Killer targets in metastasis

By Amy Donner, Senior Editor

An international team has found a new pathway in NK cells that leads to the rejection of metastatic tumors.[1] It is still unclear which components of the pathway will make the best targets.

The pathway centers around an E3 ubiquitin ligase called casitas B cell lymphoma-b (CBL-B) that is known to negatively regulate T cells.[2] In 2007, a group led by Josef Penninger showed that *Cbl-b* knockout resulted in $CD8^+$ T cell–mediated tumor rejection in several mouse models.[3]

In the following years, the group pursued strategies to target Cbl-b in T cells and understand the mechanism of Cbl-b activity in T cells. In a new study, an international team led by Penninger observed a curious result—*Cbl-b* knockouts that lacked T cells also rejected tumors. Penninger is senior scientist and scientific director of the **Institute of Molecular Biotechnology of the Austrian Academy of Sciences**.

That finding prompted Penninger to hypothesize that Cbl-b also must be operational elsewhere in the immune system and led his group to uncover NK cells as the mysterious other cell type in a CBL-B-dependent pathway that suppresses activity (*see* **Figure 1, "Strategies to activate antitumor NK cells"**).

"Since NK cells are known to be involved in rejection of metastasis, we asked whether CBL-B is a brake in NK cells, limiting their ability to kill metastatic tumors," said Penninger.

In cultured mouse and human NK cells, knockout of *CBL-B* or RNAi against the target increased tumor cell killing compared with no treatment.

In a mouse xenograft model of melanoma, *Cbl-b* knockout or expression of an inactive *Cbl-b* variant in place of the wild-type enzyme increased survival compared with wild-type Cbl-b expression. Immunodepletion of NK cells reduced survival, indicating that Cbl-b negatively regulates the antitumor activity of NK cells.

In mouse models of metastatic melanoma and breast cancer, *Cbl-b* knockout decreased lung metastasis compared with wild-type *Cbl-b* expression. Transplantation of *Cbl-b* knockout NK cells into wild-type mice decreased lung metastasis compared with transplantation of wild-type NK cells or no treatment, demonstrating that the *Cbl-b* knockout NK cells were responsible for the antimetastatic effect.

The authors next set out to identify substrates for CBL-B. "We screened more than 9,000 proteins to find targets for the E3 ligase activity of CBL-B," said Penninger. "We found TAM receptors."

TAM receptors are a small family of receptor tyrosine kinases that include TYRO3 protein tyrosine kinase (TYRO3; SKY), AXL receptor tyrosine kinase (AXL; UFO) and c-Mer proto-oncogene tyrosine kinase (MERTK).

In vitro assays showed that CBL-B added ubiquitin modifications to all three TAM family proteins. In cultured human cells, stimulation of the TAM receptors led to the recruitment of CBL-B and ubiquitination of AXL.

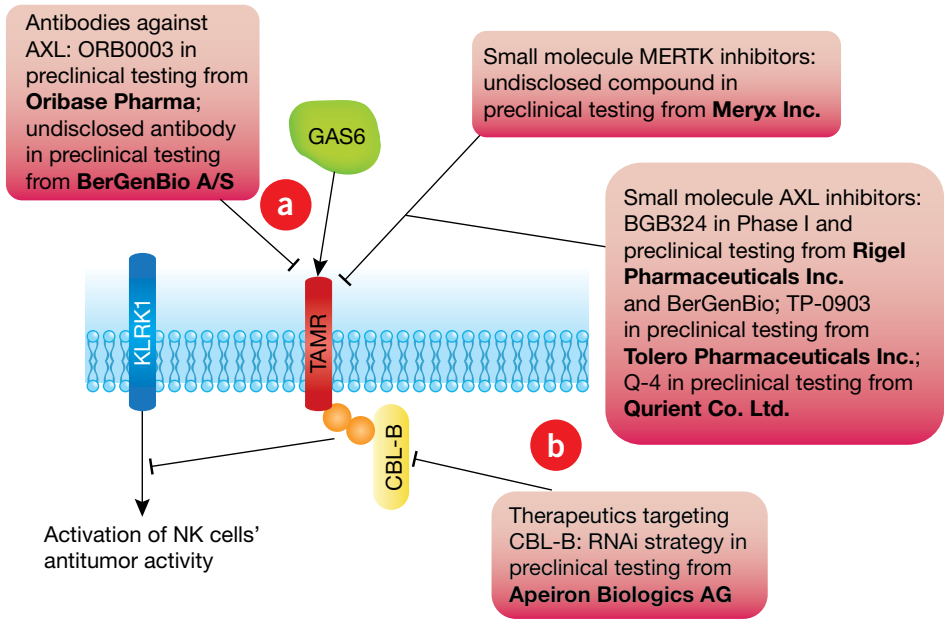

Figure 1. Strategies to activate antitumor NK cells. NK activation occurs downstream of killer cell lectin-like receptor subfamily K member 1 (KLRK1; CD314; NKG2D). In *Nature*, scientists showed that a casitas B cell lymphoma-b (CBL-B)–TAM receptor (TAMR) pathway suppresses the activation of NK cells.[1]

[**a**] TAMR kinases, including TYRO3 protein tyrosine kinase (TYRO3; SKY), AXL receptor tyrosine kinase (AXL; UFO) and c-Mer proto-oncogene tyrosine kinase (MERTK), are activated by an extracellular ligand, such as growth arrest–specific 6 (GAS6).

[**b**] Binding by GAS6 leads to the recruitment of CBL-B to TAMR and receptor ubiquitination (orange circles). Activation of this pathway inhibits NK cell antitumor activity.

A number of companies are developing therapeutics that activate antitumor immunity by interfering with this pathway.

Box 1. The development and validation of LDC1267.

In 2008, Axel Ullrich and co-workers published evidence that the TAM receptor family protein AXL receptor tyrosine kinase (AXL; UFO) promotes metastatic behavior of cancer cells in culture. The group also showed that inhibition of AXL abrogated the behavior.[6]

Ullrich is director and a professor of molecular biology at the **Max Planck Institute of Biochemistry**.

Bert Klebl, managing director and CSO of **Lead Discovery Center GmbH** (LDC), said that the findings were the starting point for a collaboration to optimize tyrosine kinase inhibitors against the TAM receptors.

Profiling and cellular characterization of the inhibitors were done in an iterative process with LDC and Max Planck scientists. "LDC optimized not only the selectivity and potency but also the physiochemical and pharmaceutical properties to yield bioavailable TAM receptor kinase inhibitor leads, which have shown proof of concept in a couple of animal models for cancer," said Klebl.

LDC1267 was rationally designed using a pharmacophore-based approach and was selected from hundreds of compounds based on activity in cell-based AXL autophosphorylation assays.

LDC1267 binds the target kinases with nanomolar affinity *in vitro*. It is selective for the TAM receptor kinases from a panel of 456 kinases tested both through cell-free KINOMEscan assays and, in cells, through quantitative proteomic assays. —*AD*

"TAM receptors can act as negative regulators in other cell types, so we asked whether TAM receptors can also inhibit NK activity," Penninger said. In cultured mouse NK cells, stimulation of the TAM receptors suppressed the activation of NK cells. In the cells, *Cbl-b* knockout restored activation.

The data collectively indicate that CBL-B works with TAM receptor kinases in a pathway to suppress the activation of NK cells.

The final step was testing whether pharmacological inhibition of the TAM receptor kinases could block the suppression of NK cells and elicit antimetastatic effects. To do this, Penninger teamed up with scientists from the **Max Planck Institute of Biochemistry** and **Lead Discovery Center GmbH** (LDC) who developed a potent and selective pan–TAM receptor kinase inhibitor called LDC1267 (*see* **Box 1, "The development and validation of LDC1267"**).

In a mouse model of metastatic melanoma, transfer of LDC1267-treated, wild-type NK cells led to antitumor responses that were comparable to those seen with transfer of *Cbl-b* knockout NK cells. Intraperitoneal injection of LDC1267 also decreased the number of metastatic lung tumors compared with vehicle injection.

Injected and oral LDC1267 yielded comparable results in mouse models of metastatic breast and liver cancer.

Results were published in *Nature*. The team also included scientists from **Medical University Innsbruck**, **Brown University**, **The University of Western Australia** and **University Hospital Bonn**.

The series of molecules described in the paper, including LDC1267, is licensed to **Qurient Co. Ltd.** from LDC and the Max Planck Institute of Biochemistry.

With these compounds, "we have achieved pharmacological validation for certain types of drug-resistant cancers, and we have seen prominent activity in some leukemias," said Bert Klebl, managing director and CSO of LDC.

Tackling the targets

Researchers had mixed views on whether CBL-B itself or the TAM receptors are better targets. Some companies have a head start on the latter family with programs against AXL.

That target is expressed in primary tumors and is an established driver of drug resistance in *epidermal growth factor receptor* (*EGFR*)-mutant lung cancer[4,5] and progression of breast cancer.[6,7]

Nevertheless, Penninger said that the company he founded, **Apeiron Biologics AG**, wants to tackle CBL-B via RNAi. The company hopes to start Phase I testing in cancer this year.

Apeiron plans to collect white blood cells from patients, use RNAi *ex vivo* to knock down *CBL-B* and then return the cells to patients.

"We have long-term experience with *Cbl-b*-mutant mice. They are quite healthy, unlike *Ctla-4*-mutant mice. Thus, the potential side effects of blocking *CBL-B* might be less than blocking CTLA-4," said Penninger.

Similar to CBL-B, CTLA-4 (CD152) negatively regulates T cells. CTLA-4 is the target of Yervoy ipilimumab, a human mAb from **Bristol-Myers Squibb Co.** that is marketed to treat metastatic melanoma. The drug's label includes a warning of severe immune-mediated adverse events due to T cell activation and proliferation.

Stephen Frye told *SciBX* that it might be better to go after the TAM receptor kinases.

"I consider the paper to be a very significant contribution to target validation for TAMs in the function of the innate immune system in oncology," he said. Frye is director of the Center for Integrative Chemical Biology and Drug Discovery at **The University of North Carolina at Chapel Hill Eshelman School of Pharmacy**. He also is cofounder of **Meryx Inc.**, which is developing MERTK inhibitors for multiple indications, including cancer and viral infection.

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> —*Stephen Frye, Meryx Inc.*

Penninger countered that "TAM receptor inhibitors probably need more preclinical studies to establish the safety profile."

Richard Godfrey said that the safety of TAM receptor inhibitors will hinge on specificity. "It is important to distinguish between compounds that inhibit specific TAM receptors and compounds that are pan–TAM kinase inhibitors. Multikinase inhibitors will be associated with more toxicity. Triple–*TAM* family knockout mice exhibit postnatal degenerative syndromes, while mice carrying specific *TAM* receptor mutations are

largely normal. Selective TAM inhibitors will not have the same effects as pan-TAM inhibitors."

Godfrey is CEO of **BerGenBio A/S**, which is developing small molecules and antibodies against AXL. Lead compound BGB324, a small molecule that was in-licensed from **Rigel Pharmaceuticals Inc.**, has completed a Phase I study. Clinical trials in acute myelogenous leukemia (AML) and lung cancer are planned for this year.

Qurient CEO Kiyean Nam said that the company is developing AXL inhibitors for drug-resistant cancers. The goal is to establish clinical proof of concept before looking for a partner.

Other companies with AXL inhibitors in preclinical development for cancer include **Tolero Pharmaceuticals Inc.** and **Oribase Pharma**.

Regardless of target choice, Penninger noted that "for many cancers, metastases are what kill people. If we can indeed use CBL-B inhibition or TAM receptor inhibition to—at least in part—control metastases, this would be tremendous."

Establishing clinical evidence for the antimetastatic activity of a therapeutic, however, is challenging because establishing that metastasis has not occurred is nearly impossible.[8]

"We could monitor NK cell activity as a surrogate marker for drug action against metastasis. This is the first step for TAM receptors to become a viable target for metastasis," said Nam.

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