. Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. *Nature Biotechnology* **29**, 1039–1045 (2011).

effectiveness all but disappears with a common mutation.

Through large library screening against mutant kinases, researchers developed a third and, most recently, a fourth-generation EGFR TKI, expanding the fight against EGFR-mutant NSCLC. Additionally, scientists are now testing the third-generation KI, AZD9291, in combination with immune checkpoint, MET and MEK inhibitors, as well as with PD-L1 antibodies. These poly-pharmacological approaches could dramatically increase available treatment options.

Implications for Kinase Profiling

As drug discovery for new and repurposed KIs grows, so will the demand for profiling against all wild type kinases and diseases associated mutations. Evaluation of off-target effects on non-target kinases and non-kinases alike is a critical safety consideration, even for repurposed drugs. Also, studies show that cytotoxicity mechanisms can be more complex than previously understood, so validating a KIs mechanism of action is more important than ever.

Over the past fifteen years, many kinase profiling methods have developed, such as luminescence detection, scintillation proximity, or competition binding, but radioisotope filter binding assays remain the gold standard. It is the only method to directly measure kinase catalytic activity and functional inhibition. Radiometric screening is also required to validate any other techniques, should a drug advance through trials.

"Of the contract research organizations (CRO) that offer radiometric kinase screening, Reaction Biology Corporation (RBC) has the largest kinase collections, including 371 wild type kinases, 17 lipid kinases, 20 atypical kinases, and 208 mutant kinases," says Haiching Ma, RBC's chief science officer. "RBC's HotSpot℠ technique is well suited to high-throughput screening and high-throughput profiling. For companies pursuing third and fourth-generation KIs against disease associated mutants, RBC's mutant kinase panel is the largest of any CRO".

RBC offers KI's mechanism of action (MOA) studies with both biochemical and biophysical techniques, including SPR, ITC and MST, whether it is an ATP competitive inhibitor, an ATP non-competitive inhibitor, or an ATP un-competitive inhibitor. RBC is also continually adding cell based assays, including target engagement, and target validations. Few CROs can provide such studies to discovery scientists.

Kinases have such immense therapeutic potential. Accurate, efficient and reliable screening is the path to unlocking it. ■

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