SELECTED OPPORTUNITIES IN IBD

Nanobody Against ELA2A to Treat Inflammatory Bowel Diseases (IBD) or Irritable Bowel Syndrome (IBS) (BIO1508601)

METHOD OF TREATMENT OF GUT INFLAMMATORY DISEASES SUCH AS INFLAMMATORY BOWEL DISEASES (IBD) OR IRRITABLE BOWEL SYNDROME (IBS) (BIO1508601)

Product factsheet

Pre-clinical, in vivo model

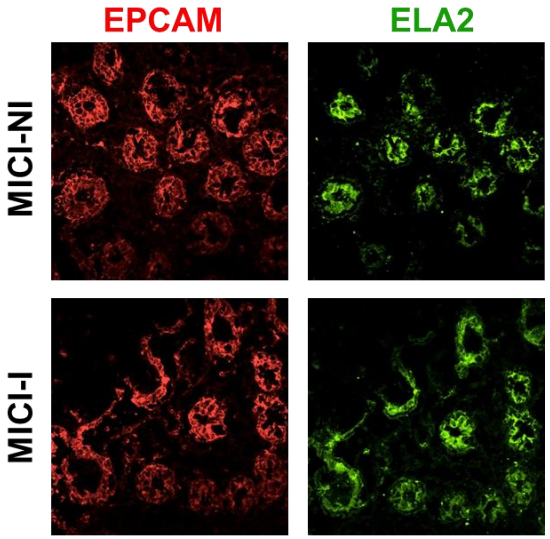
- **Target:**
  - **Elastase 2A** (ELA2A) protease secreted by gut epithelial cells.
- **Application:**
  - Treatment of gut inflammatory diseases (Inflammatory Bowel Diseases (IBD), Irritable Bowel Syndrome (IBS), celiac disease, Crohn's disease, ulcerative colitis) by restoring intestinal barrier function, intestinal homeostasis and mucosa healing.
- **Rationale:**
  - IBD, including Crohn's disease and ulcerative colitis, are characterized by chronic relapsing of intestinal inflammation.
  - Proteases released by infiltrating leukocytes during gut inflammation play a key role in mucosal lesions.
  - Elastase activity is significantly increased both in animal models of colitis and in patients suffering from IBD.
  - The inventors have identified **Elastase 2A** as the predominant protease implicated in pathophysiological pathways of IBD.
- **POC:**
  - Ex vivo: ELA2A is expressed in the epithelium of IBD patients both in inflamed and non-inflamed tissue.
  - In vivo: Overexpression of ELA2A in mice worsens inflammation.
  - In vitro: Overexpression of ELA2A disrupts intestinal barrier and induces secretion of pro-inflammatory cytokines; Inhibition of ELA2A decreases CXCL8 secretion in ELA2A-overexpressing cells.
- **Patent & publication:**
  - METHOD OF TREATMENT OF GUT DISEASES SUCH AS INFLAMMATORY BOWEL DISEASES (IBD) OR IRRITABLE BOWEL SYNDROME (IBS) - WO2017216352
  - Mucosal Immunol. 2021 May;14(3):667-678. doi: 10.1038/s41385-021-00375-w. Epub 2021 Mar 5.

Proof of concept: *ex vivo*

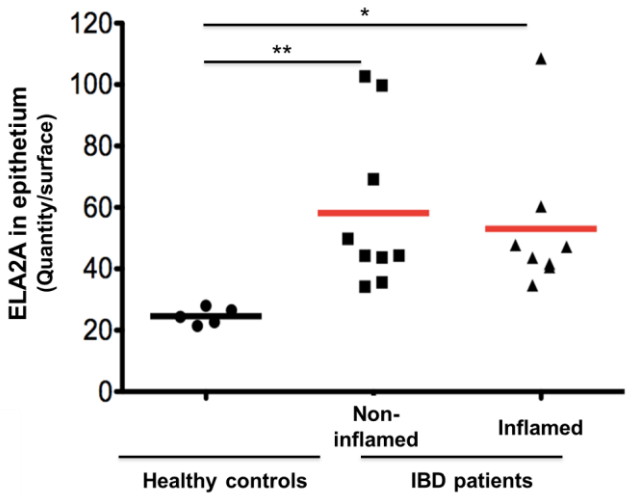
ELA2A expression is upregulated in epithelium from IBD patients

- Localization and quantification of surface ELA2A in epithelium from IBD patients

a.



b.



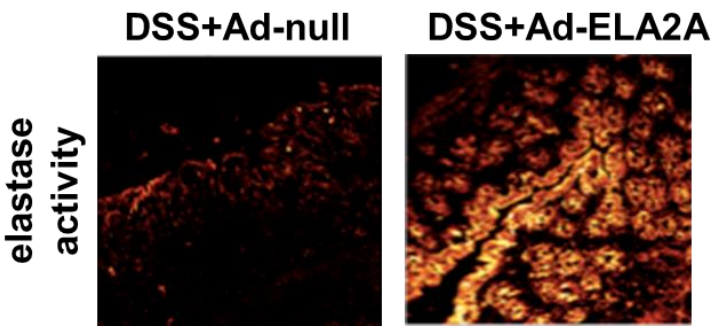
(a) ELA2A immunostaining on cross section of colonic biopsy from non inflammatory (MICI-NI) and inflammatory (MICI-I) areas from IBD patients using anti-ELA2 and anti-EPCAM antibodies, EPCAM being representative of the epithelial compartment. (b) Quantification of ELA2A signal in healthy controls (n=5) versus non-inflamed (MICI-NI) and inflamed (MICI-I) areas from IBD patients (n=9). Data represent the intensity of ELA2A signal per unit of epithelial surface.

Proof of concept: *in vivo*

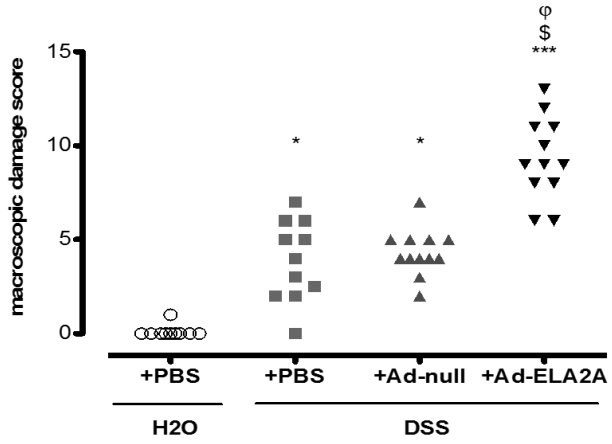
in vivo overexpression of ELA2A worsens inflammation

- Intracolonic administration of ELA2A in a mouse model of DSS-induced colitis

A.



B.



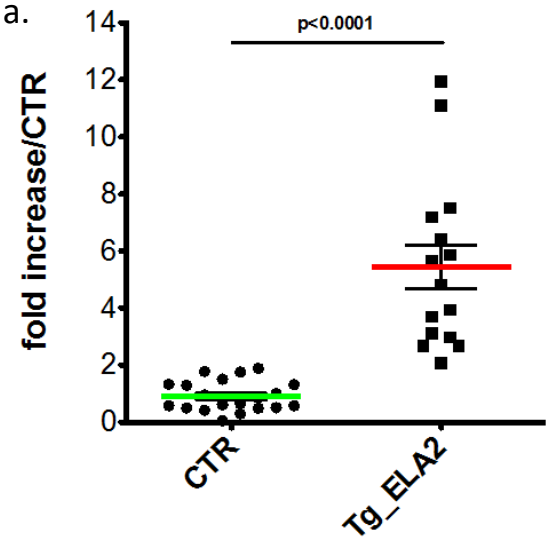
(a) In situ zymography of elastolytic activity in colon from mice receiving Ad-null (Adenovirus-null) or Ad-ELA2A during 2% DSS treatment.
(b) The macroscopic damage score in mouse colon tissue was determined.

Proof of concept: *in vitro*

Over-expression of ELA2A in Caco-2 cells disrupts epithelial barrier function and structure

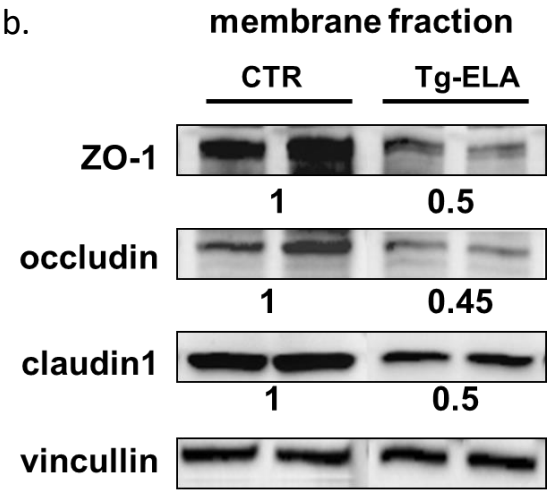
- Loss of intestinal barrier function

a.



- Loss of tight junction proteins

b.



(a) Paracellular permeability measurements in control versus ELA2-overexpressing (Tg-ELA2) Caco-2 cell monolayer. The graph indicates the fold increase in dextran flux of Tg-ELA2A cells compared to control cells.

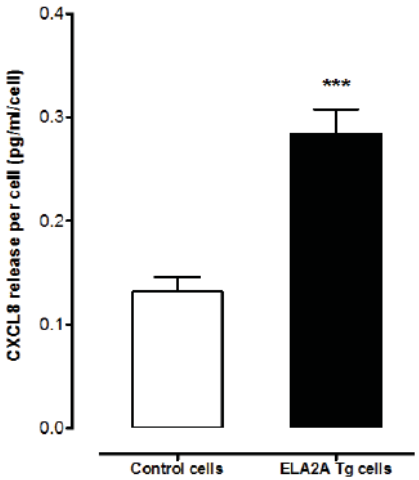
(b) Protein levels of tight junction proteins OCCLUDIN, CLAUDIN-1 and ZO-1 were assessed by Western blot analysis in insoluble and soluble fraction of protein extraction from control and Tg-ELA2A monolayer. Blot are representative of 3 independent experiments with n=3 per group.

Proof of concept: *in vitro*

Inhibition of ELA2A abrogates the amount of CXCL8 secreted by ELA2A-overexpressing intestinal epithelial cells

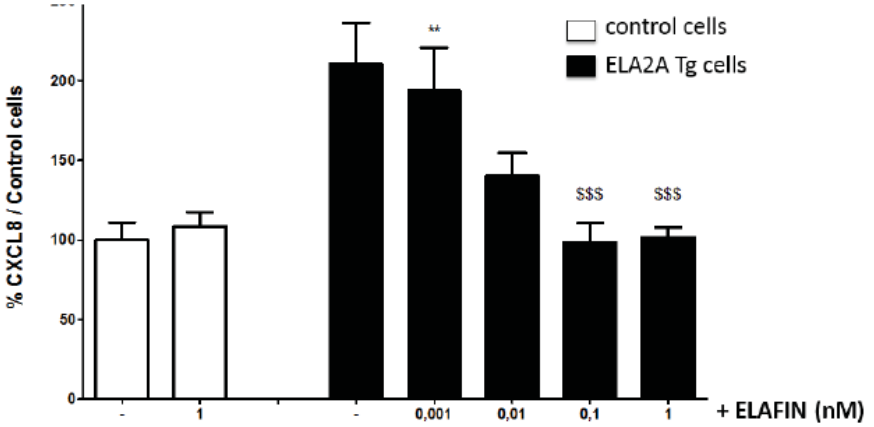
- **Secreted CXCL8 in ELA2A-overexpressing Caco2 cells**

A.



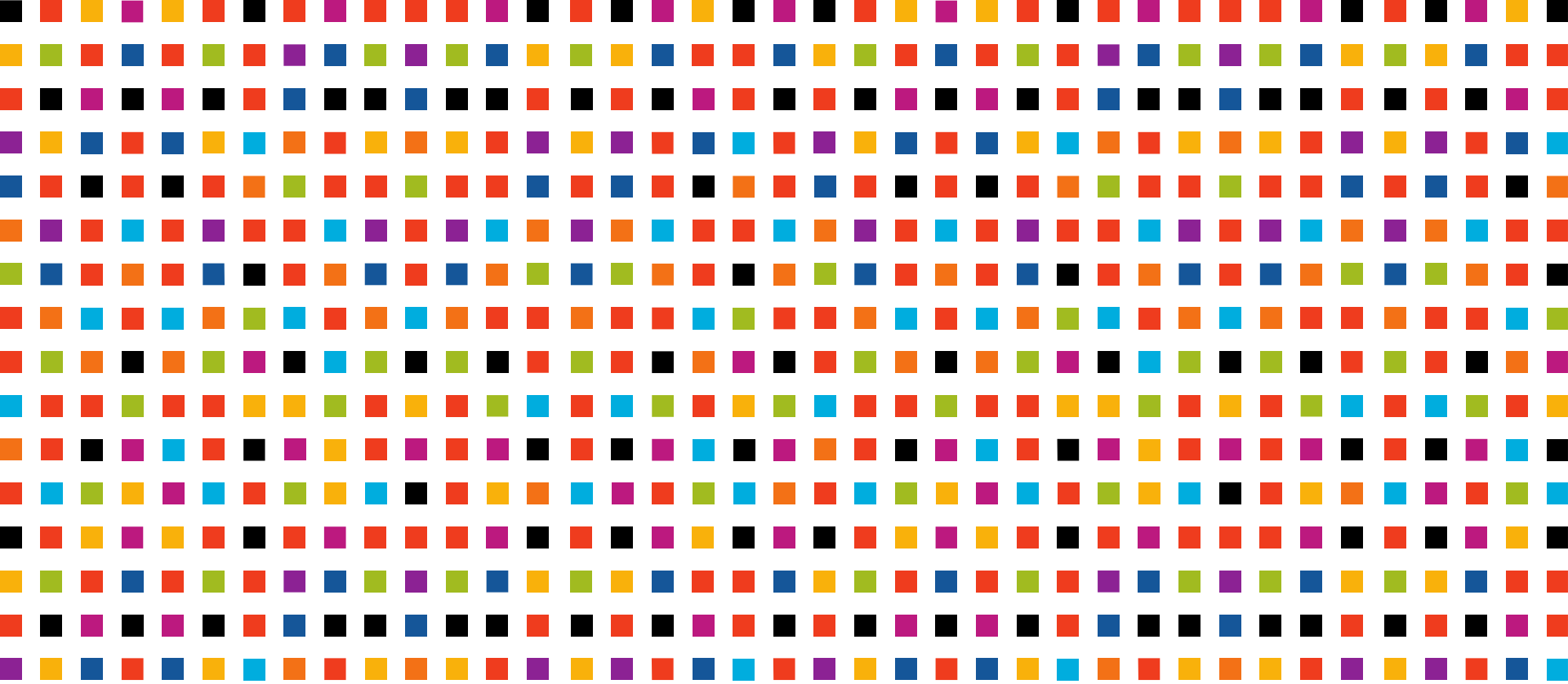
- **Secreted CXCL8 in ELA2A-overexpressing Caco2 cells treated with ELAFIN, a non-specific ELA2A inhibitor**

B.



(a) CXCL8 protein release after 24h of culture, dosed by ELISA in supernatants from wild type intestinal epithelial cells (Caco-2) (control cells) or in supernatants of Caco-2 cells transgenic for overexpressing ELA2A protein.

(b) Percentage of CXCL8 protein release compared to control cells (wild type Caco-2) in wild-type Caco-2 cells (control cells) or in Caco-2 transgenic cells overexpressing ELA2A, exposed or not (-) for 24h to different doses of ELAFIN. CXCL8 protein was dosed in cell culture supernatants by ELISA.



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