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Journal

Clinical and Translational Gastroenterology, 15(8)

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Publication Date

2024-08-01

DOI

10.14309/ctg.0000000000000751

Molecular Analysis of Persistent and Recurrent Barrett's Esophagus in the Setting of Endoscopic Therapy

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INTRODUCTION: **Early neoplastic progression of Barrett's esophagus (BE) is often treated with endoscopic therapy. Although effective, some patients are refractory to therapy or recur after apparent eradication of the BE. The goal of this study was to determine whether genomic alterations within the treated BE may be associated with persistent or recurrent disease.**

METHODS: **We performed DNA sequencing on pre-treatment esophageal samples from 45 patients who were successfully treated by endoscopic therapy and did not recur as well as pre-treatment and post-treatment samples from 40 patients who had persistent neoplasia and 21 patients who had recurrent neoplasia. The genomic alterations were compared between groups.**

RESULTS: **The genomic landscape was similar between all groups. Patients with persistent disease were more likely to have pre-treatment alterations involving the receptor tyrosine kinase pathway ($P = 0.01$), amplifications of oncogenes ($P = 0.01$), and deletions of tumor suppressor genes ($P = 0.02$). These associations were no longer significant after adjusting for patient age and BE length. More than half of patients with persistent (52.5%) or recurrent (57.2%) disease showed pre-treatment and post-treatment samples that shared at least 50% of their driver mutations.**

DISCUSSION: **Pre-treatment samples were genomically similar between those who responded to endoscopic therapy and those who had persistent or recurrent disease, suggesting there is not a strong genomic component to treatment response. Although it was expected to find shared driver mutations in pre-treatment and post-treatment samples in patients with persistent disease, the finding that an equal number of patients with recurrent disease also showed this relation suggests that many recurrences represent undetected minimal residual disease.**

KEYWORDS: Barrett's esophagus; esophageal adenocarcinoma; endoscopic therapy; radiofrequency ablation; genomics

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/B179.

Clinical and Translational Gastroenterology 2024;15:e00751. https://doi.org/10.14309/ctg.0000000000000751

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Received July 1, 2024; accepted July 4, 2024; published online July 15, 2024

INTRODUCTION

Endoscopic eradication therapy (EET) is recommended by all medical guidelines for the treatment of Barrett's esophagus (BE) with high-grade dysplasia and superficial esophageal adenocarcinoma (EAC). Therapy generally consists of mucosal resection of focal nodular disease and ablation of residual flat BE. Ablation of the mucosa in combination with acid suppression therapy can lead to complete reepithelization with neosquamous epithelium. Although endoscopic therapy is largely effective, persistence (the presence of BE/neoplasia after several endoscopic treatment attempts) or recurrence (the re-emergence of BE/neoplasia in the tubular esophagus after complete eradication has been achieved) can occur and limits the effectiveness of the procedure. Persistent and recurrent lesions have been reported in about 17%–22% of patients, with risk factors including older age, pre-treatment histology with high-grade dysplasia or cancer, longer length of initial BE segment, and a history of smoking (1,2).

Little is known as to the cause of persistence or recurrence in these patients, including a limited understanding regarding the genomic makeup of BE that persists or recurs after EET. Several studies have evaluated the molecular changes that occur in the natural progression of nondysplastic BE to EAC, including a loss or inactivation of tumor suppressor genes such as *TP53* and *CDKN2A* and amplification of oncogenes such as *MYC, ERBB2,* and others (3,4); however, few have explored how endoscopic therapy may alter this process or how these alterations may affect the efficacy of EET. A case series of 19 patients demonstrated that 5 patients with persistent postablative pathology had the same mutations before ablation (5). Additional studies by Prasad et al found that loss of 9p21 and 17p13.1, sites of *CDKN2A* and *TP53*, respectively, were predictors of decreased response to photodynamic ablative therapy (6,7). However, the utility of these markers may be limited because changes in these sites are common and occur early in BE neoplastic evolution. Furthermore, although these studies demonstrate persistence of genomic loss at 2 loci, they do not consider other genomic alterations (i.e., mutations or other copy number changes) in recurrent or persistent BE.

This study seeks to characterize the genomic makeup and clonal relationship of pre-treatment BE and post-treatment persistent and recurrent BE. Our hypothesis is that persistent/recurrent BE is clonally related to pre-treatment BE and will be genomically "advanced," i.e., contain an advanced number of molecular alterations.

METHODS

Study design

Three groups of patients were analyzed: (i) patients who were successfully treated with EET ("controls"); (ii) patients with recurrent neoplasia ("recurrent"); and (iii) patients with persistent neoplasia ("persistent") as part of this retrospective multicenter cohort study as part of the National Cancer Institute's Barrett's Esophagus Translational Research Network (BETRNet).

Subjects were potentially eligible if they had undergone EET using any combination of ablation modalities with or without associated endoscopic mucosal resection for BE with low-grade dysplasia (LGD), high-grade dysplasia (HGD), or intramucosal adenocarcinoma. Patients with esophageal cancer at baseline with depth greater than T1a, evidence of metastatic disease, or had therapy with chemotherapy or radiation to the esophagus were excluded. All procedures were performed at high-volume, expert academic centers within the United States.

Definitions for response to therapy and for recurrent and persistent disease were developed by consensus between BETRNet investigators (BETRNet consensus criteria). Controls were considered to have successful eradication without recurrence, defined as (i) no endoscopic evidence of BE and no dysplasia or intestinal metaplasia in biopsies from the gastroesophageal junction or esophagus after ≤3 ablative sessions (any type of ablative therapy allowed) and (ii) no recurrence of BE or LGD or worse at the gastroesophageal (GE) junction or in the tubular esophagus after a year of endoscopies with surveillance biopsies.

Subjects were considered to have persistent BE if they had ≥1 cm of residual BE after ≥3 endoscopic treatments, with biopsies demonstrating intestinal metaplasia or any degree of dysplasia. Recurrent BE was defined as (i) the absence of endoscopic BE and no evidence of any intestinal metaplasia (IM) or worse on ≥2 consecutive surveillance endoscopies after EET and (ii) recurrence of LGD, HGD, or EAC in the tubular esophagus or at the GE junction at least 12 months after initial complete response.

All subjects included had paraffin-embedded tissue from endoscopic biopsies before ablation, and all recurrent and persistent subjects had paraffin-embedded endoscopic biopsies from Barrett's mucosa after ablation. Post-treatment tissue was collected from those with persistent and recurrent disease. Collection of tissue blocks containing the highest degree of neoplasia from the preablation and postablation samples was prioritized. However, in some instances, either that block was unavailable or the highest grade lesional tissue had been exhausted in the block (confirmed by hematoxylin and eosin [H&E] staining), and in those cases, a lower grade of BE lesion was used. This study was approved by the respective Institutional Review Board of each participating institution.

DNA isolation and library construction

For each sample, six 8-μm or ten 4-μm sections were cut for DNA isolation as well as two 4-μm sections for H&E staining (comprising the first and last section cut). Each H&E slide was reviewed by 2 experienced gastrointestinal pathologists (M.D.S. and K.W.W.) to assess histology of the cut and used to guide macrodissection of the other unstained slides for nucleic acid isolation. Any challenging case or discrepant diagnosis was resolved through joint review. Histology of the lesions used for genomic analyses is shown in Supplementary Digital Content (see Supplementary Table 1, http://links.lww.com/CTG/B179).

Areas of BE/EAC were macrodissected using the H&E slides as a guide, and DNA was purified using the ReliaPrep formalin-fixed paraffin-embedded genomic DNA kit (Promega) from the middle tissue sections. About 20–100 ng of DNA was fragmented (Covaris sonication) to 250 bp and then ligated to specific adapters using automated library preparation (KAPA Hyper KK8504) using the Beckman FXp liquid handling robot. Libraries were pooled and sequenced on an Illumina Miseq nano flow cell to estimate each library's concentration based on the number of barcode reads per sample. The libraries were pooled and captured using a custom bait set that includes all exons from 243 gastroesophageal cancer-associated genes (see Supplementary Table 2, http://links.lww.com/CTG/B179). Captures were performed using the Agilent Sureselect XT HS Hybrid Capture kit. Captures were further pooled and sequenced on a HiSeq 2500 in Rapid Run mode as previously described (3).

Targeted gene panel sequencing

Isolated samples were sequenced using a custom-targeted sequencing panel (described above) to a target depth of ~150×.

Even with a sample purity as low as 10%, this depth provided a power of 88.9% to detect somatic mutations, assuming a sequencing error rate of 1/1,000 and maintaining a false positive rate of 5×10^{-7}. To allow broad sequencing analysis in a cost-effective manner, a custom-targeted sequencing panel consisting of 243 genes specifically selected for their relevance in Barrett's esophagus and EAC (8–11) was used.

Standard data processing and analysis pipelines that are well established for formalin-fixed paraffin-embedded DNA sequencing were used for all data processing. Pooled samples were demultiplexed using the Picard tools. Read pairs were aligned to the hg19 reference sequence using the Burrows-Wheeler Aligner (12), and data were sorted and duplicate-marked using Picard tools. The alignments were further refined using the Genome Analysis Toolkit (GATK) (13,14) for localized realignment around indel sites (15) Recalibration of quality scores was also performed using the GATK. Mutation analysis for single nucleotide variants was performed using MuTect v1.1.4 and annotated by Variant Effect Predictor. SomaticIndelDetector tool which is part of the GATK was used for indel calling.

Single nucleotide variants and Indels were annotated for gene and amino acid impact using Variant Effect Predictor v79; afterward, OncoAnnotate was used to determine the presence of the variant in external data sources such as the Exome Sequencing Project, Genome Aggregation Database (gnomAD), and Catalogue of Somatic Mutations in Cancer (COSMIC) to allow flagging common SNPs.

Criteria for filtering for pathogenic mutations

To identify likely pathogenic somatic mutations, all noncoding mutations were removed unless they were predicted to affect a splice site. For missense variants, variants identified at greater than 0.1% in any population in either the gnomAD or Exome Sequencing Project database were removed. In addition, any variant identified in previously sequenced normal control samples was removed. Genes were separated into tumor suppressors or oncogenes based on COSMIC consensus cancer gene list. For the oncogenes, we kept only recurrent mutations that are unlikely to lead to loss of the protein and which were recurrently found in cancer (previously reported in COSMIC $\geq$5 times). For the tumor suppressor genes, mutations that may lead to loss of function were kept, such as frameshift mutations, nonsense mutations, splice site mutations (within +1, −1, +2, and −2), and missense mutations reported in COSMIC $\geq$5.

As previously described, clinically validated, in-house algorithm, RobustCNV, was used for copy number assessment (3). RobustCNV relies on localized changes in the mapping depth of sequenced reads to identify changes in copy number at the loci sampled during targeted capture. Genes were assigned either as homozygous deletion (log2ration $<$ −0.7), amplification (log2ration $>$ 1), or normal copy number. Homozygous deletions in tumor suppressor genes and amplification of oncogenes were retained. Single copy deletions and low-level gains were not included unless it was determined an entire chromosomal arm was included in the copy number variant (CNV).

Statistical analysis

The outcomes of interest in this study included both preablation and postablation genomic alterations including continuous measures (number of total mutations, tumor suppressor mutations, chromosomal arm CNVs, oncogene amplifications, and tumor suppressor gene homozygous deletions) as well as the presence or absence of specific alterations that had a relatively higher frequency in the study population (present in >15% of preablative samples): *TP53*, *APC*, and *CDK2NA*. In addition, the presence of receptor tyrosine kinase (RTK) pathway alterations, namely in *BRAF*, *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *FGFR2*, *GNAS*, *IGF1R*, *IGF2R*, *KRAS*, *NRAS*, and *VEGF*, was examined because they have been implicated in the development of EAC (16). Differences in the presence of *ERBB2* alterations were also examined individually as they contributed most to RTK pathway mutations.

Kruskal-Wallis rank-sum tests, analysis of variance (ANOVA), and Wilcoxon tests were used to analyze continuous variables, and Fisher exact tests were used to compare categorical variables. Wilcoxon matched-pair tests and McNemar exact tests were used for paired analyses of preablation vs postablation. Differences in both preablation and postablation mutations were assessed across subject type (i.e., controls, persistent BE, and recurrent neoplasia) as well as by prehistological and posthistological diagnosis of the tissue sample analyzed (as noted above, in some instances, the tissue section analyzed contained a histology that did not correspond with the highest degree of neoplasia detected during the endoscopy). In secondary analyses, tissue samples that contained no dysplasia were excluded. Logistic regression analyses were also performed to assess for associations between genetic mutations with persistent BE or recurrent neoplasia compared to controls, adjusted for pre-treatment tissue histology, patient age, and BE length. The initial study design planned for analysis of samples from 40 subjects from each of the groups (controls, resistant, and recurrent). For baseline comparisons, this sample size would have had 87% power to detect a 0.7 SD difference in continuous measures and 84% power to detect a 30% difference from a baseline rate or 20% for categorical measures of genomic alterations, assuming $\alpha = 0.05$. As this was an exploratory, hypothesis-generating study, adjustment for multiple comparisons was not performed, and statistical significance was defined as $P < 0.05$. All analyses were performed in Stata version 17.0.

RESULTS

A total of 106 subjects were analyzed, of whom 45 (34 male, 11 female) were controls (achieved CE-IM and remained recurrence-free), 40 (34 male, 6 female) had persistent disease, and 21 (18 male, 3 female) had recurrent disease (see Supplementary Table 1, http://links.lww.com/CTG/B179). Although there was no statistical difference in distribution of male and female patients across categories, similar to previous reports, the median max length of BE was longer in those with persistent (7.5 cm, $P < 0.001$) and recurrent (6 cm, $P = 0.012$) disease compared with controls (4 cm). Patients with persistent disease were older than controls (68.4 vs 63.7, $P = 0.02$). There was no difference in age between those with recurrent disease and controls (see Supplementary Table 3, http://links.lww.com/CTG/B179). All patients were placed on acid suppression before endoscopic therapy. Initial analyses were performed to assess for genomic alterations based on the histology of the tissue sample analyzed. As expected, preablation tissue samples with higher grades of dysplastic BE and EAC had significantly more *TP53* and *APC* mutations and tumor suppressor gene mutations overall, as well as a nonsignificant trend for more chromosomal arm level CNVs and oncogene amplifications (Table 1). In postablation tissue, the number of CNV chromosomal arm level changes and

the proportion of samples having *ERBB2* alterations were significantly increased as grade of histology increased. There were also nonsignificant trends toward greater oncogene amplifications, *TP53* mutations, and receptor tyrosine kinase (RTK) pathway alterations overall with increasing grade of histology (Table 1).

Analyses were then performed to assess genomic alterations before and after ablation. In the baseline preablation samples, overall, the mean number of pathogenic/likely pathogenic mutations per sample was 2.21 (SD 1.18), with *TP53* mutations being the most common mutation identified (70/106 [66%] of samples) (Table 2). The mean number of chromosomal arm-level copy number changes per sample was 3.34 (SD 4.43). Pathogenic focal copy number changes were less common, with the mean number of oncogenic high-level amplifications being 0.02 (SD 0.14) and 2.07 (SD 1.16) homozygous deletions in tumor suppressor genes per sample. Alterations involving the RTK pathways were fairly common, being found in 28/106 (26.4%) of baseline samples. Alterations of *ERBB2* (through amplification or mutation) were the most common RTK event, present in 14/106 (13.2%) of samples.

Comparing preablation genomic differences across the groups, overall, the genomic profiles were similar. However, subjects with persistent BE had significantly more RTK pathway alterations at baseline compared with controls ($P = 0.01$) (see Supplementary Table 4, http://links.lww.com/CTG/B179). After ablation, both persistent BE and recurrent neoplasia had a lower mean number of tumor suppressor deletions compared with before ablation ($P = 0.02$ and 0.03, respectively). Persistent BE also had fewer amplifications in oncogenes after ablation compared with before ablation.

To determine whether any of the observed differences in genomic alterations from pre-treatment tissue samples, comparing those with persistent BE vs controls, were due to differences in histology of the tissue samples analyzed or due to the identified differences in patient characteristics (age and BE length), logistic regression analyses adjusting for these factors were performed (Table 3). When controlling for tissue histology, baseline alterations in *ERBB2* (odds ratio [OR] = 4.77, confidence interval [CI] = 1.05–21.6), total number of oncogenic amplifications (per amplification, OR = 1.64, CI = 1.12–2.4), total number of deletions in tumor suppressors (per deletion, OR = 2.01, CI = 1.11–3.63), and the presence of RTK alterations (OR = 4.52, CI = 1.44–14.14) were associated with persistent disease. Of note, however, both oncogenic amplifications and RTK alterations contain *ERBB2* alterations; neither total number of non-*ERBB2* oncogene amplifications (per amplification, OR = 2.43, CI = 0.60–9.83) nor presence of non-*ERBB2* RTK alterations (OR = 1.54, CI = 0.99–2.37) was independently associated with persistent BE. After further adjusting for patient age and BE length,

Table 1. Differences in preablation and postablation genetic alterations by histology of tissue sample analyzed across the entire study population

Before ablation	ND (n = 5)	LGD (n = 22)	HGD (n = 44)	EAC (n = 34)	*P* value
Total mutations, mean (SD)	1 (1)	2.32 (1.09)	2.43 (1.34)	2.06 (0.95)	0.06
Tumor suppressor mutations, mean (SD)	0.80 (1.10)	2.04 (1.13)	2.30 (1.23)	2.00 (0.98)	**0.05**
CNV arms, mean (SD)	0.40 (0.89)	1.86 (2.32)	3.32 (3.83)	4.44 (5.69)	0.07
Oncogene amplifications, mean (SD)	0 (0)	0.14 (0.35)	0.84 (1.16)	0.97 (1.96)	0.08
Tumor suppressor deletions, mean (SD)	1.20 (1.10)	0.45 (0.80)	0.45 (0.95)	0.41 (1.08)	0.40
TP53 mutation, n (%)	1 (20.0)	12 (54.6)	35 (79.6)	22 (64.7)	**0.02**
APC mutation, n (%)	0 (0)	9 (40.9)	5 (11.4)	8 (23.5)	**0.03**
CDK2NA mutation, n (%)	0 (0)	5 (22.7)	7 (15.9)	4 (11.8)	0.63
RTK pathway activation, n (%)	1 (20.0)	4 (18.2)	17 (38.6)	6 (17.8)	0.14
ERBB2, n (%)	0 (0)	1 (4.6)	10 (22.7)	3 (8.8)	0.16
After ablation	**ND (n = 11)**	**LGD (n = 18)**	**HGD (n = 19)**	**EAC (n = 13)**	***P* value**
Total mutations, mean (SD)	1.55 (1.29)	2.44 (1.25)	2.47 (1.43)	2.15 (0.99)	0.23
Tumor suppressor mutations, mean (SD)	1.36 (1.03)	2.28 (1.07)	2.26 (1.52)	1.92 (0.95)	0.19
CNV arms, mean (SD)	0.64 (1.80)	1.44 (1.95)	2.89 (3.46)	7.46 (5.29)	**<0.001**
Oncogene amplifications, mean (SD)	0.09 (0.30)	0.22 (0.43)	0.53 (1.17)	1.00 (1.22)	0.06
Tumor suppressor deletions, mean (SD)	0.18 (0.60)	0.22 (0.65)	0.16 (0.50)	0.15 (0.55)	0.98
TP53 mutation, n (%)	4 (36.4)	11 (61.1)	12 (63.2)	11 (84.6)	0.11
APC mutation, n (%)	0 (0)	5 (27.8)	3 (15.8)	3 (23.1)	0.27
CDK2NA mutation, n (%)	1 (0)	6 (33.3)	3 (15.8)	1 (7.7)	0.10
RTK pathway mutation, n (%)	2 (18.2)	4 (22.2)	4 (21.1)	6 (46.2)	0.39
ERBB2 mutation, n (%)	0 (0)	1 (5.6)	3 (15.8)	5 (38.5)	**0.04**

Mean (SD) or n (%) reported with corresponding ANOVA or Fisher exact *P* value reported. Bolded *P* values denote $P \leq 0.05$.
CNV, copy number variant; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; LGD, low-grade dysplasia; ND, non-dysplastic; RTK, receptor tyrosine kinase.

the associations between deletions in tumor suppressors and persistent BE (OR = 1.94, CI = 0.98–3.84) and recurrent BE (OR = 1.98, CI = 0.94–4.15) remained qualitatively unchanged, although no longer statistically significant. The associations between oncogenic amplifications as well as RTK alterations with persistent and recurrent BE were attenuated and no longer significant. There were no mutations present at baseline that were independently associated with recurrence.

To determine how genomically related pre-treatment and post-treatment samples were in relation to driver mutations, in patients with persistent and recurrent disease, we compared the number of shared likely pathogenic mutations. There were no significant differences in the percent of shared driver mutations, defined as the proportion of mutations present in preablation tissue that were also found in postablation tissue in persistent and recurrent subjects (see Supplementary Table 5, http://links.lww.com/CTG/B179). In persistent disease, 21/40 (52.5%) patients shared at least 50% of the likely pathogenic mutations comparing pre-treatment and post-treatment samples, with 10 (25%) patients sharing all mutations. About 12/40 (30%) of patients did not share any mutations between the pre-treatment and post-treatment samples, despite not achieving eradication. Interestingly, trends were very similar comparing pre-treatment and post-treatment samples from patients with recurrent disease; 12/21 (57.1%) of patients shared at least 50% of the mutations, whereas 8/21 (38.1%) patients with recurrent disease did not share any mutations between pre-treatment and post-treatment samples.

DISCUSSION

Endoscopic removal of visible HGD and early EAC followed by radiofrequency ablation is standard of care for early BE-associated neoplasia because of its high level of effectiveness and significantly decreased morbidity compared with esophagectomy. However, a clinically significant number of patients either fail to completely respond (persistent disease) or recur after initially appearing to have eradication of disease. What factors lead to some patients responding and others to respond less favorably has been understudied. It is important to understand whether recurrence or persistence is related to the genomic makeup of BE samples from a range of treatment outcomes to determine whether any genomic factors correlated with poor

Table 2. Overview of genetic alterations across the study population: mean (SD) or n (%) reported

	Before ablation				After ablation		
	Controls (n = 45)	Persistent (n = 40)	Recurrent (n = 21)	Overall (n = 106)	Persistent (n = 40)	Recurrent (n = 21)	Overall (n = 61)
Total mutations, mean (SD)	2.02 (1.06)	2.45 (1.36)	2.14 (1.06)	2.21 (1.18)	2.38 (1.41)	1.95 (0.97)	2.23 (1.28)
Oncogenic mutations, mean (SD)	0 (0)	0.05 (0.22)	0 (0)	0.02 (0.14)	0.05 (0.32)	0 (0)	0.03 (0.26)
Tumor suppressor mutations, mean (SD)	1.96 (1.04)	2.2 (1.34)	2.05 (1.02)	2.07 (1.16)	2.1 (1.36)	1.86 (0.91)	1.17 (1.37)
CNV arms, mean (SD)	3.98 (5.28)	2.25 (2.98)	4.05 (4.54)	3.34 (4.43)	2.55 (3.59)	3.95 (4.90)	3.03 (4.11)
Oncogene amplifications, mean (SD)	0.47 (1.06)	1.05 (1.84)	0.52 (0.75)	0.70 (1.38)	0.48 (0.93)	0.43 (0.98)	0.46 (0.94)
Tumor suppressor deletions, mean (SD)	0.22 (0.64)	0.70 (1.16)	0.62 (1.07)	0.48 (0.97)	0.23 (0.62)	0.10 (0.44)	0.18 (0.56)
TP53 mutation, n (%)	30 (66.7)	26 (65.0)	14 (66.7)	70 (66.0)	26 (65.0)	12 (57.1)	38 (62.3)
APC mutation, n (%)	8 (17.8)	9 (22.5)	5 (23.8)	22 (20.8)	8 (20.0)	3 (14.3)	11 (18.0)
CDK2NA mutation, n (%)	7 (15.6)	6 (15.0)	3 (14.3)	16 (15.1)	5 (12.5)	4 (23.8)	10 (16.4)
RTK pathway activation, n (%)	7 (15.6)	17 (42.5)	4 (19.1)	28 (26.4)	11 (27.5)	5 (23.8)	16 (26.2)
ERBB2	3 (6.7)	9 (22.5)	2 (9.5)	14 (13.2)	6 (15.0)	3 (14.3)	9 (14.8)
GNAS	1 (2.2)	3 (7.5)	0 (0)	4 (3.8)	4 (10.0)	0 (0)	4 (6.6)
KRAS	2 (4.4)	2 (5.0)	0 (0)	4 (3.8)	1 (2.5)	0 (0)	1 (1.6)
EGFR	0 (0)	2 (5)	1 (4.8)	3 (2.8)	0 (0)	1 (4.8)	1 (1.6)
ERBB4	0 (0)	2 (5)	0 (0)	2 (1.9)	0 (0)	0 (0)	0 (0)
BRAF	0 (0)	1 (2.5)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
ERBB3	1 (2.2)	0 (0)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
FGFR2	0 (0)	0 (0)	1 (4.8)	1 (0.9)	0 (0)	1 (4.8)	1 (1.6)
IGF1R	0 (0)	1 (2.5)	0 (0)	1 (0.9)	1 (2.5)	0 (0)	1 (1.6)
NRAS	1 (2.2)	0 (0)	0 (0)	1 (0.9)	0 (0)	0 (0)	0 (0)
VEGFA	0 (0)	1 (2.5)	0 (0)	1 (0.9)	1 (0)	0 (0)	1 (1.6)
IGF2R	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	1 (1.6)

CNV, copy number variant;RTK, receptor tyrosine kinase .

response to treatment and to determine how pre-treatment and post-treatment samples of BE neoplasia may be related.

Although the overall genomic profile of known "driver" alterations was relatively similar in pre-treatment samples across treatment outcomes, a few genomic events seemed to be more common in those who fail to completely respond to endoscopic therapy. This study found that the total number of tumor suppressor deletions in pre-treatment samples was higher in patients with persistent BE. Adjustment for histology, age, and baseline BE length did not diminish the strength of association, although it was no longer statistically significant. In addition, patients with BE that contained a likely activating event in the RTK pathways (predominately amplification of ERBB2) were more likely to manifest as persistent disease, although this finding did not remain significant after controlling clinical factors. The correlation of RTK activation with patient age and BE length is interesting and did not seem to be a function of histologic diagnosis. Regardless, it seems that these clinical factors likely play a larger role in treatment outcomes than different genomic drivers of disease.

In those patients with persistent disease, it is unknown how the genomic makeup of the post-treatment disease may change compared with pre-treatment. We found that in approximately 50% of patients, as expected, the pre-treatment and post-treatment samples were highly related. However, there was a perhaps larger than expected number of patients where there were few or no shared driver mutations. There are several possible explanations for this. It is known that all stages of BE neoplasia can be multiclonal (17). It is possible treatment selected for a small clone not detected in the pre-treatment sample either because it harbored some sort of advantageous alterations or by endoscopic removal of the previous dominant clone. Alternatively, both the pre-treatment and post-treatment BE could contain multiple clones and no shared driver mutations were identified because of sampling error.

Similarly, it is unknown how genomically related recurrent disease is to pre-treatment neoplasia. In this study, in more than 50% of patients with recurrent disease, the post-treatment recurrent sample was clonally related by pathogenic mutations to the pre-treatment sample. This would suggest that in a large percentage of recurrent cases, the patient harbors undetected persistent disease and has not developed new BE de novo. Despite not sequencing a paired germline sample, given the mutations

Table 3. Logistic regression analyses of associations between preablation genetic alterations in patients who had persistent BE or recurrent neoplasia compared with controls, adjusting for histology of the tissue sample analyzed (top) and further adjusted for patient age, and BE length (bottom)

Adjusted for histology		
	Persistent vs controls Odds ratio[a] (95% CI)	**Recurrent vs controls Odds ratio[a] (95% CI)**
TS	1.21 (0.81–1.81), $P = 0.35$	1.05 (0.62–1.79), $P = 0.85$
CNV arms	0.94 (0.82–1.08), $P = 0.40$	1.03 (0.92–1.15), $P = 0.65$
Onco amps	**1.64 (1.12–2.40), $P = 0.01$**	1.18 (0.67–2.07), $P = 0.56$
TS dels	**2.01 (1.11–3.63), $P = 0.02$**	1.76 (0.91–3.40), $P = 0.15$
TP53	1.02 (0.36–2.89), $P = 0.97$	0.83 (0.25–2.72), $P = 0.75$
APC	1.51 (0.44–5.15), $P = 0.51$	1.58 (0.41–6.11), $P = 0.51$
CDK2NA	0.87 (0.24–3.14), $P = 0.83$	0.73 (0.15–3.45), $P = 0.69$
RTK	**4.52 (1.44–14.15), $P = 0.01$**	1.00 (0.24–4.09), $P = 1.00$
ERBB2	**4.77 (1.05–21.57), $P = 0.04$**	1.40 (0.19–10.15), $P = 0.74$
Adjusted for histology, age, and BE length		
	Persistent vs controls Odds ratio[a] (95% CI)	**Recurrent vs controls Odds ratio[a] (95% CI)**
TS	0.99 (0.61–1.60), $P = 0.96$	0.90 (0.51–1.60), $P = 0.73$
CNV arms	0.94 (0.81–1.10), $P = 0.47$	1.03 (0.92–1.15), $P = 0.64$
Onco amps	1.35 (0.91–1.99), $P = 0.14$	1.14 (0.59–2.18), $P = 0.70$
TS dels	1.94 (0.98–3.84), $P = 0.06$	1.98 (0.94–4.15), $P = 0.07$
TP53	0.98 (0.31–3.12), $P = 0.98$	0.73 (0.21–2.59), $P = 0.63$
APC	1.77 (0.46–6.84), $P = 0.41$	1.38 (0.31–6.11), $P = 0.67$
CDK2NA	0.59 (0.13–2.60), $P = 0.48$	0.70 (0.15–3.36), $P = 0.66$
RTK	2.77 (0.77–10.02), $P = 0.12$	0.98 (0.21–4.66), $P = 0.98$
ERBB2	1.69 (0.31–9.27), $P = 0.55$	0.97 (0.10–9.09), $P = 0.98$

[a]Shown are odds ratios (95% CI) with bolded estimates indicating statistical significance $P < 0.05$. Each model includes the individual genomic alteration plus tissue sample histology (e.g., CNV deletions plus histology).
Amps, amplification; BE, Barrett's esophagus; CI, confidence interval; CNV, copy number variant; dels, deletion; Onco, oncogene; RTK, receptor tyrosine kinase; TS, tumor suppressor.

were stringently filtered for likely pathogenic alterations and the generally low allele fraction of the mutations (less than would be expected for a germline event), it is unlikely the number of shared mutations is artificially elevated to a significant level. Clinically, this is an important point and speaks to the need for meticulous examination at each endoscopy and treatment.

This study has several advantages and limitations that should be mentioned. First, only 1 sample in each pre-treatment and post-treatment outcome was able to be sequenced. Although every attempt was made to select the block that represented the highest histologic grade at the given endoscopy, additional genomic alterations in unsampled clones were likely present. One advantage of this study was that there were a relatively large number of samples that were collected from a wide range of academic centers across the United States. However, this also limited the amount of clinical data that were able to be collected, not allowing detailed associations between the genomics and clinical factors such as weight and smoking status to be made. The targeted sequencing panel used for this study was designed to include alterations in genes known to be altered in gastroesophageal cancers and is well validated in small formalin-fixed paraffin-embedded samples (3,18). However, the targeted nature of this technology does not allow novel drivers to be identified and limits the detailed phylogenetic relationships, which use passenger/nondriver alterations, to be performed. Regardless, from a clinical and pathologic progression standpoint, focusing on shared pathogenic/driver alterations is highly relevant.

In summary, we found pre-treatment genomic profiles are relatively similar regardless of EET outcome, with the possible exception of increased tumor suppressor deletions and RTK pathway alterations in patients with persistent disease, although after adjusting for patient age and BE length, these were no longer significant. The point estimates for the tumor suppressor deletions were nearly identical with and without adjusting for age and BE length, and although RTK point estimate was attenuated, it still seems to possibly have some effect for persistent. This finding suggests there is not a strong genomic basis for endoscopic treatment resistance, although this study was likely underpowered to detect more moderate and still meaningful associations.

Importantly, although it was expected to find shared driver mutations in pre-treatment and post-treatment samples in patients with persistent disease, the finding that an equal number of patients with recurrent disease also showed this relation suggests that many recurrences represent undetected persistent or minimal residual disease.

CONFLICTS OF INTEREST

Guarantor of the article: Matthew D. Stachler, MD, PhD.

Specific author contributions: M.D.S., J.A.A., K.K.W., J.H.R., and A.C.: conceived study. A.C., P.G.I., K.K.W., Y.L., L.K., R.S.K., E.W., M.I.C., and N.J.S.: collected and annotated samples. M.D.S., K.W.W., and M.W.: histology review and sample preparation. L.B., Y.L., M.R., and A.K.: database management. A.K., J.A.A., M.Y., and M.D.S.: data analysis. A.K., J.A.A., and M.D.S.: composed manuscript. W.M.G., A.C., A.K.R., T.C.W., J.H.R., Y.L., L.K., M.W., R.S.K., E.W., T.W., L.B., M.I.C., N.J.S., and K.K.W.: reviewed manuscript.

Financial support: BETRNet consortium (NCI U54CA163004, U54CA163059, U54CA163060, and U24CA163056), M.D.S.: Doris Duke Charitable Foundation Clinician Scientist Development award and NCI R37CA269649. J.A.A.: R01CA238433, R01CA255298, R01CA272898, and P30DK132710. K.K.W.: Russ and Kathy Van Cleve Research Professorship Fund.

Potential competing interests: A.C. is a scientific founder and stockholder of Lucid Diagnostics. A.C. is also a consultant to Lucid Diagnostics. A.C. is an inventor on patents assigned to Case Western Reserve University and licensed to Lucid Diagnostics. J.H.R. has received research funding from Lucid Diagnostics. W.M.G. has received nonfinancial research support from Lucid Diagnostics and consulting fees from Freenome, Diacarta, Guardant Health, Natera, and SEngine. M.I.C. received research grants from Pentax Medical Corporation and Endogastric Solutions, consulting fees from Castle Biosciences, Bluestar Genomics, and royalties from UpToDate. J.A.A. is a consultant to Exact Sciences and Castle Biosciences and receives research support from Pentax Medical.

ACKNOWLEDGEMENTS

The authors offer their sincere gratitude to the BETRNet NCI Program Officials Ellen Richmond, Deputy Chief Gastrointestinal and Other Cancers Research Group, and Rihab Yassin, Chief Cancer Cell Biology Branch, for their commitment and support of the BETRNet consortium and this study. The authors thank all the patients who generously allowed this study to be performed and the Center for Cancer Genomics at the Dana Farber Cancer Institute for sequencing support.

Study Highlights

WHAT IS KNOWN

- ✓ Barrett's esophagus is the precursor lesion and largest risk factor for esophageal adenocarcinoma.
- ✓ Enndoscopic therapy for early Barrett's esophagus-associated neoplasia is often effective with relatively low associated morbidity.
- ✓ Patients either fail to completely respond to endoscopic therapy or have a recurrence after apparent successful eradication.

WHAT IS NEW HERE

- ✓ Many cases of apparent recurrent disease share genomic 'driver' mutations with the pre-treatment Barrett's associated neoplasia, strongly suggesting these recurrent cases are actually residual persistent disease.
- ✓ No major genomic differences were identified between pre-treatment samples from patients who responded to endoscopic therapy, had persistent disease, or had recurrent disease.
- ✓ However, patients with persistent disease were more likely to have pre-treatment samples with alterations involving the receptor tyrosine kinase pathway, amplifications of oncogenes, and deletions of tumor suppressor genes. These associations were no longer significant after adjusting for patient age and BE length.

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