Blocking brain tumor rebound

By Michael J. Haas, Senior Writer

California researchers have uncovered a key role for the interaction of CXCR4 and SDF-1 in the recurrence of glioblastoma multiforme (GBM), handing **Genzyme Corp.** a potential new indication for its CXCR4 inhibitor, Mozobil plerixafor.¹

In most GBM patients, tumors recur within two years after a course of treatment that includes radiation and may incorporate an angiogenesis inhibitor. Radiation kills the tumor endothelial cells capable of angiogenesis. But most GBM tumors recur at the irradiated site, and the mechanisms of neovascularization that enable tumor regrowth have not been well understood.

Angiogenesis, a process in which new blood vessels sprout from existing vessels, is distinct from vasculogenesis, in which bone marrow-derived cells are recruited from the circulation to facilitate formation of brand-new vessels.

Previous studies have helped to elucidate the mechanism of vasculogenesis in ischemic tissues following hypoxia. Hypoxia upregulates hypoxia-inducible factor 1 (HIF1), inducing the expression and secretion of chemokine CXC motif ligand 12 (CXCL12; SDF-1), which in turn binds to CXC chemokine receptor 4 (CXCR4; NPY3R) on circulating monocytes, recruiting the cells to the ischemic region, where they help rebuild blood vessels.²⁻⁴

Moreover, a 2008 study led by J. Martin Brown, professor of radiation oncology and radiation biology at **Stanford University School of Medicine**, showed that murine breast and fibrosarcoma tumors implanted in pre-irradiated tissues in mice triggered the recruitment of blood monocytes, leading to neovascularization and ultimately to tumor growth.⁵

Those findings led Brown and colleagues to hypothesize that irradiation of GBM tumors might activate the same vasculogenic pathway seen in ischemic tissues and that blocking the pathway could help prevent the brain tumor from recurring.

Indeed, the latest research by Brown and his team has found that radiotherapy in mice bearing GBM xenograft tumors led to tumor hypoxia, upregulation of Hif1 and greater Hif1-stimulated expression and secretion of Sdf-1 by the tumor compared with what was seen without radiotherapy. Subsequent binding of Sdf-1 to Cxcr4 on circulating monocytes activated and recruited the cells to the irradiated tumor site, in which they targeted endothelial cells of unknown origin, promoting vasculogenesis and thus enabling tumor cell survival and tumor recurrence (see **Figure 1, “Neovascularization in irradiated GBM tumors”**).

Figure 1. Neovascularization in irradiated GBM tumors. Treatment of glioblastoma multiforme (GBM) tumors with radiation therapy triggers new blood vessel formation that enables tumor cell survival and subsequent tumor recurrence.

Radiation therapy kills tumor vascular and endothelial cells [a], leading to tumor hypoxia, upregulation of hypoxia-inducible factor 1 (HIF1) in tumor cells [b] and subsequent expression and tumor secretion of chemokine CXC motif ligand 12 (CXCL12; SDF-1) [c].

SDF-1 then binds CXC chemokine receptor 4 (CXCR4; NPY3R) [d] on bone marrow-derived cells to activate and recruit them to the tumor site [e], where they promote new blood vessel formation from endothelial cells of unknown origin [f], thus enabling tumor survival and recurrence.

The team went on to show that xenograft models treated with radiation and either a HIF1 inhibitor, a CXCR4 inhibitor (plerixafor) or an anti-CXCR4 antibody had less tumor recurrence than controls treated with radiation and a VEGF inhibitor or with radiation only.

Use of the inhibitors to treat nonirradiated xenograft GBM tumors had no effect on tumor growth or blood flow, thus showing that radiotherapy triggered the switch from angiogenesis to vasculogenesis in the tumors, the team wrote in its report in *The Journal of Clinical Investigation*.

Lastly, the team found that recurrent GBM tumors in human patients had higher levels of monocytes compared with the same tumors prior to radiotherapy or than tissue from normal control subjects, suggesting that radiotherapy triggered vasculogenesis in the human tumors.

The team also included researchers from the **University of California, San Diego** and **AntiCancer Inc.**, which supplied some of the mice used in the study.

“The study makes a very compelling argument that the almost invariable tumor recurrence following aggressive radiotherapy in GBM patients is caused by vasculogenesis rather than angiogenesis,” said Stefan Helmling, EVP of business development at **Noxxon Pharma AG**. He noted that all of the team’s approaches to blocking vasculogenesis “were substantially more effective than inhibition of VEGF” with an antiangiogenic agent.

Noxxon is developing an SDF-1 inhibitor, NOX-A12. According to Helmling, it is in Phase I testing for autologous human stem cell transplantation and/or cancer indications.

“The experiments testing the effects of an antiangiogenic compound side by side with an antivasculogenetic compound were convincing and could challenge the concept of antiangiogenic therapy” as a treatment for GBM, said Ilker Eyüpoğlu, neurosurgeon and principal investigator at the **University of Erlangen-Nuremberg**.

Charles Cobbs, an oncology and spine neurosurgeon at **California Pacific Medical Center (CPMC)**, agreed that the team had sufficiently proved the antivasculogenetic concept “in terms of the extent to which a given tumor recurrence is wholly dependent on new blood vessel formation.” Cobbs is also an associate clinical professor of neurological surgery at the **University of California, San Francisco (UCSF)** and a scientist at the **California Pacific Medical Center Research Institute**.

“This preclinical study provides adequate rationale to support a clinical trial testing the concept,” said Lance Leopold, VP of clinical development and CMO at **Ascenta Therapeutics Inc.**

Mozobil’s target is in the vasculogenic pathway and already is approved to increase mobilization of hematopoietic stem cells to the bloodstream for collection and subsequent autologous transplantation to treat multiple myeloma (MM) and non-Hodgkin’s lymphoma (NHL). The drug is also in Phase I/II testing to treat acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), MM and lymphoma. Genzyme did not comment on whether the company would be interested in developing Mozobil to treat GBM.

On trials

Cancer researchers contacted by *SciBX* pointed out that the benefits of preventing tumor recurrence would likely outweigh any risk of side

effects caused by inhibitors of vasculogenesis. But they added that further studies were needed to determine how and when to incorporate such inhibitors into existing GBM treatment regimens in order to optimize their benefits.

“My concern is that an inhibitor would need to be given chronically to prevent tumor regrowth,” Leopold said. “If I were designing a Phase I clinical trial, I would run two cohorts: one administering radiation therapy concurrent with the inhibitor and one administering the inhibitor as a single agent after the radiation therapy.” Depending on the Phase I results, “you could advance either the concurrent or sequential therapy—or both—to a Phase II trial.”

Leopold’s company has its AT-101 small molecule pan-inhibitor of the B-cell lymphoma 2 (BCL-2; BCL2) family of proteins in Phase II testing to treat progressive or recurrent GBM and other cancers.

Noxxon’s Helmling noted that “from the study it can be inferred that lower doses of radiation may be possible.”

Indeed, Cobbs said, it would be desirable to reduce the amount of radiation needed to treat GBM.

“Anyone who survives more than a few years after brain irradiation sustains significant effects of brain atrophy” and other side effects such as deterioration in mental functions, he noted. If the antivasculogenetic strategy could be combined with another potent anticancer drug, “then perhaps in time we could dispense with radiation therapy.”

Cobbs noted that mounting evidence indicates antiangiogenic drugs may in fact induce surviving tumor cells to become less dependent on angiogenesis and more motile. As an example, he cited a 2008 study by a UCSF team, which found that blocking VEGF caused residual tumor cells to become more invasive.⁶

He said it wasn’t clear whether antivasculogenetic agents would have a similar effect, but “you may find that the tumor stem cells that survive can adopt novel strategies to grow in the absence of new vascular supply. The only way to know would be to use primary human gliomal tumors in mouse models and see whether the tumor repopulated with more invasive or resistant cells over time.”

Liliana Soroceanu, an associate scientist at CPMC Research Institute, also wanted to see studies in mouse models with primary tumor-derived xenografts “because the growth pattern and response to radiation therapy is so different in primary GBM tumors” from what it is in the cell line-derived xenografts used by Brown’s team.

Helmling said it would be even better to see results “in models of spontaneous GBM tumors.”

Soroceanu also pointed out that Brown’s team did not mention that gliomal stem cell-like cells have a well-documented resistance to radiotherapy. “Ideally one would want to inhibit both regrowth due to cancer stem cells and recurrence due to vasculogenesis,” she said. “I think the Brown team’s study is useful, but it is far from completely addressing post-radiation therapy recurrence in gliomas.”

Brown agreed that radiation does not kill all tumor cells. “If it did, we would not have to inhibit blood vessel growth. That is why inhibiting the vasculogenic pathway works so well,” he said. But, Brown added, killing the tumor cell “is important because that is what stimulates the vasculogenesis pathway.”

“This preclinical study provides adequate rationale to support a clinical trial testing the concept.”

**—Lance Leopold,
Ascenta Therapeutics Inc.**

Nicolai Savaskan, professor of neuroanatomy at **Charité–University Hospital Berlin**’s Institute of Cell Biology and Neurobiology, also noted that inhibiting SDF-1 or its upstream activator, HIF1, could have a detrimental effect on immune responses in the brain because SDF-1 targets microglial cells.

Savaskan said that possibility could not be assessed from the *JCI* report because Brown’s team used an immunocompromised mouse model. Thus, “before testing the vasculogenic inhibition strategy in humans, it must be shown that immune system responses are not adversely affected” in immunocompetent mouse xenograft or spontaneous tumor models, he said.

Spontaneity and mystery

Brown said the team’s ongoing studies include testing HIF1, SDF-1 and CXCR4 inhibitors in a rat model of spontaneous GBM. “This is a much more difficult tumor to cure, but so far the results are consistent with what we found” in the *JCI* study, he said.

“We do not study xenografts from primary tumors,” he added. “Few people do such xenografts because it is difficult to work with them.”

Brown’s team is also investigating a currently unanswered question: which endothelial cell cohort is activated by monocytes to initiate vasculogenesis? “We know they do not come from the bone marrow or the surrounding normal tissue,” he said. “The most likely source is circulating endothelial cells or progenitors from other sources” that have yet to be identified.

Brown said Stanford University has patented the findings reported in *JCI* and that they are available for licensing.

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COMPANIES AND INSTITUTIONS MENTIONED

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