Development of drug for the chemoprevention and or treatment of HDGC

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Introduction

We have proposed that the loss of E-cadherin due to pathogenic *CDH1* mutations creates vulnerabilities in early-stage T1a gastric cancer cells that can be targeted with drugs. In this project, we have investigated the vulnerability of these cells to histone deacetylases (HDACs) inhibitors, a class of enzymes with multiple roles in cancer development. HDAC inhibitors are the subject of strong research interest internationally, with over 97 clinical trials in numerous cancers currently recruiting worldwide.

Our earlier studies had identified HDAC inhibitors as potential chemoprevention compounds for hereditary diffuse gastric cancer (HDGC) [1]. In this latest research funded by No Stomach for Cancer, we have extended that early work to a wider range of drugs in this class and validated their effect in an advanced pre-clinical model known as 'organoids.' Gastric organoids are 3D cell cultures derived from normal stomach tissue or stomach tumors and better recapitulate *in vivo* conditions than standard lab models (cell lines). We have used genetic engineering techniques to modify mouse stomach organoids to include a *CDH1* mutation, thus generating a novel model of early-stage HDGC. We have now tested several HDAC inhibitors in this and other related models and identified two promising HDAC inhibitors for further development as HDGC chemoprevention drugs.

Results

- Testing of 21 different HDAC inhibitors on breast and stomach cell lines identified Entinostat, Pracinostat, Mocetinostat and Vorinostat as the most effective inhibitors of *CDH1* mutant cells compared to cells with normal E-cadherin activity. These four drugs inhibit multiple different members of the HDAC enzyme family at the same time and were significantly more effective than drugs which were highly specific for any individual HDAC. Figure 1 shows the preferential inhibition of growth of a *CDH1* mutant cell line following a short (2 days) treatment with Entinostat.

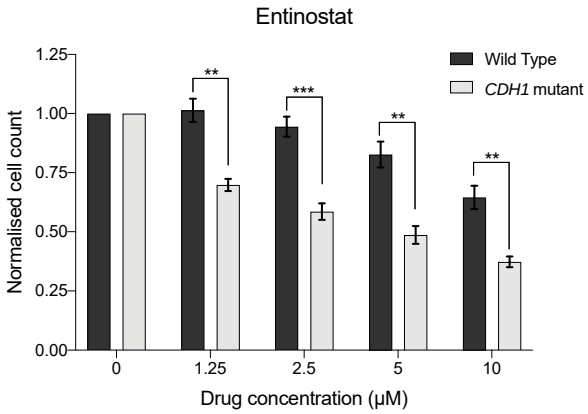

Figure 1: Graph representation of the normalized number of cells after 2 days of treatment with different concentration of Entinostat in normal cells (Wild Type) or mutated for *CDH1*. The * represents the statistical significancy of the difference between normal and mutated cells.

- Testing of Entinostat, Pracinostat, Mocetinostat and Vorinostat in gastric organoids demonstrated that each of Entinostat, Pracinostat and Mocetinostat effectively inhibited the growth of *CDH1* mutant organoids but had little observable effect on the growth of normal organoids (Figure 2). More detailed analysis using fluorescence activated cell sorting (FACS) further showed that the drugs were not only stopping cell division, but also leading to the death of these cells.

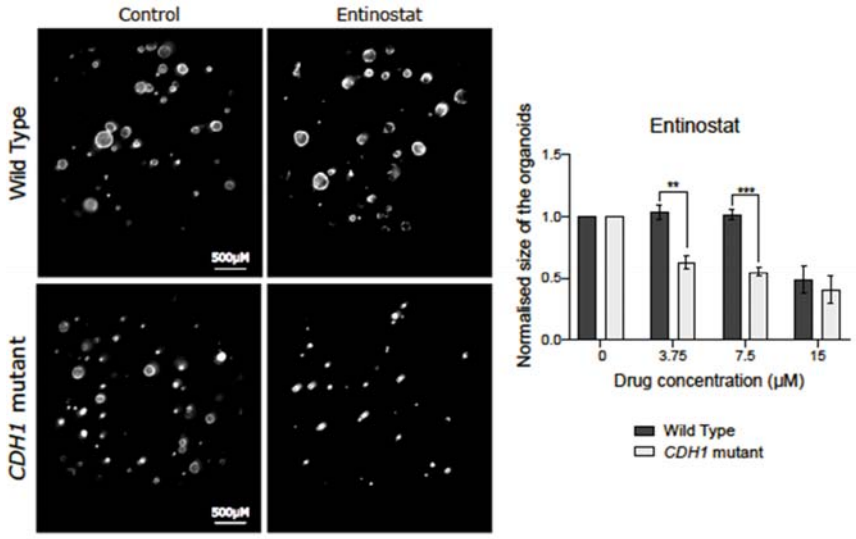

Figure 2: Pictures and graph representation of the normalized size of normal (wild type) and mutant organoids after 2 days of treatment with Entinostat.

- Entinostat and Pracinostat (but not Mocetinostat and Vorinostat) also specifically reduced the growth of *CDH1*-mutant organoids that also had an additional mutation in the p53 gene. P53 is central to cancer development, and mutations in this gene are likely to be an early event in the progression of HDGC. Consequently, we have prioritized the further validation of Entinostat and Pracinostat, with particular emphasis on Entinostat due to its more advanced state of clinical testing (29 ongoing clinical trials) and a good safety profile [2].

- A striking but well-established feature of HDAC inhibitors is their ability to promote E-cadherin's expression (activity) in cells in which it has been downregulated through means other than mutation. In *CDH1* mutation carriers, one of the two copies of *CDH1* is permanently mutated in every cell, but the 2nd copy of the gene is generally active. However, in the few stomach or breast cells in which cancers develop from, this 2nd copy of *CDH1* is 'turned off', often through a mechanism linked to the activity of the HDAC family of enzymes. Therefore, we were interested in seeing the effect of Entinostat on organoids with one mutated copy of *CDH1* and a second copy that has been reversibly downregulated. These organoids showed a disturbed appearance due to the absence of sufficient E-cadherin to hold the cells neatly together (Figure 3). Treatment with Entinostat resulted in reactivation of the 2nd copy of *CDH1*, leading to a normal rounded organoid structure and renewed E-cadherin expression (green below). This result suggests that Entinostat may be useful in preventing the development of early stage T1a lesions and eliminating those early lesions that have already developed.

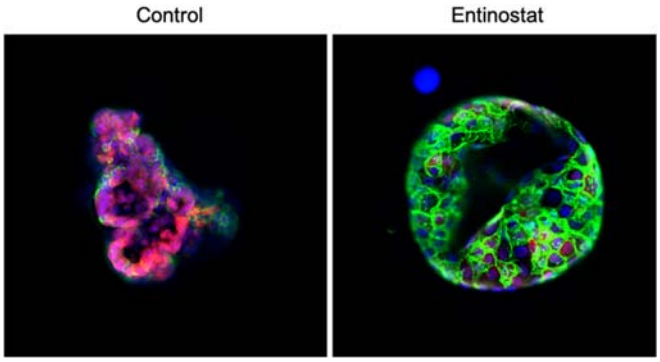

Figure 3 (Left to right): Organoid with one mutant copy of *CDH1* without drug and same type of organoid but treated with the drug. Green: staining of E-cadherin; Red: positive control for the activation of the mutation; Blue: staining of the nucleus of cells.

Summary

We have successfully demonstrated that HDAC inhibitors are able to specifically kill cells lacking E-cadherin and also restore expression of the non-mutant copy of *CDH1* in gastric organoids. This data puts Entinostat amongst our lead candidates for HDGC chemoprevention. We now propose to further validate Entinostat in additional pre-clinical models (including lobular breast cancer models), determine the minimum required dose, and explore drug combinations which may enhance Entinostat's effect at lower dose. Successful completion of this validation will pave the way for a clinical trial within 3-4 years. In a separate initiative, we are also aiming to develop a stomach-specific drug delivery system which we predict would improve drug efficacy and dramatically reduce side effects.

Plan 2021-2022

- Mammary organoids with and without *CDH1* are currently being optimized in our lab. The effect of Entinostat will be tested on this new model of organoids to see if it also preferentially targets breast tissue lacking E-cadherin. These experiments will be important for determining whether Entinostat will also reduce the risk of lobular breast cancer risk in patients with HDGC.
- Different combinations of Entinostat and other candidate drugs will be tested on the gastric and mammary organoids in order to find drug combinations that are synergistic (ie the effect of the drugs together is greater than the sum of the effect of each drug alone). This may allow us to reduce the required drug concentration, making the treatment more tolerable without impacting on efficacy.
- Entinostat (alone or in combination) will be tested on mice with a *CDH1* mutation and the impact of the drug on E-cadherin-negative cells determined using flow cytometry of disassociated stomach cells.
- In the longer term, but out of the scope of this application, we will test entinostat on a new mouse cancer model we have just developed. This mouse carries both *CDH1* and P53 mutations, and appears to develop SRCC in about 3 months. We are still characterizing this mouse, but are confident it should be ready for use in drug testing studies before the end of 2021. Once we have data in this model, we will be in a position to move to human studies and clinical trials.

References

1. Telford BJ, Chen A, Beetham H, Frick J, Brew TP, Gould CM, Single A, Godwin T, Simpson KJ, Guilford P: **Synthetic Lethal Screens Identify Vulnerabilities in GPCR Signaling and Cytoskeletal Organization in E-Cadherin-Deficient Cells**. *Mol Cancer Ther* 2015, **14**(5):1213-1223.
2. Connolly RM, Rudek MA, Piekarz R: **Entinostat: a promising treatment option for patients with advanced breast cancer**. *Future Oncol* 2017, **13**(13):1137-1148.

Budget Request 2021-2022

We request funding to continue this research as outlined above, with the amount requested outlined below:

Salary (Dr. Lyvianne DeCourtye): $US72,000

Consumables: $US 9,000

Total: $US 81,000

Start date: 21st August 2021

Finish date: 20th August 2022