Solving triptolide

By Kai-Jye Lou, Staff Writer

University of Minnesota researchers have developed a water-soluble prodrug of the poorly soluble cancer therapeutic triptolide that could give a second life to the plant-derived compound.¹ **Minneamrita Therapeutics LLC** holds a license to the new molecule and plans to start a Phase I trial within six months.

Triptolide is a diterpenoid triepoxide derived from the traditional Chinese medicine plant *Tripterygium wilfordii*.

In early 2007, researchers at the University of Minnesota led by Ashok Saluja showed that small interfering RNA-mediated knockdown of heat shock protein 70 (Hsp70) potentially killed pancreatic cancer cells,² which prompted them to search for pharmacological agents that inhibit the protein.

Later that year, Saluja and colleagues showed that triptolide potentially killed pancreatic cancer cells by downregulating Hsp70.³ However, after working with the compound and showing antiproliferative effects in two other forms of cancer,^{4,5} it became clear that triptolide was not suited for clinical development.

“Triptolide is not very soluble in water, so if we were to try to deliver it to a patient we would first need to dissolve it in solvents, which could be very harsh to the patient,” said Saluja, professor and vice chair of research in the Department of Surgery at the University of Minnesota and a cofounder of Minneamrita. “Thus, we decided to modify the compound to make it more water soluble.”

The resulting compound, called minnelide, is designed to release the parent molecule when exposed to phosphatases (see Figure 1, “Model of triptolide release from minnelide”).

An *in vitro* bioconversion assay showed that minnelide had a half-life of about two minutes in the presence of alkaline phosphatase and was rapidly converted into triptolide. In five human pancreatic cancer cell lines, minnelide in the presence of alkaline phosphatase significantly decreased cell viability at nanomolar concentrations compared with no treatment ($p < 0.05$).

In transgenic and xenograft mouse models of pancreatic cancer, intraperitoneal injection of minnelide caused tumor regression and led to a 90% survival rate, whereas saline-treated controls had a 10% survival rate. In one of the mouse xenograft models, minnelide caused more potent reductions in tumor burden than Gemzar gemcitabine, which is marketed by **Eli Lilly and Co.** for pancreatic cancer.

No supplementation of alkaline phosphatase was needed in the mouse studies as the enzyme already is present in blood and many bodily tissues.

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— Gunda Georg,
University of Minnesota

Results were published in *Science Translational Medicine*.

“The data in our current study show that minnelide is very effective for treating pancreatic cancer,” said Saluja, corresponding author of the paper. “We showed in multiple types of mouse pancreatic cancer models that treatment with minnelide causes the tumors to quickly melt away.”

“Triptolide was an attractive starting point for synthesizing analogs because the molecule had proven anticancer and antiangiogenic properties,” added Gunda Georg, coauthor of the paper, professor and department head of medicinal chemistry at the University of Minnesota and director of the university’s Institute for Therapeutics Discovery and Development. “There was also evidence in the literature to suggest that the compound acts via a mechanism of action that is distinct from that of existing chemotherapy drugs and also could potentiate the effect of other chemotherapy agents.”

Indeed, data published by a research group in Europe in 2009 showed that triptolide had a unique antiproliferative profile against the NCI-60 panel of tumor cell lines.⁶

Despite the observed downregulation of Hsp70 activity, Saluja noted that the exact molecular target of triptolide—and thus minnelide—remains unclear.

“We previously found that triptolide inhibited Hsp70 and thought that this was its primary mechanism of action, but we now know that the

compound can also inhibit multiple other cancer-associated pathways, such as NF- κ B and other antiapoptosis pathways,” he told SciBX.

A working prodrug strategy

There have been multiple past efforts to develop triptolide prodrugs to treat cancer, but none has panned out.

Georg gave the example of F60008, a prodrug developed by a group at **Erasmus Medical Center** that uses a succinate-based prodrug approach. The conversion of F60008 into triptolide was unpredictable and incomplete, and the researchers discontinued development of the prodrug after two reports of death in an investigator-led Phase I dose-escalation study in patients with advanced solid tumors.⁷

Minneamrita expects that the rapid and consistent pharmacokinetics

Figure 1. Model of triptolide release from minnelide. Minnelide consists of a phosphonoxyethyl prodrug group that increases the compound’s solubility in water.

Phosphatases, which are present in blood and many bodily tissues, cleave the phosphate group from minnelide to yield an unstable O-hydroxymethyl intermediate. This intermediate then degrades into formaldehyde and the anticancer compound triptolide.

behind minnelide's conversion into triptolide could give it an edge over previous attempts.

According to Georg, steric hindrance caused by the prodrug group being in close proximity to the active molecule may have contributed to F60008's unpredictable and incomplete conversion into triptolide.

"With minnelide, the cleavage site for the prodrug group is further out from the active molecule, so we would expect less steric hindrance and thus faster and more complete conversion of the compound into triptolide," she told *SciBX*.

Georg added that a prodrug that releases its drug reliably would allow for much better control over drug levels and toxicity than one with unpredictable release.

Saluja said Minneamrita plans to start recruiting patients in a Phase I trial of minnelide within six months. He said the company wants to position minnelide as a monotherapy to treat patients with pancreatic cancer for whom Gemzar has failed.

In parallel, Saluja said his group at the university is running preclinical studies to determine the potential of using minnelide in combination with Gemzar or inhibitors of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in pancreatic cancer. His group also is trying to determine

what other cancers could be treated with minnelide and to elucidate the mechanisms underlying the compound's effects.

The University of Minnesota has filed a patent covering triptolide prodrugs including minnelide. The IP is licensed to Minneamrita.

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COMPANIES AND INSTITUTIONS MENTIONED

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