Homing in on CML stem cells

By Kai-Jye Lou, Staff Writer

Researchers at **Lund University** have found a cell surface marker called IL-1 receptor accessory protein that could be used to isolate and kill chronic myelogenous leukemia stem cells.¹ **Cantargia AB**, a biotech founded based on the findings, is now developing a mAb to attack CML at its root by killing the stem cells, which respond poorly to standard CML treatments and are believed to spawn the leukemic cells that characterize the disease.

The majority of CML cases are characterized by cells carrying the Philadelphia chromosome, which contains the oncogenic BCR-ABL fusion protein. Tyrosine kinase inhibitors (TKIs) like Gleevec imatinib are very effective at putting the disease into remission and prolonging patient survival by inhibiting the BCR-ABL tyrosine kinase. However, CML patients require chronic daily treatment with TKIs, and interruption or discontinuation of therapy almost invariably results in disease relapse.

Novartis AG markets Gleevec to treat multiple cancers, including CML. The company also markets a second-generation TKI, Tasigna nilotinib, for Philadelphia chromosome-positive (Ph⁺) CML. **Bristol-Myers Squibb Co.** markets another second-generation TKI, Sprycel dasatinib, to treat CML and Ph⁺ acute lymphoblastic leukemia (ALL) patients who are resistant or intolerant to prior therapy, including Gleevec.

Mature and differentiated CML cells, which are readily killed by TKIs, are spawned from a small population of self-renewing stem cells that carry the Philadelphia chromosome.² Earlier studies have shown that these CML stem cells are partially resistant to TKIs and are likely to be the culprits in disease relapse.³⁻⁵

The problem is that CML stem cells have been difficult to isolate and study because there were no cell surface markers that would allow for prospective separation of normal hematopoietic stem cells from CML stem cells.

Although the Philadelphia chromosome is the putative marker for CML, the chromosome and its associated BCR-ABL tyrosine kinase are located within the cell. In general, intracellular molecules cannot be used for prospective stem cell isolation and are difficult to target with therapeutic antibodies.

The Lund researchers have now identified a potential cell surface marker by carrying out a microarray-based gene expression profiling study comparing samples from CML patients and healthy controls. The study pinpointed IL-1 receptor accessory protein (IL-1RAP) as a cell surface marker that is upregulated in CML cells but not in normal bone marrow cells.

"We were actively looking for cell surface markers that are upregulated on leukemic cells and targetable for diagnostic and therapeutic purposes," said Thoas Fioretos, a professor and senior consultant in the Department of Clinical Genetics at the university.

His group analyzed IL-1RAP expression in a stem cell-enriched subpopulation of cells isolated from five CML patients and found that 99.9% of cells in the IL-1RAP⁺ group carried the Philadelphia chromosome. In contrast, only 2.9% cells in the IL-1RAP⁻ group had the chromosome.

After showing that IL-1RAP is a highly specific marker on the surface of CML stem cells, the Lund researchers carried out a preliminary evaluation of IL-1RAP as a potential therapeutic target.

In both stem cell-enriched cell populations from patients and human leukemia cell lines, a polyclonal rabbit antibody targeting human IL-1RAP increased antibody-dependent cellular cytotoxicity (ADCC) against Ph⁺ cells compared with that against Ph⁻ cells. Importantly, the antibody did not induce ADCC in normal bone marrow cells isolated from the stem cell-enriched subpopulation.

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

"We have identified a marker on the cell surface that could make it easy to sort out candidate CML stem cells from a population of normal hematopoietic stem cells," said Fioretos, who is corresponding author on the paper. "We found that if you sort the stem cell-enriched cell population based on IL-1RAP, you could get a cell population where almost every cell contains the Philadelphia chromosome, which characterizes CML. By performing long-term cultures, we should demonstrate that the sorted population of cells contains candidate CML stem cells."

The study "is an interesting example of cell surface target discovery using microarrays," said Thomas Cirrito, director of operations at **Stemline Therapeutics Inc.** "What they've presented here is that the IL-1 receptor accessory protein appears to be a cell surface marker for Philadelphia chromosome-positive CML stem cells, which would make it a valuable tool for prospectively isolating this population of cells."

Stemline's lead program is a biologic that targets the IL-3 receptor, which is overexpressed on leukemic blasts and leukemia stem cells in multiple hematological malignancies including CML, acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). The compound is in a Phase I/II trial in patients with advanced AML and MDS. In *in vitro* and *in vivo* CML models, including samples from patients with TKI-resistant disease, SL401 has been shown to induce apoptosis in both CML blasts and CML stem cells.

Novartis declined to comment.

Therapeutic directions

Cantargia was formed by Fioretos, Marcus Järås, Kjell Sjöström and **Lund University Bioscience AB**, the biotechnology commercialization arm of the university. Järås is lead author on the *PNAS* paper and a post-doctoral fellow in the Department of Clinical Genetics at Lund University. Sjöström is director at **Innovagen AB**, which provides molecular

biology services that include the manufacturing of custom antibodies and peptides.

Allogeneic hematopoietic stem cell transplantation is currently the only known curative therapy for CML. However, patients generally have to be less than 65 years old to be eligible for the procedure and need a suitable donor.

“What we want to do at Cantargia is to develop a therapeutic antibody that will potentially cure the patient by targeting the leukemia stem cells,” said CEO Eskil Söderlind, who also is an investment director at Lund University Bioscience. “Gleevec and its follow-up compounds have been revolutionary treatments for CML and work very well. These treatments put patients into remission, but these patients are not cured. There is the risk that patients taken off the drug will experience disease relapse.”

“In theory what we could do, if we are able to show that our IL-1RAP-targeting approach works in humans, is to first give Gleevec to the patient to kill off the more mature CML cell population and then administer the IL-1RAP-targeting therapeutic antibody to kill off the CML stem cell population,” Fioretos told *SciBX*. “If our method works, then the patient will no longer need daily treatment with Gleevec.”

“From a therapeutic standpoint, it’s important to note that any therapeutic to be developed in CML today will have to be evaluated in the context of tyrosine kinase inhibitors as these have become the standard of care,” Stemline’s Cirrito noted. “Ultimately the goal for the development of new CML treatments will be to target TKI-resistant cells, which are the CML stem cells.”

Cirrito added that it will be important to determine whether IL-1RAP is shed from the cell surface. “If you have a cell surface protein that gets shed into plasma, the shed protein could act as a sink for your antibody therapeutic and thus decrease the amount of antibody that gets to the target cell,” he told *SciBX*. “This is something that is important to characterize when developing antibody therapeutics that target cell surface proteins.”

At the same time, the high selectivity of IL-1RAP for CML stem cells also could reduce the likelihood of collateral damage against normal cell populations and thus reduce the potential for side effects.

“Our work opens up for the first time the opportunity to target CML stem cells in a highly selective manner,” Fioretos told *SciBX*. “You don’t have this level of selectivity with other therapeutic antibodies that have been tested in patients with hematological malignancies, which target cell surface markers like CD33 and CD20. CD33, for example, is expressed on nonleukemic hematopoietic stem cells, and CD20 is expressed on normal B cells.”

Both Fioretos and Söderlind added that an IL-1RAP-based treatment also could become an alternative for CML patients who are unable to tolerate or fail to respond to Gleevec and other TKIs.

Cirrito, however, thinks it is still unclear whether IL-1RAP itself would be a good therapeutic target for CML. He noted that the primary

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patient samples used in the study were taken from CML patients who have yet to receive any form of treatment.

Cirrito also pointed out that one of the experiments in the *PNAS* paper showed that IL-1RAP was downregulated in a Gleevec-treated CML cell line and thinks this effect could potentially cause an IL-1RAP-targeting therapy to miss a significant number of the TKI-resistant CML stem cells in patients previously treated with TKIs.

“The question they will now have to address is whether IL-1RAP is a good therapeutic target in the context of patients who have been treated with tyrosine kinase inhibitors,” he told *SciBX*.

Fioretos said that studies evaluating the IL-1RAP-targeting antibody in primary samples taken from TKI-treated CML patients are under way. He added that the experiment showing Gleevec-mediated downregulation of IL-1RAP was part of a set of experiments to show that BCR-ABL tyrosine kinase activity regulates IL-1RAP expression. He thinks IL-1RAP should still remain upregulated in the resistant CML stem cell population given that IL-1RAP expression was shown to be induced as a consequence of BCR-ABL tyrosine kinase expression and TKIs are unable to effectively target this cell population.

Fioretos said his group is also developing mouse CML models that it will use to evaluate the IL-1RAP-targeting antibody. “We want to show that an antibody targeting IL-1RAP is not toxic and could reverse the disease. Once we have established our mouse CML models, we could test the antibody as a standalone therapy and in combination with current treatments for CML.”

Cantargia has filed for patents covering IL-1RAP’s diagnostic and therapeutic applications in leukemia. The company’s mAb against IL-1RAP is in preclinical development for CML.

Söderlind said Cantargia also is planning to evaluate the potential of targeting IL-1RAP in other types of leukemia. The company is actively seeking to out-license or partner its IL-1RAP antibody program.

Lou, K.-J. *SciBX* 3(36); doi:10.1038/scibx.2010.1080
Published online Sept. 16, 2010

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COMPANIES AND INSTITUTIONS MENTIONED

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