BIOCENTURY
Innovations
FROM IDEA TO IND

JANUARY 28, 2016

PRODUCT R&D

HITCHHIKER'S GUIDE TO THE GUT

By Selina Koch, Staff Writer

Applied Molecular Transport LLC (AMT) believes it has cracked the problem of orally delivering therapeutic proteins that has been the bane of biologics from the outset. The company has co-opted a strategy and a molecule used by bacteria that infect the gut to create a platform that shuttles proteins across the intestinal epithelium and delivers them to cells deep inside the gut or in the liver.

The gut epithelium has thwarted efforts to orally administer biologics because proteins can't diffuse across the barrier or sneak through the tight junctions, and when they are taken up by endocytosis — the only route left to them — they are typically degraded in lysosomes rather than transported into the body.

The most common solution is to use systemic administration, but that often translates into higher doses and more side effects than would take place if compounds could be selectively delivered to target cells, according to AMT CEO Tahir Mahmood.

Mahmood told BioCentury the company has developed a bacteria-derived carrier system that exploits vesicular recycling pathways in epithelial cells to shuttle even large cargo proteins across the barrier efficiently.

The technology can deliver proteins to the submucosal region of the gut mucosa, which contains Tregs, macrophages and dendritic cells. That allows the company to improve delivery for a wide range of GI-related autoimmune diseases such as ulcerative colitis, Crohn's disease, systemic lupus erythematosus (SLE), rheumatoid arthritis and psoriasis, said Mahmood.

In addition, the technology can deliver proteins fully across the GI lining to the portal vein that leads to the liver, thus creating possibilities for treating liver diseases such as non-alcoholic steatohepatitis (NASH) and metabolic diseases with liver targets such as diabetes and hypercholesterolemia.

Mahmood noted that previous attempts at shuttling biologics through the intestinal lining failed because most relied on diffusion, which cannot be increased enough to get sufficient doses of proteins across, or on opening tight junctions, which is unsafe because "it lets everything through."

BIOCENTURY PRODUCT PROFILE	
INNOVATION STAGE	
Product	"Transint": bacterial toxin-derived peptide for conjugating to biologics
Concept	A technology for delivering biologics orally by enabling transport across the GI epithelium, based on the infectivity mechanism of *Vibrio cholerae*
Disease	Autoimmune diseases related to the GI tract; liver diseases, including metabolic diseases with liver targets
Competition	(I) Injectable biologics (II) Oral peptides stabilized with scaffolds, such as disulfide-rich peptides
Differentiation	(I) Fewer side effects from systemic exposure; increased convenience and compliance (II) Not limited to peptides
Administration	Oral
Risks	Dosing limited by pill size
Development status	Preclinical
Patents	Patented
Company; lead investigator	Applied Molecular Transport LLC

"We have a natural immune-targeting vector that's based on well-characterized, receptor-mediated infection pathways," he said.

GUT REACTION

AMT's platform is based on the infection route of *Vibrio cholerae*.

CSO Randall Mrsny told BioCentury, "We looked at viruses and bacteria that know how to cross the epithelium and took it down to the simplest of materials that have solved this problem — and those are toxin proteins."

The company's platform — dubbed "Transint" — is based on cholix toxin, a protein *V. cholerae* secretes to evade immune attack. During the bacterial infection, cholix penetrates the gut by binding non-selective scavenger receptors on the luminal surface of intestinal epithelial cells. The epithelial cells then endocytose the toxin and shuttle it across the

GUT MOVEMENT

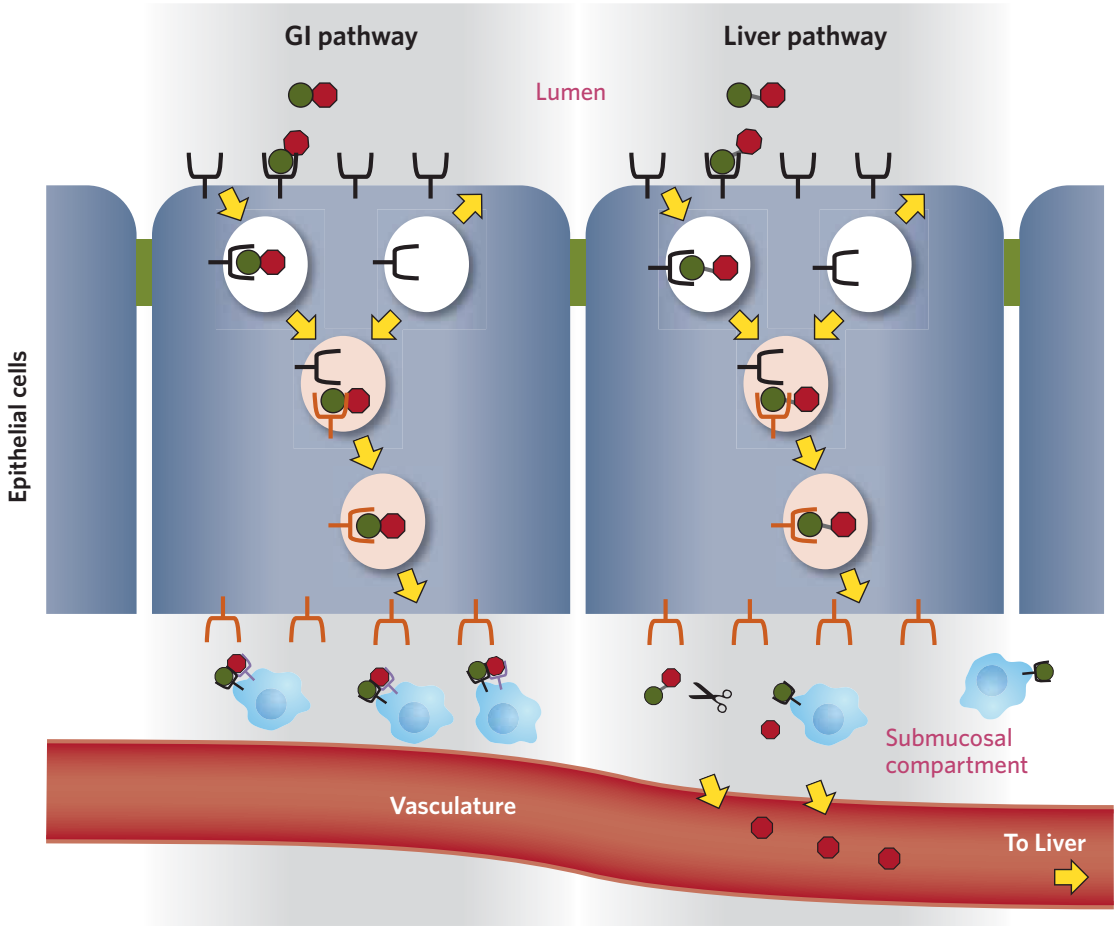
Applied Molecular Transport LLC (AMT) has co-opted the infection route of a bacterial toxin to create Transint, an oral biologics platform that can target peptides and proteins to cells in the GI tract or liver. The platform utilizes a truncated protein derived from *Vibrio cholerae*'s cholix toxin — which helps the bacteria evade immune attack by traversing the gut epithelium to deposit a toxic payload in immune cells — as a carrier for therapeutic cargo. The Transint carrier is based on the part of the toxin required for trafficking and omits the cytotoxic domain.

AMT has two versions of the platform: one targeting immune cells in the submucosal region of the GI tract **(GI pathway)**, and the other targeting cells in the liver **(Liver pathway)**.

Both versions rely on the same mechanism to transport the Transint carrier **(green)** and therapeutic cargo **(red)** across the epithelium. First, the carrier binds scavenger receptors **(black)** on the luminal surface of epithelial cells, triggering endocytosis of the carrier and cargo. The acidic pH of the early endosome then decreases the affinity of the Transint carrier for scavenger receptors. When released from the scavenger receptors, the carrier binds to ganglioside receptors **(orange)**, diverting the endosome to the basal side of the cell, where the carrier and compound are exocytosed into the submucosal compartment.

GI pathway. The Transint carrier is covalently bound to the cargo and the combined molecule is retained in the submucosal compartment by two types of interactions with GI immune cells: carrier binding to scavenger receptors and cargo binding to its target receptor **(light purple)**.

Liver pathway. The Transint carrier and therapeutic cargo are joined by a cleavable linker **(gray bar)** that is cut by an undisclosed enzyme in the submucosal compartment, freeing the cargo from the immune cell-binding carrier and allowing it to enter the mesenteric vein, which connects to the portal vein that carries it directly to the liver.



barrier where it delivers its toxic payload to local immune cells.

AMT's platform uses a truncated form of the cholix protein that contains the part required for epithelial transport and omits the cytotoxic domain. The truncated toxin is covalently bound to the therapeutic cargo. (See "Gut Movement", page 2)

Mrsny told BioCentury that while epithelial cells typically degrade scavenged material in lysosomes, the Transint system avoids that problem because the carrier loses its affinity for scavenger receptors as the endosome acidifies. Instead, it binds to ganglioside receptors, which divert the endosome to the basal surface of the cell. "Gangliosides move through the cells and these toxins hitch a ride," he said.

> "We looked at viruses and bacteria that know how to cross the epithelium and took it down to the simplest of materials that have solved this problem — and those are toxin proteins."
>
> Randall Mrsny, AMT

When the endosome fuses with the membrane and opens up on the basal side of the cell, the extracellular pH causes the ganglioside receptor to release the Transint carrier — still bound to its therapeutic cargo — into the submucosal compartment, where the molecule encounters antigen-presenting cells (APCs), macrophages and Tregs.

"The intestine is the biggest immune organ in the body," Mrsny said. "If you can deliver things to the submucosa of that immune organ you open all kinds of possibilities for therapeutic strategies."

Although binding of the Transint carrier to scavenger receptors on immune cells should trigger endocytosis, which would sequester the therapeutic protein cargo away from its intended binding partner on the surface of immune cells, Mrsny emphasized that risk has not materialized in the team's experiments. That's probably because the cargoes the company is using have much higher affinities for their receptors than the carrier has for scavenger receptors, he said. "It's sort of a horse race between the two, but the high-affinity interaction seems to be winning out because we see good biological activity."

He noted that the company has connected the Transint carrier to seven different cargo proteins, and all have made it through the epithelial cells without being damaged by the acidic pH of the endosome or by any enzymes present in the vesicles.

The company has tested undisclosed cytokines and an interferon with the system. "We're starting with molecules as cargoes that are really well known and either already have a long track record in man or should have been great drugs but had a safety problem related to systemic administration," Mrsny said.

Mahmood noted that the GI tract connects to the hepatic portal system, so any compound that doesn't bind locally in the gut will go straight to the liver and stay there if the target is highly expressed on liver cells.

To facilitate liver targeting, the company not only chooses targets with higher expression in the liver than in the GI tract, but has also designed a series of cleavable linkers that it can insert between the Transint carrier and the cargo. The linkers are cut by an enzyme on the basal side of the epithelium, freeing the cargo from the immune-interacting carrier.

The linkers also block access to the cargo before it crosses the epithelial barrier, preventing any interactions with receptors in the gut lumen.

Mahmood noted the gut-to-liver route is much more efficient than systemic administration. "With subcutaneous injection, about 10% of the drug hits the liver with every cycle; 90% goes everywhere else. With our route, nearly 100% of the drug will reach the liver."

Mrsny added that diabetes drugs are given systemically "but sugar regulation is all happening in the liver."

He noted that the hypoglycemia often caused by systemic insulin injections would be reduced by delivering insulin directly to the liver, and that using Transint to deliver GLP-1 would alleviate the nausea caused by the peptide binding receptors outside the liver.

"If things are designed to go to the liver first, it only makes sense to try and recreate that in a delivery system," he said.

MAKING BIO-BETTERS

Mahmood believes the platform can improve the biology of marketed drugs and rescue failed compounds. "We're making bio-betters, not biosimilars," he said. "They'll be NMEs."

Oral administration with Transint will also "make compounds easier to take, which has the cascading effects of better compliance and lower costs," he said.

While the company hasn't disclosed any of its preclinical programs, Mahmood said its primary focus is on inflammatory diseases of the gut.

Last year, AMT, which has been incubating at Johnson & Johnson's South San Francisco JLABS site, granted the Janssen Biotech Inc. unit of JNJ exclusive worldwide rights to a preclinical candidate to treat inflammatory bowel disease (IBD).

While the company is primarily seeking partners for indications outside the GI tract, Mahmood said it has also received "considerable interest" from pharma in other GI indications and will "explore all avenues."

COMPANIES AND INSTITUTIONS MENTIONED

Applied Molecular Transport LLC, South San Francisco, Calif.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.

TARGETS AND COMPOUNDS

GLP-1 - Glucagon-like peptide-1

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EDITORIAL & RESEARCH

NEWSROOM:
pressreleases@biocentury.com

SAN CARLOS, CA:
+1 650-595-5333; Fax: +1 650-595-5589

CHICAGO:
+1 312-755-0798; Fax: +1 650-595-5589

WASHINGTON, DC:
+1 202-462-9582; Fax: +1 202-667-2922

UNITED KINGDOM:
+44 (0)1865-512184; Fax: +1 650-595-5589

Editor: C. Simone Fishburn, Ph.D.

Associate Editor: Michael J. Haas

Senior Writers: Lauren Martz; Stephen Parmley, Ph.D.

Staff Writers: Selina Koch, Ph.D.; Mary Romeo; Karen Tkach, Ph.D.; Mark Zipkin

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BioCentury Publications, Inc.
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MAIN OFFICES
PO Box 1246
San Carlos CA 94070-1246
+1 650-595-5333; Fax: +1 650-595-5589

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