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Optimizing duodenal tissue acquisition for mechanistic studies of duodenal ablation in type 2 diabetes

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Trial registration: NCT06333093, ClinicalTrials.gov (http://www.clinicaltrials.gov/), Open-label study and double-blind sham-controlled trial.

Abstract:
Introduction
Histological analysis of regular duodenal biopsies to study morphologic changes after duodenal ablation for type 2 diabetes (T2D) and metabolic syndrome is hampered by the variability in tissue orientation. We designed an optimized tissue acquisition protocol using duodenal cold snare resections to create tissue microarrays (TMAs) and to allow for single-cell RNA sequencing (scRNA-seq).
Methods
The open-label DIRECT study included patients undergoing an upper gastrointestinal interventional endoscopy for non-duodenal indications. All underwent 1-2 single-piece duodenal cold snare resections. Endpoints were safety, adequate histological orientation of specimen and TMA, and tissue dissociation quality for scRNA-seq. The optimized tissue acquisition protocol was validated in a duodenal ablation study, EMINENT-2.
Results
In DIRECT, nine patients were included in whom a total of 16 cold snare resections were obtained. No (severe) adverse events ([S]AEs) occurred. 80% of specimens and corresponding TMAs showed optimal tissue orientation. Further improvement was achieved by reducing tissue damage during endoscopic retrieval and improving histologic evaluation by eliminating ink use and pinning the tissue on cork. High-quality tissue dissociation scores for scRNA-seq were achieved in 13/18 (72%) samples. In EMINENT-2, 38 cold snares were obtained without (S)AEs, histopathologic analysis showed good orientation in all samples and dissociation scores for scRNA-seq were qualified in 35/38 (92%) samples.
Discussion
Duodenal cold snare resection is safe and can provide high-quality tissue for optimally oriented TMAs and high-quality tissue dissociation scores for scRNA-seq;Clinicaltrials.gov, NCT06333093, NCT05984238. This approach will allow mechanistic studies into the effects of duodenal ablation on metabolic syndrome and T2D.

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1 **INTRODUCTION**

2 Endoscopic duodenal ablation for the treatment of type 2 diabetes (T2D) has emerged in
3 recent years. Several techniques, including the Revita duodenal mucosal resurfacing (DMR)
4 and recellularization via electroporation therapy (ReCET), have been shown in a number of
5 clinical trials to have a beneficial effect on glycemic control and improvement of metabolic
6 parameters in patients with T2D.[1, 2, 3] The hypothesis is that duodenal ablation improves
7 insulin sensitivity. Mechanistic assessments have looked at incretins, bile acids and the gut
8 microbiome.[4, 5, 6, 7] However, the mechanism of action is still largely unknown.

9 The endoscopic appearance and histology of biopsy samples taken before and after DMR
10 and ReCET suggest a normal duodenal mucosa. However, histomorphologic changes of the
11 duodenal mucosa after the procedures have not been extensively studied. This is partly due
12 to the limited scope for obtaining duodenal biopsies in most human studies, due to
13 concerns about influencing the clinical course after ablation in any way. The INSPIRE
14 study[2] (EudraCT 2017-00349-30) was the first duodenal ablation study in which duodenal
15 biopsies were taken before and after DMR, yet the study was hampered by a significant
16 variability in tissue orientation between biopsies, even for biopsies that were taken in the
17 same endoscopic procedure under identical conditions. This variability is probably caused by
18 the tangential orientation of the biopsy forceps onto the tissue, the immediate curling of
19 the tissue upon immersion in formalin, the random orientation of the specimens upon
20 embedding of the tissue in paraffin, and the subsequent tangential sectioning of the paraffin
21 blocks (Figure 1). As a result, a quantitative assessment of duodenal mucosa and
22 submucosa, with measurements such as villus length and crypt depth, is overwhelmed by
23 the background variability in tissue orientation. The above challenges persisted during the

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24 EMINENT study (International Clinical Trials Registry Platform, NL9482), an open-label study
25 of ReCET in patients with T2D.[1]

26 As the evidence on safety, feasibility and efficacy of duodenal ablation techniques continues
27 to grow, understanding how these techniques affect glycemic and metabolic control has
28 become even more important. For such mechanistic studies, optimal tissue acquisition
29 before and after ablation is a prerequisite, especially since our group has initiated two
30 sham-controlled randomized duodenal ablation trials (EMINENT-2 study [NCT05984238] and
31 REMIND study [NCT06092476]), in which, for the first time, mechanistic studies have been
32 incorporated. Both studies aim to evaluate changes before and after duodenal ablation
33 compared to a sham procedure using single cell ribonucleid acid sequencing (scRNA-seq)
34 and optimally orientated tissue microarrays (TMAs).

35 In recent years, cold snare polypectomy has become common practice for the removal of
36 sessile polyps in the colon.[8] Compared with standard electrocoagulation polypectomy, this
37 procedure is safer, easier, and faster.[9, 10] A cohort study indicates that larger piecemeal
38 cold snare resections are feasible in the colorectum with a negligible risk of post-resection
39 bleeding and perforation.[11] The technique has subsequently been used in the duodenum
40 for the removal of sessile polyps and piecemeal resections of larger, benign-looking flat
41 polyps.[12, 13]

42 In the INSPIRE study, we obtained 12 biopsies before and after DMR in all patients. Based on
43 the safety profile of cold snare resections, we hypothesized that performing one to two cold
44 snare resections would be as safe as the extensive biopsy protocol of the INSPIRE study, and
45 would provide more suitable material for scRNA-seq and would allow for optimally
46 orientated TMAs.

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47 In preparation for the two aforementioned sham-controlled randomized trials, we

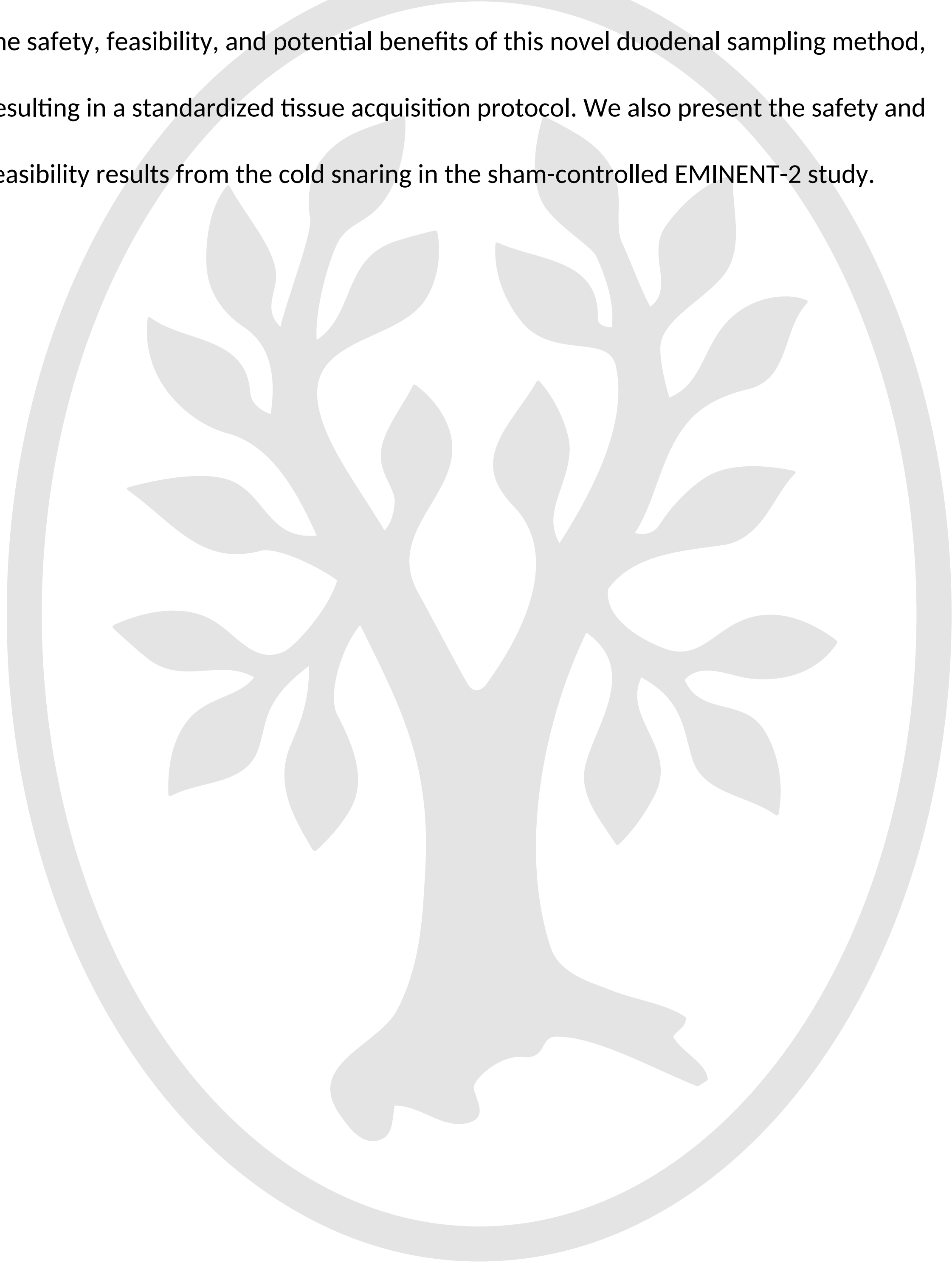
48 developed and tested a cold snare-based duodenal tissue acquisition protocol in an

49 independent cohort. This article systematically outlines our approach, placing emphasis on

50 the safety, feasibility, and potential benefits of this novel duodenal sampling method,

51 resulting in a standardized tissue acquisition protocol. We also present the safety and

52 feasibility results from the cold snaring in the sham-controlled EMINENT-2 study.



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53 **METHODS**

54 *Initial endoscopic cold snare resection technique*

55 All cold snare resections were obtained by a single endoscopist (JB) who also performs all
56 endoscopic duodenal ablation procedures in the Netherlands. A pediatric colonoscope (PCF-
57 H190TL; Olympus Corporation, Tokio, Japan)[14] was used in order to reach the horizontal
58 part of the duodenum. Saline (sodium chloride 0.9%) stained with methylene blue
59 (methylthioniniumchloride 5mg/ml) was drawn into 10 ml syringes. A 23-gauge injection
60 therapy needle (Interject; Boston Scientific, Marlborough, MA, USA)[15] was advanced
61 through the working channel of the scope, and directed to the selected site in the
62 duodenum. Subsequent to submucosal needle insertion, 3-5 ml of 'blue saline' was injected.
63 A 15 mm cold snare (Diamond Cut; Micro-Tech Endoscopy, Nanjing, China)[16] was then
64 advanced into the duodenum, positioned around the elevated duodenal mucosa, and closed
65 for cold snare cutting without forcefully pulling the snare back in the scope. Closure of the
66 mucosal defect was secured by placing 1-2 hemoclips (Resolution 360 Clip; Boston Scientific,
67 Marlborough, MA, USA)[17]. The excised tissue was aspirated through the endoscope's
68 working channel and collected in a collection box.[18] A small pea-sized piece was cut from
69 the specimen and rinsed for three times with phosphate-buffered saline, and placed in a
70 tube containing sCelLiVE Tissue Preservation Buffer (Singleron Biotechnologies, Cologne,
71 Germany). The tissue preservation buffer stimulates the physiological environment and
72 ensures high cell viability over a 72-hour time period. The tissue-filled tube was sent to
73 Singleron Biotechnologies for tissue dissociation using the Singleron PythoN Automated
74 Tissue Dissociation System. A detailed description of the tissue dissociation process at
75 Singleron Biotechnologies can be found in Supplementary Material A. The remaining tissue

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76 (approximately 10 millimeters in length) was directly placed into a cassette with an open-
77 celled foam sponge pad[19], and manually spread out by one of the investigators. The
78 cassette was then immersed in 4% buffered formalin for 24-48 hours and subsequently
79 embedded in paraffin, resulting in a formalin fixed paraffin embedded (FFPE) block. The
80 samples were embedded on edge, with the mucosal side facing sideways, to allow cutting of
81 the specimen perpendicular to the mucosal surface, creating a full mucosal thickness
82 specimen with optimal tissue orientation. Hematoxylin and eosin (H&E) stained slides were
83 obtained and evaluated by an expert pathologist, who identified the part where the slide
84 portrayed the tissue most accurately. Next, a 2.5 mm core was punched out from these
85 sections, which were used to make a TMA by Applied Biological Materials Inc. (Richmond,
86 Canada).

87 *DIRECT study: design, participants, and endpoints*

88 The open-label DIRECT study was initiated to test the envisioned tissue sampling and tissue
89 handling protocol outside clinical duodenal ablation studies. This study was conducted at
90 the Amsterdam UMC between January 2023 and May 2023. The study was approved by the
91 medical ethics committee of the Amsterdam University Medical Centers (Amsterdam UMC)
92 and registered as NCT06333093 at Clinicaltrials.gov.

93 Patients were eligible if they were scheduled for an upper gastric or esophageal
94 interventional endoscopy under deep sedation with propofol at the Amsterdam UMC.
95 Exclusion criteria included a history of gastrointestinal surgery affecting gastrointestinal
96 anatomy and impeding endoscopic access to the duodenum (e.g. Roux-en-Y gastric bypass),
97 and a history of duodenal inflammatory diseases (e.g. Crohn's disease and celiac disease).
98 Written informed consent was obtained from all patients prior to the endoscopy.

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99 The primary study endpoints were safety and feasibility. The primary safety endpoint was
100 the incidence of serious adverse events (SAEs) after cold snare resections. Feasibility was
101 evaluated based on the ability to acquire adequate histological samples, including
102 endoscopic acquisition of the duodenal tissue, and the fixation, slicing, and staining of the
103 samples. Secondary endpoints included the ability to obtain adequate orientation in the
104 tissue slides where adequate assessment of villus and crypt length, thickness of the different
105 tissue layers, and cell densities would be possible at the pathologist's discretion, the
106 feasibility of creating a TMA, and tissue dissociation quality assessment results from
107 Singleron Biotechnologies as a quality measure for future scRNA-seq. Tissue dissociation
108 quality control results were based on the standard quality criteria from Singleron
109 Biotechnologies as shown in Table 1.

110 *Method evaluation and interim analysis*

111 During the course of the DIRECT study, several optimizations on the initial endoscopic cold
112 snare resection technique were implemented. An interim analysis was conducted in
113 collaboration with the involved pathologist (EANB) after obtaining and processing four
114 tissue resection specimens. After each additional analysis, adjustments were made to
115 improve tissue quality in consultation with the involved endoscopist. The main
116 modifications are outlined in the following section.

117 *Implemented optimization steps for endoscopic cold snare resections and tissue handling*

118 1. Minimize tissue curling using pinning on cork
119 Persisted tissue curling at the edges of the tissue samples was observed when using
120 cassettes with sponge foam pads. Inspired by the preparation of endoscopic submucosal
121 dissection (ESD) specimens, tissue samples were pinned on cork. Careful stretching of the

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122 tissue, without overstretching, was performed using pins at the lateral sides of the

123 specimens.[20]

124 2. Mitigate tissue damage using a basket for endoscopic retrieval

125 Aspiration of resected tissue specimen through the working channel of the endoscope, as

126 was the general approach to retrieve cold snare resection specimens for other indications in

127 our unit, resulted in significant tissue damage and fragmentation. To address this, the

128 resected specimens were delicately captured using a 3 cm retrieval basket (Roth Net, Steris,

129 Mentor, OH, USA)[21] without complete closure of the net (Figure 2). The net, along with

130 the tissue and the endoscope, were subsequently extracted from the patient. Initially,

131 wooden sticks were used to remove tissue from the biopsy collector, followed by manual

132 spreading the tissue on cork. This was replaced by removing the specimens from the net by

133 a pair of tweezers, placing them on cork, and carefully spreading them using pins.

134 3. Optimize pathology assessment by refraining from using ink

135 We eliminated the routine application of Indian ink at the deeper margin of the resection.

136 We found this to be a disturbing factor in histopathologic analysis. Ink usually facilitates the

137 assessment of whether the specimen has clean, i.e. non-neoplastic resection margins.

138 However, this is not applicable for our research purposes.

139 *EMINENT-2 study: design, participants and endpoints*

140 The optimized protocol resulting from the initial DIRECT-study was subsequently validated in

141 our sham-controlled randomized trial, the EMINENT-2 study, which was approved by the

142 medical ethics committee of the Amsterdam UMC. Enrollment began in July 2023 and is still

143 ongoing. Written informed consent was obtained from all patients.

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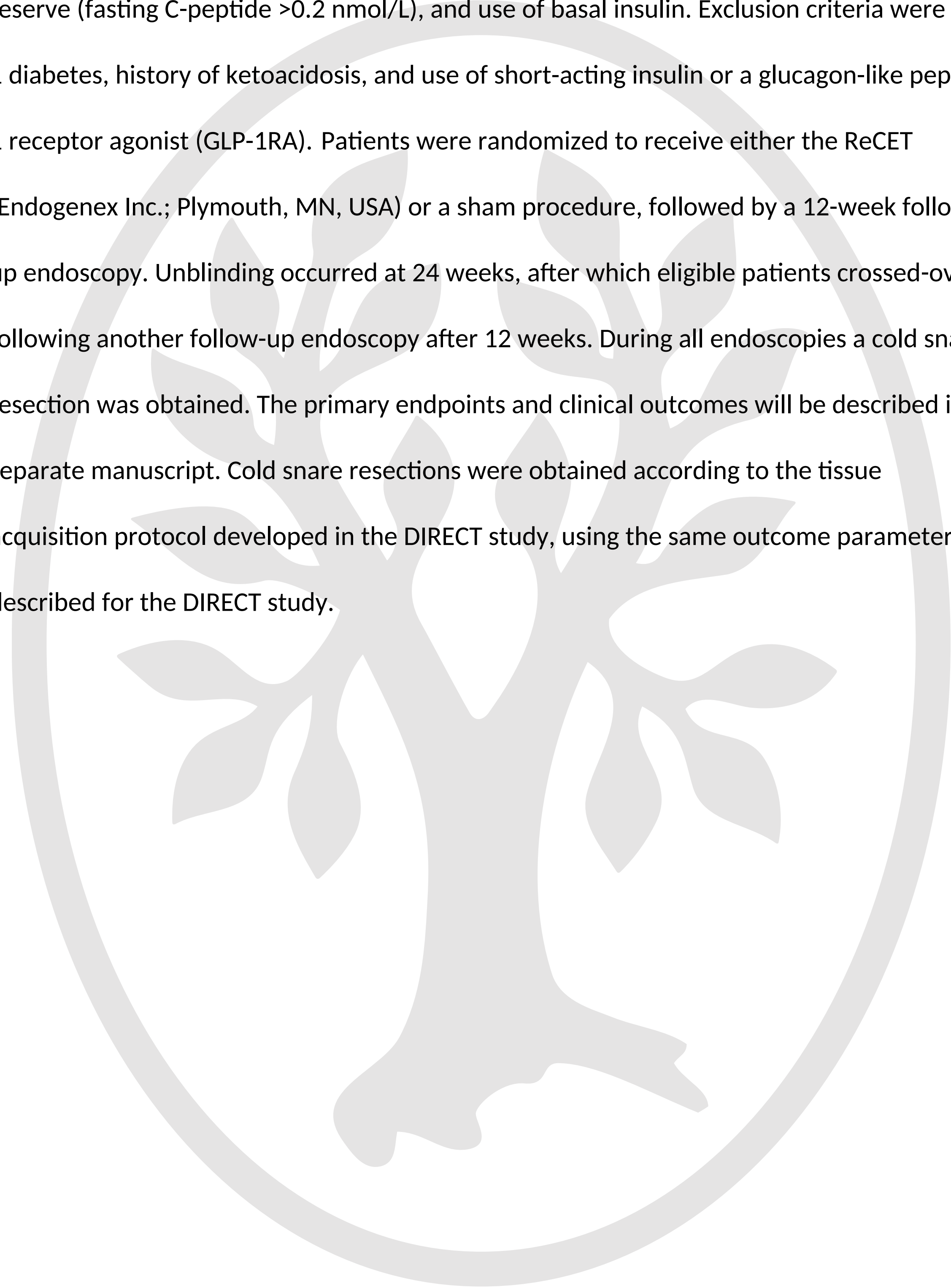
144 Inclusion criteria were T2D, age between 28 and 75 years, a body mass index (BMI) of 24 to
145 40 kg/m², a maximum hemoglobin A1c (HbA1c) of 8.0% (64 mmol/mol), adequate beta-cell
146 reserve (fasting C-peptide >0.2 nmol/L), and use of basal insulin. Exclusion criteria were type
147 1 diabetes, history of ketoacidosis, and use of short-acting insulin or a glucagon-like peptide-
148 1 receptor agonist (GLP-1RA). Patients were randomized to receive either the ReCET
149 (Endogenex Inc.; Plymouth, MN, USA) or a sham procedure, followed by a 12-week follow-
150 up endoscopy. Unblinding occurred at 24 weeks, after which eligible patients crossed-over,
151 following another follow-up endoscopy after 12 weeks. During all endoscopies a cold snare
152 resection was obtained. The primary endpoints and clinical outcomes will be described in a
153 separate manuscript. Cold snare resections were obtained according to the tissue
154 acquisition protocol developed in the DIRECT study, using the same outcome parameters as
155 described for the DIRECT study.



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156 **RESULTS**

157 *DIRECT study: baseline characteristics and primary endpoints*

158 Nine patients were included with a median age of 65 years (IQR, 60 – 71) and median body
159 mass index of 22.7 kg/m^2 (IQR, 21.3 – 25.9). Two cold snare resections were obtained from
160 seven patients and one cold snare resection from two patients, totaling 16 samples. No
161 post-procedural SAEs or AEs were observed.

162 *DIRECT study: secondary endpoints*

163 Histopathologic assessments were performed after every 4 tissue resections. After obtaining
164 twelve samples the pathologist was satisfied with the orientation of the samples. The tissue
165 could be adequately assessed, at least 80% of the specimen cuts showed optimal
166 orientation and allowed reliable measurements of the length of villi and crypts, thickness of
167 the mucosal and submucosal layers, and cell density. The FFPE samples of tissue also
168 enabled creating well-oriented TMAs (Figure 3 and Figure 4).

169 Eighteen pea-sized fresh tissue samples were obtained from eight patients, all were sent to
170 Singleron Biotechnologies. Thirteen samples were graded as A (72% of total), one sample
171 was graded B (6% of total) and two samples were graded C (11% of total). Two samples
172 could not be assessed due to a delayed transport. After several consecutive A graded
173 samples, Singleron Biotechnologies indicated that further optimization was not necessary.
174 For a detailed overview of the quality scores per sample, see Supplementary Table 1.

175 *Standardized method to obtain endoscopic large cold snare duodenal mucosectomies*

176 The findings from the DIRECT-study resulted in the following advised method of acquiring
177 cold snare resections as portrayed endoscopically in Figure 2, and graphically in Figure 5.

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178 1. Advance the pediatric colonoscope[22] into the duodenum and select the site for
179 obtaining the mucosectomy.
180 2. Insert the injection needle[15] through the working channel of the endoscope and
181 inject a sufficient amount of blue saline into the submucosa, causing tissue elevation
182 and bluish discoloration (Figure 5A).
183 3. Advance the 15-mm cold snare[16] through the scope, open the snare and position it
184 over the elevated tissue, and close the snare to excise the tissue using standard cold
185 snare polypectomy technique (Figure 5B).
186 4. Replace the cold snare with a Roth net[21], gently move the fully opened net over
187 the resection site in order to separate the specimen from the surrounding mucosa.
188 Minimal closure of the net to avoid any mechanical damage to the specimen. Close
189 the net over the tissue sample without excessive compression. Withdraw the net
190 with the entrapped tissue and the endoscope from the patient (Figure 5C).
191 5. Reintroduce the endoscope and Inspect the wound bed after resection. If necessary,
192 place an (prophylactic) endoscopic clip.[23]
193 6. Carefully transfer the tissue from the opened net to a cork using tweezers and
194 secure the tissue with one pin (Figure 5D). The luminal (or villous) side should be the
195 upper side, the submucosa on the cork. Use a 10x hand held magnifying glass to
196 recognize the villous side.
197 7. If desired, cut a pea-size tissue sample for single-cell RNA sequencing (optional)
198 (Figure 5D).
199 8. Use additional pins to spread the (remaining) tissue without overstretching (Figure
200 5E), and place the pinned tissue upside down in 4% buffered formalin.

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201 9. Have the tissue embedded on edge (Figure 5F-G). If the tissue is too big to be
202 embedded en-bloc, slice it into two or more fragments, always perpendicular to the
203 surface.
204 10. Create a H&E stained slide of each FFPE block (Figure 5H-J), identify the most
205 representative area of the mucosectomy and have it marked (Figure 5K-L).
206 11. Extract an appropriate core from the embedded specimen using a microarrayer and
207 the markings on the H&E slide as a reference (Figure 5M-N). Place the tissue core
208 into the recipient FFPE TMA block (Figure 5O).
209 12. Repeat this process with other cold snare resections (from other patients and/or
210 other time points of the same patient) until the desired number of tissue pieces is
211 placed in the recipient FFPE TMA block (Figure 5P).
212 13. Cut sections of the TMA and apply the desired stains for additional histopathologic
213 analyses (Figure 5Q).
214 *EMINENT-2: baseline characteristics and endpoints*
215 Twenty-one patients were included with a median age of 66 years (IQR, 60 – 68) and median
216 BMI of 27.5 kg/m2 (IQR, 26.6 – 30.1). To date, 16 patients have undergone the 12-week
217 follow-up endoscopy and one patient received the cross-over procedure. A total of 38 cold
218 snare resections were performed. No post-procedural SAEs or AEs were observed.

219 Tissue from 21 patients at different timepoints was sent for tissue dissociation and
220 subsequent scRNA-seq. Thirty-five samples were graded A (92% of total), three samples
221 were graded B (8% of total) and no samples were graded C. See Supplementary Table 2 for a
222 detailed overview of the quality after tissue dissociation.

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224 **DISCUSSION**

225 These studies are the first to report the use of cold snare resections in healthy duodenal
226 mucosa for scientific studies. We did not observe any (S)AEs in over 50 patients, and
227 combined with the reported safety profile of cold snare resections in colon and duodenum
228 we can conclude that our stepwise approach to acquiring duodenal tissue via a cold snare
229 resection appears safe.

230 We designed an optimized protocol for tissue resection, specimen retrieval, handling and
231 processing for research purposes and provide a detailed description with corresponding
232 illustrations. This method enables adequate and reliable histopathologic analysis, both by
233 traditional histopathologic assessment of the tissue and by immunohistochemical staining of
234 a TMA, overcoming the limitations of using regular duodenal biopsies for this purpose. In
235 addition, it provides a larger piece of tissue for a variety of other assays such as scRNA-seq.
236 In our opinion, our protocol will enable better mechanistic studies into the effect of
237 duodenal ablation in patients with T2D and metabolic syndrome. It may also find a wider
238 application for other basic and translational studies into the function of the gastrointestinal
239 tract. We used a 15-mm snare for our cold snare resections but avoided full 15-mm snare
240 resections of duodenal mucosa, and did not use the technique of forcefully pulling the
241 closed snare to the tip of the endoscope. We feel that this increases the risk of
242 complications and mechanically damages the resected specimen. In retrospect, a 10-mm
243 snare might be more appropriate for this purpose, which is in agreement with the recent
244 ESGE guideline.[24]

245 Our study has several limitations. First, the study had a relatively small sample size.
246 However, this was an optimization study and after ten patients it was decided that the

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247 optimal protocol had been achieved. Second, alternative techniques for obtaining duodenal
248 tissue were not explored, as the study focused on improving the acquisition of duodenal
249 tissue using the cold snare resection technique. Third, the generalizability of our results has
250 not yet been demonstrated, because all procedures were performed by a single
251 endoscopist. However, given the detailed description of our method, and the widespread
252 use of cold snare resections in gastrointestinal endoscopy, we are convinced this method
253 can be easily performed by other endoscopists.

254 The results of the initial DIRECT study were validated in our sham-controlled randomized
255 trial, the EMINENT-2. The optimized and standardized protocol improved the homogeneity
256 of tissue acquisition and processing. This will ultimately lead to reliable histopathologic and
257 cellular analyses comparing tissue before and after ablation in the EMINENT-2 and the
258 REMIND. This advancement is expected to enhance our understanding of the mechanism of
259 action of duodenal ablation in patients with T2D.

260 In conclusion, performing cold snare resections to retrieve small-sized mucosectomies for
261 research purposes in the duodenum using a 15 mm cold snare appears safe and feasible. For
262 widespread use we recommend using a 10 mm cold snare following the recent ESGE
263 guideline. Cold snare resections are a better alternative to multiple regular biopsies for both
264 histological and cellular activity research purposes.

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Grade	Cell number (cells)	Cell viability (%)
A (qualified)	≥20,000	≥85
B (suboptimal)	≥20,000	≥70
C (unqualified)	<20,000	<70

334 **Table 1**. Singleron Biotechnologies standard quality criteria of tissue dissociation.

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335 **Figure 1**. H&E slides of regular biopsies from one patient from the INSPIRE study.
336 Abbreviations: H&E, hematoxylin and eosin.

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337 **Figure 2**. Endoscopic photos of the acquiring of a cold snare resection in the duodenum. A)
338 Normal looking duodenum and the injection needle. B) Moments after injection of
339 submucosal saline. C) Cold snare is placed around the lifted tissue. D) Closure of the snare.
340 E) The tissue is gently held with the Roth net. F) The duodenum moments after the
341 mucosectomy has been obtained.

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342 **Figure 3**. A) Macroscopic picture of formalin-fixed paraffin embedded tissue microarray
343 block. B) Macroscopic picture of H&E stained slide of the tissue microarray. Abbreviations:
344 H&E, hematoxylin and eosin.

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345 **Figure 4.** A) Microscopic images of H&E stained slide of tissue microarray. B) 1x
346 magnification. C) 2x magnification. D) 5x magnification. E) 10x magnification. F) 20x
347 magnification. Abbreviations: H&E, hematoxylin and eosin.

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349 **Figure 5.** Step-by-step explanation of duodenal tissue processing.

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SUPPLEMENTARY MATERIAL

Supplementary material A

Tissue dissociation process Singleron Biotechnologies

Dissociation is performed using the Singleron PythoN Automated Tissue Dissociation System. The tissue section is washed 3 times in 1x HBSS (Hank's Balanced Saline Solution, Gibco, REF Nr.: 24020-117) and placed in a 2ml Eppendorf tube (Eppendorf, Hamburg, Germany) and weighed. The tissue is then briefly cut with ophthalmic scissors in 500µl of sCelLiVE Tissue Dissociation Buffer and transferred to the Singleron PythoN Dissociation Unit (DNFU). The final volume of the dissociation mixture is calculated according to the amount of tissue. In detail, 500µl, 1000µl or 2000µl of dissociation solution are used for up to 50mg, 100mg or more than 100mg of tissue, respectively. The automated dissociation is performed at 180rpm, including 36 cycles of 15 CCW and 10 CW rotations. The sample is incubated at 37°C during the whole program (15 min). Afterwards, the cell suspension is filtered using a 40µm sterile strainer (EASYstrainer, Greiner Bio-one, REF Nr.: 542040). The filtered suspension is washed twice with phosphate-buffered saline by centrifugation at 4°C, 350g for 5 minutes. The resulting pellet containing single cells is resuspended in 1000 µl cold PBS. Cells are counted using acridine orange/propidium iodide double staining with Luna FX7 automated cell counter (Logos Biosystems, Villeneuve d'Ascq, France). Tissue dissociation quality control results are based on the standard quality criteria of Singleron Biotechnologies. If necessary, removal of erythrocytes, dead cells and/or debris is performed according to Singleron Biotechnologies protocols.

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Sample	Date	Tissue weight (mg)	Cell number	Cell viability (%)	Quality Score
Di001A	12-1-2023	< 1 mg	19,080	83.3	C
Di001B	12-1-2023	< 1 mg	108,000	95.6	A
Di002A	26-1-2023	31	142,000	93.9	A
Di002B	26-1-2023	54	122,060	98.9	A
Di002C	26-1-2023	42	74,290	97.5	A
Di002D	26-1-2023	17	1,219	97.5	C
Di003A	7-2-2023	30	417,000	91.0	A
Di003B	7-2-2023	42	1,720,000	97.5	A
Di003C	7-2-2023	10	33,000	79.8	B
Di005A	18-4-2023	30	1,140,000	98.3	A
Di005B	18-4-2023	29	3,200,000	99.1	A
Di006A	3-4-2023	49	NA*	NA	NA
Di006B	3-4-2023	NA	NA*	NA	NA
Di007A	16-5-2023	83	3,328,000	97.7	A
Di007B	16-5-2023	17	1,375,000	98.1	A
Di008A	16-5-2023	40	2,685,000	97.6	A
Di008B	16-5-2023	10	595,000	98.9	A
Di009A	23-5-2023	20	1,770,000	97.3	A

Supplementary Table 1. Quality report after tissue dissociation of tissue obtained in the DIRECT study sent for single-cell RNA sequencing. *Due to a delayed transport the temperature of the tissue was too high to get reliable results.

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Sample	Timepoint	Date	Tissue weight (mg)	Cell number	Cell viability (%)	Quality Score
EM2-002	T0	21-08-2023	40	7.200.000	93.0	A
EM2-004	T0	21-08-2023	40	8.770.000	94.9	A
EM2-001	T0	25-09-2023	10	2.350.000	93.9	A
EM2-003	T0	25-09-2023	10	948.000	89.9	A
EM2-005	T0	25-09-2023	50	1.690.000	83.8	A
EM2-006	T0	25-09-2023	20	1.820.000	89.1	A
EM2-002	T12W	22-11-2023	70	556.000	97.1	A
EM2-004	T12W	22-11-2023	100	118.600	94.6	A
EM2-010	T0	22-11-2023	20	61.200	99.2	A
EM2-011	T0	22-11-2023	50	471.500	95.3	A
EM2-012	T0	22-11-2023	60	765.000	94.6	A
EM2-003	T12W	13-12-2023	70	3.460.000	87.3	A
EM2-005	T12W	13-12-2023	40	8.760.000	89.5	A
EM2-006	T12W	13-12-2023	30	3.360.000	97.3	A
EM2-001	T12W	19-12-2023	23	795.000	93.3	A
EM2-008	T0	19-12-2023	114	1.180.000	93.8	A
EM2-009	T0	19-12-2023	104	2.980.000	75.9	B
EM2-013	T0	19-12-2023	60	745.000	91	A
EM2-015	T0	31-01-2024	<10	2.583.000	94.8	A
EM2-016	T0	31-01-2024	<10	870.000	90.9	A
EM2-014	T0	12-02-2024	60	2.660.000	76.2	B
EM2-017	T0	12-02-2024	65	1.790.000	92.2	A
EM2-010	T12W	14-02-2024	50	2.510.000	97.4	A
EM2-011	T12W	14-02-2024	50	2.510.000	87.6	A
EM2-012	T12W	14-02-2024	30	2.260.000	94.2	A
EM2-008	T12W	18-03-2024	30	984.000	86.4	A
EM2-009	T12W	18-03-2024	30	2.292.000	88.1	A

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EM2-013	T12W	18-03-2024	30	882.000	71.3	B
EM2-019	T0	22-04-2024	80	1.460.000	96.4	A
EM2-023	T0	22-04-2024	40	478.000	97.0	A
EM2-016	T12W	22-04-2024	167	6.050.000	91.7	A
EM2-015	T12W	02-05-2024	50	1.100.000	86.1	A
EM2-014	T12W	08-05-2024	20	880.000	93.1	A
EM2-017	T12W	08-05-2024	31	1.030.000	99.2	A
EM2-022	T0	08-05-2024	40	880.000	93.1	A
EM2-025	T0	10-06-2024	72	1.935.000	98.2	A
EM2-026	T0	10-06-2024	50	1.415.000	94.8	A
EM2-008	T0C	10-06-2024	70	1.455.000	97.3	A

Supplementary Table 2. Quality report after tissue dissociation of tissue obtained in the EMINENT-2 study sent for single-cell RNA sequencing. Abbreviations: RNA, ribonucleic acid.

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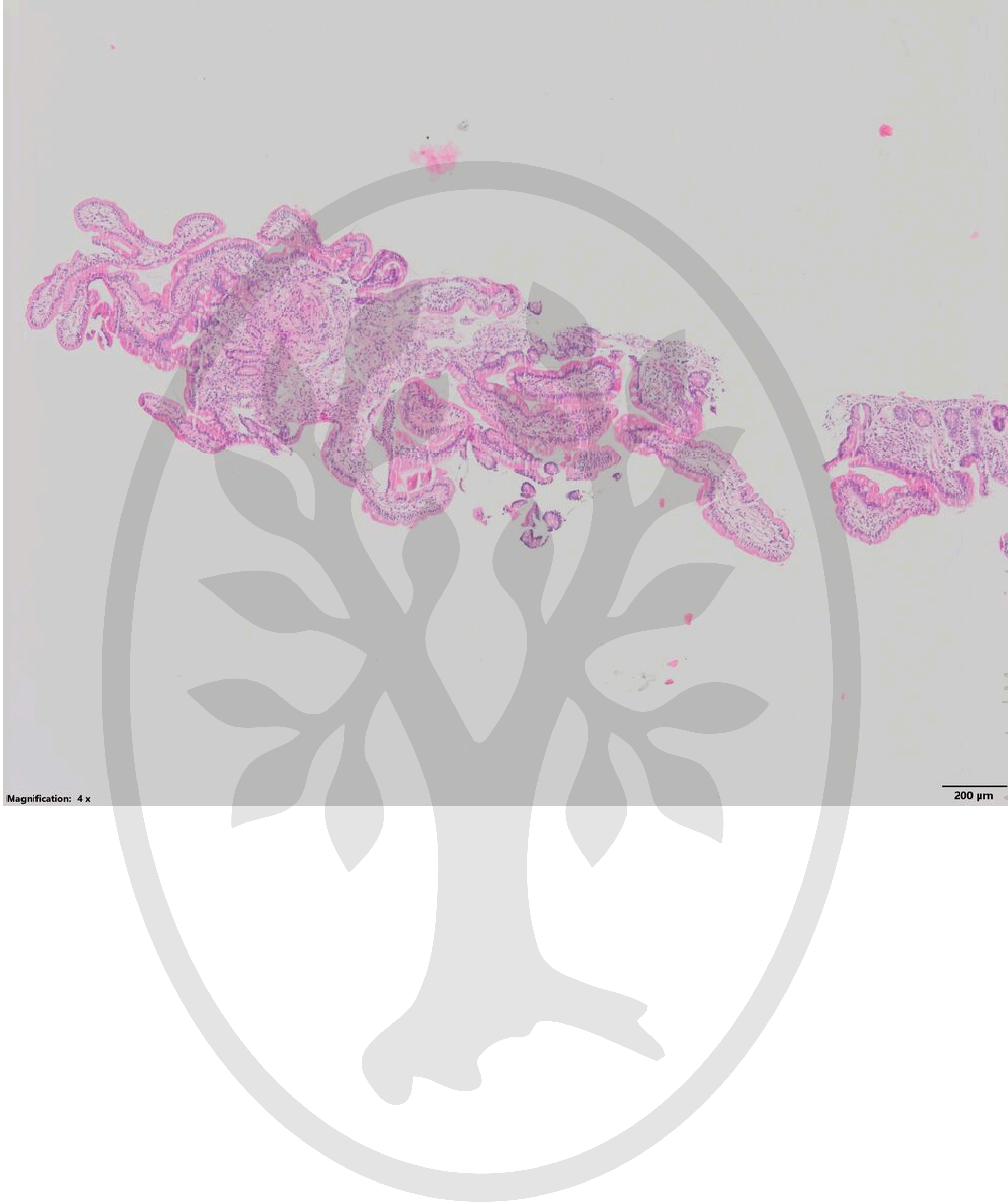

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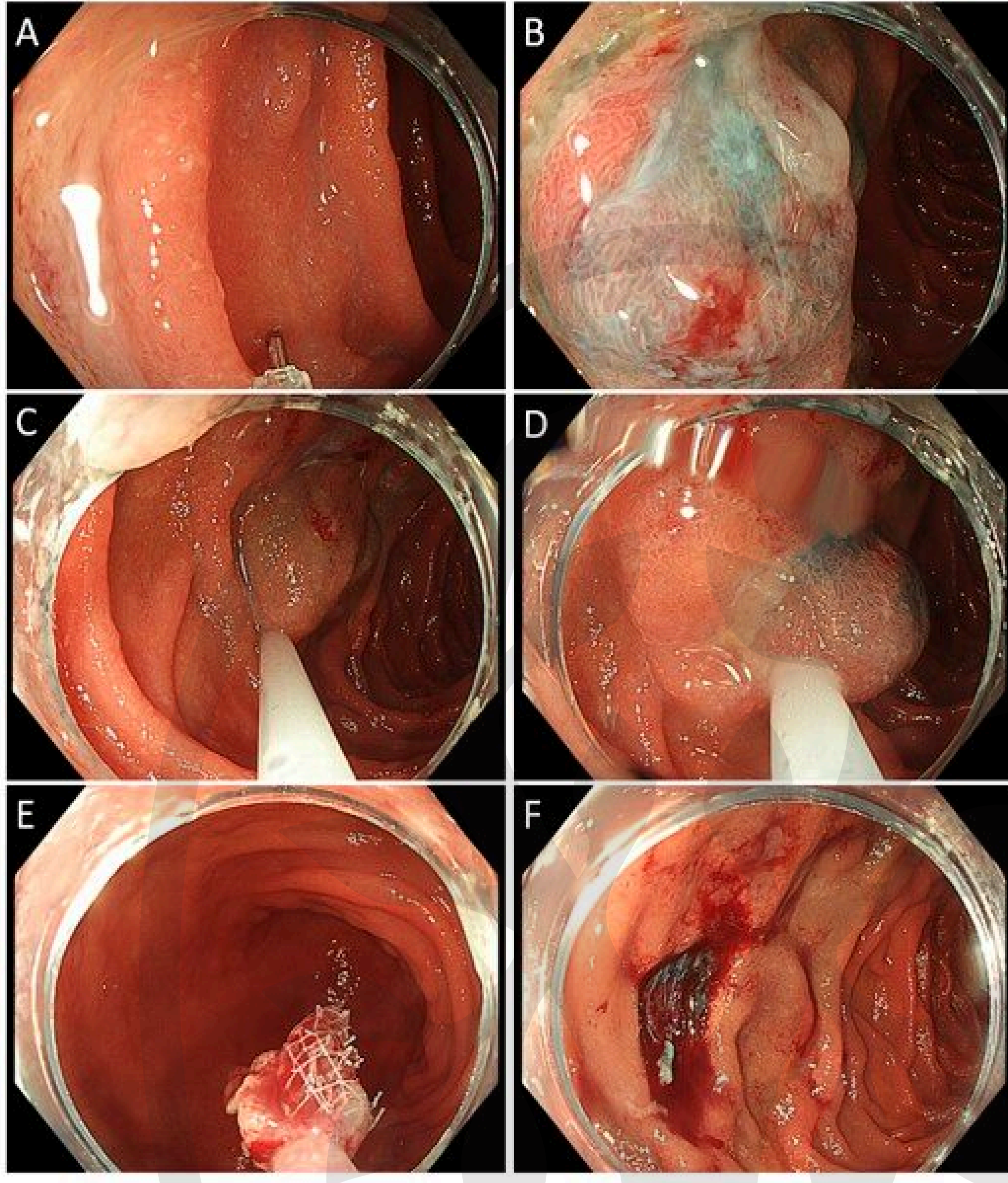

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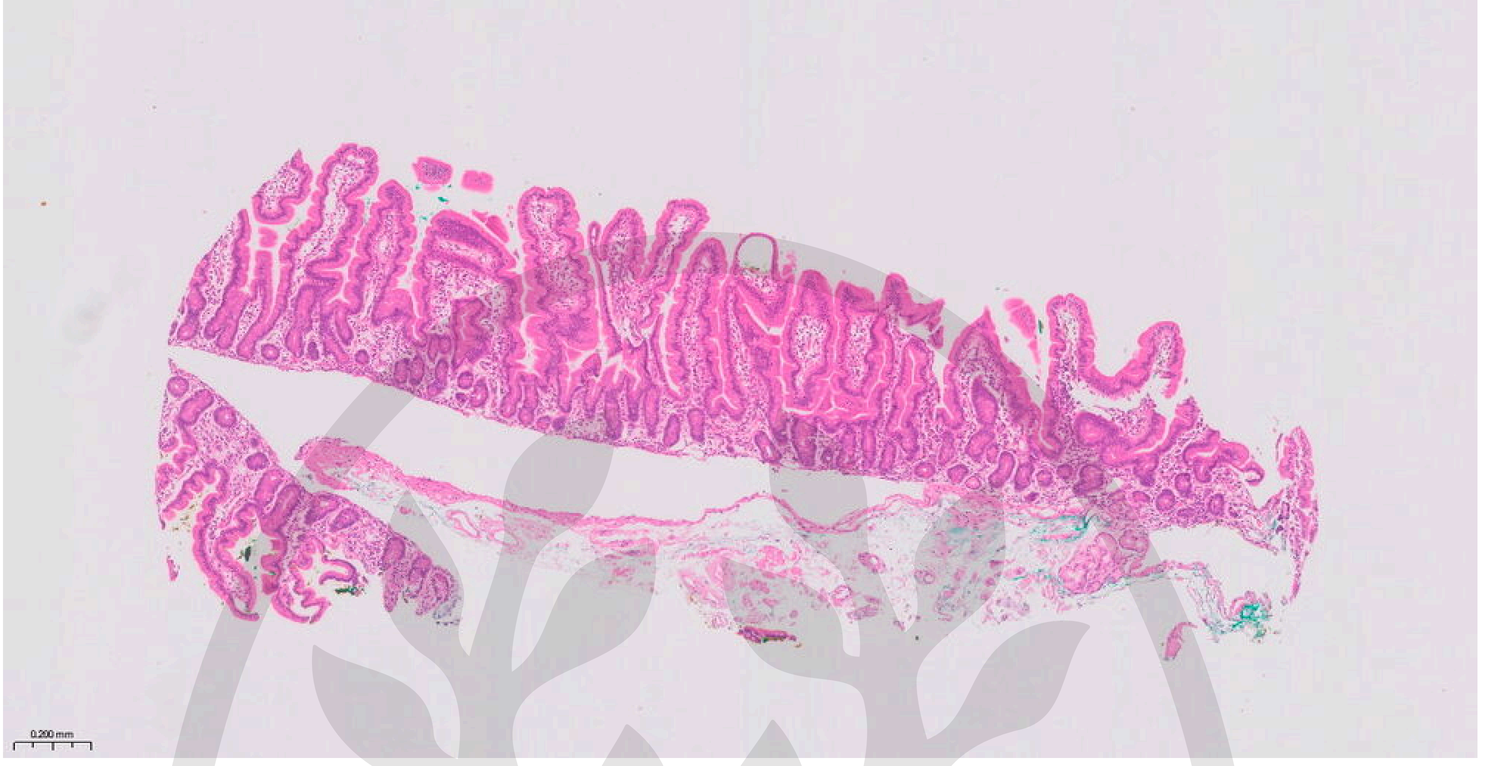

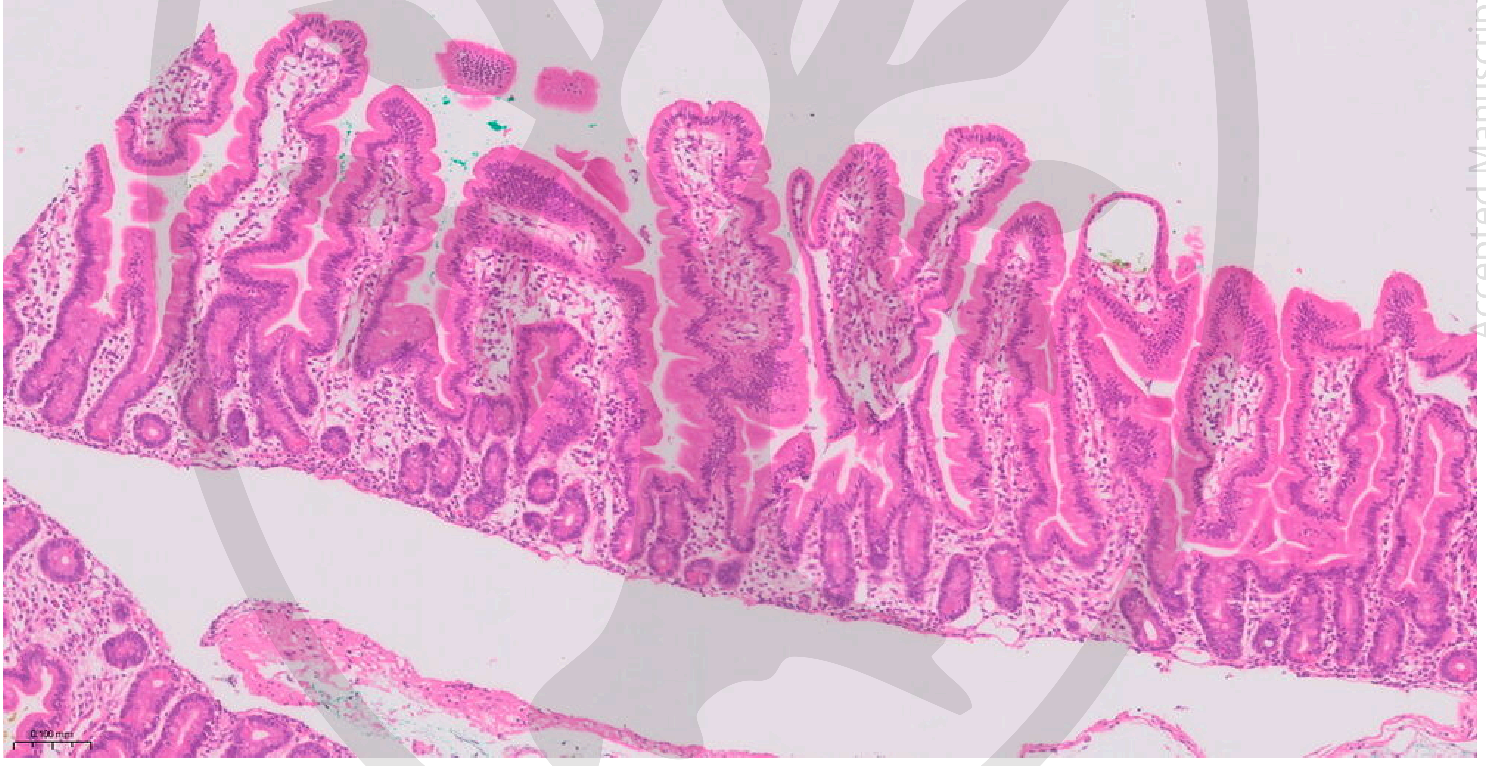

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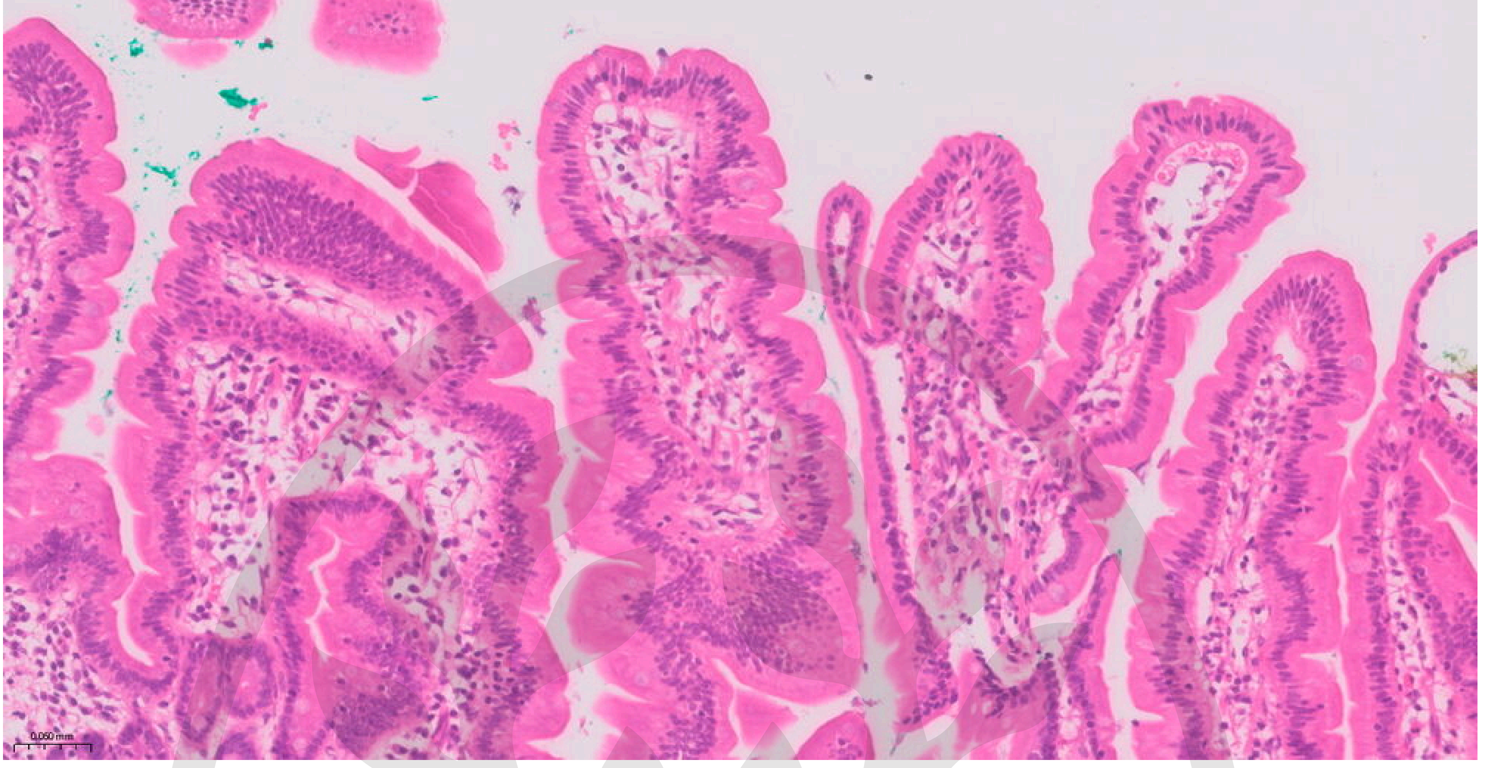

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