PlGF point-counter-point

By Kai-Jye Lou, Staff Writer

Back-to-back papers in *Cell* provide disparate views on the relevance of placental growth factor as a cancer target.^{1,2} A group at **Genentech Inc.** presented data challenging the importance of PlGF in primary tumor growth and angiogenesis, whereas a leading PlGF academic group in Europe responded with evidence to the contrary. The key to resolving the debate is likely to involve studies with anti-PlGF mAbs across multiple animal models.

Placental growth factor (PGF; PlGF) is involved in processes that drive tumor growth, including angiogenesis, neovascularization and inflammation.³

Unlike VEGF-targeted therapeutic approaches, PlGF inhibition does not affect healthy blood vessels, causes less tumor hypoxia and blocks infiltration by inflammatory cells such as macrophages. In tumors, hypoxia can stimulate an increase in the expression of angiogenic factors that contribute to resistance to VEGF inhibitors (see **Figure 1, “Resistance mechanism against anti-VEGF therapy”**).⁴

In 2007, a study published in *Cell* by Peter Carmeliet, professor of medicine and director of the Vesalius Research Center at the **Catholic University of Leuven** (KU Leuven), and colleagues showed that an anti-PlGF mAb inhibited the growth of tumors that were resistant to VEGF inhibitors without affecting healthy blood vessels.⁵ Moreover, the anti-PlGF mAb blocked macrophage infiltration into the tumor and did not cause severe tumor hypoxia.

But a research group at Genentech conducted animal studies that led to a different conclusion.

In all but one mouse tumor model, a series of anti-PlGF mAbs generated by the Genentech group failed to significantly inhibit primary tumor growth versus that seen with a control mAb. In contrast, an anti-VEGF-A mAb significantly reduced tumor growth compared with that in the control group ($p < 0.05$).

In two of the mouse models, combining an anti-VEGF-A mAb with an anti-PlGF mAb led to tumor growth inhibition that was no better than the anti-VEGF-A mAb alone.

“We have provided multiple lines of evidence indicating PlGF blockade does not result in inhibition of primary tumor growth in most models,” the Genentech researchers wrote in *Cell*. “While we cannot rule out the possibility that PlGF inhibition may have differ-

ent effects in tumor models that we did not test, our findings argue against the broad antiangiogenic or anti-tumor efficacy in anti-VEGF-resistant tumors.”

The anti-PlGF mAbs did fare better in metastatic disease. Here, the mAbs significantly reduced metastasis to the lung compared with that seen upon control mAb treatment in mice with melanoma—a result that is in line with earlier findings from other groups.

The Genentech authors suggested that the antitumor effect of the mAb from the 2007 *Cell* paper may have been the result of off-target effects, but conceded that the reasons for the discrepancies “are currently unknown.”

The study was led by Genentech fellow Napoleone Ferrara, a co-corresponding author on the *Cell* paper.

Genentech, a unit of **Roche**, declined further comment. The companies market Avastin bevacizumab, a humanized mAb against VEGF, in the U.S. for metastatic breast cancer, metastatic colorectal cancer and unresectable or metastatic non-small cell lung cancer (NSCLC), and glioblastoma multiforme (GBM).

Roche also has partnered with **ThromboGenics N.V.**, which holds exclusive licenses from KU Leuven to IP covering antibodies to PlGF.

PlGF strikes back

Carmeliet and a team of European researchers have responded to the Genentech paper with

more evidence of the antiangiogenic and antitumor effects achieved with PlGF blockade using a different set of mouse models and anti-PlGF mAbs.

The group showed that blocking PlGF signaling in xenograft and spontaneous tumor models yielded reduced tumor growth and angiogenesis compared with that seen in controls.

In a mouse model of ocular neovascularization, anti-PlGF mAbs blocked PlGF-driven choroidal neovascularization and ocular angiogenesis. A combination of an anti-PlGF mAb and a VEGF inhibitor produced stronger inhibition of choroidal neovascularization than either compound alone.

“Our new findings further provide strong genetic and pharmacological support for a disease candidate role of PlGF in cancer and ocular disease, and the therapeutic potential of anti-PlGF for such disorders,” said Carmeliet, who also is corresponding author on the latest *Cell* paper.

“Indeed,” he added, “our new studies have not only increased the mechanistic understanding of how PlGF blockage can inhibit malignant and nonmalignant disease, but also provide the first evidence that anti-PlGF is effective in spontaneous tumor models, which are more relevant as models for human cancer” than xenograft models because the animals’ immune systems remain intact.

The team found at least three anti-PlGF mAbs that were active *in vitro* and *in vivo*. But notably, it also generated a handful of antibodies

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Figure 1. Resistance mechanism against anti-VEGF therapy.

Tumor cells, stromal cells and inflammatory cells within a tumor secrete angiogenic factors that promote the growth of new tumor vasculature. Anti-vascular endothelial growth factor (VEGF) therapies such as Avastin bevacizumab have an antiangiogenic effect and work against a broad range of tumors. However, the strong antiangiogenic effect of anti-VEGF therapies can promote the development of resistance and eventual escape from such treatments.

One such resistance mechanism involves hypoxia-stimulated increases in the production of angiogenic factors coupled with the recruitment of tumor-infiltrating macrophages.

Treatment with an anti-VEGF mAb causes existing tumor vasculature to regress and thus deprives tumor cells of oxygen [a(1)]. This results in a strong hypoxic stimulus that causes cells inside the tumor to increase their production of angiogenic factors [a(2)]. Moreover, anti-VEGF therapies are not effective at blocking the recruitment of inflammatory cells, such as macrophages, into the tumor.

In contrast, blocking placental growth factor (PlGF) signaling has a weaker antiangiogenic effect than inhibiting VEGF does and thus causes a weaker hypoxic stimulus [b]. In addition, PlGF inhibition also blocks the recruitment of inflammatory cells, including macrophages, into the tumor.

These two effects suggest that an anti-PlGF strategy is less likely to promote the development of resistance and escape from treatment.

that worked in the former setting but not in the latter. Thus, the process of selecting effective anti-PlGF antibodies may be more complicated than previously thought.

“We can only speculate at this stage about possible subtle differences in the quality and characteristics of these mAbs that might explain the differences *in vivo*,” Carmeliet told *SciBX*. “Selection of anti-PlGF antibodies that are effective is critical: like the Genentech group, we also obtained anti-PlGF antibodies that block PlGF binding *in vitro* but were not effective *in vivo*.”

Carmeliet added that other groups have already confirmed the effect of one of the anti-PlGF mAbs used in the new study, and that independently generated anti-PlGF mAbs from other groups have also inhibited tumor growth in animal models.

Carmeliet said his group will be working in collaboration with other academic groups to continue testing various anti-PlGF mAbs in oncology and nononcology disease models.

KU Leuven holds multiple issued and pending patents covering anti-PlGF antibodies and their use in multiple diseases. Licensee ThromboGenics brought on development partner **BioInvent International AB** in 2005, and they then partnered with Roche in 2008 to develop anti-PlGF antibodies. Their lead anti-PlGF mAb, RG7334 (formerly TB-403), is in Phase Ib testing for solid tumors.

“We remain committed to aggressively pursuing our development plan for RG7334,” said Roche spokesperson Peter Sandbach. “We’re familiar with the results of these publications and they will not have an impact on our development plan. It is not unusual to have different

scientific results, especially when different reagents and models are used for comparison.”

At least two other PlGF-targeting compounds are in the clinic. VEGF Trap aflibercept from **Regeneron Pharmaceuticals Inc.** and partner **sanofi-aventis Group** is a fusion protein that binds PlGF and all forms of VEGF-A. It is in Phase III testing for solid cancers.

Regeneron also has an intraocular formulation called VEGF Trap-Eye in development with partner **Bayer AG**. That compound is in Phase III trials for wet age-related macular degeneration (AMD) and retinal vein occlusion, and in Phase II for diabetic macular edema (DME).

At the end of the day, the disparate data sets in *Cell* have other researchers scratching their heads.

“These results are confusing and imply that there is a complicated mechanism behind effects of PlGF,” said Yihai Cao, a professor in

the department of microbiology and tumor biology at the **Karolinska Institute**. “They suggest that targeting PlGF may not be as straightforward of an approach as we originally thought.”

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**—Peter Carmeliet,
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