Institution: University of Bath		
Unit of Assessment: A3 Allied Health Professions, Dentistry, Nursing and Pharmacy		
Title of case study: Oral Delivery of Biopharmaceuticals		
Period when the underpinning research was undertaken: 2014-2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Randall Mrsny	Professor of Epithelial Biology	July 2007 – February 2011; June 2011 – June 2017; July 2017 - present
Alistair Taverner	Research Assistant	February 2013 – present
Julia MacKay	Research Assistant	January 2011 – July 2013; September 2014 – December 2014; March 2015 to present
Period when the claimed impact occurred: 2014-2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

The efficient and reproducible **oral** delivery of biopharmaceuticals (i.e., macromolecular drugs, such as proteins) would revolutionize healthcare, but barriers presented by the intestinal epithelium has limited this possibility. Fundamental, underpinning research, led by Professor Randall Mrsny at the University of Bath, has now validated and provided proof-of-concept for an endogenous, vesicular trafficking pathway used by the bacterial exotoxin, cholix, to overcome these barriers. During the REF period, Applied Molecular Transport, Inc. (AMT), co-founded by Mrsny, has received approximately USD290,000,000 to develop this technology. AMT has a current market capitalisation of more than USD1,100,000,000.

2. Underpinning

A safe and efficient method for the oral delivery of macromolecular drugs has been a goal of the pharmaceutical industry since insulin was purified in 1921. Since that time, many more protein (and peptide) therapeutics have been identified, with all requiring administration to patients via an injection. Patient convenience and compliance would be greatly increased by an oral dosing approach and would significantly benefit therapy with these drugs. Previous efforts to facilitate oral protein delivery focused on physically altering (damaging) the intestinal barrier, which can be problematic for chronic dosing. Underpinning research at the University of Bath since 2014 has focused on examining how Mother Nature solved this challenge; Professor Mrsny hypothesized that non-pandemic pathogens present in the intestine might secrete agents that could clandestinely overcome this barrier to maintain their residence at this location.

Mrsny demonstrated that cholix, a virulence factor secreted by certain strains of *Vibrio cholera,* was capable of efficient transcytosis across an intact intestinal epithelium without modifying its barrier properties. Mrsny (including Bath research assistants Taverner and MacKay) subsequently demonstrated that a poorly immunogenic domain of cholix (lacking all cytotoxic function) was sufficient for efficient transcytosis and that biopharmaceuticals could be conjoined to this domain through genetic or chemical coupling schemes **[1]**.

Mrsny went on to identify endogenous elements within intestinal epithelial cells that are used by cholix for its efficient vesicular trafficking that allows it to enter the body. Through these studies, Mrsny identified new biological paradigms related to intestinal pathogen/host interactions and specific mechanisms for how cholix transiently alters the properties of

intestinal epithelial cells to allow its transcytosis. A fundamental hallmark of this pathway is that cholix associates with LMAN1 (a lectin-binding protein of the retrograde pathway) to direct it away from the lysosomal degradation pathway and toward the endoplasmic reticulum-Golgi intermediate complex (ERGIC) compartment of the cell. Cholix was also shown to invert cellular distributions of the coatamer proteins COPI and COPII; a result that directed cholix-containing vesicles to the basal region of the cell as opposed to recycling back to the apical surface. Third, within the basal region of the cell, cholix interacts with heparin sulphate proteoglycan, which is an extracellular matrix protein destined for basal secretion. Through the inversion of COPI/COPII, cholix is released by exocytosis at the basal surface from a recycling endosome-based pathway. This deep mechanistic understanding of cholix biology has provided the first rational and tractable approach for oral protein delivery.

Interleukin-10 (IL-10) was selected for the first clinical application of the cholix technology. IL-10 has shown promise in treating inflammatory bowel disease (ulcerative colitis and Crohn's disease) but has been limited by severe hematopoietic side effects following systemic injection. Professor Mrsny's laboratory has now shown that a genetic fusion of cholix and IL-10 efficiently delivers this anti-inflammatory cytokine to the *lamina propria* of rats and non-human primates, providing the potential to elicit the intestinal anti-inflammatory actions desired to treat IBD without the side-effects associated with IL-10 following systemic injection. These results have been published in 2020 **[2]** and this medicine is now in Phase II clinical trials in patients.

Since 2014, Applied Molecular Transport, Inc. (AMT) has fully funded two University of Bath postdoctoral research associates, a Ph.D. studentship, and an independent three-year Fellowship position. Overall, including equipment and overhead, AMT has awarded research funding to the University of Bath of approximately GBP845,000 during the REF period.

3. References to the research

[1] Taverner, A, Mackay, J, Laurent, F, Hunter, T, Liu, K, Mangat, K, Song, L, Seto, E, Postlethwaite, S, Alam, A, Chandalia, A, Seung, M, Saberi, M, Feng, W & Mrsny, R 2020, 'Cholix Protein Domain I Functions as a Carrier Element for Efficient Apical to Basal Epithelial Transcytosis', *Tissue Barriers*, vol. 8, no. 1, 1710429. https://doi.org/10.1080/21688370.2019.1710429

[2] Fay, NC, Muthusamy, BP, Nyugen, LP, Desai, RC, Taverner, A, MacKay, J, Seung, M, Hunter, T, Liu, K, Chandalia, A, Coyle, MP, Kim, HL, Postlethwaite, S, Mangat, K, Song, L, Seto, E, Alam, A, Olson, CV, Feng, W, Saberi, M, Mahmood, TA & Mrsny, RJ 2020, 'A novel fusion of IL-10 engineered to traffic across intestinal epithelium to treat colitis', *Journal of Immunology*, vol. 205, no. 11, pp. 3191-3204. https://doi.org/10.4049/jimmunol.2000848

4. Details of the impact

The research described above supports the concept that local, targeted delivery can provide effective therapy without systemic exposure thereby 'opening the door' to other biopharmaceuticals (e.g., interleukin-22 and glucagon-like peptide (GLP)-2) that have been unsuccessful in the clinic because of undesirable side-effects. Additional benefits of oral biopharmaceutical medicines include avoidance of the cold chain currently required for injectable formulations, the fact that these products do not need to be sterile, significantly reducing production costs compared to injectables (and eliminating the use of needles and syringes), and the obvious potential to improve patient convenience and compliance.

The chronology of the relevant events and actions relevant to the impact articulated here is presented in Table 1 below. The Mrsny laboratory at Bath first demonstrated the potential of the cholix transport mechanism *in vitro* using the Caco-2 cell model. This enabled Applied Molecular Transport, Inc. (AMT) to obtain funding of USD5,000,000 to explore the potential of the approach for the oral delivery of proteins to the intestinal *lamina propria*. These resources

allowed AMT - then a limited company that became incorporated in 2016 - to engage its first four employees and to support further research at Bath. This subsequent work, under Mrsny's direction, clearly showed IL-10 transcytosis *in vitro* and *in vivo* and AMT was then able to secure venture capital investments of USD35,000,000 for series A in 2016 and USD77,000,000 for series B in 2018. By 2020, the development programme for cholix-mediated, oral delivery of IL-10 had advanced through Phase Ia/Ib clinical trials and entered Phase II **[A]**; AMT had grown to 45 employees and, in June, had become a public company (AMTI (NASDAQ)) **[B**].

The CEO of AMT has stated **[C]** that the collaboration between the University of Bath and AMT, *"allowed [the company] to accelerate studies to validate the basic biological principles at the university while simultaneously advancing the clinical translation through private sector venture capital funding of its pharmaceutical technology… Outcomes from the relationship between AMT and the University of Bath is a clear demonstration that such a strategy cannot only be successful, it can be a new paradigm in delivering impact in the setting of a novel pharmaceutical technology*."

Table 1: Chronology of events and actions relevant to the impact

Date	Events and Actions relevant to the impact
09-2010	Applied Molecular Transport LLC formed
09-2010	Provisional patent application no. 61/403,394 filed eventually leading to US patent 9,090,691
06-2011	Professor R. Mrsny joins University of Bath as a full-time employee
01-2014	Alistair Taverner joins Mrsny laboratory in Bath and underpinning research at Bath expands and accelerates
01-2014	USD5,000,000 investment from Janssen Pharmaceuticals
03-2015	Julia Mackay joins Mrsny laboratory in Bath
05-2015	Continuation-in-part of… application no. 13/822,435, filed as application no. PCT/US2011 (now US 9,909,691), that becomes US patent 10,130,688
07-2015	US patent 9,090,691 issued
11-2016	Applied Molecular Transport, Inc. (AMT) is incorporated
11-2016	AMT receives USD35,000,000 Series A venture capital funding
11-2018	US patent 10,130,688 issued
2018	AMT receives USD77,000,000 Series B venture capital funding
2019	AMT commences Phase Ia/Ib clinical studies with AMT-101
12-2019	*Tissue Barriers* paper published
2020	AMT commences Phase II clinical studies with AMT-101
06-2020	AMT initial public offering with a closing of USD177,100,000
11-2020	*Journal of Immunology* paper published

The disruptive nature of the cholix technology and the potentially transformative impact of its applications was recently recognized when AMT was selected (from a field of more than 1,000) as one of three biotech innovation finalists in the Fierce Innovation Awards – Life Sciences Edition 2020 **[D]**. This is a peer-reviewed awards program based on the evaluation criteria of effectiveness, technical innovation, competitive advantage, financial impact, and true innovation of a therapeutic. Wide scientific interest in the cholix technology is corroborated (for example) by Professor Mrsny's presentation of a plenary talk, describing the science behind AMT-101, at the European Crohn's and Colitis Organisation (ECCO) held in Vienna in February 2020 **[E]**.

Impact 1: Enabling investment and job creation at AMT

The underpinning science performed at the University of Bath enabled AMT to obtain funds for the initial development of the oral IL-10 delivery system. In June 2020, AMT concluded its initial public offering (IPO) of 12,650,000 shares of common stock at USD14.00 per share, generating USD177,100,000. It has a current market capitalisation of more than USD1,100,000,000 **[F]**. The AMT head count post-IPO through the end of 2020 has increased to approximately 100 people.

Impact 2: Informing clinical trials

Venture capital funding to AMT of USD112,000,000 supported development of the oral IL-10 delivery system (now known by its codename AMT-101) through Phase Ia/Ib clinical testing involving healthy individuals and ulcerative colitis patients, respectively. These studies demonstrated that AMT-101 is safe and provided evidence for dose selection in Phase II. The significant resources generated by the IPO have permitted AMT to initiate a series of Phase II studies to test AMT-101 for the treatment of ulcerative colitis, pouchitis, anti-TNF add-on therapy in ulcerative colitis, and anti-TNF add-on therapy in rheumatoid arthritis **[A]**.

5. Sources to corroborate the impact

[A] Press release Bloomberg, 27/08/20. AMT-101 has successfully completed Phase Ia/Ib testing and has initiated Phase II testing: https://www.bloomberg.com/press-releases/2020-08-27/applied-molecular-transport-announces-first-patient-dosed-in-phase-2-study-of-oral-amt-101-in-ulcerative-colitis.

[B] Securities and Exchange Commission report, 01/06/20. To become a public company in the U.S., an S1 filing is required. Within that document is legally verified information on the history, financial status, outlook, and indices of impact that are of interest to potential investors: https://sec.report/CIK/0001801777.

[C] A letter of support from the Chief Executive Officer of AMT highlighting the key role of underpinning research at the University of Bath on the development of the cholix technology and AMT-101, 02/12/20.

[D] Press release GlobeNewswire, 05/08/20. “Applied Molecular Transport Named as One of Three Finalists for the Fierce Innovations Awards for AMT-101” 5 August 2020 https://markets.businessinsider.com/news/stocks/applied-molecular-transport-named-as-one-of-three-finalists-for-the-fierce-innovations-awards-for-amt-101-1029472285.

[E] Press release GlobeNewsire, 18/02/20. “Applied Molecular Transport Presents Preclinical Data on AMT-101, an Oral GI-selective rhIL-10 at European Crohn’s and Colitis Organisation (ECCO)” 18 February 2020 https://www.globenewswire.com/news-release/2020/02/18/1986228/0/en/Applied-Molecular-Transport-Presents-Preclinical-Data-on-AMT-101-an-Oral-GI-selective-rhIL-10-at-European-Crohn-s-and-Colitis-Organisation-ECCO.html.

[F] Marketwatch website, 15/01/21. AMT is a valued public company with a current market value of >$1.2 B USD: https://www.marketwatch.com/investing/stock/amti.