Natural & Semi-Synthetic Prodrugs as Selective Antibiotics Against Colibactin-Producing E. coli for Colorectal Cancer Prevention

Precise and preventive therapeutic strategy for patients with a high risk of developing colorectal cancer.

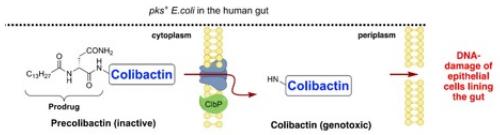

Figure 1
Schematic illustrating the enzymatic processing of colibactin from an inactive ("precolibactin") to active genotoxic form via ClbP cleavage.

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Category
Life Sciences/Biochemicals & Small Molecules
Life Sciences/Therapeutics/Antibacter
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Technology

The Healy Lab at NYU Abu Dhabi and colleagues at the Max Planck Institute have pioneered an innovative method for preventing colibactin-caused colorectal cancer (CRC) by leveraging the enzymatic machinery used by pathologic E. coli for colibactin production to activate natural and semi-synthetic antibiotic prodrugs to selectively kill colibactin-producing *E. coli* strains (e.g., *pks*$^+$ *E. coli*). As illustrated in Figure 1, *pks*$^+$ *E. coli* synthesizes colibactin intracellularly in its inactive form, termed precolibactin. Just prior to its secretion, a specialized peptidase (ClbP) converts precolibactin into its active form, colibactin, to prevent autotoxicity. An analogous prodrug activation mechanism has been described in the biosynthesis of natural antibiotics containing a fatty acid-D-Asn prodrug motif, such as amicoumacin, xenocoumacin, zwittermicin, and rhabdobranin. The innovators have recently discovered that ClbP can similarly convert these compounds from their inactive prodrug form, indicating their application as selective antibiotics for colibactin-producing *E. coli* strains. One such prodrug, pre-xenocoumacin, has been validated *in vitro* for its strain-specific activity in unpublished proof-of-principle experiments. Namely, growth curves showed a significant reduction in ClbP+ *E. coli* colonization following pre-xenocoumacin incubation. Inspired by this finding, the innovators have engineered a collection of semi-synthetic pre-rhabdobranin and pre-xenocoumacin derivatives displaying enhanced

specificity toward ClbP. By fusing the fatty acid-D-Asn prodrug motif to a known antibiotic, these derivatives pave the way for the development of a new class of strain specific compounds for *E. coli* producing colibactin. In summary, the innovators have demonstrated the feasibility of natural and semi-synthetic prodrug antibiotics for CRC prevention by exploiting the unique enzymatic process for colibactin production in *pks*$^+$ *E. coli.*

Development Status

Pre-rhabdobranin and pre-xenocoumacin derivatives are being synthesized in Dr. Healy's lab and preliminary efficacy experiments are being repeated. Once validated, the antibiotic prodrugs will be tested in more complex cellular or organoid-based models of the gut microbiome, before moving to an *in vivo* mouse model.

Background

Excluding skin cancers, colorectal cancer is the third most common cancer diagnosed in the United States, with an estimated 150,000 new cases expected each year. It is also the second leading cause of cancer-related deaths in the US. A majority of cases (60%) are diagnosed after the cancer has metastasized beyond the colon or rectum. *Pks*+ *E. coli* is a strain of commensal bacteria known for producing a genotoxin termed colibactin, which has been linked with the development and progression of conditions such as Irritable Bowel Syndrome (IBS), Inflammatory Bowel Disease (IBD), and colorectal cancer (CRC). *Pks*$^+$ *E. coli* are present in a substantial fraction of individuals (about 20% of healthy individuals, about 40% of patients with inflammatory bowel disease, and about 60% of patients with familial adenomatous polyposis or CRC). When these *E. coli* bacteria colonize the gut they secrete the mutagen colibactin leading to increased risk for CRC. Treatment options for CRC vary depending on the stage of the cancer at time of diagnosis. Typically, surgical resection is the first option, followed by chemotherapy, radiation, targeted therapy, and/or immunotherapy if the cancer has metastasized. However, these treatments often carry significant adverse side effects (e.g., chemotherapy-induced neuropathy, fatigue, nausea, hair loss, and a weakened immune system) and recurrence risk. The 5-year CRC patient survival rates are 91% for localized recurrence, 73% for regional recurrence, and 13% for distant metastatic recurrence ("advanced CRC"). Given the late stage of CRC diagnosis and high mortality rate associated with advanced CRC, there is a pressing unmet need to develop prophylactic therapeutics. The innovative method described herein for preventing CRC by inhibiting the production of the colibactin offers a precision medicine approach for patients colonized with *pks*$^+$ *E. coli*.

Application

CRC prevention and treatment: Prophylactic measure for patients colonized with *pks*$^+$ *E. coli*, including those also with a genetic predisposition to irritable bowel syndrome (IBS), inflammatory bowel disorder (IBD), or CRC.

Advantages

- **Preventive treatment:** Kills *pks*$^+$ *E. coli* before CRC-causing colibactin is produced.
- **Precision medicine:** ClbP-activated prodrug antibiotics selectivity kill ClbP-expressing *pks*$^+$ *E. coli*, limiting off-target effects.
- **Monotherapy or complementary therapy:** The species-selective prodrug antibiotics can be used alone or in combination with surgery, radiation, and chemotherapy.

Intellectual Property

NYU has filed a U.S. provisional patent application covering (1) the method of inhibiting colibactin production with natural and semi synthetic prodrug antibiotics, (2) the use of such

method as a means to prevent and treat CRC, and (3) the compositions of semi-synthetic prodrug antibiotic derivatives.