Targeting chemokines in breast cancer

By Tim Fulmer, Senior Writer

American and French researchers have identified CXC chemokine receptor 1 as a new target for blocking the formation of breast cancer stem cells that drive tumor growth and metastasis.¹ The findings could represent a repurposing opportunity for Italian biotech **Dompe Farmaceutici S.p.A.**, which is exploring new indications for its small molecule inhibitor of the receptor after the compound missed the primary endpoint in Phase II transplant dysfunction trials.

The therapeutic rationale for targeting cancer stem cells is clear: deplete the subset of cancer cells with the ability to self-renew and generate the full range of cells that make up a bulk tumor² so the disease cannot progress or relapse. The challenge has been identifying drug-gable targets unique to cancer stem cells.

Researchers at the **University of Michigan** and the **Institut National de la Santé et de la Recherche Médicale** (INSERM) have used gene expression profiling to identify a breast cancer stem cell signature.³ In 2009, the team found that *CXC chemokine receptor 1* (*CXCR1*) was among the genes almost exclusively expressed in the cancer stem cell population compared with its expression in bulk tumor cells.

CXCR1 is a receptor for IL-8 (CXCL8), a proinflammatory chemokine that has been implicated in the metastasis and progression of multiple malignancies, including glioma and prostate, breast and ovarian cancers.⁴⁻⁷ IL-8 has also been shown to stimulate self-renewal of breast cancer stem cells *in vitro*.²

The UM-INSERM team now has taken the next step and studied the effects of small molecule and antibody antagonists of CXCR1.

In a human breast cancer cell line, both the small molecule CXCR1 inhibitor reparixin and an anti-CXCR1 antibody reduced the number of breast cancer stem cells compared with no treatment.

After four days, both molecules abolished the entire cancer cell population even though CXCR1-expressing stem cells comprised about 2% of the total. That result suggested that CXCR1 blockade directly decreased the survival of cancer stem cells and indirectly induced cell death in the bulk tumor population.

Mechanistic studies showed that inhibiting CXCR1 induced the production of soluble Fas ligand (TNF superfamily, member 6; FASL) by cancer stem cells. The proapoptotic FASL then bound its receptor on bulk tumor cells, triggering apoptosis and cell death (see **Figure 1, "Chemokine blockade in breast cancer"**).

In mice with primary human breast cancer xenografts, reparixin alone or in combination with docetaxel reduced tumor growth

compared with saline control. The combination had greater efficacy than either reparixin or docetaxel alone.

Additional experiments showed that reparixin could reduce cancer metastasis. Three groups of mice were injected with breast cancer cell lines. The animals then received reparixin twice daily for 28 days. At the end of that period, two of the three groups showed significant reductions in metastasis compared with animals that received saline control ($p < 0.01$).

The team was led by Max Wicha, professor of oncology and internal medicine at the University of Michigan, director of Michigan's Comprehensive Cancer Center and cofounder of stem cell therapeutic developer **OncoMed Pharmaceuticals Inc.** The findings were published in *The Journal of Clinical Investigation*.

OncoMed develops antibodies that target pathways important to the survival of cancer stem cells. The company's lead antibody, OMP-21M18, targets an activator of the Notch signaling pathway and is in Phase I testing to treat advanced solid tumors. The compound is partnered with **GlaxoSmithKline plc**.

Wicha said he plans to study CXCR1 inhibitors as monotherapy and in combination with chemotherapy in animal models of multiple types of cancer.

Wicha told *SciBX* a first priority for his group is forming a collaboration with Dompe, which owns the IP covering reparixin and its use as a therapeutic. In the meantime, he said the researchers will attempt to synthesize other small molecule inhibitors of CXCR1. The antibody used by his group was a research tool used mainly for proof of concept.

Reparixin (formerly repertaxin) was originally designed to target CXCR1 and CXCR2 on the surface of neutrophils to prevent their migration to sites of inflammation.^{8,9} The compound showed efficacy in animal models of inflammatory disorders, including postischemic reperfusion injury and acute lung injury.^{10,11}

However, the compound missed the primary endpoint in two Phase II trials to prevent primary graft dysfunction after lung and kidney transplantation, according to Maria Candida Cesta, R&D project manager at Dompe.

Although the compound is no longer in development for preventing graft dysfunction, Cesta said reparixin was safe and the company hopes to find new indications. Indeed, she said a Phase II trial of reparixin in an undisclosed indication is expected to begin in the third quarter.

Cesta told *SciBX* that Dompe is aware of the *JCI* paper and is interested in setting up a collaboration with Wicha's team to study reparixin in cancer.

Combo platter

Regardless of the specific inhibitor used, Anil Sood of **The University of Texas M.D. Anderson Cancer Center** thinks it's worth looking at CXCR1 blockade in other types of cancer. The UM-INSERM researchers also should study CXCR1 inhibitors in chemoresistant tumors, added Sood, who is professor of gynecologic oncology, director of ovarian cancer research and co-director of M.D. Anderson's Center for RNA Interference and Non-Coding RNA.

Figure 1. Chemokine blockade in breast cancer. In *The Journal of Clinical Investigation*, researchers suggest that chemotherapy plus an inhibitor of CXC chemokine receptor 1 (CXCR1) could be a powerful treatment for breast cancer.

[a] In response to chemotherapy, bulk cancer cells release multiple signaling molecules before dying, including IL-8 (CXCL8)—the ligand of CXCR1—and the proapoptotic Fas ligand (TNF superfamily, member 6; FASL).

[b] Both IL-8 and FASL then bind their receptors on chemo-resistant cancer stem cells, activating their respective intracellular signaling pathways.

[c] Signaling through CXCR1 via the FAK/AKT/β-catenin pathway stimulates multiple self-renewal and survival genes in cancer stem cells.

[d] In the presence of activated CXCR1, signaling through the FASL receptor is blocked. As a result, cancer stem cells do not undergo apoptosis and instead survive in a quiescent state in which they can sustain long-term tumor growth even in the presence of chemotherapy.

The *JCI* paper shows that blocking CXCR1 at step [b] with a small molecule or antibody prevents self-renewal and survival of breast cancer stem cells and, in combination with chemotherapy, sensitizes the stem cells to proapoptotic FASL.

Rather than targeting CXCR1 directly, Sood and colleagues are using RNAi to target the receptor's ligand, IL-8. In mouse models of ovarian cancer, liposomes containing IL-8 siRNA reduced tumor weight compared with empty control liposomes.⁷

Wicha agreed that augmenting chemotherapy with CXCR1 blockade could be especially useful. "Bulk tumor cells are known to secrete IL-8 when they're killed by chemotherapy," he noted. "Unfortunately, the binding of IL-8 to CXCR1 can then stimulate the growth of cancer stem cells and make tumors resistant to chemotherapy over the long term."

A CXCR1 antagonist thus could allow chemotherapeutics to kill tumor cells without triggering the growth of quiescent cancer stem cells.

Wicha said his lab also is combining CXCR1 blockade with other targeted cancer stem cell therapeutics like the Notch pathway-targeted mAbs developed by OncoMed.

The company declined to comment on the *JCI* paper.

If used as a monotherapy, it will be important to find out "how durable CXCR1 blockade is and whether the tumor adapts and recurs following treatment," said Erwin Van Meir, professor of neurosurgery, hematology and oncology at **Emory University**. A straightforward way of doing that would be to study reparixin in the mouse xenograft models beyond the 30 days reported in the paper, he added.

Van Meir is Director of the Winship Cancer Institute Brain Tumor Program and the Laboratory of Molecular Neuro-Oncology at Emory.

Wicha said the University of Michigan holds a patent on targeting

CXCR1 to block proliferation of cancer stem cells and that the IP is available for licensing.

Fulmer, T. *SciBX* 3(4); doi:10.1038/scibx.2010.105

Published online Jan. 28, 2010

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COMPANIES AND INSTITUTIONS MENTIONED

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