SciBX
Science-Business eXchange

Covalent hits on KRAS

By Amy Donner, Senior Editor

University of California, San Francisco– and **Harvard Medical School**–led teams have independently synthesized the first covalent inhibitors of a common oncogenic form of KRAS.[1,2] **Araxes Pharma LLC** has licensed the UCSF team's findings and has partnered with **Johnson & Johnson** to optimize the compounds for *in vivo* testing.

Ras proteins are GTPases that act as molecular switches to control cell growth in response to extracellular signals. Three genes encode related Ras proteins: *KRAS* (*K-Ras*), *v-Ha-ras Harvey rat sarcoma viral oncogene homolog* (*HRAS*) and *neuroblastoma Ras viral (v-Ras) oncogene* (*NRAS*). Activating mutations in these genes are found in about 30% of cancers.

> **"The data say this protein is at the very heart of cancer. With such an opportunity, you cannot choose to not work on it."**
>
> *—Troy Wilson, Wellspring Biosciences LLC*

Ras proteins were considered undruggable until about two years ago when multiple groups began to describe small molecule–based strategies for targeting Ras activity.[3] But the compounds were designed against wild-type Ras, and it was unclear until now whether oncogenic, mutant forms could specifically be targeted.

Researchers also are pursuing siRNA-based approaches to inhibit aberrant KRAS activity in cancer.[4,5]

Rather than seek to generally inhibit Ras, the UCSF and Harvard teams saw an opportunity to hone in on a specific form of mutant KRAS hypothesized to be more tractable than the wild-type form.

Specifically, the teams set out to develop cysteine-targeting covalent inhibitors of the oncogenic KRAS G12C mutant, which is found in 50% of Ras-driven lung cancers and 10%–20% of Ras-driven cancers overall.

"The G12C mutation in KRAS is reasonably frequent in tumors, but more importantly the oncogenic mutation places a cysteine in the GTP-binding site that can be targeted by a suitably designed inhibitor," said Nathanael Gray, a professor of cancer biology at the **Dana-Farber Cancer Institute** and a professor of biological chemistry and molecular pharmacology at Harvard Medical School.

The UCSF team used an *in vitro*, fragment-based approach to identify lead compounds that covalently modified KRAS G12C. Structural analysis showed that one of the compounds bound to an allosteric pocket that had not been seen in previous structural studies of Ras proteins.

The group next used structure-based design to synthesize chemically distinct compounds that selectively modified KRAS G12C *in vitro*. This led to the identification of an acrylamide-based compound that inhibited KRAS G12C activity by causing the protein to favor interactions with GDP instead of GTP.

In vitro and in cells, the acrylamide compound also disrupted KRAS G12C–effector protein interactions. In a panel of cultured human lung cancer cell lines, the compound selectively killed cells expressing KRAS G12C with an EC_{50} of about 30 μM.

The study was published in *Nature* and was led by Kevan Shokat, who is chair of cellular and molecular pharmacology at UCSF and an investigator at the **Howard Hughes Medical Institute**.

A second team, composed of U.S. and Korean scientists led by Gray, reported covalent inhibitors of KRAS G12C that bound to a nearby but distinct site on the protein.[2]

In silico molecular docking aided the design of a GTP analog that covalently bound to purified, recombinant KRAS G12C but not the wild-type protein. Further *in vitro* analysis showed that the compound acted by stabilizing KRAS G12C in an inactive, GDP-bound conformation.

In a cultured human cell line, a cell-permeable prodrug version of the compound directly bound KRAS G12C and inhibited two downstream signaling pathways. In a panel of cultured human cancer cells, the prodrug selectively inhibited proliferation of KRAS G12C–dependent cells with EC_{50} values of 20–50 μM.

The work was published in *Angewandte Chemie International Edition*. The second team also included researchers from **The University of Texas Southwestern Medical Center**, **Northeastern University**, the **Broad Institute of MIT and Harvard**, **Harvard University**, the **Whitehead Institute for Biomedical Research**, the **Daegu-Gyeongbuk Medical Innovation Foundation** and the **Korea Institute of Science and Technology**.

The long road ahead

Although both groups are now working to improve potency and cell permeability, the researchers conceded that it will be a tall order to optimize compounds for *in vivo* testing.

Gray told *SciBX* that the molecules disclosed in the papers are only active at unreasonably high concentrations. "To address this for our GTP analogs, we are trying prodrug and bioisosteric replacement strategies," he said. "The first step is to get compounds active in cell culture at submicromolar concentrations, and then we will worry about pharmacology *in vivo*."

"The compounds published by the UCSF team catalyzed the formation of Wellspring. We are now looking for complementary opportunities related to G12C mutations. The Ras pathway is our primary focus," said Troy Wilson, president and CEO of **Wellspring Biosciences LLC**.

Wellspring is a drug discovery incubator launched in 2012 with

Copyright © 2014 Nature Publishing Group

programs organized in asset-centric companies. Araxes was created by Wellspring and is responsible for the Ras program.

In February 2013, Araxes signed an exclusive deal with the Janssen Biotech Inc. unit of J&J to develop therapeutics against KRAS G12C to treat cancer.

Wilson said that the priority is to understand how binding in the newly discovered allosteric pocket of KRAS G12C disrupts Ras signaling both at the molecular and cellular levels. He said knowledge of that mechanism will aid in optimizing activity.

Gray added that his team's compounds bound in the GTP-binding site, whereas UCSF's bound in a small pocket adjacent to the site. Thus, the findings could yield drug leads that inhibit oncogenic KRAS by distinct mechanisms.

Wilson, who previously founded Intellikine Inc., a phosphoinositide 3-kinase (PI3K)-focused company that was acquired in 2012 by **Takeda Pharmaceutical Co. Ltd.**, said that targeting KRAS is an ambitious goal.

"This is a challenging starting point. Kinases are well known in the industry. This is totally new ground and a new mechanism of action. But the data say this protein is at the very heart of cancer. With such an opportunity, you cannot choose to not work on it," said Wilson.

He added that many of the scientists from Intellikine are now at Wellspring. "We have worked together developing small molecules against targets in oncology before," he said.

The compounds published by the UCSF team are patented by UCSF and are licensed to Araxes. The patent and licensing status of the compounds published by Gray's team is undisclosed.

Donner, A. *SciBX* 7(1); doi:10.1038/scibx.2014.3
Published online Jan. 9, 2014

REFERENCES

1. Ostrem, J.M. *et al. Nature*; published online Nov. 20, 2013; doi:10.1038/nature12796
   Contact: Kevan M. Shokat, University of California, San Francisco, Calif.
   e-mail: kevan.shokat@ucsf.edu
2. Lim, S.M. *et al. Angew. Chem. Int. Ed.*; published online Nov. 20, 2013; doi:10.1002/anie.201307387
   Contact: Nathanael S. Gray, Dana-Farber Cancer Institute, Boston, Mass.
   e-mail: nathanael_gray@dfci.harvard.edu
3. Donner, A. *SciBX* **6**(24); doi:10.1038/scibx.2013.588
4. Khvalevsky, E.Z. *et al. Proc. Natl. Acad. Sci. USA* **110**, 20723–20728 (2013)
5. Martz, L. *SciBX* **7**(1); doi:10.1038/scibx.2014.4

COMPANIES AND INSTITUTIONS MENTIONED

Araxes Pharma LLC, San Diego, Calif.
Broad Institute of MIT and Harvard, Cambridge, Mass.
Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, South Korea
Dana-Farber Cancer Institute, Boston, Mass.
Harvard Medical School, Boston, Mass.
Harvard University, Cambridge, Mass.
Howard Hughes Medical Institute, Chevy Chase, Md.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Korea Institute of Science and Technology, Seoul, South Korea
Northeastern University, Boston, Mass.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
University of California, San Francisco, Calif.
The University of Texas Southwestern Medical Center, Dallas, Texas
Wellspring Biosciences LLC, San Diego, Calif.
Whitehead Institute for Biomedical Research, Cambridge, Mass.

Copyright © 2014 Nature Publishing Group