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LAMbasting brain cancer

By Michael J. Haas, Senior Writer

Approved therapies to treat brain cancer are not entirely tumor cell–specific and thus can have severe dose-limiting toxicities that decrease their effectiveness. A team of U.S. and German researchers thinks it has overcome this problem with a nanoconjugate that selectively targets brain tumor cells to block production of laminin-411—a proangiogenic protein that is highly expressed in cancerous but not normal brain cells.[1] **Arrogene Nanotechnology Inc.**, which already has similar nanotechnologies in preclinical development to treat cancer, has in-licensed the findings.

Laminins are a large class of trimeric extracellular matrix proteins that play roles in angiogenesis, cell adhesion and migration, and other processes in both normal and cancer cells. A team co-led by Julia Ljubimova, professor of neurosurgery and director of drug development and nanomedicine in the Department of Neurosurgery at **Cedars-Sinai Medical Center**, previously showed that laminin-411 was overexpressed in 75% of glioblastoma multiforme (GBM) tumors compared with in normal brain tissues.[2]

In addition, the protein was associated with greater invasiveness and recurrence of GBM tumors.[3] Collectively, Ljubimova said, the findings led her team to hypothesize that inhibiting laminin-411 could treat GBM.

Laminin-411 is composed of three subunits: laminin α4 (LAMA4), laminin β1 (LAMB1) and laminin γ1 (LAMC1). The trimeric nature of laminins means that "you need to block the synthesis of at least two of the protein units in order to effectively inhibit laminin production," a difficult task for a single drug compound, Ljubimova told *SciBX*.

Thus the researchers set out to develop a nanotechnology capable of delivering two laminin protein inhibitors specifically to tumor cells.

The resulting nanoconjugate consisted of a nontoxic, nonimmunogenic poly(β-L-malic) acid (PMLA) polymer backbone covalently linked to multiple bioactive components. These included anti–transferrin receptor antibodies that allowed the nanoconjugate to cross the blood brain and brain-tumor barriers, a peptide that helped it escape intact from endosomes once inside the tumor cell and two antisense oligonucleotides that targeted *LAMA4* and *LAMB1* to block synthesis of the corresponding proteins.

In a mouse model of xenograft gliomal tumors, i.v. administration of the nanoconjugate lowered tumor growth by 90% compared with administration of vehicle.

Data were reported in the *Proceedings of the National Academy of Sciences*. The team was co-led by Eggehard Holler, professor of biochemistry at the **University of Regensburg**, and included researchers from the **University of Erlangen-Nuremberg**.

Ljubimova also is cofounder, president and CSO of Arrogene, and Holler is the company's VP of advanced chemistry. Arrogene has PMLA-based nanoconjugates in Phase I testing as cancer imaging agents and in preclinical development to treat various cancers.

In April, Ljubimova's team reported that a PMLA-based nanoconjugate linked to Temodar temozolomide decreased the viability of Temodar-resistant brain cancer cell lines,[4] thereby suggesting that the technology could overcome drug resistance as well as toxicity issues.

Merck & Co. Inc. markets Temodar, a DNA alkylating agent, to treat brain cancer.

Conjugal challenges

Pieter Gaillard, CSO of **to-BBB technologies B.V.**, said the multicomponent structure of the nanoconjugate appeared to increase its overall selectivity for brain tumors, thereby increasing its potential safety. "The higher the selectivity of a technology, the fewer side effects that are to be expected," he said.

Nevertheless, Gaillard wanted to see data confirming the nanoconjugate's safety in acute and chronic dosing regimens. He also wanted to know the conjugate's half-life in blood plasma and whether it had an effect on survival in animal models of brain cancer.

Ljubimova said her team has collaborated with researchers at the **NIH** to show that the nanoconjugate is nontoxic and nonimmunogenic in preclinical models and remains stable in *ex vivo* human plasma for more than 24 hours.

But even if the nanoconjugate proves safe and stable in humans, Gaillard said its complex structure presents an obstacle to development and manufacturing.

The multiple components of the technology make it "virtually impossible to investigate the relative contribution of each to the overall effect of the nanoconjugate, which in turn would make it difficult to explain an unexpected toxic effect or to predict the effect of modifications to the product" that would inevitably be introduced during development and scale up, he said.

As an example, he said the nanoconjugate in the *PNAS* paper used an antibody against murine transferrin receptor to cross the blood brain barrier. "During development, the final product for human use will have to be tested for toxicity in a relevant species—most likely in nonhuman primates in this case," which would require modifying the anti–transferrin receptor antibody accordingly, he said.

"We do not anticipate problems at this point and have the knowledge to deal with all of these issues," Ljubimova countered, noting that the team had developed controls for each component of the nanoconjugate and that humanized antibodies against transferrin receptor could readily be used.

Gaillard also said the chemistry, assays and controls involved in scale up and manufacturing would be "extremely difficult tasks for

the manufacturing party and would therefore be an expensive process with a high risk of failure."

He added: "All of these disadvantages will make translation of this technology into a clinical and commercial reality highly unlikely. Other strategies will most likely offer better, more affordable approaches to treat brain and other cancers and attract more investment upfront."

to-BBB's 2B3-101, a liposomal formulation of doxorubicin conjugated to pegylated glutathione, is in preclinical development to treat brain cancer. The company expects to begin a Phase I/IIa trial in 1H11. The compound has Orphan Drug designation in the U.S. and EU.

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—Maurizio Vecchione, Arrogene Nanotechnology Inc.

Maurizio Vecchione, CEO of Arrogene, disagreed that the complexity of the nanoconjugate renders it too difficult and expensive to develop and market as a cancer therapeutic.

He noted that unlike synthetic nanoparticles, which can be difficult to manufacture and are highly toxic, the nanoconjugate's polymer scaffold is a natural, nontoxic compound that is readily isolated from a species of mold.

Vecchione also said Arrogene has developed the process of nanoconjugation—connecting desired drug compounds and other components to the scaffold—to a point that it is simple and reproducible.

The company also has performed scale up studies on a production line at Cedars-Sinai to show that "we can make nanoconjugates of pharmaceutical-grade purity efficiently, consistently and at reasonable cost," Vecchione said. "Our experience with nanoconjugation allows us to think of this as a typical pharmaceutical process" that is not any more difficult than synthesizing, optimizing and manufacturing a drug compound.

Vecchione acknowledged that the nanoconjugates were likely to be more expensive on a per-milligram basis than other drug compounds. "But we think the higher cost will be offset by the fact that a nanoconjugate is highly targeted to the cancer cell," he said. "This means that drugs delivered via nanoconjugates could be given at lower doses than the unconjugated drugs. Consequently, the overall cost of treatment is likely to be comparable to, or less than, that of the unconjugated drug, and nanoconjugate treatment would have the added benefits of increased efficacy and lower toxicity."

Arrogene has not decided whether to develop the brain cancer nanoconjugate described in the *PNAS* paper or another of its PMLA-based nanoconjugates to treat a different cancer as its lead compound, Vecchione said.

In addition to ongoing studies with NIH researchers, Ljubimova said her team has papers in press and in preparation that describe nanoconjugates that could help treat HER2 (ERBB2; neu)-positive and triple-negative breast cancers, respectively.

She added that the team also is conducting *in vivo* studies of the Temodar-linked nanoconjugate to treat brain cancer.

Cedars-Sinai has patented the findings reported in *PNAS* and out-licensed the technology to Arrogene.

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