Broadening TDO's base

By Kai-Jye Lou, Staff Writer

A team at the **Ludwig Institute for Cancer Research Ltd. Brussels Branch** and the **University of Namur** has shown in mice that a small molecule inhibitor of tryptophan 2,3-dioxygenase promotes immune rejection of tumors without signs of toxicity.¹ The group plans to run high throughput screens to identify a more stable inhibitor that could be advanced into clinical testing, and the institute is in discussions to spin out a company this half.

Tryptophan 2,3-dioxygenase (TDO2; TDO) catalyzes tryptophan to generate kynurenine, a metabolite that inhibits the antitumor immune response.² Last October, a group in Germany reported in *Nature* that glioblastomas could exploit the immunoregulatory properties of TDO-generated kynurenine to dampen the antitumor immune response and promote growth.³

However, Michael Platten, corresponding author on the October *Nature* paper, chose to pursue targets downstream of TDO, as there were concerns on whether inhibiting the enzyme itself would have adverse effects because normal liver and brain cells also express TDO.⁴ Platten is a professor in the Department of Neurooncology at **Heidelberg University Hospital** and head of the Experimental Neuroimmunology Unit at the **German Cancer Research Center**.

To find out, a group led by Benoît Van den Eynde, director of the LICR Brussels Branch and a professor at the **Université Catholique de Louvain**, set out to determine how widespread TDO activity is across tumors and whether inhibiting the enzyme's function would have adverse effects.

The team first showed that TDO is not just a target in glioblastoma. His team assayed a range of tumor types from 144 patient samples for TDO expression and found all 7 hepatocarcinoma samples, 10 of 20 melanoma samples and 9 of 22 bladder carcinoma samples tested to be TDO-positive.

Van den Eynde's group then addressed the toxicity concerns by treating mice injected with *Tdo*-expressing murine mastocytoma tumor cells with a small molecule TDO inhibitor. In treated mice, the inhibitor, a tetrazolyl-vinyl substituted (fluoro)indole scaffold dubbed LM10, promoted immune rejection of the cells compared with no treatment.

Animals receiving the inhibitor for more than 100 days did not show signs of toxicity, and liver enzyme levels in plasma remained within normal ranges.

Results were published in the *Proceedings of the National Academy of Sciences*.

"Our work shows that TDO could be a relevant new therapeutic target in many cancers and provides pharmacological proof of concept that the enzyme could be blocked with a small molecule," said Van den Eynde.

"The preliminary toxicity studies in the mouse model strongly suggest that TDO inhibition would not cause liver damage," added Vincenzo Cerundolo, director of the MCR Human Immunology Unit at **The Weatherall Institute of Molecular Medicine at University of Oxford** and a professor of immunology at the **University of Oxford**.

"The current findings suggest that tryptophan degradation is a mechanism employed by a broad range of tumors to suppress the antitumor immune response," said Platten.

Cerundolo agreed. "The study raises the possibility that tryptophan degradation could be a general strategy that tumors employ to hamper the immune system," he told *SciBX*. "The findings suggest

that an effective therapeutic strategy would be to combine an agent that amplifies the specific antitumor response with one that abolishes the immunosuppressive mechanisms employed by the tumor itself."

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University of Oxford

Being a good host

Whereas Platten's work suggests that TDO's tumor growth-increasing effect in glioblastomas primarily stems from kynurenine-mediated effects on the tumor cells themselves, the current findings in a different tumor model suggest that the antitumor effect of TDO inhibition is primarily mediated by the host immune system (*see Figure 1, "Dependency of TDO inhibition on the host immune system"*).

Indeed, Van den Eynde's team found that LM10 decreased tumor growth in immunocompetent mice injected with *Tdo*-expressing mastocytoma cell lines but had no effect on tumor growth in immunodeficient mice injected with the same cell lines, suggesting that the immune system is the key regulator of tumor growth in this case.

"The current paper not only is confirmatory of the *Nature* publication, but also further raises an interesting issue as to whether the underlying mechanism promoting tumorigenicity by TDO is dependent on, or independent of, the host's immune system," said Ursula Grohmann, a professor of pharmacology in the Department of Experimental Medicine and Biochemical Sciences at the **University of Perugia**.

She said Van den Eynde's findings open up the possibility of developing antitumor strategies that enhance or reinforce host immune surveillance.

Complementing immunotherapy

The ability of the TDO inhibitor to reverse tumor immune resistance and the molecule's safety profile suggest it could complement existing cancer immunotherapies.

“Because of the main mechanism of action, we would recommend testing TDO inhibitors in therapeutic settings where one wants to induce a strong immune response to attack the tumor, such as in combination with a cancer vaccine,” Van den Eynde told *SciBX*. “Our data suggest that specific tumor types where a TDO inhibitor could be useful are melanomas, bladder carcinomas and liver carcinomas.”

In addition, targeting TDO also could complement current efforts to develop inhibitors of indoleamine 2,3-dioxygenase (INDO; IDO), another tryptophan metabolism-related enzyme that also generates

kynurenine. The discovery of a link between tryptophan metabolism and immunosuppression in a variety of diseases, including cancer, was first established through IDO.²

“This work is particularly interesting because the authors show that most human tumors will express either TDO, IDO or a combination of both,” said Grohmann. “IDO is another major enzyme that catalyzes the same reaction as TDO, which points to the striking finding that aberrant tryptophan catabolism is a hallmark of most human tumors.”

Figure 1. Dependency of TDO inhibition on the host immune system. Tumors can exploit the immunoregulatory properties of tryptophan 2,3-dioxygenase (TDO2; TDO) to dampen the antitumor immune response and promote their own growth. As reported by Pilotte *et al.*, a small molecule TDO inhibitor could reverse the immune-dampening effects of TDO.

In immunocompetent mice, a small molecule TDO inhibitor promoted a rejection response against injected *Tdo*-expressing murine mastocytoma cells and decreased tumor progression compared with no inhibitor (I.1). In contrast, the TDO inhibitor had no effect on tumor growth in immunodeficient mice (I.2), suggesting TDO's tumor-promoting activity depends primarily on the enzyme's effects on the host immune system.

The results in the mastocytoma model differ from those in a study by Opitz *et al.*, which suggest TDO's tumor-promoting activity in glioblastoma hinges on the enzyme's effects in the tumor cells themselves. In that study, immunodeficient mice were transplanted with human glioblastoma cells with and without small hairpin RNA-mediated TDO knockdown.

Despite being immunodeficient, only one of six mice receiving the glioblastoma cells with TDO knockdown developed tumors compared with five of six for those receiving cells without TDO knockdown (II).

The differences between these two studies may stem from the different tumor types analyzed, but taken together, the results provide two distinct mechanistic rationales for targeting TDO in cancer.

In the *PNAS* paper, Van den Eynde's team found that out of a panel of 104 human tumor cell lines analyzed, 20 expressed only TDO, 17 expressed only IDO and 16 expressed both. The researchers did not detect expression of either enzyme in the other 51 tumor cell lines.

"TDO inhibition would be very complementary to IDO inhibition," Van den Eynde told *SciBX*. "For example, a TDO inhibitor could be used when a tumor develops resistance to an IDO inhibitor by upregulating TDO. One could also consider inhibiting both TDO and IDO simultaneously, which could increase the proportion of tumors that become responsive to an immunotherapy."

At least two biotechs—**Incyte Corp.** and **NewLink Genetics Corp.**—have IDO inhibitors in the clinic. Both programs are in Phase I testing to treat solid tumors.

Van den Eynde said his group now is looking to validate the effects of TDO inhibition in other preclinical tumor models. The team also is trying to develop a more stable small molecule TDO inhibitor that could be advanced into the clinic, which will include medicinal chemistry studies to optimize LM10 and high throughput screening efforts to identify new inhibitors.

"I envision that the first clinical trials for such an inhibitor would be in the form of combination therapy for patients receiving a cancer vaccine," he said.

LICR has filed multiple patent applications to cover the findings described in *PNAS*. LICR's planned spinoff will focus on

immunomodulatory therapies for cancer, including the TDO inhibitor program. The institute said the spinoff already has an undisclosed amount of committed financing.

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COMPANIES AND INSTITUTIONS MENTIONED

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