



**FOR IMMEDIATE RELEASE**

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University of Tsukuba  
EA Pharma Co., Ltd.  
Eisai Co., Ltd.

**POTENT NEW MECHANISM OF ACTION**  
**FOR TREATMENT OF INFLAMMATORY BOWEL DISEASE REVEALED**  
*ORALLY ACTIVE SMALL MOLECULE WITH ANTI-INFLAMMATORY EFFECT DUE TO*  
*SUPPRESSION OF INFILTRATION BY LEUKOCYTES INTO INFLAMED SITES*

**Key Points of Research**

1. Through research on the small molecule analogue of E6007 which is under clinical development as a treatment for inflammatory bowel disease (IBD)\*<sup>1</sup>, a novel mechanism of action was revealed in which this analogue inhibited the adhesion and infiltration of various leukocytes through the blockade of the interaction between calreticulin (CRT)\*<sup>2</sup> and the leukocyte adhesion molecule integrin\*<sup>3</sup>  $\alpha$  (ITGA) by associating with CRT.
2. When this compound was orally administered to IBD model mice, remarkable anti-inflammatory effects were demonstrated through the suppression of the adhesion and infiltration of leukocytes into the inflamed sites.
3. This novel mechanism of action elucidated is expected to lead to value enhancement and acceleration of the development of E6007 which aims to provide a new remedy for IBD patients.

Professor Fukamizu's research group of Life Science Center for Survival Dynamics (Tsukuba Advanced Research Alliance, TARA), University of Tsukuba, Eisai Co., Ltd. (Eisai) and its gastrointestinal business subsidiary EA Pharma Co., Ltd. (EA Pharma) have revealed a mechanism in which an analogue (ER-464195-01) of Eisai's in-house discovered E6007 inhibits integrin activation by dissociating interaction between calreticulin (CRT) and integrin  $\alpha$  4 (ITGA4), suppressing adhesion and infiltration of leukocytes overall. This mechanism was revealed through the use of a biomarker developed by University of Tsukuba that visualizes protein-protein interaction. E6007 is currently under investigation by EA Pharma in ongoing studies as a treatment for IBD.

IBD refers to a group of intractable diseases which lead to repeated inflammation in the mucus of the large or small intestines, resulting from an unidentifiable cause, and is generally classified as ulcerative colitis (UC) or Crohn's disease (CD). This joint industry-academia research group found that increased CRT-ITGA interaction influences cell adhesion and infiltration of leukocytes at the inflamed sites in large intestines affected by UC, and discovered that ER-464195-01 suppresses this protein-protein interaction using cultured cell line. Furthermore, it was demonstrated that oral administration of ER-464195-01 in IBD model mice induces anti-inflammatory effects through the suppression of infiltration by leukocytes into the inflamed sites. In addition, from transcriptome analysis\*<sup>4</sup> of the colonic structure of IBD model mice, the genetic information for programming the healthy-inflammation-recovery process was made clear.

With the continuing increase in the number of IBD patients in recent years, there is a need for an orally active treatment with a novel mechanism of action that has superior efficacy and makes it easy to comply with treatment. It is hoped that the results of this joint research on this point will lead to the provision of a new option for treating IBD.

The results of this research were disclosed in the electronic version of *Nature Communications*, dated May 17, 2018.

This research is being conducted under the Japan Science and Technology Agency's Newly extended Technology transfer Program (NexTEP) for "Treating inflammatory bowel disease using small molecules and biomarkers" (Principal Investigator: Professor Akiyoshi Fukamizu, period of research: 2014 – 2020)

[https://www.jst.go.jp/jitsuyoka/topics/saitaku\\_201403.html](https://www.jst.go.jp/jitsuyoka/topics/saitaku_201403.html) (Japanese only)

### Background to Research

IBD refers to a group of intractable diseases which lead to repeated inflammation in the mucus of the large or small intestines, resulting from an unidentifiable cause (Fig. 1). According to a survey by the Japan Intractable Diseases Information Center (<http://www.nanbyou.or.jp> (Japanese only)) in 2013, IBD had the greatest incidence among young people (in their 20's and 30's), and among IBD, it is reported that the number of patients with UC and CD was 166,060 and 39,799, respectively, which means IBD is the intractable disease with the greatest number of patients. Currently, in addition to observing infiltration of various leukocytes into the inflamed sites of IBD, ITGA4 is strongly expressed, and therefore treatment consists of leukocyte apheresis therapy or monoclonal antibody treatment targeting ITGA4. However, with the number of IBD patients increasing year after year, development of a small molecule treatment that makes it easy to comply with treatment and has superior efficacy is highly anticipated.

Eisai and EA Pharma are engaged in development of the small molecule compound E6007, as a new IBD treatment with a mechanism of action for inhibiting integrin activity (Reference document 1), and using an analogue of this E6007 (ER-464195-01), the joint research group utilized a biomarker technology developed by University of Tsukuba which visualizes protein-protein interaction in an attempt to reveal the mechanism expressing anti-inflammatory effects.

### Outline and Results of Research

CRT, a molecular chaperone<sup>\*5</sup>, binds to integrin subunits and promotes cell adhesion (Reference document 2). Using a biomarker to investigate CRT and ITGA4 interaction in the colonic structure of UC patients, the joint research group found that interaction at inflamed sites significantly increases compared to healthy areas. Given that dissociation of CRT and ITGA4 interaction could suppress activation of leukocytes, high-throughput screening assay was conducted on Eisai's compound library. Consequently, ER-464195-01 was identified as a small molecule that suppresses leukocyte adhesion by binding to CRT and inhibiting CRT-ITGA4 interaction (Fig. 2).

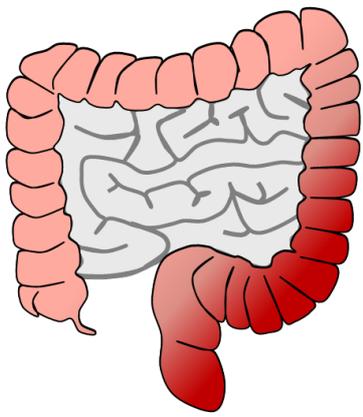
When mice were orally administered ER-464195-01 as a prophylactic treatment, in addition to exhibiting remarkable anti-inflammatory effects in dextran sodium sulfate (DSS)-induced colitis, from a comprehensive analysis of gene expression using RNA sequencing found that inflammatory cytokines and expression of inflammatory response signaling factors were significantly suppressed. Furthermore, when ER-464195-01 was therapeutically administered to mice with DSS-induced colitis, it was interesting that mucosal barrier injury<sup>\*6</sup> as well as infiltration of inflamed cells was remarkably improved. This novel mechanism of action revealed through this joint research is expected to lead to the provision of a new IBD treatment option.

### Future Development

ER-464195-01, which possesses this mechanism of action revealed through this joint research, is an analogue of E6007, and it is believed that E6007 also has the same novel mechanism of action. Therefore, our finding is expected to lead to value enhancement and acceleration of the development of E6007 which aims to provide a new treatment method for IBD patients.

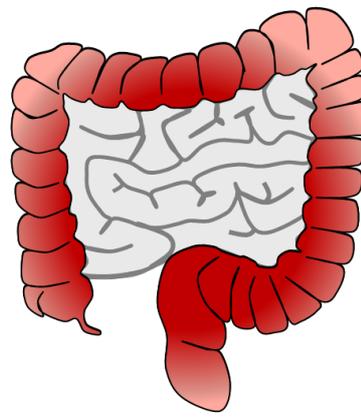
### Reference figures

## Inflammatory Bowel Disease (IBD)



### Ulcerative Colitis (UC)

A disease of unidentified cause which leads to inflammation in the colonic mucosa and successive ulcers and abscesses.



### Crohn's Disease (CD)

Centered on the small and large intestines, inflammation occurs in the alimentary canal from the mouth to the anus. A disease of unidentified cause which leads to ulcers and granuloma.

Figure 1) IBD is a group of diseases which cause inflammation and ulcers in the alimentary canal, as well as symptoms such as bleeding, diarrhea, weight loss and fever. Narrowly defined as UC or CD.

Inflammation process: leukocytes which have infiltrated attack the cells of the intestinal mucosa

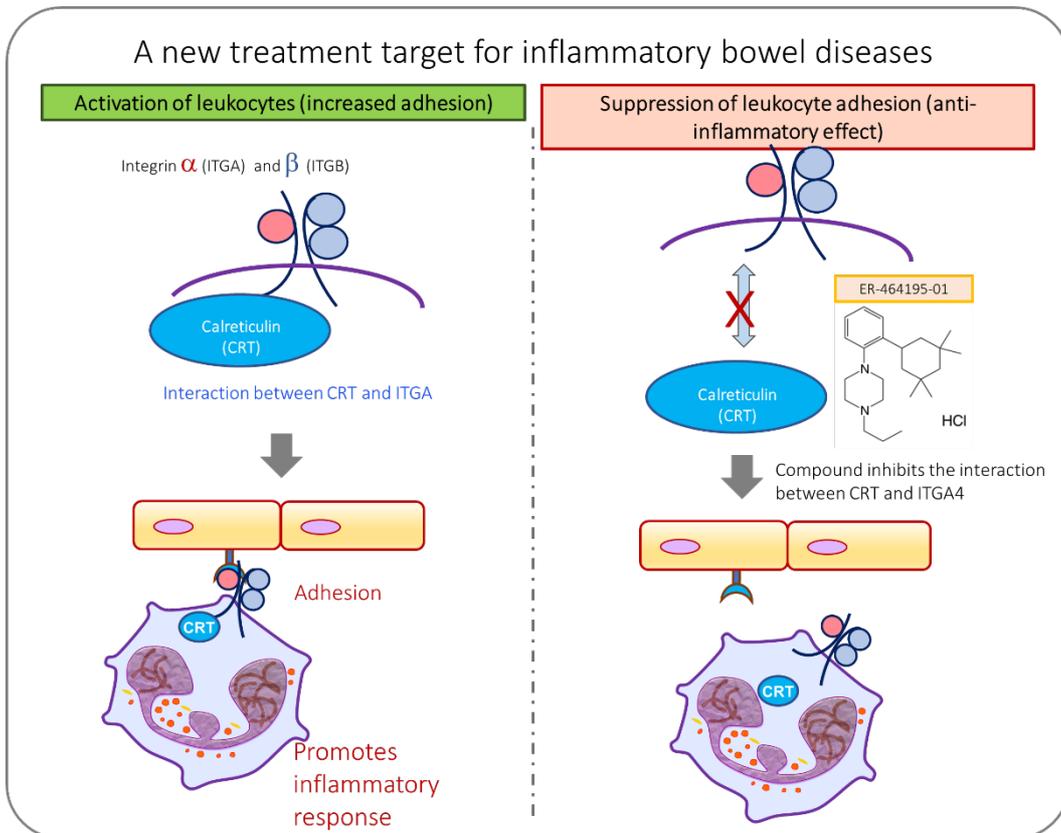
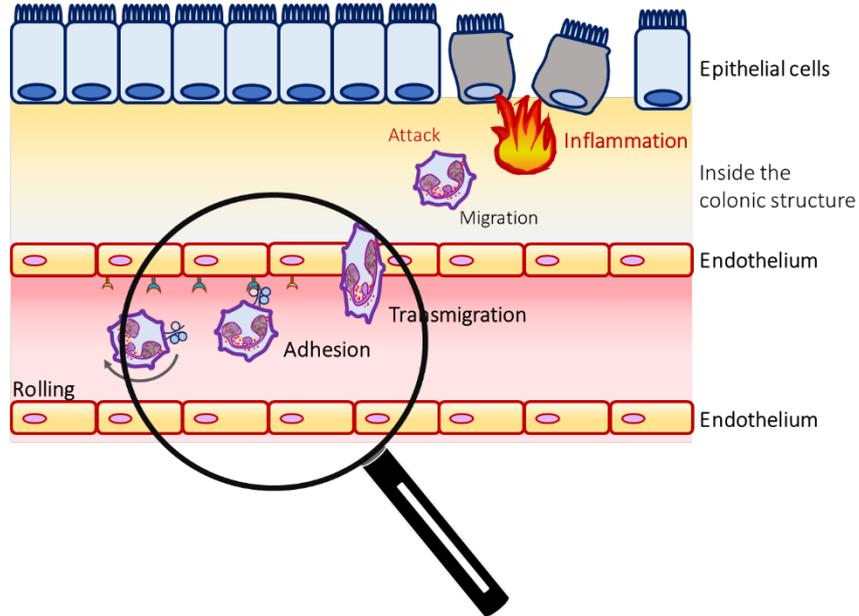


Figure 2) The small molecule compound being developed through this research (ER-464195-01) binds to CRT, and by inhibiting CRT-ITGA interaction, suppresses adhesion of leukocytes to endothelial cells which occurs at the initial stage of the the inflammatory response.

## Glossary of Terms

### \*1 Inflammatory Bowel Disease (IBD)

Referring to ulcerative colitis and Crohn's disease, IBD leads to ulcers and inflammation in the mucosal membrane of the large intestine, and causes symptoms such as bleeding, diarrhea, weight loss and fever, without any identifiable cause.

### \*2 Calreticulin (CRT)

CRT is a molecular chaperone binding to calcium ions ( $\text{Ca}^{2+}$ ) that are located in the endoplasmic reticulum. It is a protein with diverse biological functions within cells including cell adhesion, homeostasis of calcium ions, information signaling between cells, gene expression, and glycoprotein synthesis.

### \*3 Integrin

Integrins are adhesion molecules comprised of heterodimers for  $\alpha$  and  $\beta$  subunits. From the combination of 18 types of  $\alpha$  subunits and 8 types of  $\beta$  subunit, there are 24 different types of integrins known. CRT binds to amino acid sequences within cells of integrin  $\alpha$  subunits.

### \*4 Transcriptome analysis

The transcriptome is the set of all mRNA (messenger RNA) within a cell. In recent years, with the development of next generation sequencing technology, it has become possible to rapidly analyze gene sequences and expression. Among these developments, it has also become possible to rapidly analyze a large volume of RNA sequence information through the development of RNA sequencing methods.

### \*5 Molecular chaperon

Within cells, proteins are folded into their structural arrangement. A molecular chaperon is a protein that assists with this folding so that various proteins can acquire functionality.

### \*6 Mucosal barrier injury

Intestinal mucosal barriers are made of intestinal epithelium, and their function is to block pathogens, toxic substances, viruses ingested through the mouth and other substances from being absorbed into the body through the intestinal wall. If the intestinal membrane experiences severe inflammation, the mucosal membrane will be damaged, causing injury to the mucosal barrier.

## Reference documents

- 1) Press release issued by Eisai Co., Ltd. and University of Tsukuba, dated May 7, 2014.  
<https://www.eisai.com/news/news201420.html>
- 2) Coppelino MG, et al. *Nature* 386,843-847,1997

## Published Paper

Title: Calreticulin and integrin alpha dissociation induces anti-inflammatory programming in animal models of inflammatory bowel disease.

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