Celgene skips SKP2

By Joanne Kotz, Senior Editor

Celgene Corp. and academic collaborators have designed the first small molecule inhibitor of an E2 component in the ubiquitin pathway.¹ Despite positive data *in vitro*, the team thinks it will be difficult to optimize the cancer therapeutic for the clinic and has abandoned its program targeting the ubiquitin pathway. Instead, Celgene is setting its sights on other post-translational modifications that regulate protein degradation.

For more than a decade, researchers have been developing ways to target the ubiquitin-proteasome pathway in cancer. Only one marketed therapy actually hits the pathway—the proteasome inhibitor Velcade bortezomib, which is marketed in the U.S. by **Takeda Pharmaceutical Co. Ltd.’s Millennium Pharmaceuticals Inc.** subsidiary to treat multiple myeloma (MM) and mantle cell lymphoma.

Velcade’s broad side-effect profile has prompted a search for alternatives, including a family of targets immediately upstream of the proteasome: E3 ubiquitin ligases.

Although Celgene set out to screen for an inhibitor of a cancer-associated E3 ubiquitin ligase, it ended up identifying the first inhibitor of an E2 ubiquitin-conjugating enzyme.

The ubiquitination cascade includes three protein subunits denoted E1, E2 and E3, which function in tandem to attach ubiquitin molecules to proteins that are then transferred to the proteasome for degradation.

Unlike the proteasome, which is essentially identical in healthy and cancer cells, the ubiquitin pathway components occur in a variety of combinations, with 2 E1, 40 E2 and at least 600 E3 subunits identified in the human genome thus far.

Selectively targeting a particular E3 ligase associated with a specific cancer has the potential to achieve an anticancer effect with less off-target toxicity than Velcade. The ongoing challenge has been figuring out how to effectively target the protein-protein interactions that hold the E3 ligases together.

The Celgene-led team initially focused on the S-phase kinase-associated protein 2 (SKP2) E3 ligase, which has been associated with multiple cancers.²

The first priority was setting up a screen to identify inhibitors of the SKP2 E3 ligase.

To do so, the researchers developed an *in vitro* assay of the ubiquitin pathway, which leads to ubiquitination of cyclin-dependent kinase inhibitor 1B (CDKN1B; p27; Kip1), a tumor suppressor protein targeted for degradation by the SKP2 E3 ligase complex.

A high throughput screen using the assay identified hits that were optimized to CC0651, an inhibitor of p27 ubiquitination with an IC₅₀ of 2 μM.

The next task was to identify the precise target of CC0651. For this, Celgene collaborated with teams led by Frank Sicheri, professor of molecular genetics at the **University of Toronto**, and Mike Tyers, professor of systems biology at **The University of Edinburgh**.

The surprise was that CC0651 worked even in the absence of SKP2, suggesting it was actually modulating an upstream target. Additional studies revealed the target was an E2 enzyme called cell division cycle 34 homolog (CDC34).

Indeed, CC0651 had inhibitory activity in assays involving human CDC34 but had no effects when the assays used other E2s or even yeast cdc34.

The team then confirmed biochemically and structurally that CC0651 binds directly to CDC34.

Finally, the researchers tested cellular activity of the compound. In prostate cancer cells, a less potent but more cell-permeable analog of CC0651 increased levels of p27, suggesting a decrease in p27 ubiquitination, and lowered cell proliferation compared with vehicle.

Data were reported in *Cell*.

“The importance of this paper is that it is the first small molecule targeting an E2 conjugating enzyme that has been described. This is an important first step, but clearly much more potent, drug-like molecules would be needed for therapy,” said Philip Cohen, director of the MRC Protein Phosphorylation Unit at the **University**

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“Nobody is targeting E2s, so in that sense it is a very interesting molecule” for a target class that previously was off the map, said Tauseef Butt, president and CEO of **Progenra Inc.** Progenra is targeting E3 ligases and the deubiquitinating enzymes that remove ubiquitins from proteins.

Hitting E2 obstacles

Despite identifying an inhibitor that worked in cell culture, David Webb, formerly VP of research at Celgene and now an adjunct professor in the Department of Molecular Biology at **The Scripps Research Institute**, told *SciBX* the company does not plan to pursue CC0651 as a drug lead or CDC34 as a target.

“One of the reasons we decided not to patent the compound was that it was difficult to see a way forward to get below a micromolar IC₅₀,” said Webb. “The chemistry didn’t look to us like it was going to get us to the potency needed for a drug.”

“Celgene was excited about this area,” said Webb. “This was a seven-year effort. This program used an 11-protein assay and we screened an enormous library twice to get to the compound you see in the paper.” The only other hit from the two screens was a direct SKP2 ligase inhibitor that also could not be advanced due to potency issues.

“Thus after two failed attempts to produce a nanomolar-potency E3 ligase inhibitor, the program was stopped. The ubiquitin ligases themselves are incredibly difficult to drug; we consider them relatively undruggable,” said Webb.

Rakesh Kumar, director of biology in the Cancer Research group at **GlaxoSmithKline plc**, commented that although only minimal medicinal chemistry was shown in the publication, there was no apparent improvement in potency over CC0651. Nonetheless, the binding site, which is outside of the active site, has not been extensively investigated, and it is possible that further chemical modifications to optimize, particularly hydrophobic, interactions could provide compounds with improved potency, he noted.

In February 2010, GSK entered into a collaboration with **Proteologics Inc.** involving six drug discovery projects targeting E3 ligases.

A remaining question is whether other E2s might prove more targetable.

Sicheri and Tyers intend to continue to develop inhibitors of other E2s. “It’s our goal to find analogs that work on other E2s. In the place where the small molecule binds on CDC34, there are partial depressions on other E2s. If one could find a way to identify molecules that bind in this area it might be a more general way to inhibit these targets,” said Sicheri.

But Kumar also noted that based on the structural information reported in *Cell*, it is unclear that the binding site, even if present on other E2s, will be able to bind CC0651 or other similar small molecules with high affinity.

Celgene is continuing to pursue targets in protein degradation

pathways, but the company is no longer directly targeting E2s or E3s and intends to take new approaches, said Webb. “There are other players in the ubiquitin ligase pathways and the general post-translational modification of proteins for degradation, and Celgene has nondisclosed targets in that general area,” he added.

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