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Impact of paired genetic testing for patients with gastric and esophageal cancers and their families

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Impact of paired genetic testing for patients with gastric and esophageal cancers and their families

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Abstract (250 words):

The prognosis of gastric and esophageal cancers is poor but may be improved by leveraging genetic testing to identify a personalized treatment. While testing is increasingly performed, it most often

focuses on the tumor alone. Identifying tumor mutations and a cancer genetic predisposition could increase access to clinical trials, predict response to therapy, and outcomes.

In this study, retrospective registry and claims data was analyzed for patients with gastric and esophageal cancers (GEC). Subsequently, patients newly diagnosed with GEC between 2021 and 2022 were offered clinical paired tumor-normal testing and we recorded demographic data, testing results, treatments received, and clinical outcomes.

Of 556 patients with GEC in the Puget Sound region from 2015 to 2019, 233 patients (41.9%) had an advanced stage, no treatment was documented for 132 patients (23.7%), and less than 11 (<2.0%) had documentation of a genetics referral. Between 2021 and 2022, 42/58 (72.4%) patients with newly diagnosed GEC seen were offered paired genetic testing. 27 patients were males (64.3%), 26 (61.9%) had an advanced GEC diagnosis, and 12 (28.6%) were positive for Helicobacter pylori. 32 patients (76.2%) were eligible for at least one targeted therapy and 19 patients received adjuvant immunotherapy. 6 patients were identified to carry an actionable hereditary cancer syndrome.

This project highlights utility of paired genetic testing, and for integrating paired genetic testing results as early as possible in treatment decisions for GEC. Uncovering a cancer genetic predisposition also prompted cascade testing, tailored screening, risk reduction, and early cancer detection for at-risk relatives.

Summary Box:

What is already known on this topic:

Patients with gastric and esophageal cancers could draw a long-term survival benefit from targeted therapies like patients with colon cancers. Often tumor-only testing is performed missing an opportunity for early detection and prevention in relatives.

What this study adds:

Here we report results of paired genetic testing for these patients where 32 patients (76.2%) were eligible for at least one targeted therapy and 19 patients received adjuvant immunotherapy. 6 patients were found to carry an actionable highly penetrant cancer predisposition to cancer and almost all would have been missed outside of this study.

How this study might affect research, practice or policy:

Results highlight the need to ensure patients meeting testing criteria are offered genetic testing.

Introduction (385 words):

Thousands of patients diagnosed with gastroesophageal cancers (GEC) face a dire prognosis[1,2,3] every year impelling we develop better methods for early diagnosis and treatments.

A subset of GEC exhibits mismatch repair (MMR) or homologous DNA damage repair deficiency (dHRD)[39]. Treatments targeting deficient DNA-repair damage pathways such as immunotherapy and/or poly (ADP-ribose) polymerase (PARP) inhibitors are associated with better tolerance, fewer long-term side effects, and better outcomes than conventional cytotoxic chemotherapy and radiation [10,11,12,13]. A recent study in advanced gastric cancer where patients with dHRD were treated with neoadjuvant durvalumab (Programmed death Ligand -1 inhibitor), paclitaxel and olaparib (PARP inhibitor) demonstrated promising results [40,41].

The etiology of GEC is heterogeneous and population-dependent [20,23]. Familial case studies of GEC suggest a hereditary component for up to 15% of patients [15,16,19]. Drawing from the overall survival benefit gained with PARP inhibitors in germline mutated breast and ovarian cancer, understanding inherited genetic factors in GEC would augment our ability to identify the most appropriate targeted therapy and predict response [30]. There are rare genetic predispositions to GEC including hereditary diffuse gastric cancer syndrome, tylosis with esophageal cancer syndrome, or chromosome breakage disorders [14,17,18,25,26,27]. However, patients with more common hereditary cancer syndromes such as Lynch syndrome and hereditary breast and ovarian cancer syndrome (HBOC), have an increased lifetime risk of upper gastrointestinal malignancies[14,21,24,28]. Uncovering HBOC would unlock access to targeted treatment with a PARP inhibitor[40,41]. Furthermore, delay in identifying a hereditary cancer syndrome at the time of a patient's GEC diagnosis closes a window of opportunity for early detection and prevention of hereditary cancers for at-risk relatives. National treatment guidelines, including the National Comprehensive Cancer Network (NCCN) guidelines, have not yet specified guidance for appropriateness of genetics referral at GEC diagnosis limiting access and insurance coverage of genetic services.

Our question was centered around contribution of tumor profiling results in guiding choice of therapy compared to foundational data on treatments and testing offered in our Puget Sound region in the years prior, and prevalence of hereditary cancer syndrome in this cohort. With this project, we set to 1) review

retrospective registry and claims data for patients with GEC diagnosed between 2015 and 2019, and 2) offer paired clinical tumor and germline testing to newly diagnosed patients to characterize the utility of tumor mutation profiles and germline test results in GEC.

Methods (378 words):

This project was implemented in two overlapping phases. First phase focused on collecting and analyzing de-identified health metrics from retrospective Surveillance, Epidemiology, End Results (SEER) data for the 13 counties of the Puget Sound region and claims submitted to Center for Medicare & Medicaid Services (CMS), Washington state Medicaid, Premera Blue Cross, and Regence Blue Shield with the Hutchinson Institute for Cancer Outcomes Research (HICOR) database. Our study team received demographic and ethnicity, cancer diagnosis and treatment data, family history, payor, area of deprivation index[46,47], and potential referral to genetics or reimbursement for genetic testing for patients diagnosed with GEC between 2015 and 2019. During the second phase of the study, we estimated number of new patients with a GEC diagnosis at Fred Hutch by querying an institutionally generated de-identified dashboard of annual completed appointments (see supplemental data). Between 01/01/2021 and 12/31/2022, oncology providers referred new patients with GEC for a cancer genetics evaluation. Their visit included a comprehensive genetics' evaluation with collection of demographic information and ancestry, construction of a 3-generation family history, pre-test counseling, review of the purpose of the study as well as documentation of interest for genetic testing and research participation. Following the genetic counseling visit, patients were contacted by research coordinator who obtained informed consent to participate. Paired somatic and germline genetic testing was performed using the clinical tests called Oncoplex and BROCA[5,6] developed by Laboratory Medicine at the University of Washington in Seattle, WA. Study team performed chart review and recorded participant demographics, personal risk factors, cancer diagnosis, pathology reports, treatment sequence, genetic test results, and vital status at follow up. Post-test genetic counseling visit included result disclosure, gastrointestinal cancer risk assessment, and recommendations for familial cascade testing if indicated. Patients and family members confirmed to have a hereditary cancer syndrome were offered a referral to a gastrointestinal cancer high-risk program and enrollment in a long-term surveillance program. We received institutional review board approval for this study. Data was stored in a password-protected REDCap database only accessible to the study team. The last six months of this project was focused on tracking outcomes, follow-up, and data analysis. Our study team performed

descriptive data analysis using Excel version 2307 and no complex statistical tests were performed. Authors of this manuscript have no competing interests.

Results (1425 words):

Patients with gastroesophageal cancers in the Puget Sound region.

Between 2015 and 2019, 556 patients were diagnosed with GEC in the Puget Sound region, including 375 males (67.4%) and 181 females. 50 patients (9.0%) reported Non-Hispanic Asian ancestry, and the remaining majority was of Non-Hispanic White ancestry. 233 patients (41.9%) were diagnosed at an advanced stage. Area Deprivation Index was 3 or lower for 212 patients (38.1%) and 6 or greater for 197 patients (35.4%) when most of the inhabitants of the Puget Sound region have an Area Deprivation Index of 3 or lower, see Figure 1. Data on familial and personal risk factors was incomplete. A referral to genetics was documented for less than 11 (<2.0%). There was no documentation of treatment for 132 patients (23.7%). Of the 424 patients who received treatment, 194 patients (34.9%) were treated for esophageal cancer with 73 (37.6%) receiving surgery, and 230 patients (41.4%) were treated for gastric cancer with 114 (49.6%) receiving surgery (Figure 2).

Patients newly diagnosed with gastric and esophageal cancers.

Between 01/01/2021 and 12/31/2022, fifty-eight patients completed an appointment at Fred Hutch for a new GEC diagnosis, see Figure 3. Forty-three patients were referred to our cancer genetics service, and one was excluded given diagnosis of laryngeal cancer extending into the upper esophagus rather than a primary GEC. Median age at diagnosis was 59.5 years [range, 33-81 years] with 9 patients (21.4%) aged 30-49; 27 patients (64.3%) were male sex; 29 patients (69%) of White or European ancestry. Of these 42 patients, 14 (33.3%) had esophageal cancer, 21 (50.0%) had gastric cancer, and 21 (50.0%) had stage 4 disease at time of diagnosis. Twelve patients (28.6%) had a prior *Helicobacter pylori* infection, and 10 (23.8%) had Barrett's esophagus. 13 patients (31.0%) had a previous primary cancer diagnosis. Of the 39 patients (92.9%) who had a family history of cancer, 35 patients (81.0%) met the NCCN guideline for genetic testing for breast and ovarian cancer and/or for Lynch syndrome, 24 patients would have been missed logistically at time of GEC diagnosis if not systematically referred to cancer genetics, see supplemental Figure 1. 37 patients had Medicare/Medicaid or Tricare, and 30 had a commercial or another insurance. All patients in this study received treatment (Table 1).

Tumor profiling and germline genetic results

Through our study, 39 out of 42 patients received tumor genetic testing, see Table 2. Six GEC (14.3%) were reported to have microsatellite instability (MSI-H), 28 (66.7%) were reported microsatellite stable (MSS). Of the 6 GEC with MSI-H, 3 patients had documented hypermethylation of the *MLH1* promoter, one had somatic biallelic inactivation of *MLH1*, one with somatic biallelic inactivation of *MSH6*, and hypermethylation studies was cancelled at patient death. All 6 had negative germline genetic testing. Six GEC (14.3%) had a high Tumor Mutational Burden (TMB >5), TMB for them was between 9 and 50 mutations/Mb. All 6 of them had concurrent MSI-H. We had no reported MSI status and TMB for 8 and 9 patients respectively. Reasons for missing tumor profiling data included insufficient tumor content, lost to follow-up, second opinion at Fred Hutch, and patient death.

Most common somatic pathogenic variants identified were in the gene *TP53* (53.1%, n=17) followed by *KRAS, GRAS*, and *NRAS* grouped together (n=8, 25.0%), *HER2* (n=6, 18.8%), and *MLH1* promoter hypermethylation (n=5, 15.6%). Interestingly, three patients had a somatic pathogenic variant in *PIK3CA*, one patient with gastroesophageal junction cancer had the *PIK3CA* c.1634 A>G (p.E545G) along with somatic biallelic inactivation of *PTEN,* and *KRAS* c.175G>A (p.A59T), two patients with gastric cancer, one with *PIK3CA* c.3140A>G (p.H1047R), and one with *PIK3CA* c.323G>A (p.R108H) and *KRAS* c.38G>A (p.G13D) highlighting the potential benefit of targeting this molecular pathway in gastric and esophageal cancer. Five patients (11.9%) had an amplification of *CCND1*, one in *CCNE1*, and one in *CCND2* highlighting potential benefit of CDK4-CDK6 inhibitors. No patients received a *KRAS* inhibitor such as Sotorasib (Lumakras®), a PIK3CA inhibitor such as Alpelisib (Piqray®) and one of our patients was prescribed the CDK4/6 inhibitor Abemaciclib (Verzenio®) that was denied by insurance. One patient was found to have an incidental pathogenic variant in the gene *CSF3R* at variant allele fraction (VAF) 37% that was suspected but not confirmed germline given this gene is not on BROCA. *CSF3R* encodes the receptor for granulocyte-colony stimulating factor (G-CSF), is involved in myeloid cell differentiation, and this variant has been associated with lower *CSF3R* messenger RNA, receptor, and response to G-CSF [42]. Patient did receive 5'Florouracil based chemotherapy, required granulocyte colony stimulating factor (G-CSF) when his absolute white count nadired below 0.5, and mounted a normal white blood cell count response.

Of 42 patients, 39 (92.8%) received germline genetic testing and 3 died prior to providing a sample. Six patients (14.3%) had a pathogenic variant, 2 were heterozygous carrier for autosomal recessive conditions, 4 (9.5%) had a variant of uncertain significance (VUS), and 29 (69.0%) had negative results. One patient with esophageal cancer before age 50 had a pathogenic variant in the gene *ERCC2* called

c.1972C>T (p.R658C) with loss of heterozygosity in the tumor. There was no history of blistering sunburns, this patient had a big family with longevity, no relatives with cancer and no relatives with Xeroderma pigmentosum. One patient with gastric cancer had a pathogenic variant in the gene *FANCA* called c.216_217del (p.L72Ffs*7) and a variant of uncertain significance in the gene *FANCI* called c.839 A>G was identified at VAF 49% on Oncoplex, *FANCI* not being on the hereditary cancer gene panel, this finding wasn't confirmed to be germline in origin. There was no documented family history of bone marrow failure or leukemia, no head and neck or other anogenital cancer in relatives. One patient with gastric cancer before age 50 and their father with history of gastric cancer shared the same VUS in the *PDGFRA* called c.470C>T (p.T157I). One patient with gastric esophageal junction cancer had three VUSs, one in *CTNNA1* called c.1726A>G (p.T576A) which is at a highly evolutionarily conserved position but with limited population and functional data, one splice site variant in the gene *USP7* called c.1839+5G>A, and one in the gene *FBXW7* called c.1076A>G (p.H359R). The gene *FBXW7* is a tumor suppressor gene known to be downregulated in gastric cancers, it is being evaluated as a marker for poor prognosis [43]. Surprisingly, we found more patients with GEC meeting NCCN guidelines for breast and ovarian cancer than for Lynch syndrome. Five patients had germline alterations in the homologous recombination DNA damage/repair pathway with pathogenic variants in *BRCA2, ATM, BRCA1,* and biallelic *FANCA*. Of the 3 patients who couldn't receive paired testing, one patient diagnosed with metastatic diffuse gastric adenocarcinoma before age 40 had their tumor sent to a tumor-only commercial laboratory. An in-frame deletion in the gene *CDH1* called c.1747_1749del (p.L583del) was identified at 47.8% VAF and classified as a VUS. Given the high suspicion for hereditary diffuse gastric cancer syndrome, multiple attempts were made to follow up with this patient for additional testing without success.

Treatment and targeted therapies

Most patients received surgery alone or neoadjuvant chemotherapy and radiation before surgery when they were eligible. Molecular tumor profiling unlocked access to at least 1 adjuvant targeted therapy approved by the US Food and Drug Administration (FDA) for 32 of the 42 patients (76.2%). Targeted therapy was known to be beneficial for 17 patients (40.5%) and potentially beneficial for 21 patients (50.0%) as efficacy was not established yet in GEC but reported in other cancer types. An example of this was having an *FGFR2* amplification or a fusion with the potential benefit of Erdafitinib (Balversa®). Overall, 24 patients (57.1%) received at least one targeted therapy such as Pembrolizumab (Keytruda®),

Nivolumab (Opdivo®), Trastuzumab (Herceptin®), and Ramucirumab (Cyramza®) as part of their treatment.

NCCN guideline encourages screening for eligibility to adjuvant immune checkpoint inhibitors[34] in all patients with GEC by obtaining a Combined Positive Score (CPS) [44] from a tumor sample by measuring the ratio of tumor cells expressing Programmed Death – Ligand 1 (PD-L1) over the total number of viable tumor cells. A CPS score was documented for 31 of the 42 GEC (73.8%). 19 of the 31 patients (61.3%) received adjuvant immunotherapy, 16 of the 26 patients (61.5%) had a GEC reported with CPS score > 1, and 3 of the 5 GEC had a CPS score ≤ 1. Eleven patients' GEC didn't have a CPS score documented and 6 of them (54.5%) received immunotherapy. Should they need further therapy, 17 patients (40.5%) would be eligible for future clinical trials with regimen containing a WEE1 kinase inhibitor given tumor alterations in *TP53*.

Discussion (844 words):

In our study, we report on clinical utility of paired genetic testing in GEC. More than 75% of patients who received tumor genetic testing were eligible for a targeted therapy. Almost three quarters of patients' GEC were submitted for a CPS score when reporting a CPS score is strongly encouraged for all GEC. 26 patients were eligible for adjuvant immunotherapy and 16 received it, and for 9 patients, benefit of immunotherapy was unknown given absent or CPS ≤ 1. Our data highlights importance of improving access to tumor – normal genetic testing as part of the staging work up for tailored treatment decision making[30] in GEC. By January 1st, 2024, 18 patients (42.9%) had died of complications of their cancer emphasizing the mortality burden from GEC and the need for better treatment options in the future.

Less than 1% of patients with GEC diagnosed between 2015 and 2019 in the Puget sound region had any documentation of claims related to genetic counseling, all patients would have met eligibility criteria for this study based on documentation of a family history. Family history is likely significantly underreported in claims data given that 1) many patients with GEC don't see a genetic counselor, and 2) genetic counseling is not always billable or billed as a service. Efforts to increase awareness of the higher prevalence of HCS in patients with a cancer diagnosis irrespective of the cancer type will be useful [19]. Findings from our newly diagnosed cohort aligns with other research showing that 1 in 6 patients with GEC have an actionable hereditary cancer syndrome [36]. When planning this study, we anticipated to find more patients with Lynch syndrome than HBOC. More than 80% of patients in our cohort met the breast and ovarian cancer and/or the Lynch syndrome guideline for genetic testing. Of those identified to meet

guidelines, less than a third would have been identified at diagnosis alone if not universally referred. This may justify the need to update NCCN genetic testing guidelines for GEC, consider expanding guidance on appropriateness of genetic testing, or emphasize a short list of high-yield and actionable genes in each cancer type. Moving towards including point-of-care genetic testing may also help comprehensively identify patients with an actionable hereditary cancer syndrome. It would also guide screening for at-risk relatives when they are in a window of opportunity for risk reduction or early detection.

Lastly, it is difficult to know for sure whether hereditary genetic testing we provide for GEC today is comprehensive. We assume that all cancers develop mutations in the same DNA repair, growth factors, and cell cycle pathways. It is possible, however, that inherited alterations in pathways that repair damage caused by alcohol or immunodeficiency that prevent healing from chronic inflammation plays a role in carcinogenesis in GEC. The BROCA panel test, for example, didn't cover the gene *RHBDF2* known to cause autosomal dominant tylosis with esophageal cancer syndrome for example making even this expert test an incomplete genetic evaluation for GEC. Pathogenic variants in *RHBDF2* are associated with gain-of-function with sustained *EGFR* signaling and dysregulated wound healing in the epidermis and nonkeratinized epithelium of the upper gastrointestinal tract [37,38]. No patients in our study presented with characteristic features of palmoplantar keratoderma, oral lesions or recurring esophageal strictures lowering the probability we missed this extremely rare diagnosis. Understanding interactions between genetic predispositions affecting chronic healing or repair from environmental exposures would bring powerful insights for cancer treatment and early detection in the future.

Limitations of our project include studying the Puget Sound region not including all 39 counties of the state of Washington, a small sample size, short study period during the COVID19 pandemic, and many patients being of White or European ancestry. It is possible we would have identified additional genetic, personal, or environmental risk factors if the study was performed in a broader group of patients of Chinese or Japanese Ancestry. Further studies are also needed to understand novel monogenic causes versus polygenic risk markers of GEC along with interaction between genetic factors and environmental exposures that increase the risk of developing GEC. Another limitation was that a subset of patients with a new GEC eligible for the study weren't offered participation. Reasons for why 15 patients were not referred to our study for genetic counseling and enrollment are unknown. We hypothesize that they were not included because they were diagnosed before January 1st, 2021; they had a second opinion but did not establish care; they declined referral; they had testing already; the biopsy was sent to

another laboratory for testing among other reasons. We could see that patients with GEC were referred more often by our main campus oncologists (88.1%, n=37) compared to oncologists from our community sites (11.9%, n=5). Lastly, patients came to the clinic with an advanced stage, poor nutritional status, and many died before being able to complete their genetic test. Having the ability to store a patient's DNA in a Clinical laboratory Improvement Amendments (CLIA)-certified biobank for the future would permit completion of clinical hereditary testing later for the benefit of at-risk relatives.

Conclusion (87 words):

Our study highlighted the yield and downstream impact of paired tumor – normal genetic testing in patients with GEC. Identifying tumor markers unlocked targeted therapeutic options with the hope they improve overall survival. Uncovering a hereditary cancer syndrome in patients with GEC allowed for cascade testing, tailored screening, risk reduction, and early detection for a broad range of cancers for family members. Further research is needed in stratification of risk to develop GEC, genetic modifiers of GEC, response to targeted therapy, and novel blood-based disease recurrence surveillance tools.

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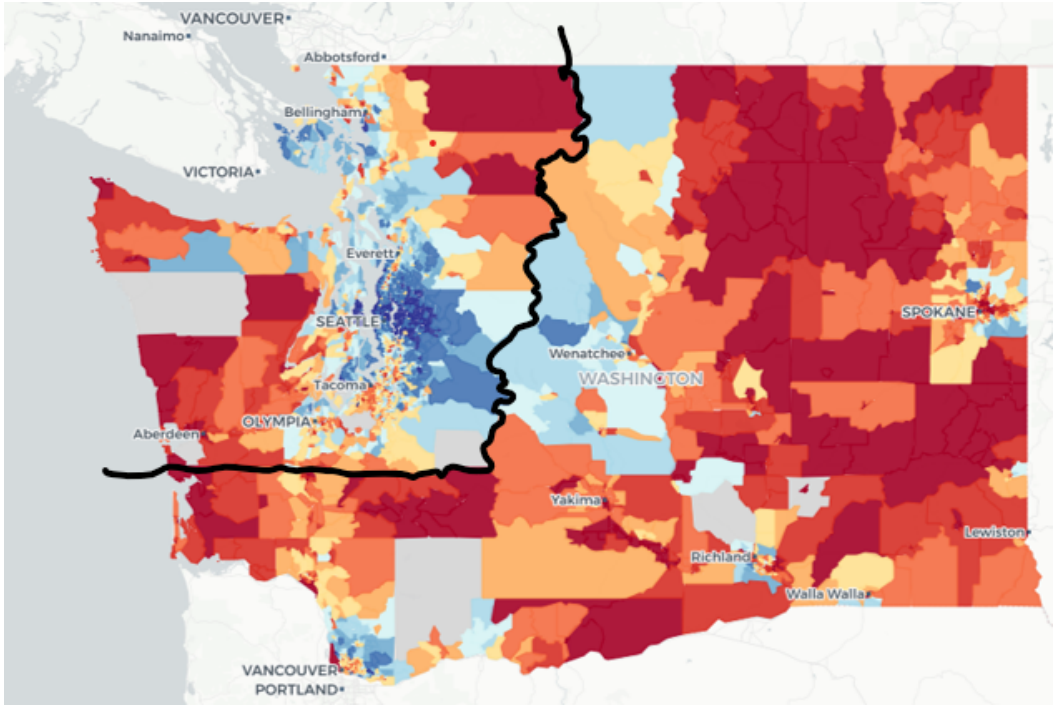
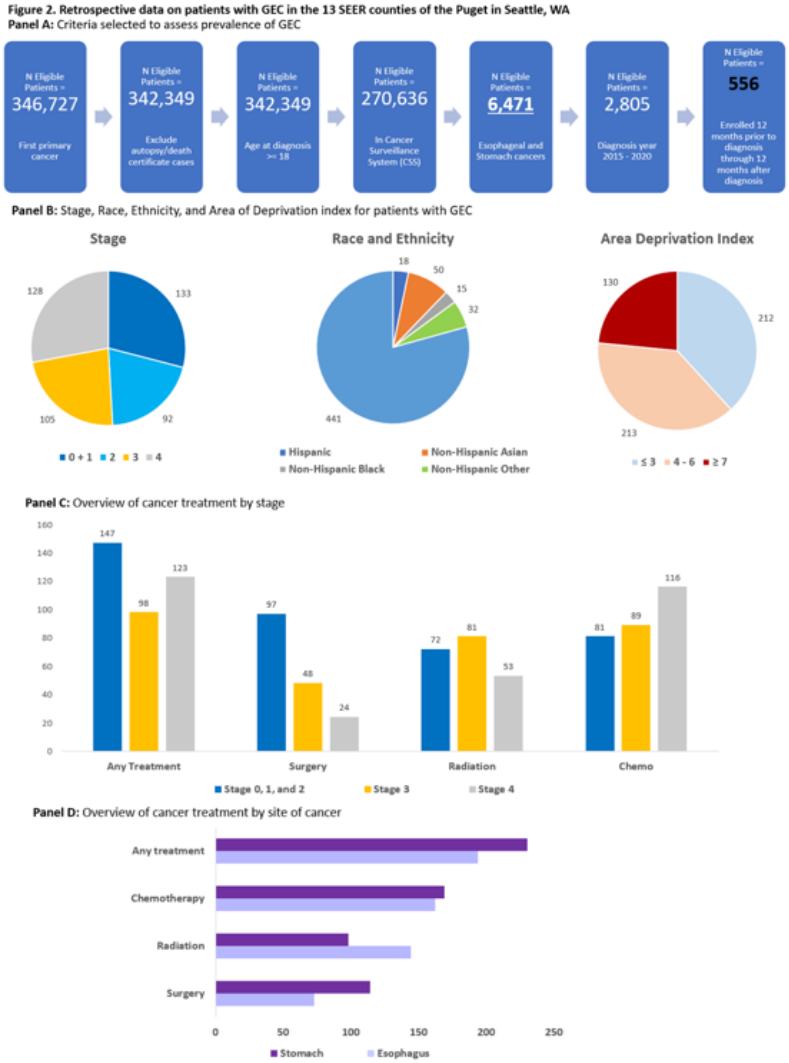
Figures for BBI GEC paper

August 2023

HICOR Figures

Figure 1. Map of area of deprivation index for the 13 SEER counties (black line) and the state of Washington





BBI-GEC Tables

Figure 3. Consort diagram for BBI-GEC study

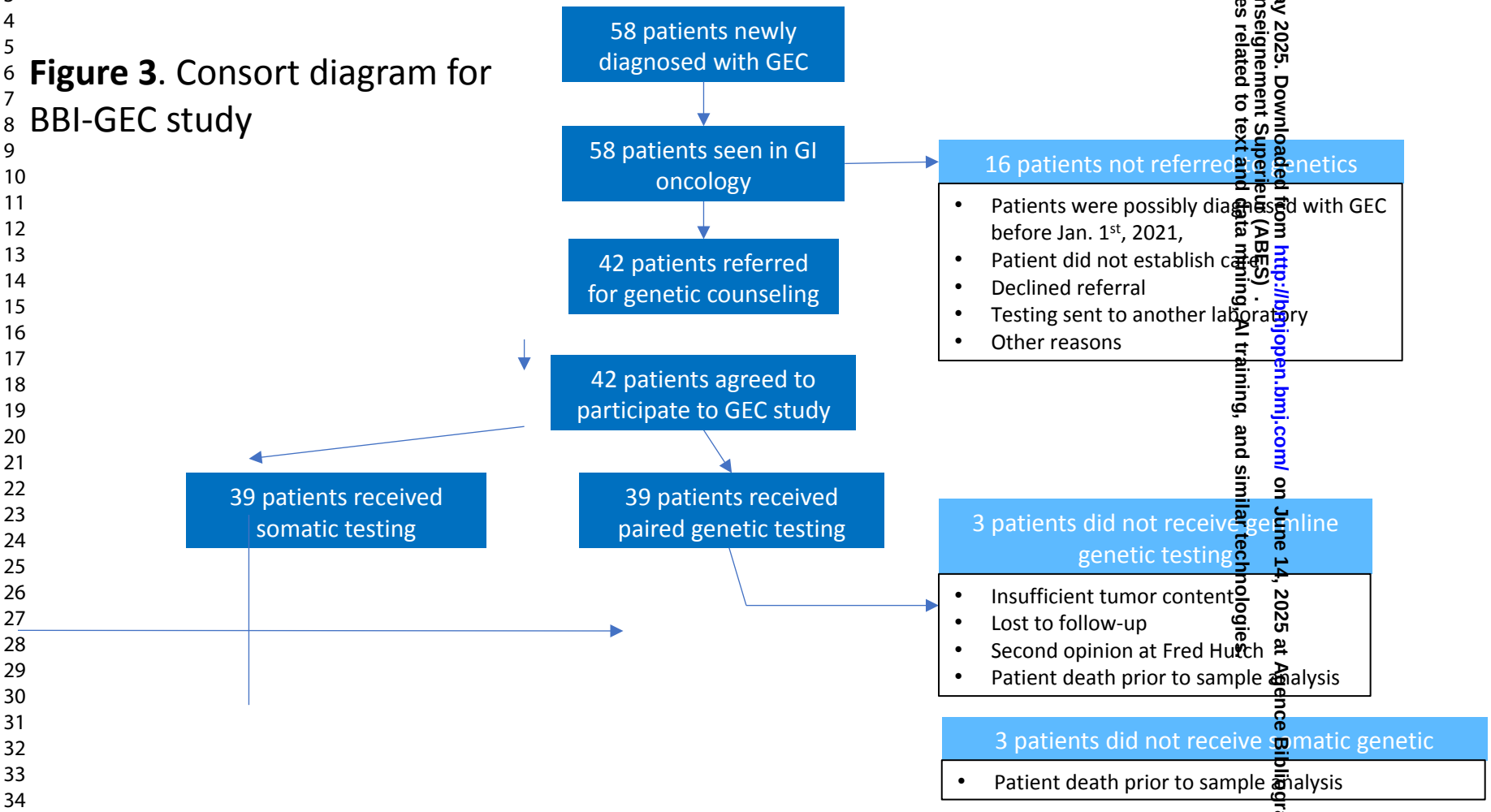


Table 1. Demographics and risk factors for GEC in patients newly diagnosed with GEC

Variable	N Population	% Population
Age		
30-39	2	4.8%
40-49	7	16.7%
50-59	12	28.6%
60-69	10	23.8%
70-79	8	19.0%
80 and older	3	7.1%
Sex		
Female	15	35.7%
Male	27	64.3%
Race		
White/European	29	69.0%
African American/Black	1	2.4%
Asian	8	19.0%
American Indian/Alaskan Native	0	0.0%
Native Hawaiian/Pacific Islander	0	0.0%
Other	2	4.8%
Unknown	1	2.4%
Declined to Answer	1	2.4%
Ethnicity		
Hispanic/Latino	7	16.7%
Non-Hispanic/Latino	33	78.6%
Unknown	2	4.8%
Cancer Type		
Esophageal, ICD-10 Code C15	14	33.3%
Gastroesophageal Junction, ICD-10 Code C16.0	7	16.7%
Gastric, ICD-10* Code C16.1-9	21	50.0%
Stage		
I	4	9.5%
II	12	28.6%
III	5	11.9%
IV	21	50.0%
Past Cancer Diagnosis		
Yes	13	31.0%
No	29	69.0%
BMI		
BMI <25	18	42.9%
BMI 25-30	17	40.5%
BMI >30	7	16.7%
Smoking History		
Never	26	61.9%
Current	2	4.8%
Former	14	33.3%
Alcohol Use		
Yes	20	47.6%
No	22	52.4%
GI medical conditions		
Helicobater pylori Infection	12	28.6%
Inflammatory condition	0	0.0%
Polyps	12	28.6%
Barrett's esophagus	10	23.8%
Other (cns, pns, head and neck, liver, pancreas, kidney, gyn, breast, immune system, psyche)	38	90.5%
Family History of Cancer		
Yes	39	92.9%
Number of patients who met NCCN guidelines	34	81.0%
Number of patients identified by MD Oncology team alone	10	23.8%
No	3	7.1%

*ICD-10 is the International Classification of Diseases, Tenth Revision.

Table 2. Tumor and germline genetic testing results

Record ID	Cancer Type	Somatic Mutations	MSI	TMB	Germline Mutations	Follow-up
10 [a]	Esophageal	FGFR2-TACC2 fusion, TP53 c.824G>A (p.C275Y), JAK3 c.475C>T (p.Q159*)	Stable	Low	Negative	No
16	Lower third of esophagus	CSF3R mutation c.1640G>A (p.W547*), ERBB2 and EGFR amplification	Stable	Low	Negative	No
17 [b]	Esophageal cancer at the GEC	COG7-PLK1 and MRPS15-CSF3R rearrangements, deletion in CDKN2A	Stable	Low	Negative	No
20	Esophageal	N/A	High	High	Negative	No
24	Esophageal	TP53 c.422G>T (p.C141F), ARID1A mutation c.5131_5132del (p.K1711Efs*16)	Stable	Low	Negative	No
25	Esophageal	KRAS, ETV6, and CCND2 amplification, TP53 mutation c.844C>T (p.R282W)	Stable	Low	Negative	No
29 [aa]	Esophagus, unspecified	2 pathogenic variants in FANCA [1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40)], TP53 mutation c.949C>T (p.Q317*), CDKN2A mutation c.247C>T (p.H83Y)	Stable	Low	FANCA [1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40)]	Yes
31	Esophageal	N/A	Stable	Unknown	Negative	No
34	Esophageal	TP53 c.1024C>T (p.R342*), APC c.4666dup (p.T1556Nfs*3)	Stable	Low	Negative	No
37 [bb]	Squamous Cell Esophogeal	BRCA2 c.9076C>T (p.Q3026*), TP53 c.637C>T (p.R213*), CDKN2A, CDKN2B, MTAP deletion, APC [1. c.7744G>T (p.E2582*), and 2. 65bp del at exon 7-intron 7 boundary)	Stable	Low	BRCA2 c.9076C>T (p.Q3026*)	Yes
40 [c]	Esophageal Adenocarcinoma	KRAS c.38_40dup (p.G13dup), TP53 c.797G>A (p.G266E), AXIN2 c.2406-2A>G, ANKRD26	Stable	Low	Negative	Yes
41	Adenosquamous Carcinoma of Esophagus	N/A	Not Reported	Unknown	Negative	Yes
42	Esophageal Adenocarcinoma	KRAS amplification, ERCC2 c.1972C>T (p.R658C), CCND1 amplification, MET, TP53 c.586C>T (p.R196*)	Stable	Low	Negative	Yes
43	Esophageal Adenocarcinoma	ERB2, ARID1B c.1543-2A>G, MPL, CDK12	Stable	Low	Negative	Yes
12	Gastroesophageal Junction	KRAS and MYC amplification, ARID1A mutation c.1459C>T (p.Q487*)	Stable	Low	VUS: GNNA11 c.1726A>G (p.T576A)	No
15 [cc]	Gastric cancer	ATM mutation c.103C>T (p.R35*), MTOR mutation c.6959A>T (p.Y2320F), CCND1 amplification	Stable	Low	ATM c.103C>T (p.R35*)	Yes
18 [dd]	Gastroesophageal	KRAS c.182A>T (p.Q61L), CDKN2A c.247C>T (p.H83Y) and BRCA1 mutation c.68_69del (p.E23Vlfs*17), MDM2 amplification	Stable	Low	BRCA1 c.68_69del (p.E23Vlfs*17), ATM c.90+1G>T (splicing)	No
26	Gastroesophageal	TP53 c.438G>A (p.W146*), ARID1A mutation c.1636C>T (p.Q546*), CCND1 amplification	Stable	Low	Negative	No
27	Gastroesophageal	NF1 c.4733C>T (p.S1578F), STK11 c.408_425del (p.M136_S142delinsI), TP53 mutations c.155_164del (p.Q52Lfs*68); EGFR and KRAS amplification	Stable	Low	Negative	Yes

Record ID	Cancer Type	Somatic Mutations	MSI	TMB	Germline Mutations	Follow-up
28	Gastroesophageal	N/A	Not Reported	Unknown	Negative	No
30	Gastric cancer	ERBB2 copy number gain	Stable	Low	Negative	No
1	Gastric adenocarcinoma	CDH1 [1. c.539C>T (p.S180F), and 2. c.689T>G (p.L230R)], FGFR2 amplification, JAK2 amplification, CDKN2A focal copy loss	Stable	Low	Negative	No
3 [d]	Advanced gastric adenocarcinoma	PIK3CA c.3140A>G (p.H1047R)	High	High	Negative	No
4	Diffuse gastric cancer	CDH1 c.1944_1952del (p.E648_I651delinsD)	Not Reported	Unknown	Negative	No
5 [e]	Gastric cancer	PMS2 c.1239dup (p.D414Rfs*44), ASXL2 c.2255C>A (p.P752H), MUTYH c.85C>T (p.Q29*), DICER1 c.5186C>T (p.P1729L)	High	High	Negative	No
6	GI adenocarcinoma	TP53 (42bp deletion in exon 7)	Not Reported	Unknown	Negative	No
7	Diffuse gastric cancer	TP53 c.524G>A (p.R175H), RB1 c.1072C>T (p.R358*), MUTYH c.1187G>A (p.G396D)	Stable	Low	MUTYH c.1187G>A (p.G396D)	No
8	Gastric adenocarcinoma	TGFBR2 c.1658G>A (p.R553H)	Stable	Low	Negative	No
9	Signet ring cell gastric cancer	CCND1 amplification	Stable	Low	VUS: ATM c.7375C>G (p.R2459G)	No
11	Gastric adenocarcinoma, WHO grade II oligodendroglioma	HER2 amplification, TP53 mutation c.844C>T (p.R282W)	Stable	Low	Negative	No
13 [e]	Gastric cancer	PRKACA-DNAJB1 fusion, VUS: PMS2 c.755G>T (p.C252F)	Stable	Low	Negative	No
14	Gastric cancer of the antrum, diffuse type with focal signet ring cells	TP53 mutation c.638G>A (p.R213Q), MYC amplification	Stable	Low	VUS: SETD2 c.608C>T (p.P203L)	Yes
19 [f]	MDI-high gastric adenocarcinoma	HER2 mutation c.2524G>A (p.V842I)	High	High	Negative	No
21	Gastric adenocarcinoma	CDH1 (1. c.1008+1G>A 2. c.1320G>T) and TP53 mutations c.844C>T (p.R282W), CCND1 amplification	Stable	Low	Negative	Yes
22 [g]	Gastric cancer	CTNNA1, ARID1A [1. c.4624G>T (p.E1542*) and 2. c.5221G>T (p.E1741*)], TP53 c.782+1G>A	Stable	Low	Negative	No
23 [ee, h]	Gastric cancer	KRAS c.38G>A (p.G13D), FANCA c.216_217del (p.L72Ffs*7) , PIK3CA c.323G>A (p.R108H), VUS: FANCI c.839 A>G (p.K280R)	High	High	FANCA c.216_217del (p.L72Ffs*7)	Yes
32	Gastric cancer	N/A	Not Reported	Unknown	VUS: PDGFRA c.470C>T (p.T157I)	No

Record ID	Cancer Type	Somatic Mutations	MSI	TMB	Germline Mutations	Follow-up
33	Gastric cancer	BAP1 c.178C>T (p.R60*)	Stable	Low	Negative	No
35	Gastric Adenocarcinoma	N/A	Not Reported	Unknown	Negative	No
36	Gastric Adenocarcinoma	N/A	Not Reported	Unknown	Negative	Yes
38	Adenocarcinoma of Stomach	N/A	Not Reported	Unknown	Negative	Yes
39 [i]	Adenocarcinoma, Nos	KRAS c.175G>A (p.A59T), PIK3CA c.1634A>G (p.E545G), PTEN [1. c.188del (p.N63Tfs*36) and 2. c.1034T>C (p.L345P)]	High	High	Negative	No

*VUSs were excluded from the Somatic Mutations as most are not relevant today.

*a: FGFR2-TACC2 fusion approximate genomic coordinates in hg19 chr10:123239366 and chr10:123985019

*b: COG7-PLK1: (approximate hg19 breakpoint coordinates: chr16:23429670 and chr16:23690241), MRPS15-CSF3R: (approximate hg19 breakpoint coordinates:chr1:36929818 a

*f: ANKRD26: (rearrangement, approximate hg19 genomic coordinates are chr10:27355570 and chr10:27691183)

*e: PRKACA-DNAJB1: (approximate hg19 genomic coordinates of the fusion are chr19:g.14226115 and chr19:g.14628283)

*f: CTNNA1: (exon 11 deletion, approximate hg19 genomic breakpoints are chr5:g.138266746-138269256del)

*d, e, f, h, i: Given patient tumor's MSI status, many more mutations were identified and not reported here.

*aa: This patient had a known diagnosis of Fanconi Anemia, family history was consistent. Patient was found to have an additional stage 1 lung adenocarcinoma on surgery. Treatment included a 3-incision esophagectomy, a video assisted Thoracic Surgery (VATS) wedge resection of right middle lobe, and 17 cycles of adjuvant immunotherapy. Genetic testing revealed 2 germline pathogenic variants in FANCA, one of which is well-characterized as a disease-causing variant in other patients with FA. Patient has no evidence of esophageal cancer, is currently receiving surveillance for FA along with Danazol for slowly progressive bone marrow failure.

*bb: Patient was diagnosed with esophageal squamous cell carcinoma without Helicobacter pylori on immunohistochemistry staining. Patient received 8 cycles of FLOT chemotherapy, weekly carboplatin, and paclitaxel and 50.4 Gy to the esophagus and left neck. Patient was placed on adjuvant immunotherapy with Nivolumab infusion. Tumor profiling results were released after adjuvant treatment decision and were significant for biallelic inactivation of the gene BRCA2 with one pathogenic variant of germline origin. Patient has no evidence of disease at two years and therapy with a PARP inhibitor would be considered for a future line of therapy.

*cc: Patient was diagnosed with invasive adenocarcinoma at the gastroesophageal junction. He was a non-smoker, negative for Helicobacter pylori on immunohistochemistry staining, and without any known exposures. Pathogenic variant in the gene ATM with associated loss of heterozygosity in the tumor. The UW laboratory included this tumor sample in the validation of their assay measuring a homologous repair damage deficiency (dHRD) score 43 by assessment of genome-wide burden of loss of heterozygosity. dHRD score in the pre-treated gastro-esophageal junction cancer was 21%, 5% above the laboratory's current threshold of 16% for a positive dHRD score suggesting at least a partial causative role for ATM. Chromosome losses and gains being common in gastroesophageal junction cancers and this may be manifested as an elevated LOH score in the absence of HRD deficiency, further studies measuring dHRD in gastro-intestinal tumors are needed.

*dd: Patient with gastroesophageal junction adenocarcinoma was found to have both a germline founder BRCA1 pathogenic variant and a likely pathogenic ATM splice site variant. Treatment included 4 cycles of neoadjuvant Fluorouracil, Leucovorin, Oxaliplatin and Docetaxel (FLOT) chemotherapy, a trans hiatal esophagectomy, and 4 cycles of adjuvant Fluorouracil, Leucovorin, Oxaliplatin and Irinotecan (FOLFIRI) chemotherapy. Patient had no evidence of disease at the 3-year mark and screening for other cancers is negative. The UW laboratory included this tumor sample in the validation of their assay measuring a homologous repair damage deficiency (dHRD) score 43 by assessment of genome-wide burden of loss of heterozygosity. As the patient had a near complete response from neoadjuvant therapy, there was more residual necrosis/fibrosis than tumor content making it insufficient for HRD score analysis.

*ee: Our fifth patient that was diagnosed with poorly differentiated gastric adenocarcinoma with signet ring features. Patient was negative for Helicobacter pylori. Patient was found to have a germline pathogenic variant in the gene FANCA and a variant of uncertain significance (VUS) in the gene FANCI on Oncoplex. The gene FANCI is not on BROCA but somatic test documented likelihood this variant was germline with a Varian Allele Fraction (VAF) of 49%. Patient died of progression of disease. We don't know the significance of these results given that there are limited studies on the risk of developing solid malignancies in adults with FA. Confirming the diagnosis of FA in this family would have implications for siblings' bone marrow failure and cancer risks.

Supplemental figures

Record ID	Met Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	Met Lynch Syndrome	Met other guideline
4	1	2	2
5	1	2	2
6	2	1	2
7	1	2	2
8	1	2	2
9	1	2	2
10	2	2	2
11	1	2	2
12	2	2	1
13	1	2	1
14	2	2	1
15	1	1	1
16	1	2	2
17	2	2	2
18	1	2	2
19	2	1	2
20	1	1	2
22	1	2	1
23	2	2	2
24	2	1	2
25	2	2	2
26	1	2	2
27	2	2	2
28	1	2	2
29	2	2	1
30	1	2	2
31	1	2	2
32	2	2	1
33	1	2	2
34	2	2	2
35	1	1	2
36	2	2	2
37	1	2	2
38	2	2	2
39	2	2	2
40	2	1	2
41	2	2	2
42	2	2	2
43	1	2	2
Total	21	7	7
Total met guidelines	35		
Total identified by MD	10		

Figure 4. Patients with GEC meeting NCCN criteria for genetic testing

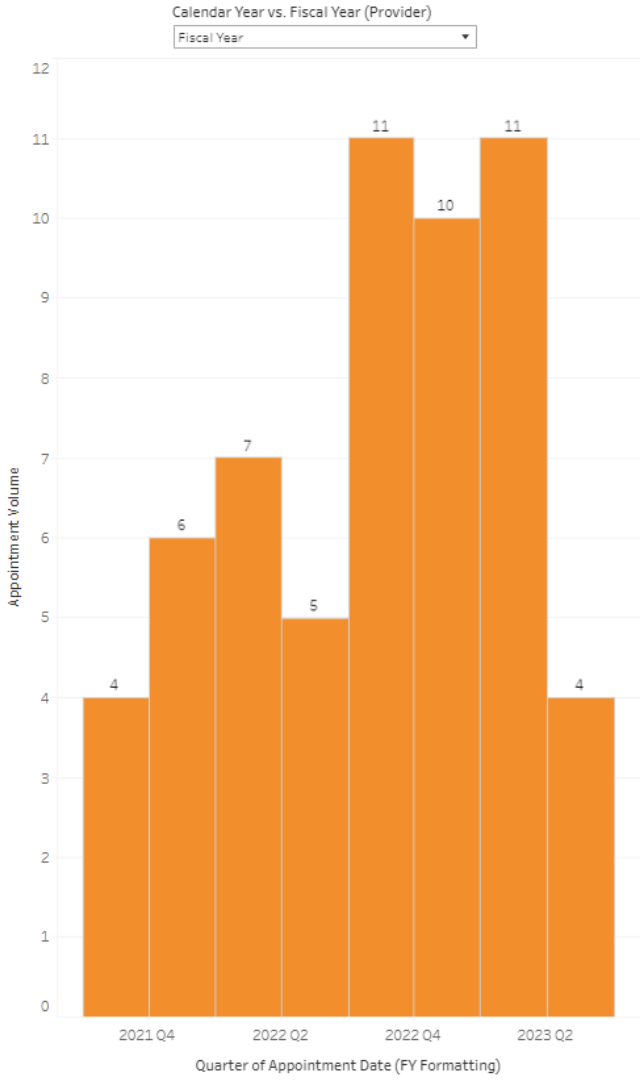
Supplemental Figure 1. Patients with GEC meeting NCCN criteria for genetic testing is now

KEY
1 Yes
2 No

	Met Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	Met Lynch Syndrome	Met other guideline
Total	21	7	7
Total met guidelines	35		
Total identified by MD	10		

Supplemental figures

Supplemental Figure 2. Appointment volume for new GEC diagnosis at FHCC between 01/01/2021 and 12/31/2022



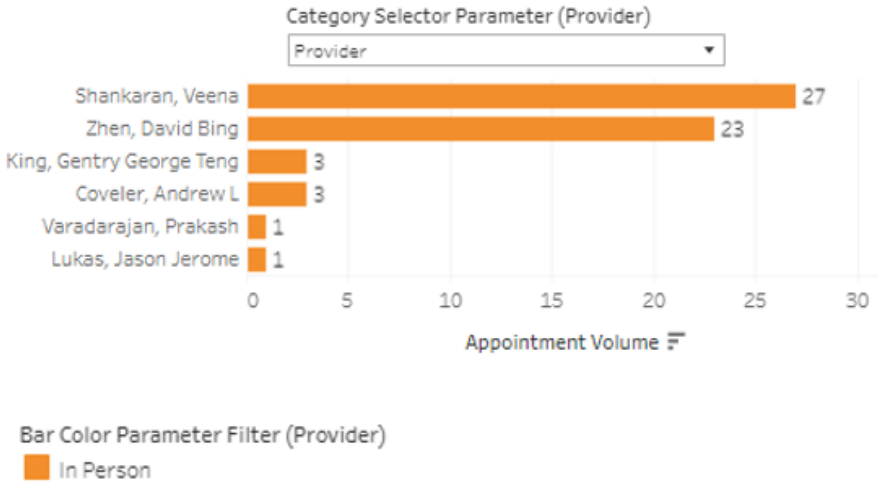
Supplementary tables

Supplemental Figure 3. Criteria to identify new GEC diagnosis at FHCC between 01/01/2021 and 12/31/2022

Dashboard Variables Chosen to Create Figure 1	
Variable	**Selection**
Appointment Date	01/01/2021 to 12/31/2022
Appt Status	Completed
Appt Name	New, New 30, New 30 + Lab Draw, New 30 Min, New 40, New 40 + Lab Draw, New 60, New 90, New Med Onc, New Patient, New Patient with Blood Draw, New Patient with Fellow
Appt Type Category	All
Appt Type Service	Clinic
Appt Type Modality	All
Provider Display Name	All
Provider Type	Physician
Provider Department	FHCC EH General Onc, FHCC ISQ General Onc, FHCC NWH General Onc, FHCC GI Onc Neighborhood, FHCC Pen General Oncology
Appointment Department	All
Appointment Location Abbr	EVG, ISQ, NWH, PEN, SLU
Case Supervisor Program	GI
Financial Class	All
Disease Group	Gastrointestinal
Disease Subgroup	Upper GI
Disease Type	Esophagus, Stomach
Service Line	Gastrointestinal

Supplementary Figures

Supplemental Figure 4. Physicians seeing patients with new GEC diagnosis at FHCC between 01/01/2021 and 12/31/2022



BMJ Open

Does paired genetic testing improve targeted therapy choices and screening recommendations for patients with upper gastro-intestinal cancers and their families? A prospective cohort of 42 patients.

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Does paired genetic testing improve targeted therapy choices and screening recommendations for patients with upper gastro-intestinal cancers and their families? A prospective cohort of 42 patients.

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Total words: 3,460

Abstract (240 words):

Objectives: Our study was designed to assess whether paired normal-tumor testing increased access to targeted therapy, clinical trials, and influenced cancer screening recommendations given to patient and their families.

Design: Prospective cohort study. No clinical trial number.

Setting: Academic cancer center in the Pacific Northwest region of the United States

Participants: Patients newly diagnosed between 01/01/2021 and 12/31/2022 with cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS) were included. All other cancer diagnoses such as head and neck, duodenal, and lower gastrointestinal tract were excluded.

Intervention: paired germline and tumor genetic test within 90 days of new patient visit.

Primary outcome measures: Number of targeted therapies received (or not) when eligible, follow up treatment data, and number of inherited predispositions to cancers identified. No secondary outcome measures.

Results: Of 42 patients, 32 (76.2%) were eligible for at least one targeted therapy. 19 patients received immunotherapy when 16 had a biomarker predicting immunotherapy benefit and benefit of immunotherapy was unclear for 3. Another 11 didn't have this biomarker, 6 of them received immunotherapy. Six pathogenic variants were identified in 4 high-risk genes. By 01/01/2024, 18 patients (42.9%) had died of complications of cancer.

Conclusion: More than 75% of patients who received tumor testing were eligible for a targeted therapy regardless of their stage at diagnosis emphasizing the need to expand access to testing with staging workup to improve survival outcomes. Six families received personalized screening recommendations thanks to this study.

Strengths and limitations of this study:

- This is a prospective cohort characterizing 42 patients newly diagnosed with upper gastrointestinal cancers between 01/01/2021 and 12/31/2022.
- Retrospective review of claims from major payors was performed to assess characteristics of prior patients with upper gastro-intestinal cancers and frequency of genetics referral in our region
- We offered paired germline and genetic testing and assessed its impact on choice of targeted therapy, access clinical trials, and cancer screening recommendations
- Our study is limited to one large academic cancer center and to genetic testing that is clinically available in 2024.
- Sample size was small limiting our ability to perform comparative analyses between subgroups

Introduction (373 words):

Thousands of patients diagnosed with cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS) face a dire prognosis[1,2,3] every year impelling we develop better methods for early diagnosis and treatments.

A subset of CEGEJS exhibits mismatch repair (MMR) or homologous DNA damage repair deficiency (dHRD)[39]. Treatments targeting deficient DNA-repair damage pathways such as immunotherapy and/or poly (ADP-ribose) polymerase (PARP) inhibitors are associated with better tolerance, fewer long-term side effects, and better outcomes than conventional cytotoxic chemotherapy and radiation [10,11,12,13]. A recent study in advanced gastric cancer where patients with dHRD were treated with neoadjuvant durvalumab (Programmed death Ligand -1 inhibitor), paclitaxel and Olaparib (PARP inhibitor) demonstrated promising results [40,41].

The etiology of CEGEJS is heterogeneous and population-dependent [20,23]. Familial CEGEJS case studies suggest a hereditary component for up to 15% of patients [15,16,19]. Drawing from the overall survival benefit gained with PARP inhibitors in germline mutated breast and ovarian cancer, understanding inherited genetic factors in CEGEJS would augment our ability to identify the most appropriate targeted therapy and predict response [30]. There are rare genetic predispositions to CEGEJS including hereditary diffuse gastric cancer syndrome, tylosis with esophageal cancer syndrome, or chromosome breakage disorders [14,17,18,25,26,27]. However, patients with more common hereditary cancer syndromes such as Lynch syndrome and hereditary breast and ovarian cancer syndrome (HBOC), have an increased lifetime risk of upper gastrointestinal malignancies[14,21,24,28]. Uncovering HBOC would unlock access to targeted treatment with a PARP inhibitor[40,41]. Furthermore, delay in identifying a hereditary cancer syndrome at the time of a patient's diagnosis closes a window of opportunity for early detection and prevention of hereditary cancers for at-risk relatives. National treatment guidelines, including the National Comprehensive Cancer Network (NCCN) guidelines, did not specify guidance for appropriateness of genetics referral for all CEGEJS diagnoses in 2021 limiting access and insurance coverage of genetic services.

The goal of this project was to report on the clinical utility of paired normal-tumor profiling results in guiding choice of therapy, access to clinical trials, and assess the prevalence of hereditary cancer syndrome in patients with CEGEJS. With this project, we reviewed retrospective registry and claims data for patients with CEGEJS diagnosed between 2015 and 2019, and we prospectively followed newly diagnosed patients with CEGEJS after their received paired clinical normal-tumor testing.

Methods (632 words):

This project included a retrospective review of registry and payor claims, and a prospective cohort study of patients newly diagnosed with CEGEJS . For the retrospective review, we collected and analyzed de-identified health metrics from the Surveillance, Epidemiology, End Results (SEER) data for the 13 counties of the Puget

Sound region (see Figure 1 of Supplemental data) and claims data submitted to Center for Medicare & Medicaid Services (CMS), Washington state Medicaid, Premera Blue Cross, and Regence Blue Shield and shared with Hutchinson Institute for Cancer Outcomes Research (HICOR) between 2015 and 2019. Retrospective dataset contained demographic and ethnicity information, cancer diagnosis and treatment data, family history, payor, area of deprivation index[46,47], and reports of referral to genetics or reimbursement for genetic testing for patients diagnosed with CEGEJS. During the prospective cohort study, we estimated number of patients newly diagnosed with a CEGEJS diagnosis at Fred Hutch by querying an institutionally generated de-identified dashboard of annual completed appointments (see Supplemental material file). Two weeks prior to study start date, we met with the Fred Hutch gastro-intestinal oncologists at each location to share the protocol, eligibility criteria, and how to refer to the study. We sent a departmental update on this study after one year of enrollment. Between 01/01/2021 and 12/31/2022, gastrointestinal oncologists referred new patients with CEGEJS for a cancer genetics evaluation and study participation. The visit with genetics included a collection of demographic information and ancestry, confirmation of histology, construction of a 3-generation family tree, pre-test counseling, review of the purpose of the study, documentation of interest for genetic testing and research participation. Following the genetic visit, patients were contacted by research coordinator who obtained informed consent to participate. Paired somatic and germline genetic testing was ordered by genetics team and performed using the clinical genetic tests called Oncoplex and BROCA[5,6] developed by Laboratory Medicine at the University of Washington in Seattle, WA (See Figure 7 of Supplemental data for list of genes tested). Post-test genetic counseling visit included result disclosure, and recommendations for familial cascade testing if indicated. Patients and family members confirmed to have a hereditary cancer syndrome were offered a referral to a gastrointestinal cancer high-risk program and enrollment in a long-term surveillance program. Study team performed periodic chart review and recorded participant demographics, personal risk factors, cancer diagnosis based on histology report, treatment sequence, genetic test results, and vital status at follow up. All histology were included. We also assessed whether each patient met criteria for genetic testing per the National Comprehensive Cancer Network (NCCN) guidelines for genetic testing available in January 2021. Testing for MSI was performed with next generation sequencing[50], testing for mismatch MMR repair deficiency with immunohistochemistry (IHC), and testing for HER overexpression with IHC and Fluorescence In Situ Hybridization (FISH). Testing for Programmed Death – Ligand 1 (PD-L1) in a tumor sample was performed by measuring the ratio of tumor cells expressing PD-L1 over the total number of viable tumor cells and reported under a combined Positive Score (CPS) [44].

The study was approved by the IRB of the University of Washington with IRB no 11490. No ethics approval was obtained for this study as ethics review is included in our institution review board when needed. Data was stored in a password-protected REDCap database only accessible to the study team. Our study team performed

descriptive data analysis using Excel version 2307 and no complex statistical tests were performed. Authors of this manuscript have no competing interests.

Patient and Public Involvement

The Institutional Review Board team of the University of Washington includes unaffiliated community members of the Seattle area. They reviewed the protocol for this study. Genetics results for each patient obtained during the study were shared with them, ample time for review and questions was provided. Results of the study will be shared with patients and their families after publication.

Results (1288 words):

Characteristics of patients newly diagnosed with CEGEJS compared to patients diagnosed between 2015-2019 in the Puget Sound.

Between 01/01/2021 and 12/31/2022, fifty-eight patients completed an appointment at Fred Hutch for a new diagnosis of CEGEJS, see Figure 1. Forty-three patients were referred to our cancer genetics service, and one was excluded given diagnosis of laryngeal cancer extending into the upper esophagus. Median age at diagnosis was 59.5 years [range, 33-81 years] with 21 patients (50.0%) aged 30-59; 27 patients (64.3%) were male sex compared to 67.4% in our registry from 2015 to 2019; 29 patients (69.0%) were reported of White or European ancestry and 8 patients of Asian descent (19.0%) compared to 79.3% and 9.0% respectively in our registry (see Table 1 and Supplemental material file).

Table 1. Demographics and risk factors for GEC in patients newly diagnosed with CEGEJS.

Variable	N Population	% Population
Age		
30-39	2	4.8%
40-49	7	16.7%
50-59	12	28.6%
60-69	10	23.8%
70-79	8	19.0%
80 and older	3	7.1%
Sex		
Female	15	35.7%
Male	27	64.3%
Race		
White/European	29	69.0%
African American/Black	1	2.4%
Asian	8	19.0%
American Indian/Alaskan Native	0	0.0%

Native Hawaiian/Pacific Islander	0	0.0%
Other	2	4.8%
Unknown	1	2.4%
Declined to Answer	1	2.4%
Ethnicity		
Hispanic/Latino	7	16.7%
Non-Hispanic/Latino	33	78.6%
Unknown	2	4.8%
Cancer Type		
Esophageal, ICD-10 Code C15	14	33.3%
Gastroesophageal Junction, ICD-10 Code C16.0	7	16.7%
Gastric, ICD-10* Code C16.1-9	21	50.0%
Stage		
I	4	9.5%
II	12	28.6%
III	5	11.9%
IV	21	50.0%
Past Cancer Diagnosis		
Yes	13	31.0%
No	29	69.0%
BMI		
BMI <25	18	42.9%
BMI 25-30	17	40.5%
BMI >30	7	16.7%
Smoking History		
Never	26	61.9%
Current	2	4.8%
Former	14	33.3%
Alcohol Use		
Yes	20	47.6%
No	22	52.4%
GI medical conditions		
Helicobater pylori Infection	12	28.6%
Inflammatory condition	0	0.0%
Polyps	12	28.6%
Barrett's esophagus	10	23.8%
Comorbidities	38	90.5%
Family History of Cancer		
Yes	39	92.9%
Patients who met NCCN guidelines	34	81.0%
Patients identified by MD Oncology team if not referred	10	23.8%
No	3	7.1%

Of these 42 patients, 14 (33.3%) had esophageal cancer, 21 (50.0%) had gastric cancer, and 26 (61.9%) had stage 3 or 4 disease at time of diagnosis compared to 41.9% in our registry. Twelve patients (28.6%) had a prior *Helicobacter pylori* infection, and 10 (23.8%) had Barrett's esophagus. 13 patients (31.0%) had a previous primary cancer diagnosis, breast cancer being the most common prior cancer. Of the 39 patients (92.9%) who had a family history of cancer, 35 patients (81.0%) met the NCCN guideline for genetic testing for hereditary breast and ovarian cancer syndrome (HBOC) and/or for Lynch syndrome, 24 patients would have not received germline testing around time of CEGEJS diagnosis if not referred to cancer genetics through this study, see supplemental material file. 37 patients had Medicare/Medicaid or Tricare, and 30 had a commercial or another insurance. Area Deprivation Index was collected in our payor claims data but not for our prospective cohort as zip codes were not recorded. It was 6 or greater for 197 patients (35.4%) when most of the inhabitants of the Puget Sound region have an Area Deprivation Index of 3 or lower, see supplemental material file. All patients in our prospective cohort received treatment compared to 424 of 556 patients (76.3%) received treatment in our registry (see supplemental material file). By January 1st, 2024, 18 patients (42.9%) had died of complications of CEGEJS.

Tumor profiling and germline genetic results

Through our study, 39 out of 42 patients received tumor genetic testing, see Table 2.

Table 2. Tumor and germline genetic testing results

Record ID	Organ Type	Somatic Mutations	MSI	TMB	Germline Mutations	Follow-up
10	**Esophagus**	FGFR2-TACC2 fusion, TP53 c.824G>A (p.C275Y), JAK3 c.475C>T (p.Q159*)	Stable	Low	Negative	No
16	**Esophagus**	CSF3R c.1640G>A (p.W547*), ERBB2 and EGFR amplification	Stable	Low	Negative	No
17	**Esophagus**	COG7-PLK1 and MRPS15-CSF3R rearrangements, deletion in CDKN2A	Stable	Low	Negative	No
20	**Esophagus**	N/A	High	High	Negative	No
24	**Esophagus**	TP53 c.422G>T (p.C141F), ARID1A c.5131_5132del (p.K1711Efs*16)	Stable	Low	Negative	No
25	**Esophagus**	KRAS, ETV6, and CCND2 amplification, TP53 c.844C>T (p.R282W)	Stable	Low	Negative	No
29 [aa]	**Esophagus**	2 PV in FANCA [1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40)], TP53 c.949C>T (p.Q317*), CDKN2A c.247C>T (p.H83Y)	Stable	Low	**FANCA** [1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40)]	Yes
31	**Esophagus**	N/A	Stable	N/A	Negative	No
34	**Esophagus**	TP53 c.1024C>T (p.R342*), APC c.4666dup (p.T1556Nfs*3)	Stable	Low	Negative	No
37 [bb]	**Esophagus**	BRCA2 c.9076C>T (p.Q3026*), TP53 c.637C>T (p.R213*), CDKN2A, CDKN2B, MTAP deletion, APC [1. c.7744G>T (p.E2582*), and 2. 65bp del at exon 7-intron 7 boundary)	Stable	Low	**BRCA2** c.9076C>T (p.Q3026*)	Yes
40	**Esophagus**	KRAS c.38_40dup (p.G13dup), TP53 c.797G>A (p.G266E), AXIN2 c.2406-2A>G, ANKRD26	Stable	Low	Negative	Yes
41	**Esophagus**	N/A	N/A	N/A	Negative	Yes
42	**Esophagus**	KRAS amplification, ERCC2 c.1972C>T (p.R658C), CCND1 amplification, MET, TP53 c.586C>T (p.R196*)	Stable	Low	Negative	Yes

43	**Esophagus**	ERB2, ARID1B c.1543-2A>G, MPL, CDK12	Stable	Low	Negative	Yes
12	GEJ	KRAS and MYC amplification, ARID1A c.1459C>T (p.Q487*)	Stable	Low	VUS: CTNNA1 c.1726A>G (p.T576A)	No
15 [cc]	GEJ	ATM mutation c.103C>T (p.R35*), MTOR c.6959A>T (p.Y2320F), CCND1 amplification	Stable	Low	**ATM** c.103C>T (p.R35*)	Yes
18 [dd]	GEJ	KRAS c.182A>T (p.Q61L), CDKN2A c.247C>T (p.H83Y) and BRCA1 c.68_69del (p.E23Vlfs*17), MDM2 amplification	Stable	Low	**BRCA1** c.68_69del (p.E23Vlfs*17), **ATM** c.901+1G>T (splicing)	No
26	GEJ	TP53 c.438G>A (p.W146*), ARID1A c.1636C>T (p.Q546*), CCND1 amplification	Stable	Low	Negative	No
27	GEJ	NF1 c.4733C>T (p.S1578F), STK11 c.408_425del (p.M136_S142delinsI), TP53 c.155_164del (p.Q52Lfs*68); EGFR and KRAS amplification	Stable	Low	Negative	Yes
28	GEJ	N/A	N/A	N/A	Negative	No
30	GEJ	ERBB2 copy number gain	Stable	Low	Negative	No
1	**Stomach**	CDH1 [1. c.539C>T (p.S180F), and 2. c.689T>G (p.L230R)], FGFR2 amplification, JAK2 amplification, CDKN2A focal copy loss	Stable	Low	Negative	No
3	**Stomach**	PIK3CA c.3140A>G (p.H1047R)	High	High	Negative	No
4	**Stomach**	CDH1 c.1944_1952del (p.E648_I651delinsD)	N/A	N/A	Negative	No
5	**Stomach**	PMS2 c.1239dup (p.D414Rfs*44), ASXL2 c.2255C>A (p.P752H), MUTYH c.85C>T (p.Q29*), DICER1 c.5186C>T (p.P1729L)	High	High	Negative	No
6	**Stomach**	TP53 (42bp deletion in exon 7)	N/A	N/A	Negative	No
7	**Stomach**	TP53 c.524G>A (p.R175H), RB1 c.1072C>T (p.R358*), MUTYH c.1187G>A (p.G396D)	Stable	Low	MUTYH c.1187G>A (p.G396D)	No
8	**Stomach**	TGFBR2 c.1658G>A (p.R553H)	Stable	Low	Negative	No
9	**Stomach**	CCND1 amplification	Stable	Low	VUS: ATM c.7375C>G (p.R2459G)	No
11	**Stomach**	HER2 amplification, TP53 c.844C>T (p.R282W)	Stable	Low	Negative	No
13	**Stomach**	PRKACA-DNAJB1 fusion, VUS: PMS2 c.755G>T (p.C252F)	Stable	Low	Negative	No
14	**Stomach**	TP53 c.638G>A (p.R213Q), MYC amplification	Stable	Low	VUS: STK11 c.608C>T (p.P203L)	Yes
19	**Stomach**	HER2 c.2524G>A (p.V842I)	High	High	Negative	No
21	**Stomach**	CDH1 (1. c.1008+1G>A 2. c.1320G>T) and TP53 c.844C>T (p.R282W), CCND1 amplification	Stable	Low	Negative	Yes
22	**Stomach**	CTNNA1, ARID1A [1. c.4624G>T (p.E1542*) and 2. c.5221G>T (p.E1741*)], TP53 c.782+1G>A	Stable	Low	Negative	No
23 [ee]	**Stomach**	KRAS c.38G>A (p.G13D), FANCA c.216_217del (p.L72Ffs*7) , PIK3CA c.323G>A (p.R108H), VUS: FANCI c.839 A>G (p.K280R)	High	High	**FANCA** c.216_217del (p.L72Ffs*7)	Yes
32	**Stomach**	N/A	N/A	Unknown	VUS: PDGFRA c.470C>T (p.T157I)	No
33	**Stomach**	BAP1 c.178C>T (p.R60*)	Stable	Low	Negative	No
35	**Stomach**	N/A	N/A	Unknown	Negative	No
36	**Stomach**	N/A	N/A	Unknown	Negative	Yes
38	**Stomach**	N/A	N/A	Unknown	Negative	Yes
39	**Stomach**	KRAS c.175G>A (p.A59T), PIK3CA c.1634A>G (p.E545G), PTEN [1. c.188del (p.N63Tfs*36) and 2. c.1034T>C (p.L345P)]	High	High	Negative	No

GEJ: Gastro Esophageal Junction.

***aa:** This patient had a known diagnosis of Fanconi Anemia (FA). Additional de-identified details on cancer diagnosis and treatment course can be shared upon request. Genetic testing revealed 2 germline *FANCA* pathogenic variants, one of which is well-characterized as a disease-causing variant in other patients with FA.

***bb:** Patient was diagnosed with esophageal squamous cell carcinoma without Helicobacter pylori on immunohistochemistry staining. Additional de-identified details on cancer diagnosis and treatment course can be shared upon request. Tumor profiling results were released after adjuvant treatment decision and were significant for biallelic inactivation of the gene *BRCA2* with one pathogenic variant of germline origin. Patient has no evidence of disease at two years.

***cc:** Patient was diagnosed with invasive adenocarcinoma at the gastroesophageal junction. Additional de-identified details on cancer diagnosis and treatment course can be shared upon request. Pathogenic variant in the gene ATM was associated with loss of heterozygosity in the tumor. The UW laboratory included this tumor sample in the validation of their assay measuring a homologous repair damage deficiency (dHRD) score by assessment of genome-wide burden of loss of heterozygosity [43]. dHRD score in the pre-treated gastro-esophageal junction cancer was 21%, 5% above the laboratory's current threshold of 16% for a positive dHRD score suggesting at least a partial causative role for ATM. Further studies measuring dHRD in gastro-intestinal tumors are needed as chromosome losses and gains are common in gastroesophageal junction cancers and this may manifest as an elevated LOH score in the absence of HRD deficiency.

***dd:** Patient with gastroesophageal junction adenocarcinoma was found to have both a germline *BRCA1* pathogenic variant and a likely pathogenic *ATM* splice site variant. Additional de-identified details on cancer diagnosis and treatment course can be shared upon request. Patient had no evidence of disease at the 3-year mark and screening for other cancers was negative. The UW laboratory included this tumor sample in the validation of the assay described above. As the patient had a near complete response from neoadjuvant therapy, there was insufficient tumor content for HRD score analysis.

***ee:** Our fifth patient that was diagnosed with poorly differentiated gastric adenocarcinoma with signet ring features. Additional de-identified details on cancer diagnosis and treatment course can be shared upon request. Patient was found to have a germline *FANCA* pathogenic variant and a variant of uncertain significance (VUS) in the gene FANCI with a Varian Allele Fraction (VAF) of 49% on tumor profiling test. Patient died of progression of disease without further germline confirmation testing. We don't know the significance of results given that there are limited studies on the risk of developing solid malignancies in adults with FA.

Six CEGEJS (14.3%) had microsatellite instability (MSI-H), 28 (66.7%) were microsatellite stable (MSS). Of the 6 CEGEJS with MSI-H, 3 patients had documented hypermethylation of the *MLH1* promoter, one had somatic biallelic inactivation of *MLH1*, one with somatic biallelic inactivation of *MSH6*, and hypermethylation studies was cancelled at patient death for the last patient. All 6 had negative germline genetic testing. Six CEGEJS (14.3%) had a high Tumor Mutational Burden (TMB >5), TMB for them was between 9 and 50 mutations/Mb. All 6 of them had concurrent MSI-H. We had no reported MSI status and TMB for 8 and 9 patients respectively. Reasons for missing tumor profiling data included insufficient tumor content, lost to follow-up, second opinion at Fred Hutch, and patient death. A combined Positive Score (CPS) score was documented for 31 of the 42 GCEGEJS (73.8%), 26 tumors had a with CPS score > 1 and 5 a CPS score ≤ 1.

Most common somatic pathogenic variants identified were in the gene *TP53* (53.1%, n=17) followed by *KRAS, GRAS*, and *NRAS* grouped together (n=8, 25.0%), *HER2* (n=6, 18.8%), and *MLH1* promoter hypermethylation (n=5, 15.6%). Interestingly, 3 patients had a somatic pathogenic variant in *PIK3CA*. One patient had a gastroesophageal junction cancer and a *PIK3CA* c.1634 A>G (p.E545G) along with somatic biallelic inactivation of *PTEN,* and *KRAS* c.175G>A (p.A59T). Two patients had gastric cancer, one with *PIK3CA* c.3140A>G (p.H1047R), and one with *PIK3CA* c.323G>A (p.R108H) and *KRAS* c.38G>A (p.G13D). Five patients (11.9%) had an amplification of *CCND1*, one in *CCNE1*, and one in *CCND2*. No patients received a *KRAS* inhibitor such as Sotorasib (Lumakras®)or a PIK3CA inhibitor such as Alpelisib (Piqray®), one was prescribed the CDK4/6 inhibitor Abemaciclib (Verzenio®) that was denied by the insurance. One patient was found to have an incidental pathogenic variant in the gene *CSF3R* at variant allele fraction (VAF) of 37% that was suspected but not confirmed germline. *CSF3R* encodes the receptor for granulocyte-colony stimulating factor (G-CSF), is involved in myeloid cell differentiation, and this variant has been associated with lower *CSF3R* messenger RNA, receptor, and response to G-CSF [42]. Patient did receive 5'Florouracil based chemotherapy, required granulocyte colony stimulating factor (G-CSF) when his absolute white count nadired below 0.5, and mounted a normal white blood cell count response.

Of 42 patients, 39 (92.8%) received germline genetic testing and 3 died prior to providing a sample. Six pathogenic variants (PV) were identified, 2 patients had PVs in genes associated with autosomal recessive conditions, 4 (9.5%) had one or more variant of uncertain significance (VUS), and 29 (69.0%) had negative results. Four patients had germline alterations in the homologous recombination DNA damage/repair pathway with PV in *BRCA2, ATM, BRCA1,* and biallelic *FANCA*. One patient with esophageal cancer before age 50 had a tumor PV in the gene *ERCC2* called c.1972C>T (p.R658C) with loss of heterozygosity, there was no history of Xeroderma pigmentosum. One patient with gastric cancer had a PV in the gene *FANCA* called c.216_217del (p.L72Ffs*7) and a VUS in the gene *FANCI* called c.839 A>G was identified at VAF 49% on tumor testing, finding in *FANCI* wasn't confirmed to be germline in origin. One patient with gastric cancer before age 50 and their father with history of gastric cancer shared the same VUS in the *PDGFRA* called c.470C>T (p.T157I), gene for which there are no functional assay to help clarify significance of certain variants. One patient with gastric esophageal junction cancer had 3 VUSs, one in *CTNNA1* called c.1726A>G (p.T576A) which is at a highly evolutionarily conserved position but with limited population and functional data, one splice site variant in the gene *USP7* called c.1839+5G>A, and one in the gene *FBXW7* called c.1076A>G (p.H359R). The gene *FBXW7* is a tumor suppressor gene known to be downregulated in gastric cancers, it is being evaluated as a marker for poor prognosis [43]. Of the 3 patients who couldn't receive paired testing, one patient was diagnosed with metastatic diffuse gastric adenocarcinoma with signet ring cells before age 40. Their tumor was sent to a tumor-only commercial laboratory and an in-frame deletion in the gene *CDH1* called c.1747_1749del (p.L583del) was

identified at 47.8% VAF and classified as a VUS. Given the high suspicion for hereditary diffuse gastric cancer syndrome, multiple attempts were made to follow up without success.

Treatment and targeted therapies

Most patients received surgery alone or neoadjuvant chemotherapy and radiation before surgery when they were eligible. Molecular tumor profiling unlocked access to at least 1 adjuvant targeted therapy approved by the US Food and Drug Administration (FDA) for 32 of the 42 patients (76.2%). Targeted therapy was known to be beneficial for 17 patients (40.5%) and potentially beneficial for 21 patients (50.0%) as efficacy was not established yet in GEC but reported in other cancer types. An example of this was having an *FGFR2* amplification or a fusion with the potential benefit of Erdafitinib (Balversa®). Of the 42 patients, 31 patients (61.3%) had a CPS score documented. 19 of them received adjuvant immunotherapy, 16 of the 26 patients (61.5%) whose tumors had a CPS score >1, and 3 a CPS score ≤ 1. Eleven CEGEJS didn't have a CPS score documented and 6 patients (54.5%) received immunotherapy anyway. Overall, 24 patients (57.1%) received at least one targeted therapy such as Pembrolizumab (Keytruda®), Nivolumab (Opdivo®), Trastuzumab (Herceptin®), and Ramucirumab (Cyramza®) as part of their first line treatment. Should they need further therapy, 17 patients (40.5%) would be eligible for future clinical trials with regimen containing a WEE1 kinase inhibitor given *TP53* tumor alterations.

Discussion (1070 words):

In our study, we report on the clinical utility of paired normal-tumor genetic testing when performed for all patients newly diagnosed with CEGEJS. In 2021, the NCCN guideline encouraged screening CEGEJS with multiple biomarker tests for eligibility for targeted therapies as part of the standard of care for patients with an advanced diagnosis [14, 15]. Biomarker testing included testing for HER2 overexpression to prompt considering treatment with Trastuzumab[48], testing for microsatellite instability (MSI) or mismatch repair (MMR) deficiency, and PD-L1 to prompt eligibility for adjuvant immune checkpoint inhibitors[34], and testing with next generation sequencing panel, when possible, for eligibility to receive a novel tyrosine kinase inhibitors. More than 75% of patients who received testing in our study were eligible for a targeted therapy regardless of their stage at diagnosis. Six patients received Trastuzumab, all had HER2 overexpression in their tumors. Almost three quarters of CEGEJS cases were submitted for a CPS score. 26 patients had a CPS score >1 and only 16 patients received immunotherapy. For the remaining 9 patients, benefit of immunotherapy was unknown given absent CPS score or CPS score ≤1. Furthermore, a quarter of our patients were found eligible for a novel targeted therapy based on our paired testing that went beyond what is recommended by the NCCN guidelines. Neither CDK4-CDK6 inhibitors nor PIK3CA inhibitors have approval for CEGEJS today. Our data highlights the importance of

improving access and utilization of normal-tumor genetic testing for every CEGEJS to guide treatment decision making[30] and to identify better treatment options in the future.

We identified 6 germline pathogenic variants in high-risk genes that would change patients' eligibility for clinical trials and screening and early detection for their at-risk relatives. Five additional findings were suspicious but lacked either functional data or further work up (*CSF3R*, *CTNNA1, PDGFRA*, *FANCI,* and *CDH1*). More than 80% of patients in our cohort met the HBOC and/or the Lynch syndrome guideline for germline genetic testing. We expected that more patients with CEGEJS would meet the NCCN guidelines for genetic testing for Lynch syndrome given it is associated with a stronger risk of upper gastrointestinal malignancy compared to HBOC. Of those meeting criteria, less than a third would have been offered germline genetic testing at CEGEJS diagnosis without this study. Still, the number of genetic tests ordered by oncologists was significantly higher than what was found in our retrospective payor data. Less than 2% of patients with CEGEJS diagnosed between 2015 and 2019 in the Puget sound region had any claims for genetic counseling and/or testing. For those who did, they all met eligibility criteria based on the documented personal or family history. Receipt of genetic counseling in CEGEJS was likely significantly underreported in the claims data given that 1) many patients with CEGEJS don't need to see a genetic counselor to obtain genetic testing through their oncologist or a research study, and 2) genetic counseling is not always billable or billed as a service. Findings from this cohort aligns with other research showing that 1 in 6 patients with CEGEJS have an actionable hereditary cancer syndrome[36]. As more data highlight the prevalence of inherited cancer predispositions for patients with CEGEJS, the NCCN guidelines have updated their recommendations for germline genetic testing. Adding broader guidance on appropriateness of germline genetic testing for each organ or listing the high-yield and actionable genes in each cancer type may help increase testing uptake. Point-of-care genetic testing may also accelerate the timely identification of patients and relatives with an actionable hereditary cancer syndrome and guide screening for at-risk relatives when they are in a window of opportunity for risk reduction or early detection.

Lastly, it is difficult to know for sure whether the hereditary genetic testing we provide for CEGEJS today is comprehensive. We assume that all cancers develop mutations in the same DNA repair, growth factors, and cell cycle pathways. It is possible, however, that inherited alterations in pathways that repair damage caused by alcohol or immunodeficiency that prevent healing from chronic inflammation plays a role in carcinogenesis for CEGEJS. The BROCA panel test, for example, didn't cover the gene *RHBDF2* known to cause autosomal dominant tylosis with esophageal cancer (TEC) syndrome making even this expert test an incomplete genetic evaluation for CEGEJS. Gain-of-function pathogenic variants in *RHBDF2* are associated with sustained *EGFR* signaling and dysregulated wound healing in the epidermis and nonkeratinized epithelium of the upper gastrointestinal tract [37,38]. No patients in our study presented with characteristic features of palmoplantar keratoderma, oral lesions

or recurring esophageal strictures lowering the probability we missed this extremely rare diagnosis. Understanding interactions between genetic predispositions affecting chronic healing or repair from environmental exposures would bring powerful insights for cancer treatment and early detection in the future.

Limitations of our project include studying a small sample at one large cancer center, a short study period during the COVID-19 pandemic, many patients being of White or European ancestry, and our claims and SEER data including 13 but not all 39 counties of the state of Washington. It is possible we would have identified additional genetic, personal, or environmental risk factors if the study was performed in a broader group of patients of Chinese or Japanese Ancestry. Further studies are also needed to understand novel monogenic causes versus polygenic risk markers for CEGEJS along with interaction between genetic factors and environmental exposures that increase the risk of developing CEGEJS. A subset of patients with a new CEGEJS eligible for the study weren't offered participation. Reasons for why 15 patients were not referred to our study are unknown. We hypothesize that they were not included because they were diagnosed before 01/01/2021 and came for follow up care without updated diagnosis codes (from diagnosis of cancer to history of cancer); they had a second opinion but did not establish care; they declined referral or died before being scheduled; they had testing already, or the biopsy was sent to another laboratory for tumor testing among other reasons. We noticed that patients with CEGEJS were referred more often by our main campus oncologists (88.1%, n=37) compared to our community oncologists (11.9%, n=5). Lastly, many patients came to the clinic with advanced stage, poor nutritional status, and many died before being able to complete their genetic test. Having the ability to store a patient's DNA in a Clinical laboratory Improvement Amendments (CLIA)-certified biobank for the future would permit completion of clinical hereditary testing later for the benefit of at-risk relatives.

Conclusion (97 words):

Our study highlights the yield and downstream impact of paired normal-tumor genetic testing in patients with CEGEJS. Identifying biomarkers unlocked targeted therapeutic options for most of our patients and we hope they will derive improved survival outcomes from these therapies. Uncovering a hereditary cancer syndrome in patients with CEGEJS also allowed for cascade testing, tailored screening, risk reduction, and early detection for a broad range of cancers for family members. Further research is needed in stratification of the risk to develop CEGEJS, genetic modifiers of risk, response to targeted therapy, and novel blood-based disease recurrence surveillance tools.

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Contribution statement:

KT, SAC, MYL and MDG designed the concept for study. LVN, MYL, and MDG prepare our site for this project. SAC, LVN, CLH, VS, BO, WMG, BS, EL, LF and MDG helped with patient care and consent. QS performed the primary analysis from the SEER data for the Puget Sound region. AJ, EQK, CP analyzed genetic test results and AJ, EQK, CP, and MDG reviewed and interpreted all genetic results. KT and MDG completed data analysis. All authors participated in developing the manuscript, all edited and approved the final version of the manuscript. MDG is the guarantor for this study.

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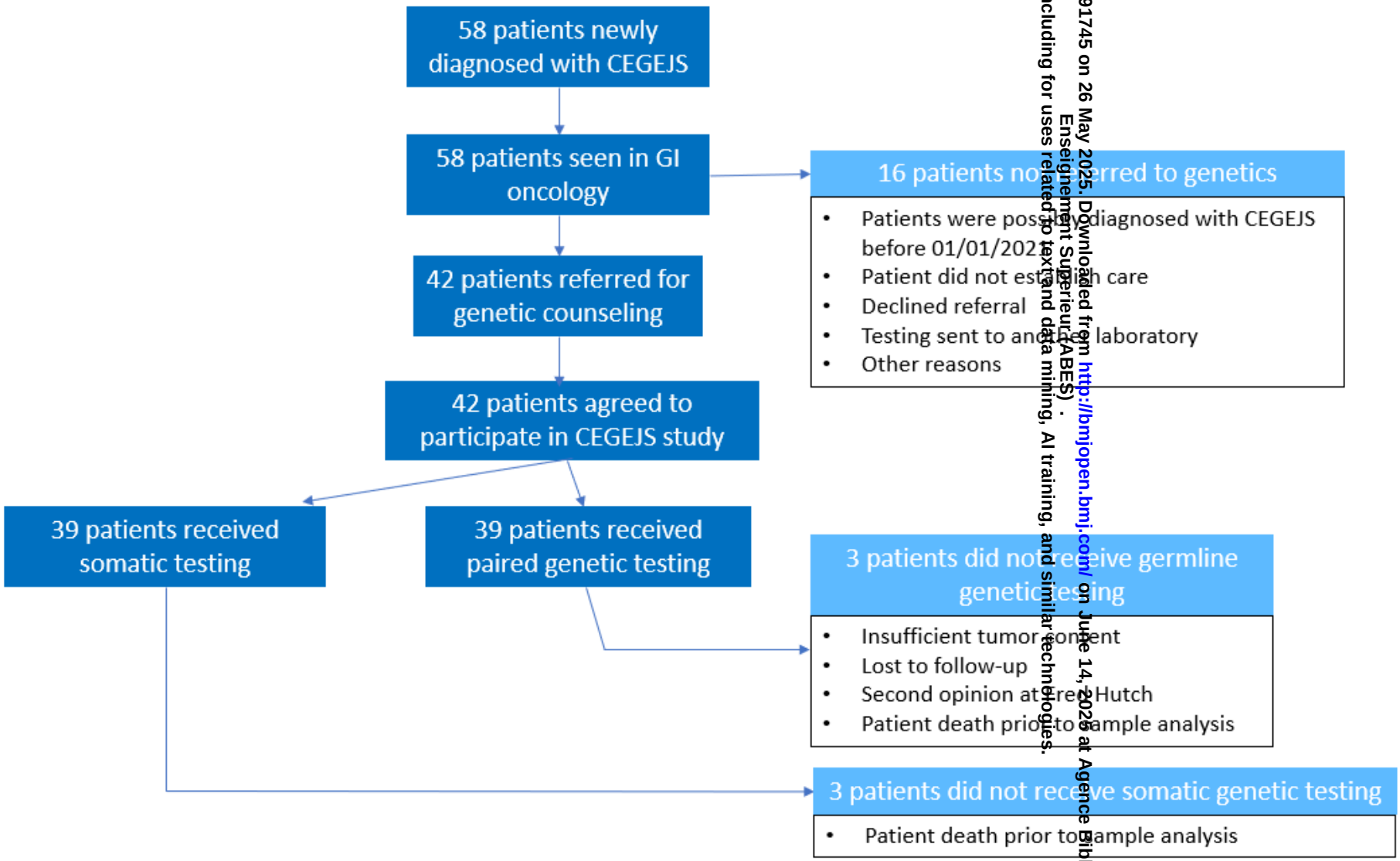
Figure 1. Consort diagram for the study on cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS)

Table 1. Demographics and risk factors for GEC in patients newly diagnosed with CEGEJS

Table 2. Tumor and germline genetic testing results

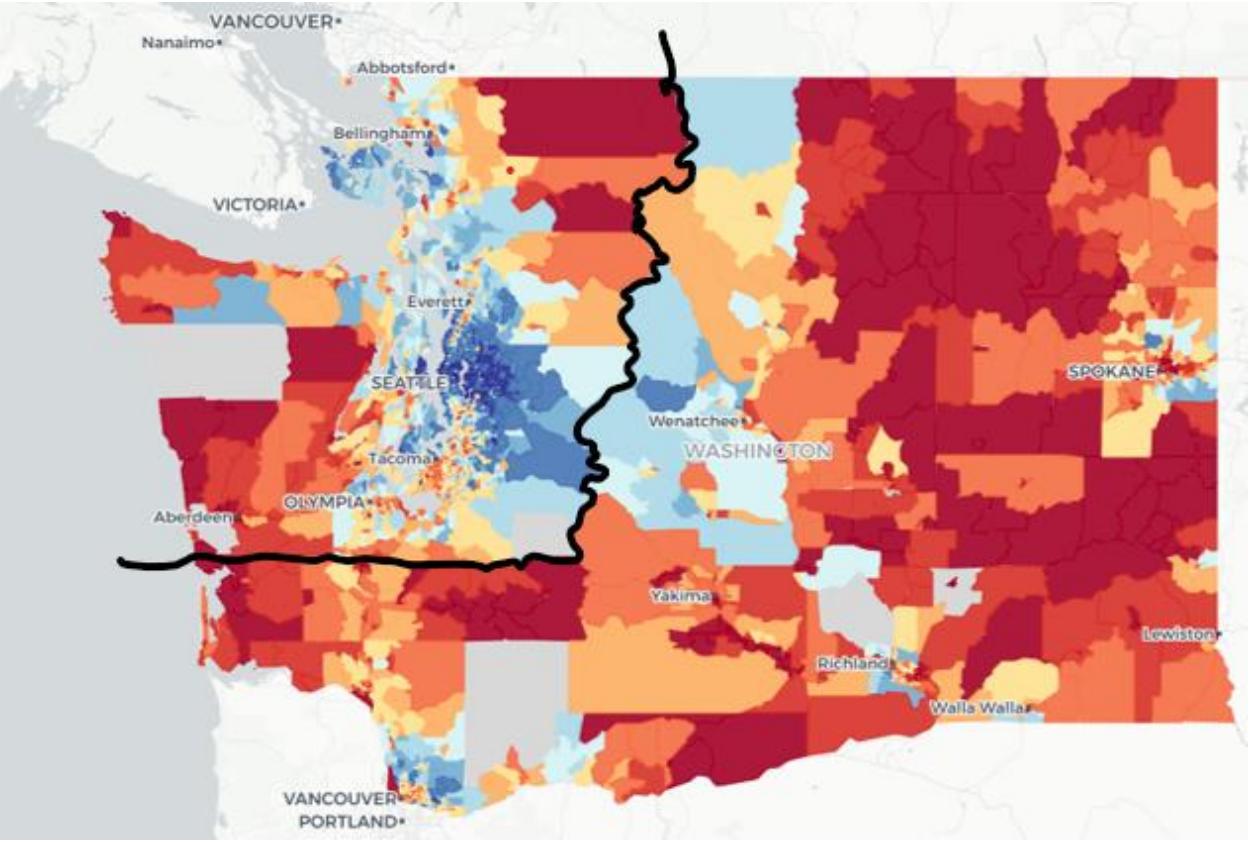
Figure 1. Consort diagram for the study on cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS)



Supplemental file

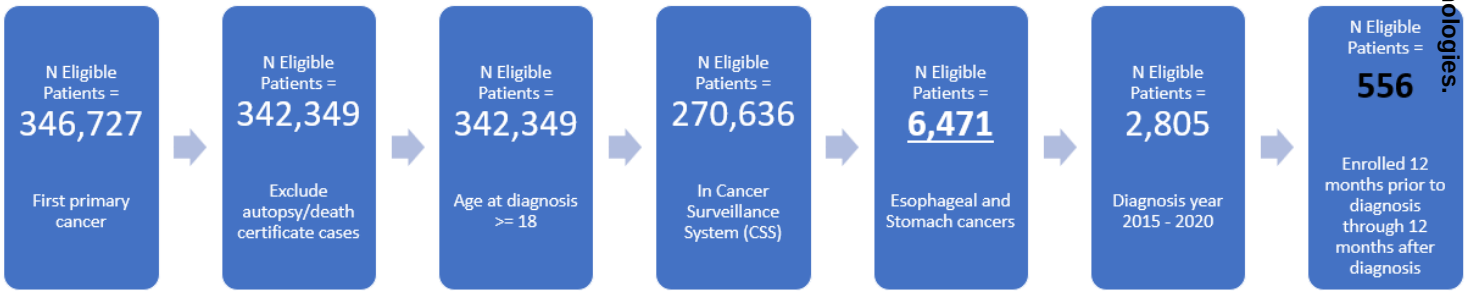
Supplemental figures

Supplemental Figure 1. Map of area of deprivation index for the 13 SEER counties (black line) and the state of Washington

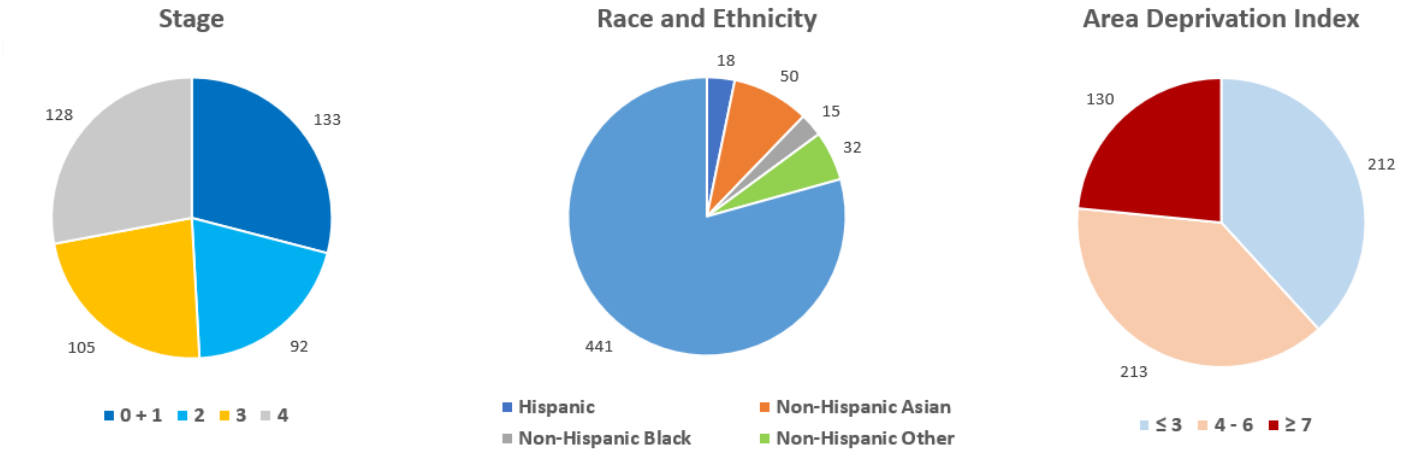


Supplemental Figure 2. Retrospective data on patients with CEGEJS in the 13 SEER counties of the Puget Sound in Seattle, WA. **Panel A:** Criteria selected to assess prevalence of CEGEJS. **Panel B:** Stage, Race and Ethnicity, and Area of Deprivation index for patients with CEGEJS. **Panel C:** Overview of cancer treatment by stage. **Panel D:** Overview of cancer treatment by site of cancer.

Panel A:



Panel B



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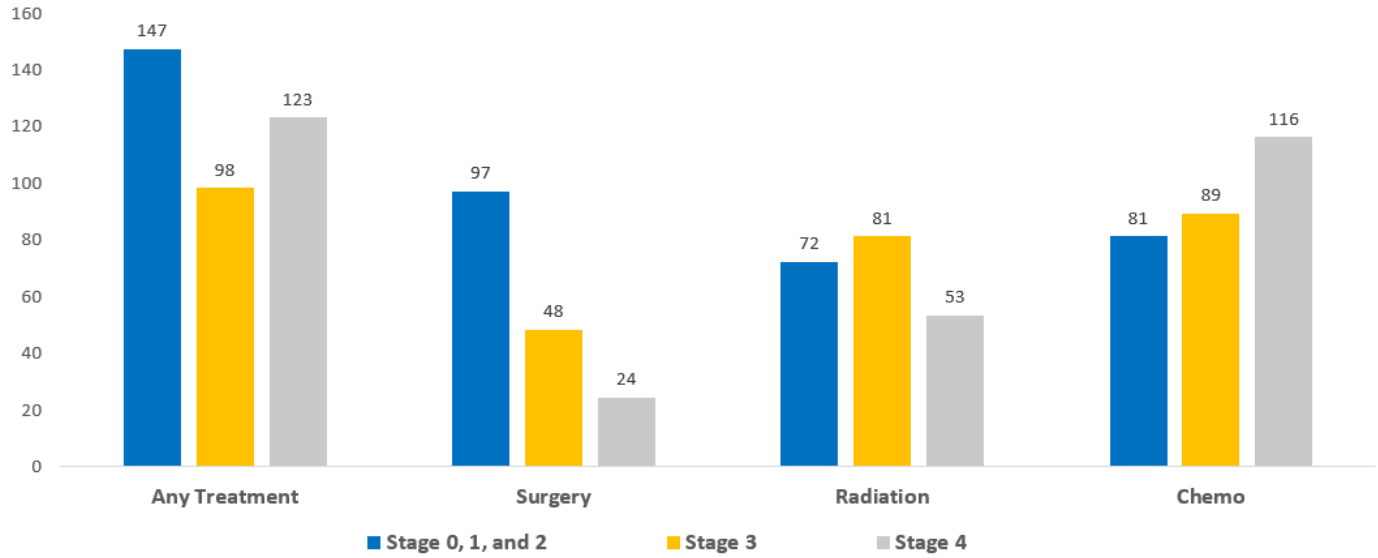
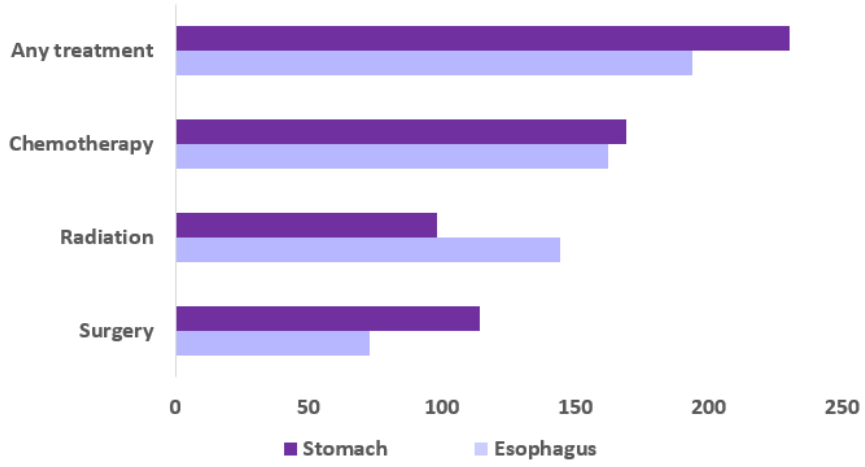


Panel D:

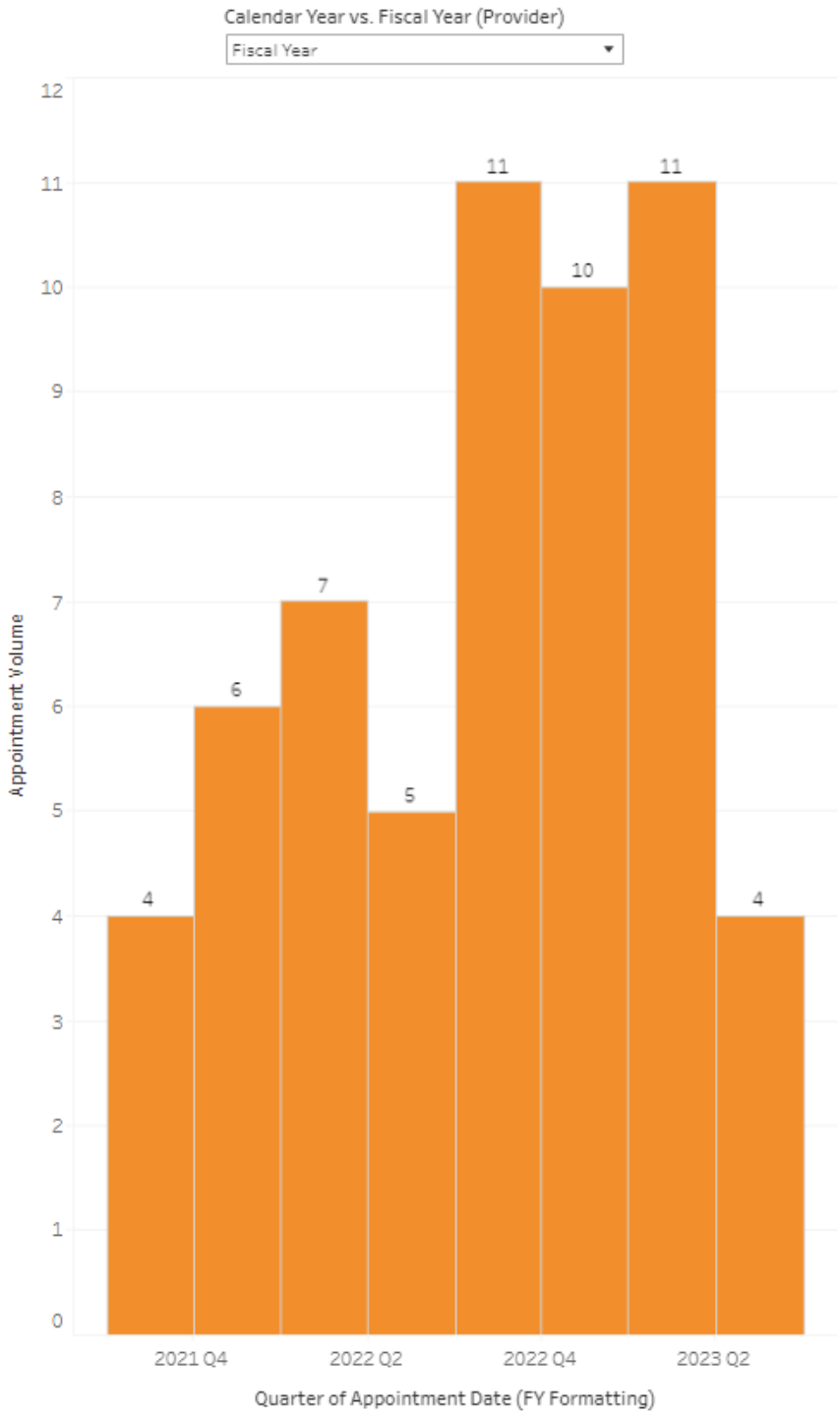


Supplemental Figure 3. Patients with CEGEJS who met NCCN criteria for genetic testing

Record ID	Met Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	Met Lynch Syndrome	Met other guideline
4	1	2	2
5	1	2	2
6	2	1	2
7	1	2	2
8	1	2	2
9	1	2	2
10	2	2	2
11	1	2	2
12	2	2	1
13	1	2	1
14	2	2	1
15	1	1	1
16	1	2	2
17	2	2	2
18	1	2	2
19	2	1	2
20	1	1	2
22	1	2	1
23	2	2	2
24	2	1	2
25	2	2	2
26	1	2	2
27	2	2	2
28	1	2	2
29	2	2	1
30	1	2	2
31	1	2	2
32	2	2	1
33	1	2	2
34	2	2	2
35	1	1	2
36	2	2	2
37	1	2	2
38	2	2	2
39	2	2	2
40	2	1	2
41	2	2	2
42	2	2	2
43	1	2	2
Total	21	7	7
Total met guidelines	35		
Total identified by MD	10		

KEY 1 Yes 2 No	Met Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	Met Lynch Syndrome	Met other guideline
Total	21	7	7
Total met guidelines	35		
Total identified by MD	10		

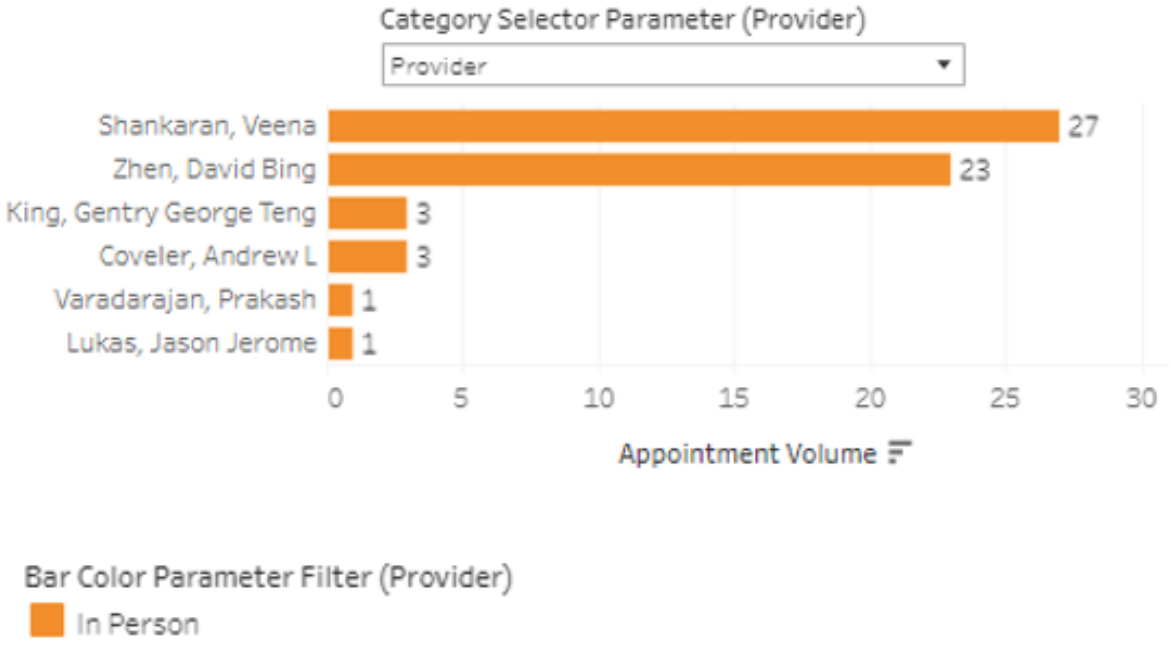
Supplemental Figure 4. Appointment volume for new CEGEJS diagnosis at FHCC between 01/01/2021 and 12/31/2022



Supplemental Figure 5. Criteria to identify new CEGEJS diagnosis at FHCC between 01/01/2021 and 12/31/2022

Dashboard Variables Chosen to Create Figure 1	
Variable	**Selection**
Appointment Date	01/01/2021 to 12/31/2022
Appt Status	Completed
Appt Name	New, New 30, New 30 + Lab Draw, New 30 Min, New 40, New 40 + Lab Draw, New 60, New 90, New Med Onc, New Patient, New Patient with Blood Draw, New Patient with Fellow
Appt Type Category	All
Appt Type Service	Clinic
Appt Type Modality	All
Provider Display Name	All
Provider Type	Physician
Provider Department	FHCC EH General Onc, FHCC ISQ General Onc, FHCC NWH General Onc, FHCC GI Onc Neighborhood, FHCC Pen General Oncology
Appointment Department	All
Appointment Location Abbr	EVG, ISQ, NWH, PEN, SLU
Case Supervisor Program	GI
Financial Class	All
Disease Group	Gastrointestinal
Disease Subgroup	Upper GI
Disease Type	Esophagus, Stomach
Service Line	Gastrointestinal

Supplemental Figure 6. Physicians seeing patients with new CEGEJS diagnosis at FHCC between 01/01/2021 and 12/31/2022



Supplemental Figure 7. List of genes on clinical genetic tests called Oncoplex and BROCA as of 01/01/2021

List of genes on Oncoplex: *ABCA10, ABCA12, ABCC9, ABL1, ABL2, ABRAXAS1 (FAM175A), ACVR1, AKAP9, AKT1, AKT2, AKT3, ALK, ANGPTL1, ANKRD26, APC, AR, ARAF, ARID1A, ARID1B, ASPH, ASXL1, ASXL2, ATM, ATR, ATRX, AURKA, AURKB, AXIN2, AXL, BABAM1, BAK1, BAP1, BARD1, BCL2, BCL2L11, BCOR, BCORL1, BCR, BICRA (GLTSCR1), BIRC3, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRIP1, BRWD3, BTK, C19MC, CALR, CARD11, CBL, CBLB, CBLC, CCL2, CCND1, CCND2, CCNE1, CD19, CD274, CD33, CD74, CD79B, CDC25A, CDC27, CDH1, CDK12, CDK4, CDK6, CDK8, CDK9, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CEBPA, CHD1, CHD3, CHD4, CHD8, CHEK1, CHEK2, COG5, CRADD, CREBBP, CRLF2, CRX, CRYBG1, CSF1R, CSF3R, CTCF, CTNNA1, CTNNB1, CUX1, CXCR4, DAXX, DDR2, DDX41, DEPDC5, DICER1, DIS3L2, DNAJB1, DNMT3A, DOCK7, EBF1, EED, EGFR, EGLN1, EIF3E, ELF1, ELP1, EML4, ENG, ENPP3, EP300, EPAS1, EPCAM, EPHA3, EPHA5, EPHB2, EPHB6, EPO, EPOR, ERBB2, ERBB3, ERBB4, ERCC2, ERG, ESR1, ESR2, ETNK1, ETV6, EZH2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FKBP1A, FLCN, FLT1, FLT3, FLT4, FOLR1, FOXA1, FOXL2, FOXR2, FUBP1, GAB2, GALNT12, GATA1, GATA2, GATA3, GEN1, GFAP, GLI1, GLI2, GLI3, GNA11, GNAQ, GNAS, GNB1, GPC3, GREM1, GRIN2A, GRM3, H3-3A, H3-3B, H3C2 (HIST1H3B), H3C3, HDAC4, HDAC9, HEPACAM, HIF1A, HNF1A, HNRNPU, HOOK3, HOXB13, HRAS, HSPH1, ID3, IDH1, IDH2, IGF1R, IKZF1, IL7R, JAK1, JAK2, JAK3, KCNJ8, KDM2B, KDM6A, KDR, KIF1B, KIF5B, KIT, KLF4, KMT2A, KMT2C, KMT2D, KRAS, KTN1, LYST, LZTR1, MAP2K1, MAP2K2, MAP2K4, MAP7, MAPK1, MAX, MBD4, MC1R, MCL1, MDM2, MDM4, MED12, MEGF6, MEN1, MET, MIOS, MITF, MLH1, MLH3, MN1, MPL, MRE11, MSH2, MSH3, MSH6, MSLN, MTAP, MTOR, MUTYH, MYB, MYC, MYCL, MYCN, MYD88, MYOD1, NAB2, NAT2, NBN, NF1, NF2, NKX2-1, NOP53 (GLTSCR2), NOTCH1, NOTCH2, NOTCH3, NOTCH4, NPM1, NPRL2, NPRL3, NR4A3, NRAS, NRG1, NRP1, NSD1, NT5C2, NTHL1, NTRK1, NTRK2, NTRK3, NUDT15, OFD1, PAK1, PALB2, PARP1, PAX5, PBRM1, PDCD1LG2, PDGFB, PDGFRA, PDGFRB, PHF6, PHOX2B, PIGA, PIK3CA, PIK3CB, PIK3R1, PIK3R2, PLCG2, PLK1, PLK2, PLK3, PLK4, PML, PMS2, POLD1, POLE, POT1, PPM1D, PPP1CB, PRKAR1A, PRPF40B, PRPF8, PRPS1, PTCH1, PTEN, PTPN11, PTPRD, QKI, RAC1, RAD21, RAD50, RAD51B, RAD51C, RAD51D, RAD54L, RAF1, RARA, RASA1, RB1, RECQL, RELA, RET, RHEB, RHOA, RICTOR, RINT1, RIT1, RNF43, ROR1, ROS1, RPL10, RPL31, RPS14, RPS15, RPS20, RPTOR, RRM1, RRM2, RSPO2, RSPO3, RUNX1, SAMD9, SAMD9L, SDHA, SDHAF2, SDHB, SDHC, SDHD, SETBP1, SETD2, SF1, SF3B1, SH2B3, SHH, SIGLEC10, SLC25A13, SLX4, SMAD2, SMAD3, SMAD4, SMARCA4, SMARCB1, SMARCE1, SMC1A, SMC3, SMO, SNAPC3, SOS1, SOS2, SPOP, SPRED1, SPRY4, SRC, SRP72, SRSF2, STAG2, STAT3, STAT5B, STAT6, STK11, STRADA, SUFU, SUZ12, TACC3, TACSTD2, TAFA2 (FAM19A2), TCF3, TERC, TERT, TET1, TET2, TET3, TFE3, TFG, TGFBR2, TLX1, TMEM127, TMPRSS2, TNFAIP3, TNFRSF14, TP53, TP53BP1, TP73, TRAF7, TRRAP, TSC1, TSC2, TTYH1, TYMS, U2AF1, U2AF2, UBA1, UBR5, USP7, VHL, WRN, WT1, XPO1, XRCC2, YAP1, ZBTB16, ZFTA (c11orf95), ZRSR2*

List of genes on BROCA: *ALK, APC, ATM, ATR, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDK12, CDKN2A, CHEK2, CTNNA1, DICER1, EPCAM, FANCM, FH, FLCN, GEN1, GREM1, HOXB13, MEN1, MET, MITF, MLH1, MLH3, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PHOX2B, PIK3CA, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51B, RAD51C, RAD51D, RB1, RECQL, RET, RNF43, RPS20, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL*

BMJ Open

Does paired genetic testing improve targeted therapy choices and screening recommendations for patients with upper gastro-intestinal cancers and their families? A prospective cohort of 42 patients.

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Does paired genetic testing improve targeted therapy choices and screening recommendations for patients with upper gastro-intestinal cancers and their families? A prospective cohort of 42 patients.

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Total words: 3,457

Abstract (237 words):

Objectives: Our study was designed to assess whether paired normal-tumor testing increased access to targeted therapy, clinical trials, and influenced cancer screening recommendations given to patient and their families.

Design: Prospective cohort study.

Setting: Academic cancer center in the Pacific Northwest region of the United States

Participants: Patients newly diagnosed between 01/01/2021 and 12/31/2022 with cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS) were included. All other cancer diagnoses such as head and neck, duodenal, and lower gastrointestinal tract were excluded.

Intervention: paired germline and tumor genetic test within 90 days of new patient visit.

Primary outcome measures: Number of targeted therapies received (or not) when eligible, follow up treatment data, and number of inherited predispositions to cancers identified. No secondary outcome measures.

Results: Of 42 patients, 32 (76.2%) were eligible for at least one targeted therapy. 19 patients received immunotherapy when 16 had a biomarker predicting immunotherapy benefit and benefit of immunotherapy was unclear for 3. Another 11 didn't have this biomarker, 6 of them received immunotherapy. Six pathogenic variants were identified in 4 high-risk genes. By 01/01/2024, 18 patients (42.9%) had died of complications of cancer.

Conclusion: More than 75% of patients who received tumor testing were eligible for a targeted therapy regardless of their stage at diagnosis emphasizing the need to expand access to testing with staging workup to improve survival outcomes. Six families received personalized screening recommendations thanks to this study.

Strengths and limitations of this study:

- This is a prospective cohort characterizing 42 patients newly diagnosed with upper gastrointestinal cancers between 01/01/2021 and 12/31/2022.
- Retrospective review of claims from major payors was performed to assess characteristics of prior patients with upper gastro-intestinal cancers and frequency of genetics referral in our region
- We offered paired germline and genetic testing and assessed its impact on choice of targeted therapy, access clinical trials, and cancer screening recommendations
- Our study is limited to one large academic cancer center and to genetic testing that is clinically available in 2024.
- Sample size was small limiting our ability to perform comparative analyses between subgroups

Introduction (373 words):

Thousands of patients diagnosed with cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS) face a dire prognosis[1,2,3] every year impelling we develop better methods for early diagnosis and treatments.

A subset of CEGEJS exhibits mismatch repair (MMR) or homologous DNA damage repair deficiency (dHRD)[39]. Treatments targeting deficient DNA-repair damage pathways such as immunotherapy and/or poly (ADP-ribose) polymerase (PARP) inhibitors are associated with better tolerance, fewer long-term side effects, and better outcomes than conventional cytotoxic chemotherapy and radiation [10,11,12,13]. A recent study in advanced gastric cancer where patients with dHRD were treated with neoadjuvant durvalumab (Programmed death Ligand -1 inhibitor), paclitaxel and Olaparib (PARP inhibitor) demonstrated promising results [40,41].

The etiology of CEGEJS is heterogeneous and population-dependent [20,23]. Familial CEGEJS case studies suggest a hereditary component for up to 15% of patients [15,16,19]. Drawing from the overall survival benefit gained with PARP inhibitors in germline mutated breast and ovarian cancer, understanding inherited genetic factors in CEGEJS would augment our ability to identify the most appropriate targeted therapy and predict response [30]. There are rare genetic predispositions to CEGEJS including hereditary diffuse gastric cancer syndrome, tylosis with esophageal cancer syndrome, or chromosome breakage disorders [14,17,18,25,26,27]. However, patients with more common hereditary cancer syndromes such as Lynch syndrome and hereditary breast and ovarian cancer syndrome (HBOC), have an increased lifetime risk of upper gastrointestinal malignancies[14,21,24,28]. Uncovering HBOC would unlock access to targeted treatment with a PARP inhibitor[40,41]. Furthermore, delay in identifying a hereditary cancer syndrome at the time of a patient's diagnosis closes a window of opportunity for early detection and prevention of hereditary cancers for at-risk relatives. National treatment guidelines, including the National Comprehensive Cancer Network (NCCN) guidelines, did not specify guidance for appropriateness of genetics referral for all CEGEJS diagnoses in 2021 limiting access and insurance coverage of genetic services.

The goal of this project was to report on the clinical utility of paired normal-tumor profiling results in guiding choice of therapy, access to clinical trials, and assess the prevalence of hereditary cancer syndrome in patients with CEGEJS. With this project, we reviewed retrospective registry and claims data for patients with CEGEJS diagnosed between 2015 and 2019, and we prospectively followed newly diagnosed patients with CEGEJS after their received paired clinical normal-tumor testing.

Methods (609 words):

This project included a retrospective review of registry and payor claims, and a prospective cohort study of patients newly diagnosed with CEGEJS . For the retrospective review, we collected and analyzed de-identified health metrics from the Surveillance, Epidemiology, End Results (SEER) data for the 13 counties of the Puget

Sound region (see Figure S1) and claims data submitted to Center for Medicare & Medicaid Services (CMS), Washington state Medicaid, Premera Blue Cross, and Regence Blue Shield and shared with Hutchinson Institute for Cancer Outcomes Research (HICOR) between 2015 and 2019. Retrospective dataset contained demographic and ethnicity information, cancer diagnosis and treatment data, family history, payor, area of deprivation index[46,47], and reports of referral to genetics or reimbursement for genetic testing for patients diagnosed with CEGEJS. During the prospective cohort study, we estimated number of patients newly diagnosed with a CEGEJS diagnosis at Fred Hutch by querying an institutionally generated de-identified dashboard of annual completed appointments (see Figures S4, S5, and S6). Two weeks prior to study start date, we met with the Fred Hutch gastro-intestinal oncologists at each location to share the protocol, eligibility criteria, and how to refer to the study. We sent a departmental update on this study after one year of enrollment. Between 01/01/2021 and 12/31/2022, gastrointestinal oncologists referred new patients with CEGEJS for a cancer genetics evaluation and study participation. The visit with genetics included a collection of demographic information and ancestry, confirmation of histology, construction of a 3-generation family tree, pre-test counseling, review of the purpose of the study, documentation of interest for genetic testing and research participation. Following the genetic visit, patients were contacted by research coordinator who obtained informed consent to participate. Paired somatic and germline genetic testing was ordered by genetics team and performed using the clinical genetic tests called Oncoplex and BROCA[5,6] developed by Laboratory Medicine at the University of Washington in Seattle, WA (see Figure S7). Post-test genetic counseling visit included result disclosure, and recommendations for familial cascade testing if indicated. Patients and family members confirmed to have a hereditary cancer syndrome were offered a referral to a gastrointestinal cancer high-risk program and enrollment in a long-term surveillance program. Study team performed periodic chart review and recorded participant demographics, personal risk factors, cancer diagnosis based on histology report, treatment sequence, genetic test results, and vital status at follow up. All histology were included. We also assessed whether each patient met criteria for genetic testing per the National Comprehensive Cancer Network (NCCN) guidelines for genetic testing available in January 2021. Testing for MSI was performed with next generation sequencing[50], testing for mismatch MMR repair deficiency with immunohistochemistry (IHC), and testing for HER overexpression with IHC and Fluorescence In Situ Hybridization (FISH). Testing for Programmed Death – Ligand 1 (PD-L1) in a tumor sample was performed by measuring the ratio of tumor cells expressing PD-L1 over the total number of viable tumor cells and reported under a combined Positive Score (CPS) [44].

The study was approved by the IRB of the University of Washington with IRB no 11490. Data was stored in a password-protected REDCap database only accessible to the study team. Our study team performed descriptive data analysis using Excel version 2307 and no complex statistical tests were performed. Authors of this manuscript have no competing interests.

Patient and Public Involvement

The Institutional Review Board team of the University of Washington includes unaffiliated community members of the Seattle area. They reviewed the protocol for this study. Genetics results for each patient obtained during the study were shared with them, ample time for review and questions was provided. Results of the study will be shared with patients and their families after publication.

Results (1294 words):

Characteristics of patients newly diagnosed with CEGEJS compared to patients diagnosed between 2015-2019 in the Puget Sound.

Between 01/01/2021 and 12/31/2022, fifty-eight patients completed an appointment at Fred Hutch for a new diagnosis of CEGEJS, see Figure 1. Forty-three patients were referred to our cancer genetics service, and one was excluded given diagnosis of laryngeal cancer extending into the upper esophagus. Median age at diagnosis was 59.5 years [range, 33-81 years] with 21 patients (50.0%) aged 30-59; 27 patients (64.3%) were male sex compared to 67.4% in our registry from 2015 to 2019; 29 patients (69.0%) were reported of White or European ancestry and 8 patients of Asian descent (19.0%) compared to 79.3% and 9.0% respectively in our registry (see Table 1 and Figures S1 and S2).

Table 1. Demographics and risk factors for GEC in patients newly diagnosed with CEGEJS.

Variable	N Population	% Population
Age		
30-39	2	4.8%
40-49	7	16.7%
50-59	12	28.6%
60-69	10	23.8%
70-79	8	19.0%
80 and older	3	7.1%
Sex		
Female	15	35.7%
Male	27	64.3%
Race		
White/European	29	69.0%
African American/Black	1	2.4%
Asian	8	19.0%
American Indian/Alaskan Native	0	0.0%
Native Hawaiian/Pacific Islander	0	0.0%
Other	2	4.8%
Unknown	1	2.4%

Declined to Answer	1	2.4%
Ethnicity		
Hispanic/Latino	7	16.7%
Non-Hispanic/Latino	33	78.6%
Unknown	2	4.8%
Cancer Type		
Esophageal, ICD-10 Code C15	14	33.3%
Gastroesophageal Junction, ICD-10 Code C16.0	7	16.7%
Gastric, ICD-10* Code C16.1-9	21	50.0%
Stage		
I	4	9.5%
II	12	28.6%
III	5	11.9%
IV	21	50.0%
Past Cancer Diagnosis		
Yes	13	31.0%
No	29	69.0%
BMI		
BMI <25	18	42.9%
BMI 25-30	17	40.5%
BMI >30	7	16.7%
Smoking History		
Never	26	61.9%
Current	2	4.8%
Former	14	33.3%
Alcohol Use		
Yes	20	47.6%
No	22	52.4%
GI medical conditions		
Helicobater pylori Infection	12	28.6%
Inflammatory condition	0	0.0%
Polyps	12	28.6%
Barrett's esophagus	10	23.8%
Comorbidities	38	90.5%
Family History of Cancer		
Yes	39	92.9%
Patients who met NCCN guidelines	34	81.0%
Patients identified by MD Oncology team if not referred	10	23.8%
No	3	7.1%

Of these 42 patients, 14 (33.3%) had esophageal cancer, 21 (50.0%) had gastric cancer, and 26 (61.9%) had stage 3 or 4 disease at time of diagnosis compared to 41.9% in our registry (see Figure S2). Twelve patients (28.6%) had a prior *Helicobacter pylori* infection, and 10 (23.8%) had Barrett's esophagus. 13 patients (31.0%) had a

previous primary cancer diagnosis, breast cancer being the most common prior cancer. Of the 39 patients (92.9%) who had a family history of cancer, 35 patients (81.0%) met the NCCN guideline for genetic testing for hereditary breast and ovarian cancer syndrome (HBOC) and/or for Lynch syndrome, 24 patients would have not received germline testing around time of CEGEJS diagnosis if not referred to cancer genetics through this study, see Figure S3. 37 patients had Medicare/Medicaid or Tricare, and 30 had a commercial or another insurance. Area Deprivation Index was collected in our payor claims data but not for our prospective cohort as zip codes were not recorded. It was 6 or greater for 197 patients (35.4%) when most of the inhabitants of the Puget Sound region have an Area Deprivation Index of 3 or lower, see Figure S2. All patients in our prospective cohort received treatment compared to 424 of 556 patients (76.3%) received treatment in our registry (Figure S2). By January 1st, 2024, 18 patients (42.9%) had died of complications of CEGEJS.

Tumor profiling and germline genetic results

Through our study, 39 out of 42 patients received tumor genetic testing, see Table 2.

Table 2. Tumor and germline genetic testing results

Record ID	Organ Type	Somatic Mutations	MSI	TMB	Germline Mutations	Follow up
10	**Esophagus**	FGFR2-TACC2 fusion, TP53 c.824G>A (p.C275Y), JAK3 c.475C>T (p.Q159*)	Stable	Low	Negative	No
16	**Esophagus**	CSF3R c.1640G>A (p.W547*), ERBB2 and EGFR amplification	Stable	Low	Negative	No
17	**Esophagus**	COG7-PLK1 and MRPS15-CSF3R rearrangements, deletion in CDKN2A	Stable	Low	Negative	No
20	**Esophagus**	N/A	High	High	Negative	No
24	**Esophagus**	TP53 c.422G>T (p.C141F), ARID1A c.5131_5132del (p.K1711Efs*16)	Stable	Low	Negative	No
25	**Esophagus**	KRAS, ETV6, and CCND2 amplification, TP53 c.844C>T (p.R282W)	Stable	Low	Negative	No
29 [aa]	**Esophagus**	2 PV in FANCA [1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40)], TP53 c.949C>T (p.Q317*), CDKN2A c.247C>T (p.H83Y)	Stable	Low	**FANCA** [1. exon 15-17del, and 2. c.1505dup (p.Y503Vfs*40)]	Yes
31	**Esophagus**	N/A	Stable	N/A	Negative	No
34	**Esophagus**	TP53 c.1024C>T (p.R342*), APC c.4666dup (p.T1556Nfs*3)	Stable	Low	Negative	No
37 [bb]	**Esophagus**	BRCA2 c.9076C>T (p.Q3026*), TP53 c.637C>T (p.R213*), CDKN2A, CDKN2B, MTAP deletion, APC [1. c.7744G>T (p.E2582*), and 2. 65bp del at exon 7-intron 7 boundary)	Stable	Low	**BRCA2** c.9076C>T (p.Q3026*)	Yes
40	**Esophagus**	KRAS c.38_40dup (p.G13dup), TP53 c.797G>A (p.G266E), AXIN2 c.2406-2A>G, ANKRD26	Stable	Low	Negative	Yes
41	**Esophagus**	N/A	N/A	N/A	Negative	Yes
42	**Esophagus**	KRAS amplification, ERCC2 c.1972C>T (p.R658C), CCND1 amplification, MET, TP53 c.586C>T (p.R196*)	Stable	Low	Negative	Yes
43	**Esophagus**	ERB2, ARID1B c.1543-2A>G, MPL, CDK12	Stable	Low	Negative	Yes
12	GEJ	KRAS and MYC amplification, ARID1A c.1459C>T (p.Q487*)	Stable	Low	VUS: CTNNA1 c.1726A>G (p.T576A)	No
15 [cc]	GEJ	ATM mutation c.103C>T (p.R35*), MTOR c.6959A>T (p.Y2320F), CCND1 amplification	Stable	Low	**ATM** c.103C>T (p.R35*)	Yes

18 [dd]	GEJ	KRAS c.182A>T (p.Q61L), CDKN2A c.247C>T (p.H83Y) and BRCA1 c.68_69del (p.E23Vlfs*17), MDM2 amplification	Stable	Low	**BRCA1** c.68_69del (p.E23Vlfs*17), **ATM** c.901+1G>T (splicing)	No
26	GEJ	TP53 c.438G>A (p.W146*), ARID1A c.1636C>T (p.Q546*), CCND1 amplification	Stable	Low	Negative	No
27	GEJ	NF1 c.4733C>T (p.S1578F), STK11 c.408_425del (p.M136_S142delinsI), TP53 c.155_164del (p.Q52Lfs*68); EGFR and KRAS amplification	Stable	Low	Negative	Yes
28	GEJ	N/A	N/A	N/A	Negative	No
30	GEJ	ERBB2 copy number gain	Stable	Low	Negative	No
1	**Stomach**	CDH1 [1. c.539C>T (p.S180F), and 2. c.689T>G (p.L230R)], FGFR2 amplification, JAK2 amplification, CDKN2A focal copy loss	Stable	Low	Negative	No
3	**Stomach**	PIK3CA c.3140A>G (p.H1047R)	High	High	Negative	No
4	**Stomach**	CDH1 c.1944_1952del (p.E648_I651delinsD)	N/A	N/A	Negative	No
5	**Stomach**	PMS2 c.1239dup (p.D414Rfs*44), ASXL2 c.2255C>A (p.P752H), MUTYH c.85C>T (p.Q29*), DICER1 c.5186C>T (p.P1729L)	High	High	Negative	No
6	**Stomach**	TP53 (42bp deletion in exon 7)	N/A	N/A	Negative	No
7	**Stomach**	TP53 c.524G>A (p.R175H), RB1 c.1072C>T (p.R358*), MUTYH c.1187G>A (p.G396D)	Stable	Low	MUTYH c.1187G>A (p.G396D)	No
8	**Stomach**	TGFBR2 c.1658G>A (p.R553H)	Stable	Low	Negative	No
9	**Stomach**	CCND1 amplification	Stable	Low	VUS: ATM c.7375C>G (p.R2459G)	No
11	**Stomach**	HER2 amplification, TP53 c.844C>T (p.R282W)	Stable	Low	Negative	No
13	**Stomach**	PRKACA-DNAJB1 fusion, VUS: PMS2 c.755G>T (p.C252F)	Stable	Low	Negative	No
14	**Stomach**	TP53 c.638G>A (p.R213Q), MYC amplification	Stable	Low	VUS: STK11 c.608C>T (p.P203L)	Yes
19	**Stomach**	HER2 c.2524G>A (p.V842I)	High	High	Negative	No
21	**Stomach**	CDH1 (1. c.1008+1G>A 2. c.1320G>T) and TP53 c.844C>T (p.R282W), CCND1 amplification	Stable	Low	Negative	Yes
22	**Stomach**	CTNNA1, ARID1A [1. c.4624G>T (p.E1542*) and 2. c.5221G>T (p.E1741*)], TP53 c.782+1G>A	Stable	Low	Negative	No
23 [ee]	**Stomach**	KRAS c.38G>A (p.G13D), FANCA c.216_217del (p.L72Ffs*7) , PIK3CA c.323G>A (p.R108H), VUS: FANCI c.839 A>G (p.K280R)	High	High	**FANCA** c.216_217del (p.L72Ffs*7)	Yes
32	**Stomach**	N/A	N/A	Unknown	VUS: PDGFRA c.470C>T (p.T157I)	No
33	**Stomach**	BAP1 c.178C>T (p.R60*)	Stable	Low	Negative	No
35	**Stomach**	N/A	N/A	Unknown	Negative	No
36	**Stomach**	N/A	N/A	Unknown	Negative	Yes
38	**Stomach**	N/A	N/A	Unknown	Negative	Yes
39	**Stomach**	KRAS c.175G>A (p.A59T), PIK3CA c.1634A>G (p.E545G), PTEN [1. c.188del (p.N63Tfs*36) and 2. c.1034T>C (p.L345P)]	High	High	Negative	No

GEJ: Gastro Esophageal Junction.

***aa:** De-identified details on cancer diagnosis and treatment course can be shared upon request. Genetic testing revealed 2 germline *FANCA* pathogenic variants, one of which is well-characterized as a disease-causing variant in other patients with FA.

***bb:** De-identified details on cancer diagnosis and treatment course can be shared upon request. Tumor profiling results were released after adjuvant treatment decision and were significant for biallelic inactivation of the gene *BRCA2* with one pathogenic variant of germline origin. Patient has no evidence of disease at two years.

***cc:** De-identified details on cancer diagnosis and treatment course can be shared upon request. Pathogenic variant in the gene ATM was associated with loss of heterozygosity in the tumor. The UW laboratory included this tumor sample in the validation of their assay measuring a homologous repair damage deficiency (dHRD) score by assessment of genome-wide burden of loss of heterozygosity [43]. dHRD score in the pre-treated cancer was 21%, 5% above the laboratory's current threshold of 16% for a positive dHRD score suggesting at least a partial causative role for ATM. Further studies measuring dHRD in gastro-intestinal tumors are needed as chromosome losses and gains are common in gastroesophageal junction cancers and this may manifest as an elevated LOH score in the absence of HRD deficiency.

***dd:** De-identified details on cancer diagnosis and treatment course can be shared upon request. Patient had no evidence of disease at the 3-year mark and screening for other cancers was negative. The UW laboratory included this tumor sample in the validation of the assay described above. As the patient had a near complete response from neoadjuvant therapy, there was insufficient tumor content for HRD score analysis.

***ee:** De-identified details on cancer diagnosis and treatment course can be shared upon request. Patient was found to have a germline *FANCA* pathogenic variant and a variant of uncertain significance (VUS) in the gene FANCI with a Varian Allele Fraction (VAF) of 49% on tumor profiling test. Patient died of progression of disease without further germline confirmation testing. We don't know the significance of results given that there are limited studies on the risk of developing solid malignancies in adults with FA.

Six CEGEJS (14.3%) had microsatellite instability (MSI-H), 28 (66.7%) were microsatellite stable (MSS). Of the 6 CEGEJS with MSI-H, 3 patients had documented hypermethylation of the *MLH1* promoter, one had somatic biallelic inactivation of *MLH1*, one with somatic biallelic inactivation of *MSH6*, and hypermethylation studies was cancelled at patient death for the last patient. All 6 had negative germline genetic testing. Six CEGEJS (14.3%) had a high Tumor Mutational Burden (TMB >5), TMB for them was between 9 and 50 mutations/Mb. All 6 of them had concurrent MSI-H. We had no reported MSI status and TMB for 8 and 9 patients respectively. Reasons for missing tumor profiling data included insufficient tumor content, lost to follow-up, second opinion at Fred Hutch, and patient death. A combined Positive Score (CPS) score was documented for 31 of the 42 GCEGEJS (73.8%), 26 tumors had a with CPS score > 1 and 5 a CPS score ≤ 1.

Most common somatic pathogenic variants identified were in the gene *TP53* (53.1%, n=17) followed by *KRAS, GRAS*, and *NRAS* grouped together (n=8, 25.0%), *HER2* (n=6, 18.8%), and *MLH1* promoter hypermethylation (n=5, 15.6%). Interestingly, 3 patients had a somatic pathogenic variant in *PIK3CA*. One patient had a gastroesophageal junction cancer and a *PIK3CA* c.1634 A>G (p.E545G) along with somatic biallelic inactivation of *PTEN,* and *KRAS* c.175G>A (p.A59T). Two patients had gastric cancer, one with *PIK3CA* c.3140A>G (p.H1047R), and one with *PIK3CA* c.323G>A (p.R108H) and *KRAS* c.38G>A (p.G13D). Five patients (11.9%) had an amplification of *CCND1*, one in *CCNE1*, and one in *CCND2*. No patients received a *KRAS* inhibitor such as Sotorasib (Lumakras®)or a PIK3CA inhibitor such as Alpelisib (Piqray®), one was prescribed the CDK4/6 inhibitor Abemaciclib (Verzenio®) that was denied by the insurance. One patient was found to have an incidental pathogenic variant in the gene *CSF3R* at variant allele fraction (VAF) of 37% that was suspected but not

confirmed germline. *CSF3R* encodes the receptor for granulocyte-colony stimulating factor (G-CSF), is involved in myeloid cell differentiation, and this variant has been associated with lower *CSF3R* messenger RNA, receptor, and response to G-CSF [42]. Patient did receive 5'Florouracil based chemotherapy, required granulocyte colony stimulating factor (G-CSF) when his absolute white count nadired below 0.5, and mounted a normal white blood cell count response.

Of 42 patients, 39 (92.8%) received germline genetic testing and 3 died prior to providing a sample. Six pathogenic variants (PV) were identified, 2 patients had PVs in genes associated with autosomal recessive conditions, 4 (9.5%) had one or more variant of uncertain significance (VUS), and 29 (69.0%) had negative results. Four patients had germline alterations in the homologous recombination DNA damage/repair pathway with PV in *BRCA2, ATM, BRCA1,* and biallelic *FANCA*. One patient with esophageal cancer before age 50 had a tumor PV in the gene *ERCC2* called c.1972C>T (p.R658C) with loss of heterozygosity, there was no history of Xeroderma pigmentosum. One patient with gastric cancer had a PV in the gene *FANCA* called c.216_217del (p.L72Ffs*7) and a VUS in the gene *FANCI* called c.839 A>G was identified at VAF 49% on tumor testing, finding in *FANCI* wasn't confirmed to be germline in origin. One patient with gastric cancer before age 50 and their father with history of gastric cancer shared the same VUS in the *PDGFRA* called c.470C>T (p.T157I), gene for which there are no functional assay to help clarify significance of certain variants. One patient with gastric esophageal junction cancer had 3 VUSs, one in *CTNNA1* called c.1726A>G (p.T576A) which is at a highly evolutionarily conserved position but with limited population and functional data, one splice site variant in the gene *USP7* called c.1839+5G>A, and one in the gene *FBXW7* called c.1076A>G (p.H359R). The gene *FBXW7* is a tumor suppressor gene known to be downregulated in gastric cancers, it is being evaluated as a marker for poor prognosis [43]. Of the 3 patients who couldn't receive paired testing, one patient was diagnosed with metastatic diffuse gastric adenocarcinoma with signet ring cells before age 40. Their tumor was sent to a tumor-only commercial laboratory and an in-frame deletion in the gene *CDH1* called c.1747_1749del (p.L583del) was identified at 47.8% VAF and classified as a VUS. Given the high suspicion for hereditary diffuse gastric cancer syndrome, multiple attempts were made to follow up without success.

Treatment and targeted therapies

Most patients received surgery alone or neoadjuvant chemotherapy and radiation before surgery when they were eligible. Molecular tumor profiling unlocked access to at least 1 adjuvant targeted therapy approved by the US Food and Drug Administration (FDA) for 32 of the 42 patients (76.2%). Targeted therapy was known to be beneficial for 17 patients (40.5%) and potentially beneficial for 21 patients (50.0%) as efficacy was not established yet in GEC but reported in other cancer types. An example of this was having an *FGFR2* amplification or a fusion with the potential benefit of Erdafitinib (Balversa®). Of the 42 patients, 31 patients (61.3%) had a CPS

score documented. 19 of them received adjuvant immunotherapy, 16 of the 26 patients (61.5%) whose tumors had a CPS score >1, and 3 a CPS score ≤ 1. Eleven CEGEJS didn't have a CPS score documented and 6 patients (54.5%) received immunotherapy anyway. Overall, 24 patients (57.1%) received at least one targeted therapy such as Pembrolizumab (Keytruda®), Nivolumab (Opdivo®), Trastuzumab (Herceptin®), and Ramucirumab (Cyramza®) as part of their first line treatment. Should they need further therapy, 17 patients (40.5%) would be eligible for future clinical trials with regimen containing a WEE1 kinase inhibitor given *TP53* tumor alterations.

Discussion (1070 words):

In our study, we report on the clinical utility of paired normal-tumor genetic testing when performed for all patients newly diagnosed with CEGEJS. In 2021, the NCCN guideline encouraged screening CEGEJS with multiple biomarker tests for eligibility for targeted therapies as part of the standard of care for patients with an advanced diagnosis [14, 15]. Biomarker testing included testing for HER2 overexpression to prompt considering treatment with Trastuzumab[48], testing for microsatellite instability (MSI) or mismatch repair (MMR) deficiency, and PD-L1 to prompt eligibility for adjuvant immune checkpoint inhibitors[34], and testing with next generation sequencing panel, when possible, for eligibility to receive a novel tyrosine kinase inhibitors. More than 75% of patients who received testing in our study were eligible for a targeted therapy regardless of their stage at diagnosis. Six patients received Trastuzumab, all had HER2 overexpression in their tumors. Almost three quarters of CEGEJS cases were submitted for a CPS score. 26 patients had a CPS score >1 and only 16 patients received immunotherapy. For the remaining 9 patients, benefit of immunotherapy was unknown given absent CPS score or CPS score ≤1. Furthermore, a quarter of our patients were found eligible for a novel targeted therapy based on our paired testing that went beyond what is recommended by the NCCN guidelines. Neither CDK4-CDK6 inhibitors nor PIK3CA inhibitors have approval for CEGEJS today. Our data highlights the importance of improving access and utilization of normal-tumor genetic testing for every CEGEJS to guide treatment decision making[30] and to identify better treatment options in the future.

We identified 6 germline pathogenic variants in high-risk genes that would change patients' eligibility for clinical trials and screening and early detection for their at-risk relatives. Five additional findings were suspicious but lacked either functional data or further work up (*CSF3R*, *CTNNA1, PDGFRA*, *FANCI,* and *CDH1*). More than 80% of patients in our cohort met the HBOC and/or the Lynch syndrome guideline for germline genetic testing. We expected that more patients with CEGEJS would meet the NCCN guidelines for genetic testing for Lynch syndrome given it is associated with a stronger risk of upper gastrointestinal malignancy compared to HBOC. Of those meeting criteria, less than a third would have been offered germline genetic testing at CEGEJS diagnosis without this study. Still, the number of genetic tests ordered by oncologists was significantly higher than what was found in our retrospective payor data. Less than 2% of patients with CEGEJS diagnosed between 2015 and

2019 in the Puget sound region had any claims for genetic counseling and/or testing. For those who did, they all met eligibility criteria based on the documented personal or family history. Receipt of genetic counseling in CEGEJS was likely significantly underreported in the claims data given that 1) many patients with CEGEJS don't need to see a genetic counselor to obtain genetic testing through their oncologist or a research study, and 2) genetic counseling is not always billable or billed as a service. Findings from this cohort aligns with other research showing that 1 in 6 patients with CEGEJS have an actionable hereditary cancer syndrome[36]. As more data highlight the prevalence of inherited cancer predispositions for patients with CEGEJS, the NCCN guidelines have updated their recommendations for germline genetic testing. Adding broader guidance on appropriateness of germline genetic testing for each organ or listing the high-yield and actionable genes in each cancer type may help increase testing uptake. Point-of-care genetic testing may also accelerate the timely identification of patients and relatives with an actionable hereditary cancer syndrome and guide screening for at-risk relatives when they are in a window of opportunity for risk reduction or early detection.

Lastly, it is difficult to know for sure whether the hereditary genetic testing we provide for CEGEJS today is comprehensive. We assume that all cancers develop mutations in the same DNA repair, growth factors, and cell cycle pathways. It is possible, however, that inherited alterations in pathways that repair damage caused by alcohol or immunodeficiency that prevent healing from chronic inflammation plays a role in carcinogenesis for CEGEJS. The BROCA panel test, for example, didn't cover the gene *RHBDF2* known to cause autosomal dominant tylosis with esophageal cancer (TEC) syndrome making even this expert test an incomplete genetic evaluation for CEGEJS. Gain-of-function pathogenic variants in *RHBDF2* are associated with sustained *EGFR* signaling and dysregulated wound healing in the epidermis and nonkeratinized epithelium of the upper gastrointestinal tract [37,38]. No patients in our study presented with characteristic features of palmoplantar keratoderma, oral lesions or recurring esophageal strictures lowering the probability we missed this extremely rare diagnosis. Understanding interactions between genetic predispositions affecting chronic healing or repair from environmental exposures would bring powerful insights for cancer treatment and early detection in the future.

Limitations of our project include studying a small sample at one large cancer center, a short study period during the COVID-19 pandemic, many patients being of White or European ancestry, and our claims and SEER data including 13 but not all 39 counties of the state of Washington. It is possible we would have identified additional genetic, personal, or environmental risk factors if the study was performed in a broader group of patients of Chinese or Japanese Ancestry. Further studies are also needed to understand novel monogenic causes versus polygenic risk markers for CEGEJS along with interaction between genetic factors and environmental exposures that increase the risk of developing CEGEJS. A subset of patients with a new CEGEJS eligible for the study weren't offered participation. Reasons for why 15 patients were not referred to our study are unknown. We hypothesize

that they were not included because they were diagnosed before 01/01/2021 and came for follow up care without updated diagnosis codes (from diagnosis of cancer to history of cancer); they had a second opinion but did not establish care; they declined referral or died before being scheduled; they had testing already, or the biopsy was sent to another laboratory for tumor testing among other reasons. We noticed that patients with CEGEJS were referred more often by our main campus oncologists (88.1%, n=37) compared to our community oncologists (11.9%, n=5). Lastly, many patients came to the clinic with advanced stage, poor nutritional status, and many died before being able to complete their genetic test. Having the ability to store a patient's DNA in a Clinical laboratory Improvement Amendments (CLIA)-certified biobank for the future would permit completion of clinical hereditary testing later for the benefit of at-risk relatives.

Conclusion (97 words):

Our study highlights the yield and downstream impact of paired normal-tumor genetic testing in patients with CEGEJS. Identifying biomarkers unlocked targeted therapeutic options for most of our patients and we hope they will derive improved survival outcomes from these therapies. Uncovering a hereditary cancer syndrome in patients with CEGEJS also allowed for cascade testing, tailored screening, risk reduction, and early detection for a broad range of cancers for family members. Further research is needed in stratification of the risk to develop CEGEJS, genetic modifiers of risk, response to targeted therapy, and novel blood-based disease recurrence surveillance tools.

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Contribution statement:

KT, SAC, MYL and MDG designed the concept for study. LVN, MYL, and MDG prepare our site for this project. SAC, LVN, CLH, VS, BO, WMG, BS, EL, LF and MDG helped with patient care and consent. QS performed the primary analysis from the SEER data for the Puget Sound region. AJ, EQK, CP analyzed genetic test results and AJ, EQK, CP, and MDG reviewed and interpreted all genetic results. KT and MDG completed data analysis. All authors participated in developing the manuscript, all edited and approved the final version of the manuscript. MDG is the guarantor for this study.

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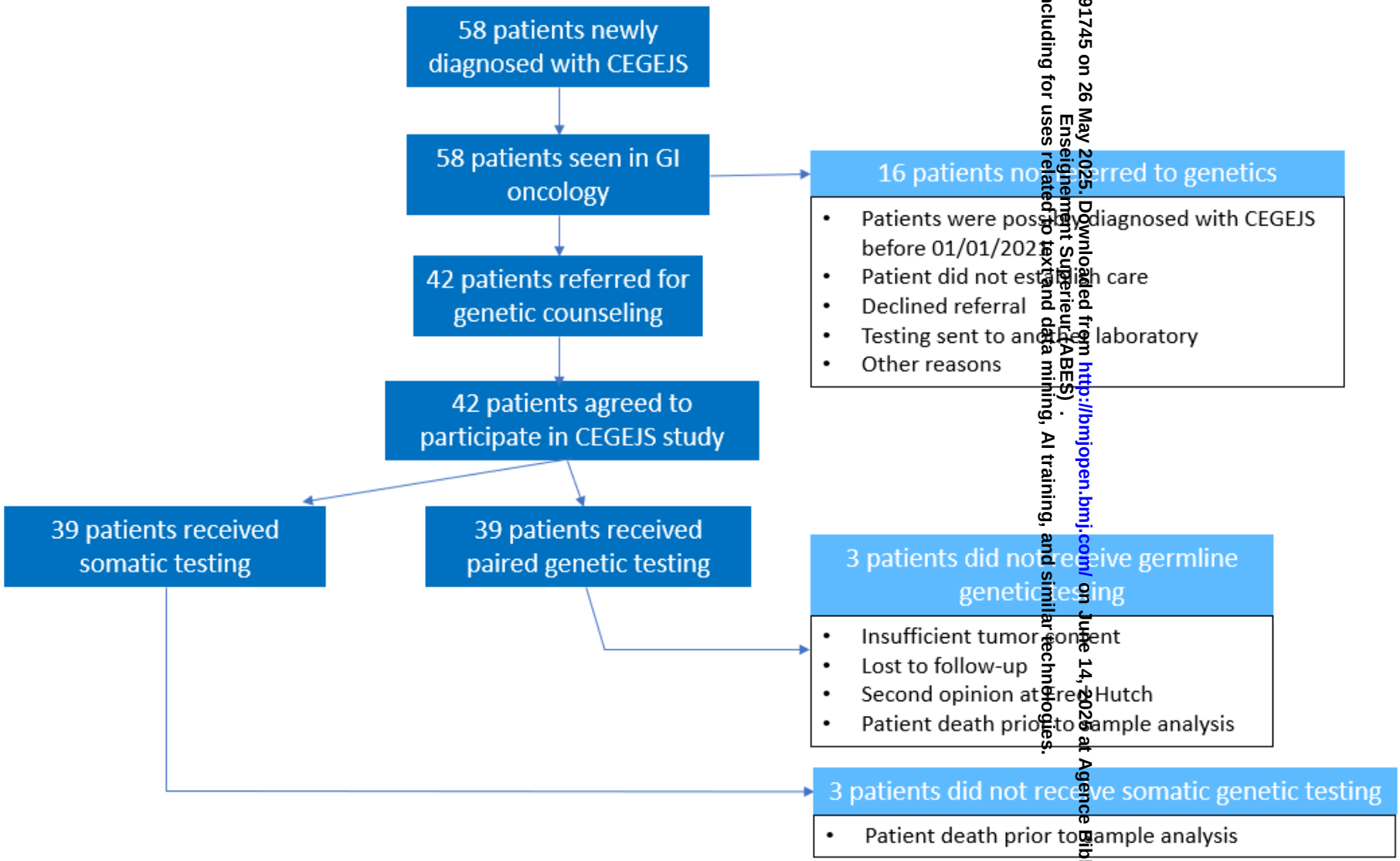
Figure 1. Consort diagram for the study on cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS)

Table 1. Demographics and risk factors for GEC in patients newly diagnosed with CEGEJS

Table 2. Tumor and germline genetic testing results

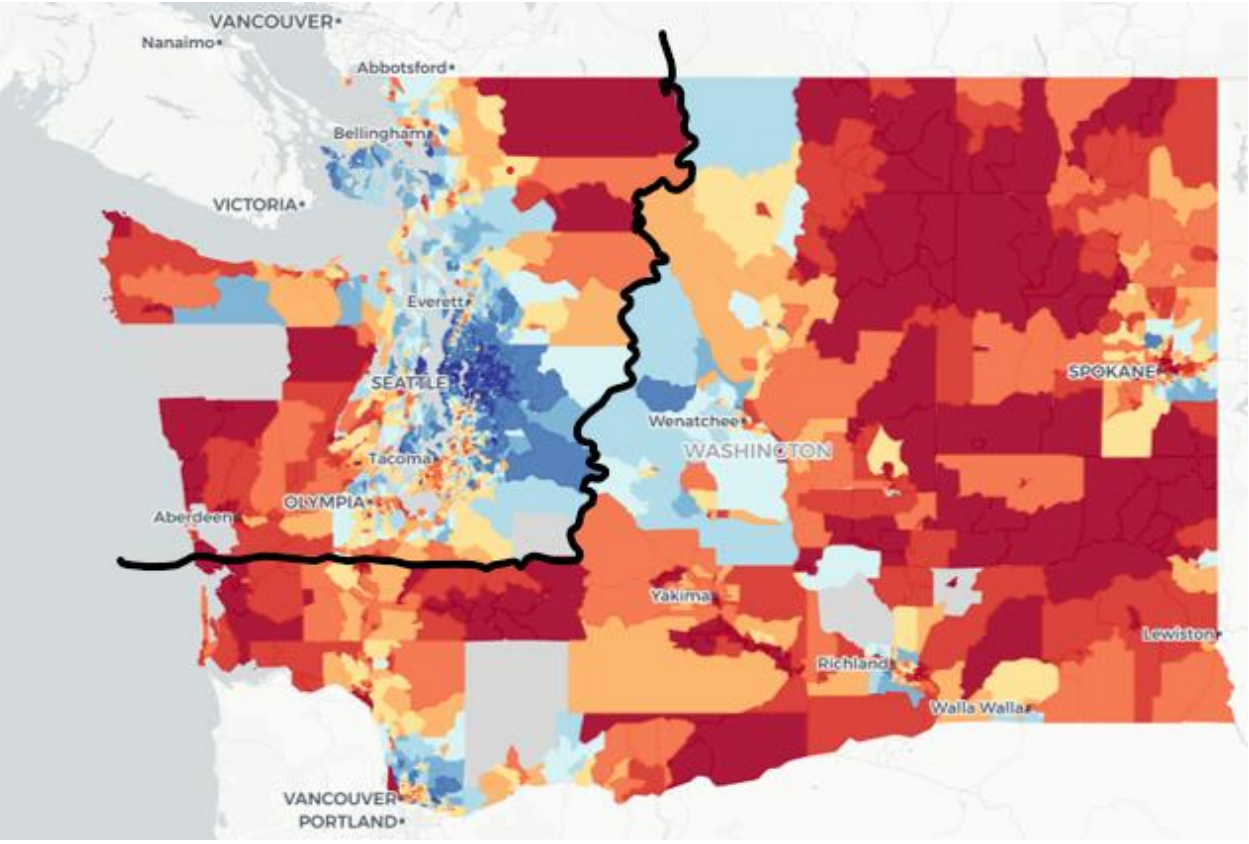
Figure 1. Consort diagram for the study on cancers of the esophagus, gastroesophageal junction, and stomach (CEGEJS)



Supplemental file

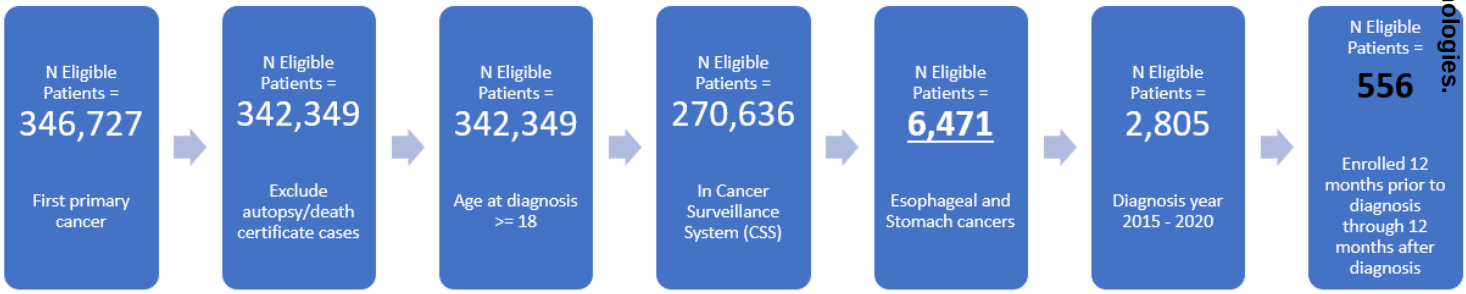
Supplemental figures

Supplemental Figure 1. Map of area of deprivation index for the 13 SEER counties (black line) and the state of Washington

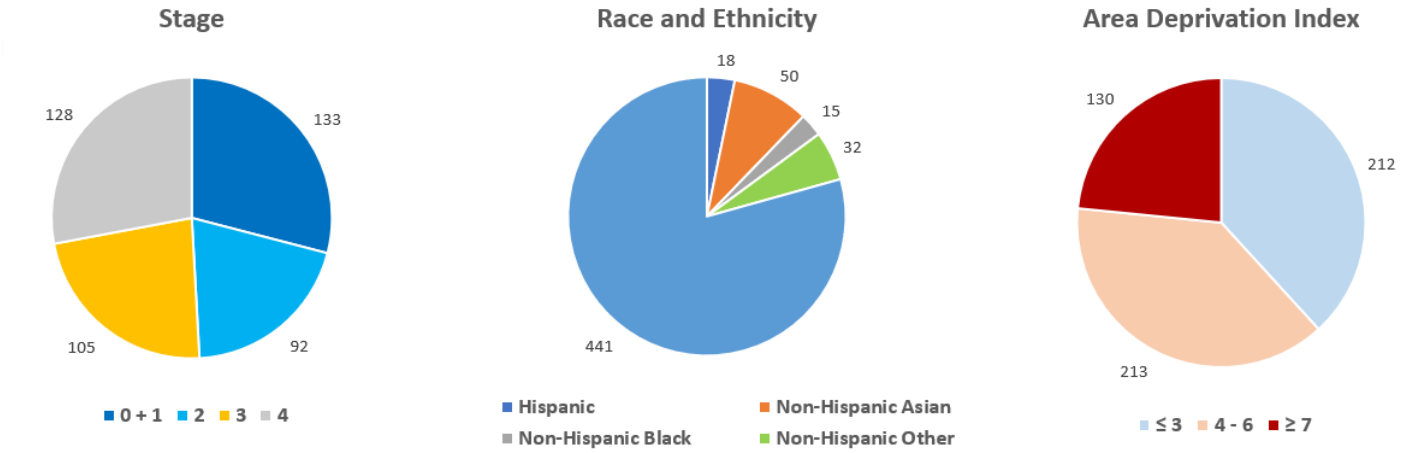


Supplemental Figure 2. Retrospective data on patients with CEGEJS in the 13 SEER counties of the Puget Sound in Seattle, WA. **Panel A:** Criteria selected to assess prevalence of CEGEJS. **Panel B:** Stage, Race and Ethnicity, and Area of Deprivation index for patients with CEGEJS. **Panel C:** Overview of cancer treatment by stage. **Panel D:** Overview of cancer treatment by site of cancer.

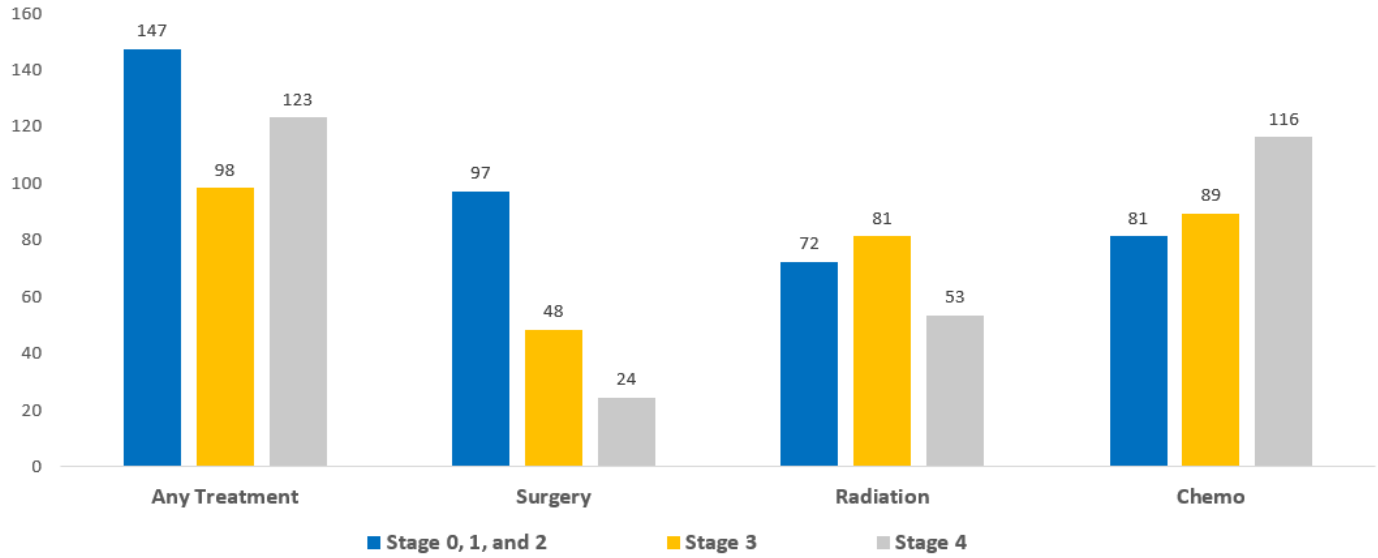
Panel A:



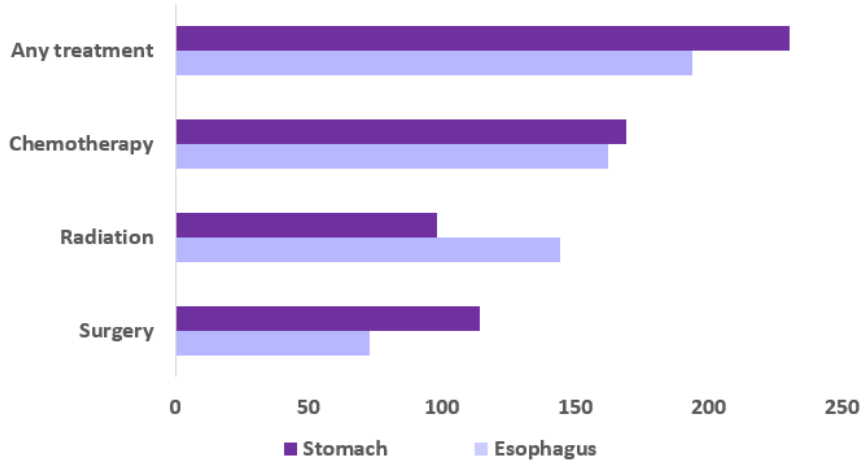
Panel B



Panel C:



Panel D:

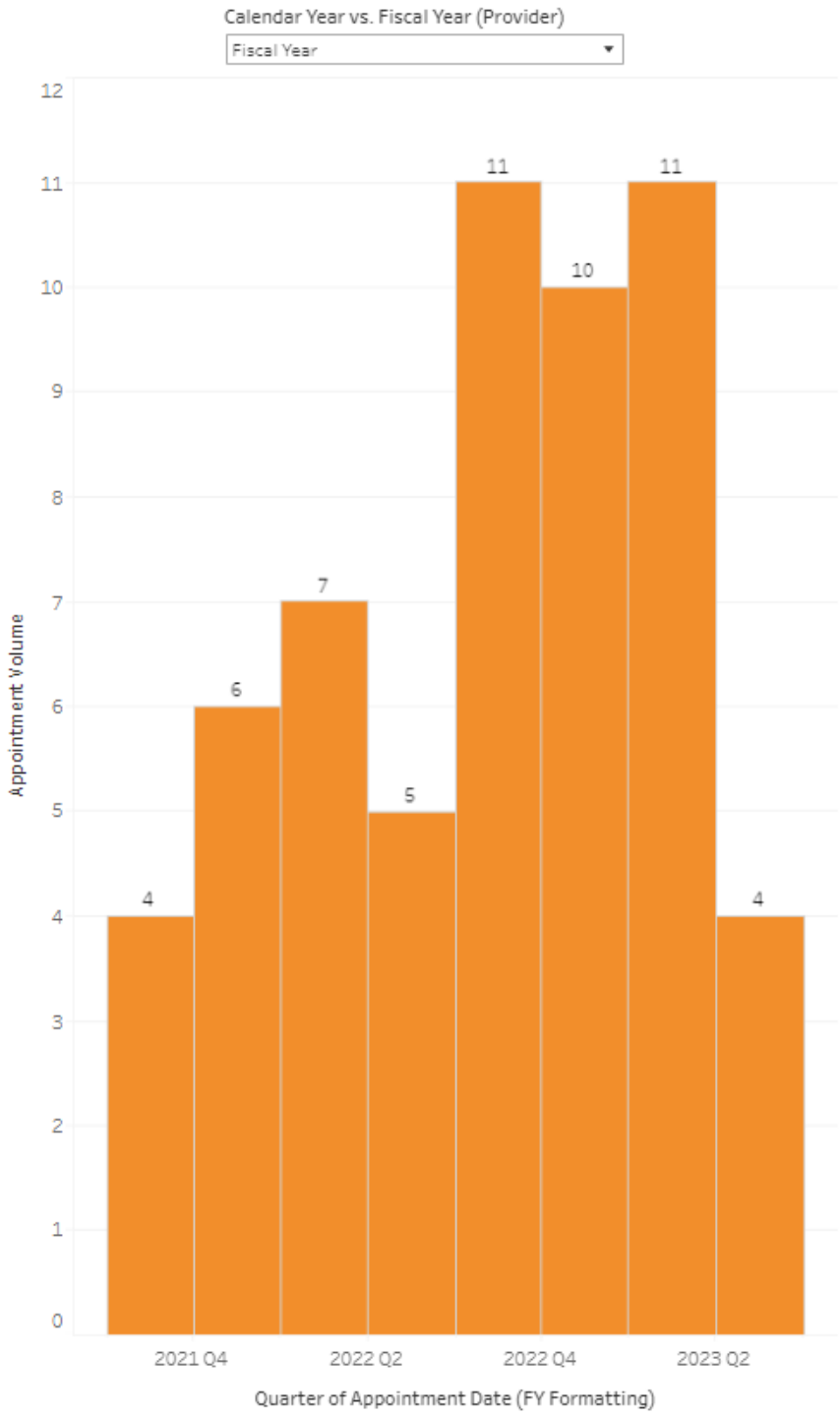


Supplemental Figure 3. Patients with CEGEJS who met NCCN criteria for genetic testing

Record ID	Met Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	Met Lynch Syndrome	Met other guideline
4	1	2	2
5	1	2	2
6	2	1	2
7	1	2	2
8	1	2	2
9	1	2	2
10	2	2	2
11	1	2	2
12	2	2	1
13	1	2	1
14	2	2	1
15	1	1	1
16	1	2	2
17	2	2	2
18	1	2	2
19	2	1	2
20	1	1	2
22	1	2	1
23	2	2	2
24	2	1	2
25	2	2	2
26	1	2	2
27	2	2	2
28	1	2	2
29	2	2	1
30	1	2	2
31	1	2	2
32	2	2	1
33	1	2	2
34	2	2	2
35	1	1	2
36	2	2	2
37	1	2	2
38	2	2	2
39	2	2	2
40	2	1	2
41	2	2	2
42	2	2	2
43	1	2	2
Total	21	7	7
Total met guidelines	35		
Total identified by MD	10		

KEY 1 Yes 2 No	Met Hereditary Breast and Ovarian Cancer Syndrome (HBOC)	Met Lynch Syndrome	Met other guideline
Total	21	7	7
Total met guidelines	35		
Total identified by MD	10		

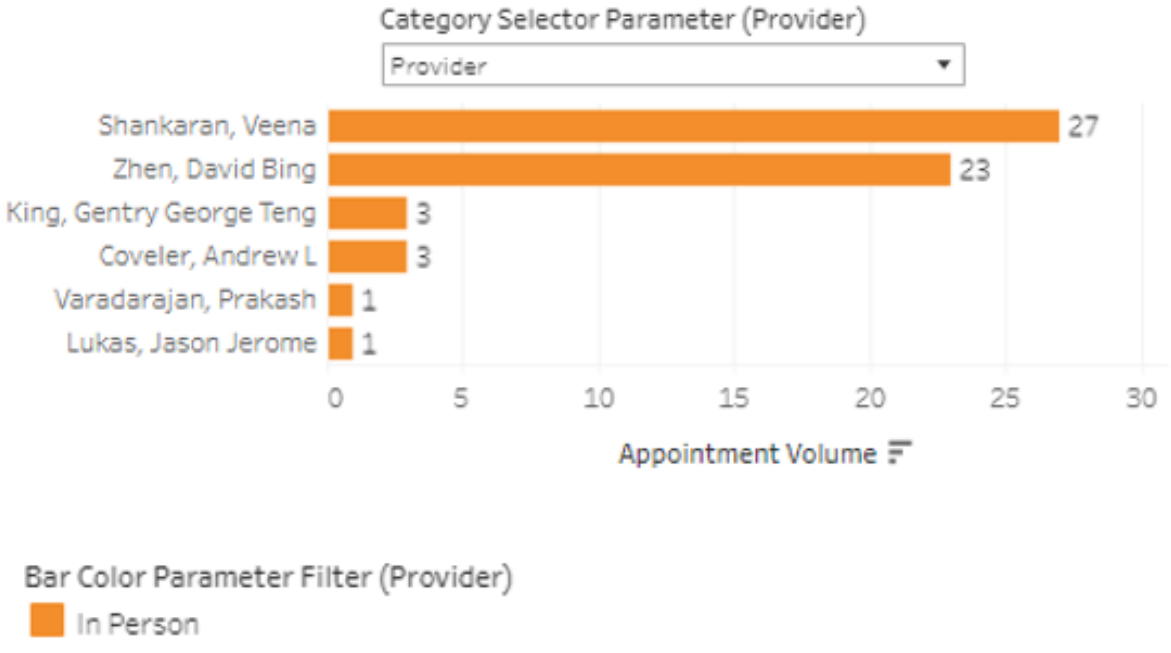
Supplemental Figure 4. Appointment volume for new CEGEJS diagnosis at FHCC between 01/01/2021 and 12/31/2022



Supplemental Figure 5. Criteria to identify new CEGEJS diagnosis at FHCC between 01/01/2021 and 12/31/2022

Dashboard Variables Chosen to Create Figure 1	
Variable	**Selection**
Appointment Date	01/01/2021 to 12/31/2022
Appt Status	Completed
Appt Name	New, New 30, New 30 + Lab Draw, New 30 Min, New 40, New 40 + Lab Draw, New 60, New 90, New Med Onc, New Patient, New Patient with Blood Draw, New Patient with Fellow
Appt Type Category	All
Appt Type Service	Clinic
Appt Type Modality	All
Provider Display Name	All
Provider Type	Physician
Provider Department	FHCC EH General Onc, FHCC ISQ General Onc, FHCC NWH General Onc, FHCC GI Onc Neighborhood, FHCC Pen General Oncology
Appointment Department	All
Appointment Location Abbr	EVG, ISQ, NWH, PEN, SLU
Case Supervisor Program	GI
Financial Class	All
Disease Group	Gastrointestinal
Disease Subgroup	Upper GI
Disease Type	Esophagus, Stomach
Service Line	Gastrointestinal

Supplemental Figure 6. Physicians seeing patients with new CEGEJS diagnosis at FHCC between 01/01/2021 and 12/31/2022



Supplemental Figure 7. List of genes on clinical genetic tests called Oncoplex and BROCA as of 01/01/2021

List of genes on Oncoplex: *ABCA10, ABCA12, ABCC9, ABL1, ABL2, ABRAXAS1 (FAM175A), ACVR1, AKAP9, AKT1, AKT2, AKT3, ALK, ANGPTL1, ANKRD26, APC, AR, ARAF, ARID1A, ARID1B, ASPH, ASXL1, ASXL2, ATM, ATR, ATRX, AURKA, AURKB, AXIN2, AXL, BABAM1, BAK1, BAP1, BARD1, BCL2, BCL2L11, BCOR, BCORL1, BCR, BICRA (GLTSCR1), BIRC3, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BRIP1, BRWD3, BTK, C19MC, CALR, CARD11, CBL, CBLB, CBLC, CCL2, CCND1, CCND2, CCNE1, CD19, CD274, CD33, CD74, CD79B, CDC25A, CDC27, CDH1, CDK12, CDK4, CDK6, CDK8, CDK9, CDKN1A, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CEBPA, CHD1, CHD3, CHD4, CHD8, CHEK1, CHEK2, COG5, CRADD, CREBBP, CRLF2, CRX, CRYBG1, CSF1R, CSF3R, CTCF, CTNNA1, CTNNB1, CUX1, CXCR4, DAXX, DDR2, DDX41, DEPDC5, DICER1, DIS3L2, DNAJB1, DNMT3A, DOCK7, EBF1, EED, EGFR, EGLN1, EIF3E, ELF1, ELP1, EML4, ENG, ENPP3, EP300, EPAS1, EPCAM, EPHA3, EPHA5, EPHB2, EPHB6, EPO, EPOR, ERBB2, ERBB3, ERBB4, ERCC2, ERG, ESR1, ESR2, ETNK1, ETV6, EZH2, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FH, FKBP1A, FLCN, FLT1, FLT3, FLT4, FOLR1, FOXA1, FOXL2, FOXR2, FUBP1, GAB2, GALNT12, GATA1, GATA2, GATA3, GEN1, GFAP, GLI1, GLI2, GLI3, GNA11, GNAQ, GNAS, GNB1, GPC3, GREM1, GRIN2A, GRM3, H3-3A, H3-3B, H3C2 (HIST1H3B), H3C3, HDAC4, HDAC9, HEPACAM, HIF1A, HNF1A, HNRNPU, HOOK3, HOXB13, HRAS, HSPH1, ID3, IDH1, IDH2, IGF1R, IKZF1, IL7R, JAK1, JAK2, JAK3, KCNJ8, KDM2B, KDM6A, KDR, KIF1B, KIF5B, KIT, KLF4, KMT2A, KMT2C, KMT2D, KRAS, KTN1, LYST, LZTR1, MAP2K1, MAP2K2, MAP2K4, MAP7, MAPK1, MAX, MBD4, MC1R, MCL1, MDM2, MDM4, MED12, MEGF6, MEN1, MET, MIOS, MITF, MLH1, MLH3, MN1, MPL, MRE11, MSH2, MSH3, MSH6, MSLN, MTAP, MTOR, MUTYH, MYB, MYC, MYCL, MYCN, MYD88, MYOD1, NAB2, NAT2, NBN, NF1, NF2, NKX2-1, NOP53 (GLTSCR2), NOTCH1, NOTCH2, NOTCH3, NOTCH4, NPM1, NPRL2, NPRL3, NR4A3, NRAS, NRG1, NRP1, NSD1, NT5C2, NTHL1, NTRK1, NTRK2, NTRK3, NUDT15, OFD1, PAK1, PALB2, PARP1, PAX5, PBRM1, PDCD1LG2, PDGFB, PDGFRA, PDGFRB, PHF6, PHOX2B, PIGA, PIK3CA, PIK3CB, PIK3R1, PIK3R2, PLCG2, PLK1, PLK2, PLK3, PLK4, PML, PMS2, POLD1, POLE, POT1, PPM1D, PPP1CB, PRKAR1A, PRPF40B, PRPF8, PRPS1, PTCH1, PTEN, PTPN11, PTPRD, QKI, RAC1, RAD21, RAD50, RAD51B, RAD51C, RAD51D, RAD54L, RAF1, RARA, RASA1, RB1, RECQL, RELA, RET, RHEB, RHOA, RICTOR, RINT1, RIT1, RNF43, ROR1, ROS1, RPL10, RPL31, RPS14, RPS15, RPS20, RPTOR, RRM1, RRM2, RSPO2, RSPO3, RUNX1, SAMD9, SAMD9L, SDHA, SDHAF2, SDHB, SDHC, SDHD, SETBP1, SETD2, SF1, SF3B1, SH2B3, SHH, SIGLEC10, SLC25A13, SLX4, SMAD2, SMAD3, SMAD4, SMARCA4, SMARCB1, SMARCE1, SMC1A, SMC3, SMO, SNAPC3, SOS1, SOS2, SPOP, SPRED1, SPRY4, SRC, SRP72, SRSF2, STAG2, STAT3, STAT5B, STAT6, STK11, STRADA, SUFU, SUZ12, TACC3, TACSTD2, TAFA2 (FAM19A2), TCF3, TERC, TERT, TET1, TET2, TET3, TFE3, TFG, TGFBR2, TLX1, TMEM127, TMPRSS2, TNFAIP3, TNFRSF14, TP53, TP53BP1, TP73, TRAF7, TRRAP, TSC1, TSC2, TTYH1, TYMS, U2AF1, U2AF2, UBA1, UBR5, USP7, VHL, WRN, WT1, XPO1, XRCC2, YAP1, ZBTB16, ZFTA (c11orf95), ZRSR2*

List of genes on BROCA: *ALK, APC, ATM, ATR, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDK12, CDKN2A, CHEK2, CTNNA1, DICER1, EPCAM, FANCM, FH, FLCN, GEN1, GREM1, HOXB13, MEN1, MET, MITF, MLH1, MLH3, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PHOX2B, PIK3CA, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51B, RAD51C, RAD51D, RB1, RECQL, RET, RNF43, RPS20, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL*