RON's new role

By Chris Cain, Senior Writer

A Utah team has shown that inhibiting the kinase RON could fight cancer metastasis by stimulating an antitumor immune response.[1] The findings build a case for directed drug discovery efforts against the target, which has long been neglected in favor of its more famous relative, MET.

In the last few years, two drugs have been approved that inhibit MET (c-Met proto-oncogene; HGFR): Xalkori crizotinib from **Pfizer Inc.** to treat EML4-ALK oncogenic fusion protein–positive lung cancer and Cometriq cabozantinib from **Exelixis Inc.** to treat medullary thyroid cancer.

At least 14 additional companies are developing small molecules or antibodies against the target.

RON (macrophage stimulating 1 receptor c-Met–related tyrosine kinase; MST1R; CD136) shares considerable structural and sequence homology with MET but nevertheless has not been as closely scrutinized as a cancer target. Exelixis CSO and EVP of discovery research Peter Lamb told *SciBX* that the choice to prioritize MET was based on the vast amount of preclinical evidence that supports its role in driving cancer.

"When we started out on the work that led to the development of cabozantinib, there was evidence that *MET* expression, *MET* amplification and upregulation of its ligand, hepatocyte growth factor/scatter factor (HGF/SF), contributed to cancer metastasis and had effects on survival," he said. "It was simply a much more intensively studied target, and there was much less data on RON, so it was not a difficult choice to focus on MET."

Indeed, researchers initially studied RON primarily for its role in regulating inflammation. Only recently has overexpression of RON or its ligand, macrophage stimulating protein (MST1; MSP), been found in many malignancies.[2] The only disclosed anti-RON–specific program in development is narnatumab (IMC-RON8), an anti-RON mAb from **Eli Lilly and Co.** that is in Phase I trials for solid tumors.

In 2010, **Aveo Pharmaceuticals Inc.** and **Johnson & Johnson** partnered to develop anti-RON mAbs,[3] but that partnership was terminated late last year for undisclosed reasons.

Aveo, J&J and Lilly declined interview requests.

Some small molecule MET inhibitors, including cabozantinib, have some inhibitory activity against RON because of homology between the proteins' kinase domains. However, Lamb said Exelixis was agnostic about the activity of cabozantinib against RON during the development of the compound and does not have data on whether RON is a clinically relevant target of cabozantinib.

Now, researchers at the **Huntsman Cancer Institute at The University of Utah** have linked the inflammatory- and cancer-associated functions of RON and provided new evidence to support the rational design of inhibitors of this target.

The group built on 2007 postdoctoral work by Alana Welm and J. Michael Bishop at the **University of California, San Francisco**. Welm and Bishop showed that overexpression of RON or MSP was linked to poor prognosis in patients with breast cancer and further demonstrated that increased MSP expression could drive metastasis in a mouse model for breast cancer.[4]

Welm is now an assistant professor in the Department of Oncological Sciences at Huntsman and is corresponding author of the new study. Bishop is chancellor emeritus of UCSF and remains a professor in the Department of Microbiology and Immunology at UCSF.

To better understand how RON and MSP drive metastasis—and to explore the therapeutic potential of RON inhibition—her team implanted MSP-overexpressing mouse breast cancer cells into either wild-type mice or knockout mice in which Ron's intracellular kinase domain had been deleted. In knockout mice lacking Ron activity, compared with in wild-type mice, spontaneous lung metastases were dramatically decreased and survival was significantly prolonged ($p<0.05$).

Importantly, the model used tumor cells implanted into genetically matched immunocompetent hosts. Unlike commonly used immunodeficient xenograft mouse models, this approach allowed the researchers to analyze the effect of the immune system on tumor growth and metastasis.

Additionally, more $CD8^+$ T cells were associated with tumors in the *Ron* kinase domain knockout mice than in the wild-type mice. These T cells and macrophages produced higher levels of proinflammatory tumor necrosis factor-α (TNF-α), suggesting the reduction of metastasis was caused by an antitumor immune response.

Indeed, antibody-mediated depletion of $CD8^+$ T cells increased metastasis in the knockout mice, whereas transplantation of $CD8^+$ T cells from the knockout mice into immunodeficient mice reduced metastasis.

Finally, to test the therapeutic relevance of inhibiting RON, the team turned to ASLAN002 (formerly BMS-777607), a small molecule RON and MET inhibitor from **Aslan Pharmaceuticals Pte. Ltd.** Mice receiving ASLAN002 before tumor cell injection had decreased metastasis compared with controls given vehicle. This effect was prevented by a $CD8^+$ T cell–depleting antibody.

In mice in which metastasis was allowed to begin prior to treatment, a model with more clinical relevance, ASLAN002 decreased metastatic outgrowth about fourfold compared with vehicle.

Results were published in *Cancer Discovery*.

Humanizing RON

Welm told *SciBX* that the results will change the way RON is viewed as a cancer target.

"Inhibiting Ron kinase activity allows an effective antitumor $CD8^+$ T cell response, which inhibits outgrowth of seeded metastatic tumor colonies. This dramatically changes the way that we think about RON inhibitors in the clinic," she said. "Currently, RON inhibitors are explored as agents that might directly shrink tumor growth. Our work shows that RON inhibitors may actually work as bona fide immune-stimulating agents, much like ipilimumab. This could impact clinical development of RON inhibitors, including rational clinical trial design, patient selection and monitoring of clinical efficacy."

Bristol-Myers Squibb Co. markets Yervoy ipilimumab to treat metastatic melanoma. The human mAb promotes cytotoxic T cell–mediated killing of tumor cells by blocking the CTLA-4 (CD152) receptor.

Welm said understanding the mechanism underlying RON inhibition is crucial for properly evaluating compounds. For example, she noted that blocking RON activity did not affect primary tumor growth in her study, and thus the approach could appear ineffective based on traditional response rate criteria.

As a result, measuring immune response markers, metastatic growth and overall survival could be important in evaluating the effects of a RON inhibitor. She added that expression of MSP could provide a tumor biomarker for sensitivity to RON inhibition.

Indeed, Welm is collaborating with Aslan to continue study of ASLAN002, which is currently being tested in a Phase I trial for solid tumors. She told *SciBX* that ASLAN002 is the only small molecule she knows of that is more selective for RON than for MET and is being tested clinically.

Aslan CSO and cofounder Mark McHale told *SciBX* that the company licensed the compound from Bristol-Myers for its anti-MET activity but is exploring new uses based on Welm's preclinical results.

"Based on Alana's work and work by our own investigators, we are actively looking at testing the compound in patients with bone metastases and will be looking for biomarkers such as macrophage activation and TNF-α expression," he said. McHale noted that inhibiting MET signaling also has been shown to have antimetastatic effects through distinct mechanisms of action.

Exelixis' Lamb said the Utah team's studies suggest it may be worthwhile to look at markers such as tumor-infiltrating lymphocytes and macrophages if patient biopsy samples are available from trials of cabozantinib or other MET and RON inhibitors.

Exelixis' cabozantinib is in two Phase III trials to treat prostate cancer, both of which are examining bone scan response as a secondary endpoint.

He added that the results increase the attractiveness of RON as a drug target and emphasized that the work reinforces scientific rationale for testing whether cabozantinib can prevent bone metastases.

Ravi Salgia, professor of medicine, pathology and dermatology at **The University of Chicago**, said Welm's results, along with work from his own lab showing RON plays a role in gastroesophageal and lung cancers,[5,6] argue for a renewed focus on RON as a drug target.

"RON is no longer the little brother of MET; it has come into its own," he said. "We are absolutely convinced RON is a therapeutic target in and of itself."

Salgia is most interested in seeing RON inhibition combined with cytotoxic chemotherapies. Welm is planning such studies, and McHale said Aslan is considering testing ASLAN002 in combination with other compounds including its pan–epidermal growth factor receptor (EGFR) inhibitor, ASLAN001.

Sandra Demaria, associate professor of pathology at the **New York University Langone Medical Center**, agreed that combination studies are a key next step and said that this study published in *Cancer Discovery* provides another example in which a therapeutic strategy that was not designed to be an immunotherapy in fact drives important antitumor immune responses.

As another recent example, she pointed to a 2011 study that showed that the tyrosine kinase inhibitor Gleevec imatinib unexpectedly induced antitumor immune responses in gastrointestinal stromal tumors (GISTs) by reducing expression of *indoleamine 2,3-dioxygenase* (*INDO*; *IDO*).[7]

Gleevec is marketed by **Novartis AG** to treat multiple cancers.

Demaria added that the new findings provide additional evidence that immunocompetent mouse models are critical to understanding how therapeutics work. "*In vivo* everybody has relied on immunodeficient mice, but this really brings a biased view of how therapeutics act," she said.

The findings of the Utah team are not patented.

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COMPANIES AND INSTITUTIONS MENTIONED

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Aveo Pharmaceuticals Inc. (NASDAQ:AVEO), Cambridge, Mass.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Eli Lilly and Co. (NYSE:LLY), Indianapolis, Ind.
Exelixis Inc. (NASDAQ:EXEL), South San Francisco, Calif.
Huntsman Cancer Institute at The University of Utah, Salt Lake City, Utah
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
New York University Langone Medical Center, New York, N.Y.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Pfizer Inc. (NYSE:PFE), New York, N.Y.
University of California, San Francisco, Calif.
The University of Chicago, Chicago, Ill.