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ORIGINAL ARTICLE

Integrated clinico-molecular analysis of gastric cancer in European and Latin American populations: LEGACY project

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Background: Gastric cancer (GC) is recognized for intrinsic heterogeneity, although it is similarly approached in Europe and Latin America (LATAM). The LEGACY project aimed to deepen GC molecular understanding through multi-omics analysis in Europe and LATAM GC samples.

Patients and methods: Tumor samples were centrally reviewed for histology, human epidermal growth factor receptor 2 (HER2) expression, and mismatch repair-deficient (dMMR)/microsatellite instability (MSI) status. In addition, we assessed Epstein—Barr virus (EBV) status, programmed death-ligand 1 (PD-L1) combined positive score (CPS), and carried out tissue genomic profiling including tumor mutation burden (TMB) quantification plus targeted transcriptomics for immune microenvironment and cancer cell signaling scores.

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Results: In total, 328 GC patients were enrolled. HER2-positive GC and high PD-L1 CPS were more frequent in Europe than in LATAM (9% versus 3% and 15% versus 3%, respectively), whereas EBV was mainly found in LATAM (7%, versus 3% in Europe), and dMMR/MSI tumors were equally distributed (16%). High TMB was enriched in dMMR/MSI and EBV tumors. Mutations in homologous recombination repair (HRR) genes were frequent in both cohorts (24.8% and 14.7% in Europe and LATAM, respectively), and mostly found in dMMR/MSI (63.6%) and intestinal HER2-negative (18.7%) tumors. The prognosis was poor in diffuse HER2-negative GC patients, whose tumors presented an immunosuppressive microenvironment and other distinct pathway activation signatures.

Conclusions: Our findings relate specific molecular alterations of GC tumors from Europe and LATAM to actionable biomarkers for precision cancer therapies. The proposed GC stratification can be implemented in routine care and guide drug development strategies.

Key words: gastric cancer, gastric cancer epidemiology, gastric cancer biomarkers, precision medicine

INTRODUCTION

Gastric cancer (GC) including the gastroesophageal junction is an aggressive disease often diagnosed at advanced stages, with a 5-year survival rate of ∼20%.[1] GC differs according to epidemiology,[2] and heterogeneity has been defined at the histological and molecular levels, with an impact on disease presentation, treatment selection, and patient prognosis[3,4] being a major cause of treatment failure. At the histological level, both Lauren and the World Health Organization (WHO) systems are used for disease classification,[5,6] with Lauren's intestinal and diffuse GC subtyping widely adopted in pathology laboratories. Different classifications have been proposed at the molecular level. The Cancer Genome Atlas (TCGA) defined four subtypes of GC based on complex multi-omics profiling: (i) microsatellite instability (MSI); (ii) Epstein—Barr virus (EBV) positivity; (iii) genomic stability [GS, enriched for *CDH1* (E-cadherin) mutations]; and (iv) chromosomal instability (CIN, enriched for *TP53* mutations and *HER2* amplifications).[7] The Asian Cancer Research Group (ACRG) also defined four subtypes of GC and used immunohistochemistry (IHC) protein expression status of p53 (TP53) and CDH1 for disease stratification: (i) MSI; (ii) TP53-active; (iii) TP53-inactive; and (iv) mesenchymal-like (CDH1 loss of expression).[8] These classifications provided important insights identifying some immunogenic subgroups, i.e. MSI or mismatch repair-deficient (dMMR) tumors, which typically feature dense lymphocyte infiltration and widespread expression of immune checkpoint proteins, and EBV tumors, which have a high immune cytotoxic microenvironment. In clinical practice, beyond dMMR/MSI and occasionally EBV, only biomarkers with therapeutic relevance in metastatic GC are routinely assessed. These include human epidermal growth factor receptor 2 (HER2) overexpression/amplification by IHC and *in situ* hybridization (ISH) and programmed death-ligand 1 (PD-L1) combined positive score (CPS) expression by IHC, which guide first-line regimens combining chemotherapy and targeted anti-HER2 or anti-programmed cell death protein 1 agents.[1]

On top of the GC molecular diversity described previously, inter-patient heterogeneity at the global scale may be even more pronounced. It is known that GC is more prevalent in Asian populations than in Western countries.[9] Interestingly, EBV-positive tumors have a higher prevalence in Chile than in European cohorts.[10] However, there is an incomplete understanding of GC's geographical differences in molecular features. We designed the LEGACY project to develop and propose a simplified classification system for GC based on histology with IHC and ISH assays that could be used to evaluate molecular subtypes in European and Latin American (LATAM) countries.[11] In addition, we further investigated emerging genomic and transcriptomic biomarkers with technologies adapted for formalin-fixed paraffin-embedded (FFPE) tissue samples. By carrying out an integrative analysis of clinical, epidemiological, and multi-omics data from geographically diverse samples, we aimed to (i) assess molecular hallmarks of GC using validated and emerging biomarkers; (ii) compare their distribution in European versus LATAM cohorts; and (iii) explore biological differences of the most prevalent GC subtypes between continents.

PATIENTS AND METHODS

LEGACY study on molecular profiling of gastric cancer

The LEGACY project is a European and LATAM consortium funded by the European Union's Horizon 2020 research and innovation program under grant agreement number 825832. For this LEGACY molecular substudy (ClinicalTrials.gov identifiers NCT04015466 and NCT03957031; 11 July 2019) we prospectively recruited adult patients with confirmed diagnoses of GC from eight organizations in Spain, The Netherlands, Portugal, Mexico, Chile, Paraguay, and Argentina between 2020 and 2023. The study members and centers can be found in Figure 1. Patients were eligible if primary tissue was available for molecular diagnosis as per surgical resection or large endoscopic biopsies carried out in routine care. INCLIVA from Valencia, Spain, was the coordinator institution of the project. In the first 3 years of recruitment, the IPATIMUP center in Portugal served as the central laboratory for histopathological confirmation of GC plus IHC and ISH assays. In the last year of recruitment, IPATIMUP was the reference laboratory for European countries, INCAN was a local laboratory for Mexico, and GENPAT was the reference laboratory for the remaining LATAM countries. Upon confirmation of sufficient FFPE tissue for additional molecular tests, samples were shipped to diagnostic labs VHIO in Spain [for broad next-generation sequencing (NGS) panel] and VUMC in The Netherlands (for

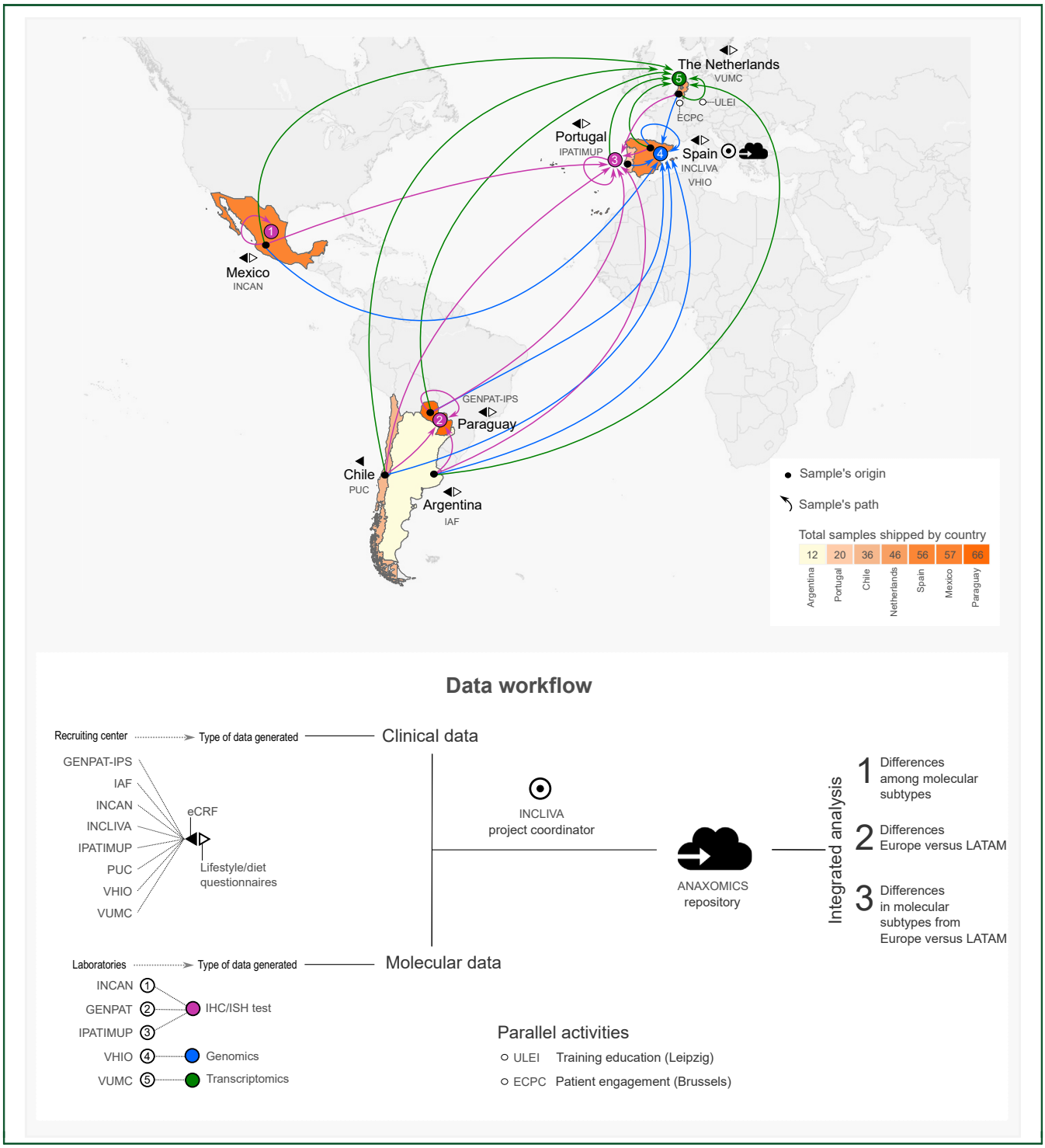

Figure 1. LEGACY project summary with samples tracking map and data workflow.
ECPC, European Cancer Patient Coalition, Brussels; eCRF, electronic case report form; GENPAT-IPS, GENPAT laboratory + Instituto de Previsión Social, Paraguay; IAF, Instituto Alexander Fleming, Argentina; IHC, immunohistochemistry; INCAN, Instituto Nacional de Cancerología, Mexico; INCLIVA, Instituto de Investigación Sanitaria INCLIVA, Spain; IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto; ISH, *in situ* hybridization; LATAM: Latin America; PUC, Pontificia Universidad Católica de Chile; ULEI, University of Leipzig, Germany; VHIO, Vall d´Hebron Institute of Oncology, Spain; VUMC, Vrije Universiteit University Medical Center, The Netherlands.

immune profiling with transcriptomics and flow cytometry). Recruiting sites completed an electronic case report form (eCRF) specifically designed for the project with patient demographics, stage at diagnosis, first-line treatment, and survival outcomes, when available. A subset of the patients filled lifestyle and diet questionnaires, previously validated with support from a patient association (ECPC).[12] *Helicobacter pylori* infection assessment was also part of the strategy in the IPATIMUP central laboratory. A common laboratory handbook was developed, and training across

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sites was specially carried out to ensure precision in the protocol implementation. Figure 1 illustrates samples and data flow from participating organizations to different diagnostics labs. Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2025.104482, details the number of samples and laboratories that carried out molecular testing.

Molecular profiling

All samples were reviewed to confirm gastric adenocarcinoma diagnosis and histological profile according to the Lauren classification. For IHC and ISH analysis, we used the Ventana® Benchmark ULTRA (Roche, Basel, Switzerland) system. We followed pre-established protocols according to the manufacturer's instructions. The following antibodies were tested: HER2 (clone 4B5), PD-L1 (22C3), MSH2 (G219-1129), MSH6 (SP93), MLH1 (M1), PMS2 (A16-4), and KI67 (30-9). We used the EBV Early RNA (EBER) assay for EBV detection. The same protocol was used at all the pathology facilities (IPATIMUP, GENPAT, and INCAN). Genomics was carried out using an ISO15189-certified assay at VHIO. The assay covers mutations and copy number alterations in 425 cancer genes. Tumor mutation burden (TMB) high was defined as $\geq$15 mutations/megabase (mut/Mb), which is equivalent to 10 mut/Mb when cross-validated with FoundationOne CDx assay. Transcriptomics profiling was carried out in the VUMC research laboratory using NanoString nCounter® analysis system with the NanoString PanCancer Immune Profiling panel (NanoString Technologies, Seattle, Washington) to extract cell type and pathway activation scores. Fresh frozen gastric biopsy samples from diagnostic endoscopy were used for microbiota characterization using 16S rRNA gene amplification and sequencing technology and the abundance of the *Helicobacter* genus was obtained from the analysis of the microbiota sequencing data. More detailed technical information on molecular profiling can be found in Supplementary Methods, available at https://doi.org/10.1016/j.esmoop.2025.104482.

Statistical analysis and ethics

All clinical, epidemiological, and molecular data were checked for completeness and accuracy (conformance, plausibility, and consistency) by the data analysis group at VHIO (Oncology Data Science). We carried out a descriptive analysis of the variables collected in the study. Continuous variables were expressed as mean and standard deviation or as median and ranges. Categorical variables were expressed as absolute values and/or percentages. For the univariate analysis, we used Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables. Statistical significance was accepted at the conventional two-sided $P < 0.05$ threshold. For exploratory transcriptomics analysis (cell type infiltration and pathway activation scores according to molecular subtypes) we compared the median scores of different molecular subtypes with the remaining samples. The Benjamini–Hochberg method was used to adjust for multiple testing. Overall survival analysis was calculated from diagnosis of metastatic disease or relapse/recurrence until death from any cause or last follow-up using the Kaplan–Meier method. Data analyses were carried out using R version 4.2.3 statistical software package. This manuscript adheres to the checklist items outlined in the STROBE statement. The project was approved by local ethics committees of each recruiting site: the ethics committee of University Clinical Hospital of Valencia, Spain (reference number 2018/205); the institutional review board of VU University Medical Center Amsterdam (reference number 2019.355. NL 69480.02919); the ethics committee of Instituto de Previsión Social, Asuncion-Paraguay (reference number CA N°11-020/19); the ethical research committee of Instituto Alexander Fleming, Buenos Aires Argentina (Resolution 25 July 2019, for LEGACY study 1 and 2 and 3 October 2019, for LEGACY study 3); the ethical committee of Instituto Nacional de Cancerología (INCAN, Mexico, reference number INCAN/CEI/0486/19); the ethics committee of the University Center of Sao Joao and Medicine Faculty of Porto University, Portugal (reference 100/019); the scientific ethical Committee of the Pontifical Catholic University of Chile, reference 180806007; and the Drug research ethics committee of Vall d'Hebron University Hospital, Barcelona, Spain with references PR (AG)387/2019 approved on 29 October 2019 for LEGACY study 1, PR (AG) 388/2019 approved on 13 December 2019 for LEGACY study 2 and PR (AG)419/2019 approved on 30 January, respectively. The study was in agreement with the ethical guidelines for the 1975 Declaration of Helsinki (sixth revision, 2008; Fortaleza, Brazil, October 2013), following the Medical Research Involving Human Subjects Act and Good Clinical Practice standards as well as personal data protection [General Data Protection Regulation (RGPD-Regulation; EU 2016/679)]. All participants provided written informed consent before study enrollment. Each data-contributing partner has undergone online ethical and data training before the beginning of data collection and has managed access to the data of their center through this security system. Inside this system, a patient ID generator has generated a unique code for each participating patient to maintain data privacy.

RESULTS

Clinicopathological characteristics

Out of 328 patients recruited in this LEGACY molecular substudy, 293 (89%) had sufficient tissue for histological diagnosis and at least one validated biomarker for molecular stratification (IHC or ISH). As shown in Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2025.104482, clinical data were available in 222 (76%) patients, lifestyle/diet questionnaires in 192 (66%) patients, genomics data in 172 (59%) cases, and transcriptomic profiling in 141 (48%) samples. Samples were eligible for genomics and/or transcriptomics profiling based on DNA/RNA quantity or quality metrics. Table 1 summarizes patient and tumor characteristics for the entire cohort and stratifies

Table 1. Patient and tumor characteristics for the entire LEGACY molecular substudy stratified by Europe or LATAM countries

	All, $N = 293$	Europe, $n = 122$	LATAM, $n = 171$	n
Center LEGACY, *n* (%)				293
GENPAT	66 (22.5)		66 (38.6)	
IAF	12 (4.10)		12 (7.02)	
INCAN	57 (19.5)		57 (33.3)	
INCLIVA	43 (14.7)	43 (35.2)		
IPATIMUP	20 (6.83)	20 (16.4)		
PUC	36 (12.3)		36 (21.1)	
VHIO	13 (4.44)	13 (10.7)		
VUMC	46 (15.7)	46 (37.7)		
Country (origin), *n* (%)				293
Argentina	12 (4.10)		12 (7.02)	
Chile	36 (12.3)		36 (21.1)	
Mexico	57 (19.5)		57 (33.3)	
Paraguay	66 (22.5)		66 (38.6)	
Portugal	20 (6.83)	20 (16.4)		
The Netherlands	46 (15.7)	46 (37.7)		
Spain	56 (19.1)	56 (45.9)		
Median age at diagnosis (range), years	65.0 (28.0-91.0)	67.0 (30.0-91.0)	64.0 (28.0-85.0)	221
Sex, *n* (%)				222
Female	85 (38.3)	46 (41.1)	39 (35.5%)	
Male	137 (61.7)	66 (58.9)	71 (64.5%)	
Race, *n* (%)				222
Asian	1 (0.45)	1 (0.89)	0 (0.00)	
Black or African American	5 (2.25)	5 (4.46)	0 (0.00)	
White Hispanic or Latino	120 (54.1)	26 (23.2)	94 (85.5)	
White not-Hispanic or Latino	76 (34.2)	73 (65.2)	3 (2.73)	
Not reported	17 (7.66)	5 (4.46)	12 (10.9)	
Unknown	3 (1.35)	2 (1.79)	1 (0.91)	
Mean height (standard deviation), cm	166 (10.1)	168 (9.88)	163 (9.79)	196
Median weight (range), kg	65.0 (35.0-158)	67.0 (45.0-138)	62.5 (35.0-158)	198
Histology subtype Laurén, *n* (%)				293
Diffuse	98 (33.4)	36 (29.5)	62 (36.3)	
Intestinal	149 (50.9)	74 (60.7)	75 (43.9)	
Mixed	34 (11.6)	11 (9.02)	23 (13.5)	
Unknown	12 (4.10)	1 (0.82)	11 (6.43)	
MMR/MSI status, *n* (%)				293
dMMR/MSI	46 (15.7)	21 (17.2)	25 (14.6)	
pMMR/MSS	246 (84.0)	101 (82.8)	145 (84.8)	
Unknown	1 (0.34)	0 (0.00)	1 (0.58)	
EBV status, *n* (%)				293
Negative	272 (92.8)	114 (93.4)	158 (92.4)	
Positive	15 (5.12)	4 (3.28)	11 (6.43)	
Unknown	6 (2.05)	4 (3.28)	2 (1.17)	
LEGACY class, *n* (%)				276
EBV+	15 (5.43)	4 (3.39)	11 (6.96)	
dMMR/MSI	44 (15.9)	19 (16.1)	25 (15.8)	
HER2+	15 (5.43)	11 (9.32)	4 (2.53)	
Diffuse HER2−	82 (29.7)	31 (26.3)	51 (32.3)	
Intestinal/other HER2−	120 (43.5)	53 (44.9)	67 (42.4)	
Tumor grade, *n* (%)				215
G1 Well differentiated	18 (8.37)	17 (16.0)	1 (0.92)	
G2 Moderately differentiated	40 (18.6)	18 (17.0)	22 (20.2)	
G3 Poorly differentiated (includes Signet Ring)	125 (58.1)	43 (40.6)	82 (75.2)	
GX Unknown	32 (14.9)	28 (26.4)	4 (3.67)	
Tumor stage at diagnosis, *n* (%)				222
I	4 (1.80)	4 (3.57)	0 (0.00)	
II	5 (2.25)	5 (4.46)	0 (0.00)	
III	71 (32.0)	38 (33.9)	33 (30.0)	
IV	138 (62.2)	63 (56.2)	75 (68.2)	
Unknown	4 (1.80)	2 (1.79)	2 (1.82)	
Helicobacter pylori status, *n* (%)				159
Negative	22 (13.8)	15 (15.3)	7 (11.5)	
Positive	137 (86.2)	83 (84.7)	54 (88.5)	
Relapse/recurrence status, *n* (%)				222
No	58 (26.1)	34 (30.4)	24 (21.8)	
Yes	153 (68.9)	75 (67.0)	78 (70.9)	
Unknown	11 (4.95)	3 (2.68)	8 (7.27)	
Survival status, *n* (%)				222
Alive	113 (50.9)	70 (62.5)	43 (39.1)	
Deceased	108 (48.6)	41 (36.6)	67 (60.9)	
Unknown	1 (0.45)	1 (0.89)	0 (0.00)	
Death cause, *n* (%)				108
Other malignancy (not stomach cancer related)	1 (0.93)	0 (0.00)	1 (1.49)	
Other non-malignant disease	4 (3.70)	2 (4.88)	2 (2.99)	
Stomach cancer	88 (81.5)	39 (95.1)	49 (73.1)	
Unknown cause of death	15 (13.9)	0 (0.00)	15 (22.4)	

them by Europe or LATAM countries. Most patients were male (62%), white Hispanic or Latino (54%), and presented with stage IV disease at diagnosis (62%). Intestinal was the most common Lauren subtype (51%), as were G3 poorly differentiated tumors (58%). *H. pylori* infection was detected in a large proportion of the samples (86%) for both European and LATAM cohorts.

Regarding epidemiological comparative analyses, we analyzed the results of lifestyle and diet questionnaires in Europe versus LATAM cohorts. The most significant associations are illustrated in Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2025.104482, and detailed results are presented in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.104482. Alcohol and smoking consumption were more frequent in Europe, as were diets rich in margarine and vinegar. Frequent consumption of fruits, vegetables, and seafood was higher in Europe, while processed cereals and chili paste were more commonly part of the diet in LATAM.

Gastric cancer subtypes and validated biomarkers across European and LATAM countries

We applied the GC molecular stratification algorithm detailed in Figure 2, which combines histological classification of the disease and readily available biomarkers of clinical relevance at the time of the study design in 2019 including HER2 and dMMR/MSI expression, on top of EBV

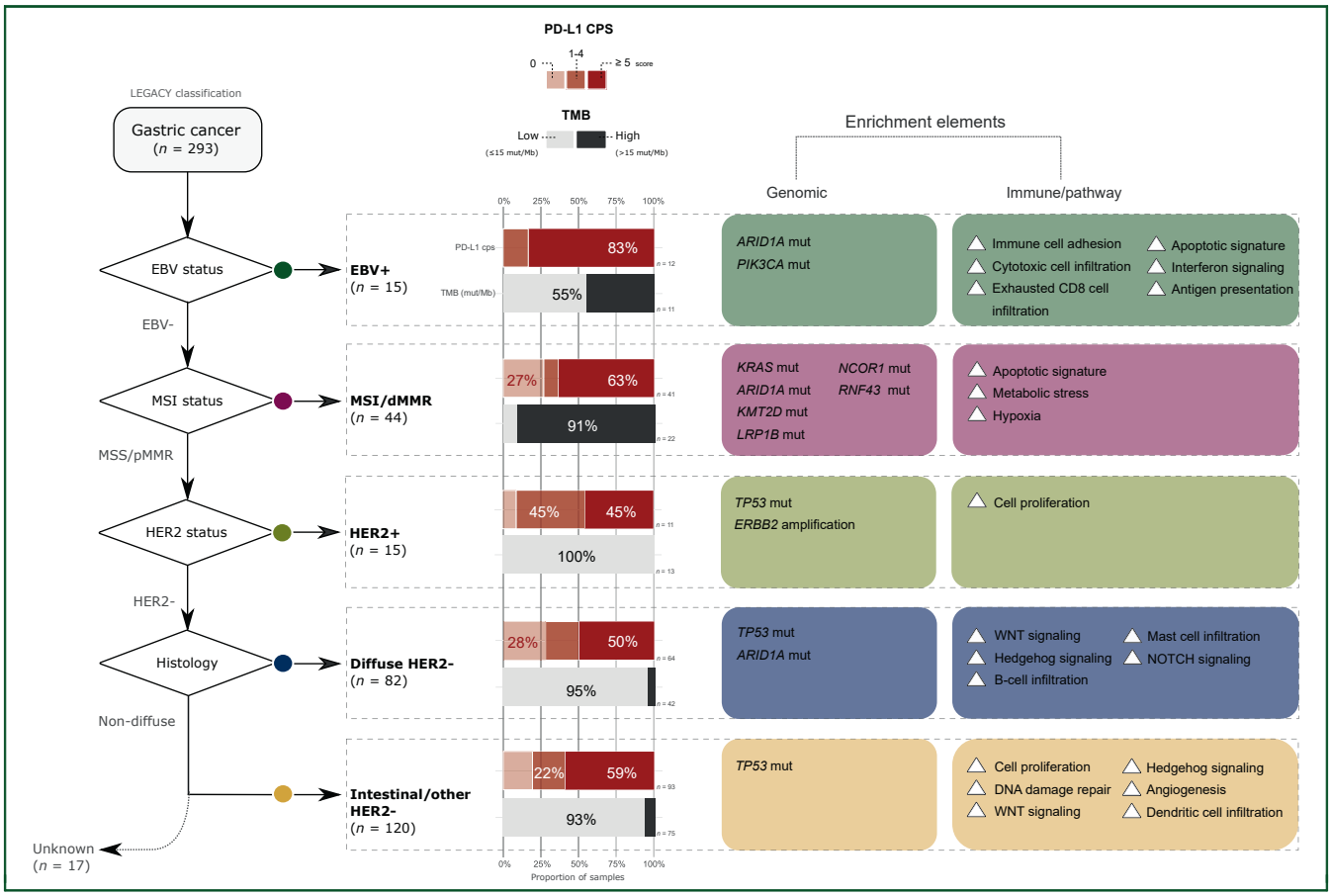

Figure 2. LEGACY project gastric cancer molecular subtyping with genomic and transcriptomic (immune/pathway) enrichments.
CPS, combined positive score; dMMR, mismatch repair-deficient; EBV, Epstein—Barr virus; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; MSS, microsatellite stability; PD-L1, programmed death-ligand 1; pMMR, mismatch repair-proficient; TMB, tumor mutation burden.

status. In parallel, we carried out correlative analyses with PD-L1 CPS and exploratory TMB estimation. EBV positivity was low (5.5%) but more prevalent in LATAM (7%) than in Europe (3%). A large proportion of EBV-positive tumors (83%) had PD-L1 high expression (CPS $\geq$5) and were TMB high (45%). Tumors with dMMR/MSI represented 16% of the populations in both LATAM and Europe and presented high TMB (91%) although with moderate PD-L1 CPS $\geq$5 expression (63%). HER2 positivity was considerably low (5.5%) but more prevalent in Europe (9%) than in LATAM (3%). All HER2-positive tumors were of intestinal histological subtype and presented TMB low, while showing a modest expression of PD-L1 CPS $\geq$5 (45% of the samples). There were no discordances of HER2 positivity by IHC/ISH analysis and genomics profiling (gene amplification by broad NGS panel). Samples with diffuse histological subtype HER2-negative represented 30% of the cohort, without major differences between Europe (26%) and LATAM (32%). Half of the diffuse HER2-negative GC subtype samples had PD-L1 CPS $\geq$5 and 95% were TMB low. Finally, the most common GC subtype was intestinal/other histology HER2-negative (44%), equally distributed in Europe (45%) and LATAM (42%). Most were PD-L1 CPS $\geq$5 (59%) and TMB low (93%). The prevalence of GC molecular subtypes as per the LEGACY study in each participating country is illustrated in Figure 3A and detailed in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2025.104482. Of note, the prevalence of EBV-positive tumors was numerically higher in Chile (19%), as was HER2-positive GC in Spain (13%). When comparing IHC markers in samples from Europe versus LATAM, as illustrated in Figure 3B and C, we found significant differences in the percentage of KI67-positive cells (83% versus 72%, respectively) and PD-L1 CPS scores (15 versus 3, respectively). When excluding dMMR/MSI samples, the median TMB was 8.2 mut/Mb, significantly higher in GC samples from LATAM (8.9 mut/Mb) versus Europe (6.7 mut/Mb), as illustrated in Figure 3D. These differences were maintained when EBV-positive samples were also excluded from the analysis, with a median TMB of 8.2 mut/Mb in GC samples from LATAM versus 6.7 mut/Mb in Europe.

Finally, with a median follow-up of 1 year, we found numerically worse overall survival in the metastatic setting for patients with diffuse HER2-negative tumors and those with dMMR/MSI GC (Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2025.104482). Of note, palliative immunotherapy was not approved for dMMR/MSI GC at the time of patient recruitment in the LEGACY project. No correlations were found specifically related to risk factors, lifestyle, and dietary habits.

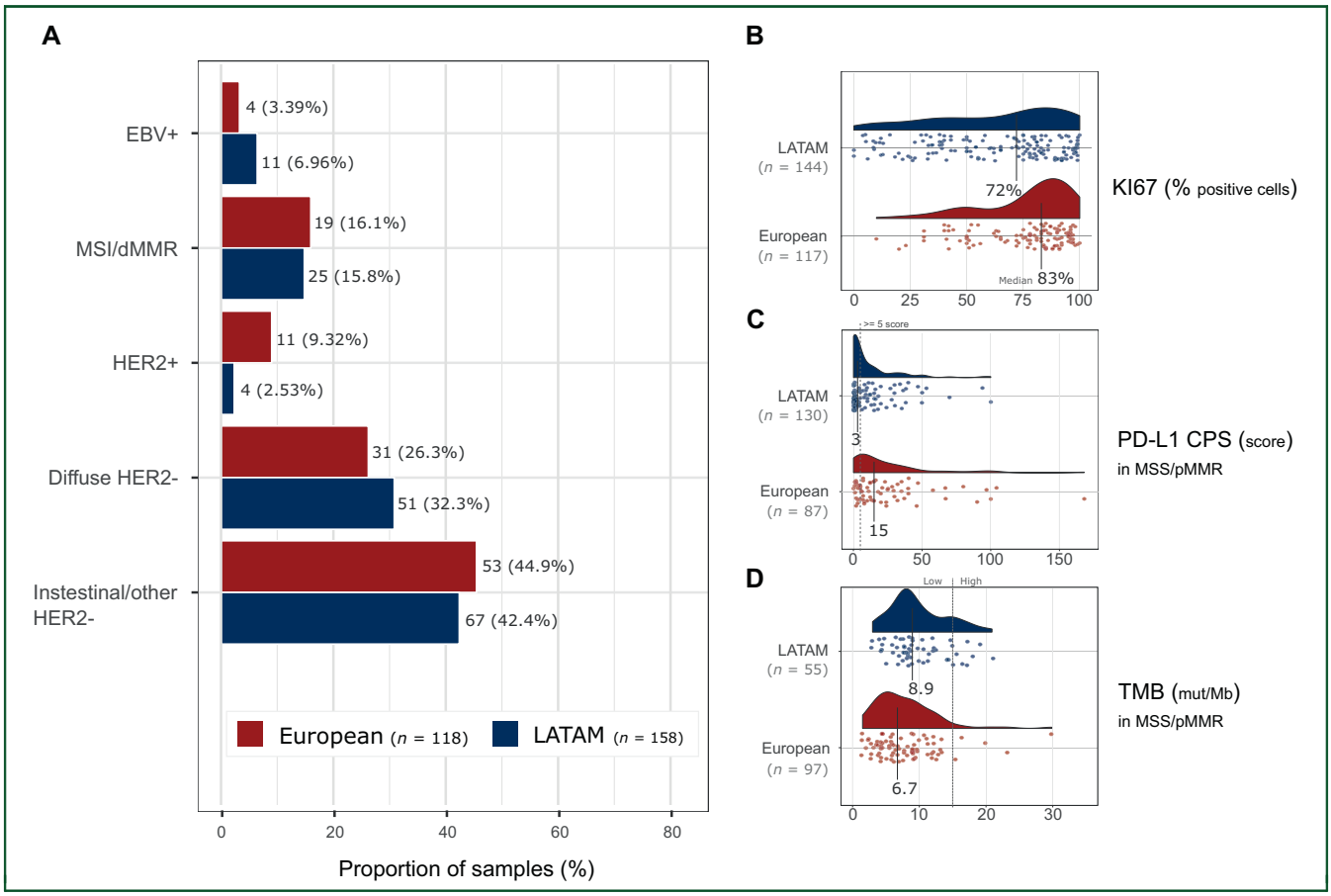

Figure 3. LEGACY project molecular data in samples from European (EU) or Latin America (LATAM) countries.
(A) LEGACY molecular subtypes classification. (B) KI67. (C) PD-L1 CPS score in MSS/pMMR samples. (D) TMB counts in MSS/pMMR samples.
CPS, combined positive score; dMMR, mismatch repair-deficient; EBV, Epstein—Barr virus; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; MSS, microsatellite stability; PD-L1, programmed death-ligand 1; pMMR, mismatch repair-proficient; TMB, tumor mutation burden.

Emerging gastric cancer biomarkers across European and LATAM countries

At the genomic level, the most frequent pathogenic mutations were found in *TP53* (45%), followed by *ARID1A* (19%), *PIK3CA* (12%), *KRAS* (11%), *APC* (9%), *RNF43* (9%), and *LRP1B* (9%). *CDH1* mutations were detected in only 5% of the samples. Figure 2 and Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2025.104482, show the most prevalent mutations in each GC molecular subtype. Overall, 20.7% of the samples had pathogenic mutations in homologous recombination repair (HRR) genes, most commonly *ATM* (6%), followed by *BRCA2* (5%), *CDK12* (3%), *BRCA1* (2%), *RAD54L* (2%), *CHEK2* (1%), and others (<1% in *BARD1*, *RAD51C,* and *RAD51D*). HRR mutations were more common in dMMR/MSI (63.6%), followed by intestinal HER2-negative (18.7%), EBV-positive (18.2%), intestinal HER2-positive (15.4%), and diffuse HER2-negative (4.8%). Overall, mutations in HRR genes were found in 24.8% of GC samples from Europe versus 14.7% in LATAM. Supplementary Figure S5 and Table S4, available at https://doi.org/10.1016/j.esmoop.2025.104482, show mutation profiles in European versus LATAM cohorts.

At the transcriptomic level, EBV-positive tumors had high scores of apoptosis, antigen presentation, interferon signaling, epigenetic regulation, immune cell adhesion, and migration, infiltration with cytotoxic and exhausted CD8 cells. dMMR/MSI tumors were enriched for apoptosis, metabolic stress, and hypoxia and had low scores for Wnt, Hedgehog, and Notch signaling pathways. HER2-positive tumors had high scores for cell proliferation. Diffuse HER2-negative tumors were enriched for Wnt, Hedgehog, and Notch signaling pathways, and display low signature scores for cell proliferation and DNA damage repair, and high infiltration with B cells and mast cells. Intestinal HER2-negative tumors had high scores for cell proliferation, angiogenesis, Hedgehog signaling, DNA damage repair, and dendritic cell infiltration. These associations are shown in Figures 2 and 4 and detailed in Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2025.104482.

Finally, we compared the transcriptomic profiling of the most prevalent GC subtypes in Europe versus LATAM. In intestinal HER2-negative tumors, European samples had higher hypoxia, apoptosis, and matrix remodeling pathway activation scores, as illustrated in Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2025.104482. On the other hand, in diffuse HER2-negative tumors, European samples had higher PI3K/AKT activation scores as

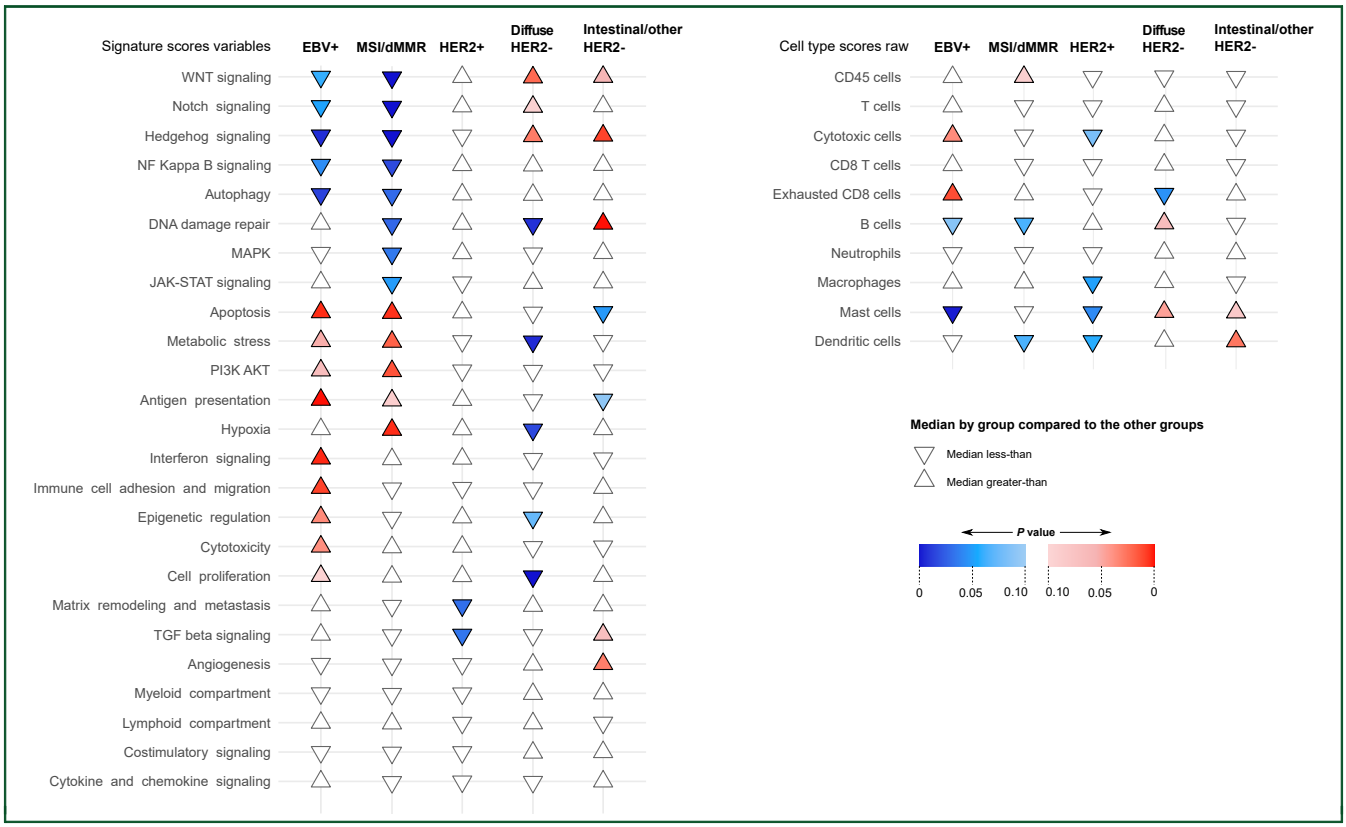

Figure 4. Transcriptomic enrichments in LEGACY project gastric cancer molecular subtyping.
dMMR, mismatch repair-deficient; EBV, Epstein—Barr virus; HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; TGF, transforming growth factor.

well as immune cell adhesion and migration, interferon and transforming growth factor-beta signaling, and antigen presentation, as shown in Supplementary Figure S7, available at https://doi.org/10.1016/j.esmoop.2025.104482.

DISCUSSION

Despite important insights into GC molecular differences, the current disease stratification approach is limited to selected IHC biomarkers with therapeutic impact in metastatic disease, i.e. HER2, PD-L1 CPS, and MMR/MSI. In the LEGACY project, we conducted a prospective clinico-molecular study of GC samples in reference institutions from Europe and LATAM, with shared protocols and standard operating procedures for biomarker testing. Considering that main GC molecular profiling studies have been conducted mainly in North American or Asian populations,[7,8,13,14] with limited data from other countries,[15] our study contributes to the GC molecular understanding in Europe and LATAM. We proposed a simple method for molecular classification of GC applicable to routine care in the advanced setting, considering accessibility to IHC/ISH technologies at the global scale. The clinical implementation of many previous GC classification schemes was hindered by the feasibility issue, particularly the need for high-burden NGS panels and the lack of clear IHC assessment criteria or cut-offs for p53 and E-cadherin positivity.[10] Therefore, in the LEGACY stratification algorithm, we have combined the molecular biomarkers HER2, dMMR/MSI, and EBV status with the Lauren histologic diagnosis, knowing the distinct biology of diffuse versus intestinal tumors.[16]

Complementing the evidence from previous studies, the LEGACY study found the following associations:

1. EBV-positive tumors had high infiltration of cytotoxic cells, high expression of interferon signaling, and immune checkpoints such as PD-L1, high TMB, and antigen presentation, and were more prevalent in LATAM, particularly in Chile. Exploratory data have shown good responses to immune checkpoint inhibitors in EBV-positive tumors,[17,18] but further validation is lacking in part due to the low prevalence in North American and European GC cohorts. The higher prevalence of this concrete molecular subtype in LATAM may stimulate regional clinical trials with the use of EBV as the biomarker to guide immunotherapy.
2. dMMR/MSI tumors had high TMB, mutations in HRR genes, modestly higher PD-L1 expression, and tended to present higher pathway activation scores for hypoxia and metabolic stress. In contrast to other types of dMMR/MSI tumors,[19] dMMR/MSI GC presents lower response rates to immunotherapy, which could be related to a heterogenic loss of the MMR proteins.[17] For instance, pathological response rates in the perioperative setting have been reported only in up to 60% of the cases,[20] and subgroup analysis of the main phase III

trials in the metastatic setting showed inconsistent results.[21,22] Future studies may consider selecting patients with dMMR/MSI tumors for immunotherapy agents considering also coexisting biomarkers, such as PD-L1 expression, or investigating novel combinations considering immune checkpoint inhibitors with drugs targeting metabolic stress pathways.[23-25] Of note, high TMB was almost exclusively found in EBV-positive and dMMR/MSI tumors, thus reflecting its deputy value for immune sensitivity. In fact, TMB alone is not predictive of response to immune checkpoint inhibitors in GC.[12,26]

3. Aligned with the literature, HER2 positivity was found in tumors with Lauren's intestinal histology, harbored high cell proliferation scores, and was more prevalent in European countries. These findings align with literature supporting how chromosomally unstable intestinal tumors frequently activate downstream proliferation pathways and other tyrosine kinase receptors (RTKs) upon resistance to the HER2 inhibition.[18,27,28] On the other hand, the lower prevalence of HER2 positivity in LATAM may explain the little evidence of treatment response to HER2 target therapies in this region. LATAM countries were clearly underrepresented in the pivotal TOGA phase III trial,[29] totally missed in the DESTINY-01[30] and DESTINY-02[31] trials, and included as an integrated subgroup as the 'rest of the world' region in the KEYNOTE-811 trial.[32]
4. Diffuse HER2-negative tumors had unique pathway activation signatures, such as strong Wnt or Notch signaling, and low cell proliferation and DNA damage repair scores. Interestingly, the immune infiltration was largely driven by immunosuppressive B cells and mast cells. These associations may be linked to the poor outcome with standard chemotherapy in the metastatic setting[33] but suggest a potential niche for novel targeted therapies accounting for the referred signaling pathways or the immunosuppressive microenvironment.[34,35]
5. Intestinal HER2-negative tumors had modestly high PD-L1 expression, high cell proliferation, and angiogenesis score, and were enriched for DNA damage repair pathways and infiltration with dendritic cells. In unselected patients, adding the antiangiogenic agent ramucirumab to chemotherapy improved outcomes,[36] although many other studies targeting the VEGF pathway had negative results.[37,38] The identification of a specific GC subtype with high angiogenic activation scores may help select patients who are tributary for these strategies and subgroup analysis of clinical trials.

Also, we found that broad NGS panels did not have added value in terms of identification of actionable tumor-agnostic mutation or fusion drivers, such as *BRAF* V600E mutations and *NTRK1-3* or *RET* fusions, which is in line with the recent European Society for Medical Oncology (ESMO) guidelines on multi-gene testing not recommending this technology in advanced GC.[39] Nevertheless, the high intratumor heterogeneity of GC may have compromised the results, and the impact of sequential liquid biopsies for genomic characterization is an active research field.[40] Importantly, we show that tumors from LATAM countries have higher median KI67-positive cells and higher TMB estimates [in the mismatch repair-proficient (pMMR)/ microsatellite stable (MSS) EBV-negative subgroup] and lower PD-L1 CPS scores (in the pMMR/MSS subgroup) when compared with European samples. However, the high TMB in tumors from LATAM patients may be partially explained by technical issues with incomplete bioinformatic adjustment for germline background mutations, which are underrepresented in public datasets, falsely increasing tumor mutation counts. In addition, diffuse HER2-negative tumors diagnosed in LATAM countries had particularly low immune pathway activation and antigen presentation, which may further impact prognosis and response to immunotherapies. These associations could be linked to distinct carcinogenesis paths, such as lifestyle and diet. As for the HER2-positive disease, LATAM countries were underrepresented in the pivotal phase III clinical trials with immune checkpoint inhibitors in the first-line setting,[21,22] thus limiting the analysis of potential interactions between GC molecular subtypes, geography, and immunotherapy efficacy.[41] Finally, we identified a high percentage of GC with HRR pathway alterations when compared with the literature,[42] mainly within the dMMR/MSI and intestinal HER2-negative subtypes. Novel drug combinations with poly(ADP-ribose) polymerase inhibitors (PARPi) in genomically selected GC tumors may increase the therapeutic benefit of this targeted approach, which has been modest to date.[43] Multiple phase Ib trials are actively recruiting patients with HRR-mutated tumors on expansion cohorts of PARPi combined with immunotherapies or novel targeted agents of the DNA damage repair pathway.

Our study has the limitations of being a relatively small cohort and not representative of the diversity of GC in European or LATAM countries. For instance, some LATAM or Central American countries with high prevalence and incidence of GC such as Colombia, Costa Rica, or Peru[2] were not included in this study due to budget limitations. Additional validation of GC molecular associations in other ethnic backgrounds would be ideal. On top of that, our study only incorporated cases of advanced GC, thus limiting the extrapolation of our findings to early-stage disease. Another weakness of our study is the lack of Claudin 18.2 (CDLN18.2) IHC staining, which was recently added to ESMO guidelines for routine testing in advanced GC[1] based on positive survival data with cytolytic antibody therapy combined with chemotherapy in HER2-negative disease.[44] The interaction of CLDN18.2 with other validated biomarkers remains poorly studied. Likewise, emerging biomarkers such as fibroblast growth factor receptor 2b (FGFR2b) IHC expression[45] were not investigated in our cohort.[4] Furthermore, information was deficient regarding clinical outcomes in the metastatic setting for close to half the patients recruited. Despite this, we were able to propose a GC molecular stratification system feasible in routine care with strong biological, therapeutic, and prognostic

rationale. Finally, we demonstrated notable differences in GC molecular underpinnings between European and LATAM samples, which may impact the response to approved targeted therapies and immunotherapeutic agents.

Given the molecular differences in GC subtypes and the validated and emerging biomarkers described in LEGACY, we highlight the need for geographic diversity in the populations recruited in pivotal clinical trials to support the best therapeutic approaches adapted to each region. Exploratory clinical trials with novel targeted agents and immunotherapy combinations should consider the biomarker enrichments described in our project. In conclusion, we provided unique insights into tumor biology from Europe and LATAM populations and proposed an accessible and affordable GC subtyping algorithm that could reproduce TCGA, ACRG, and integrated classifications, using techniques available in routine diagnosis thus supporting a precision medicine approach applicable worldwide.

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DATA SHARING

The data presented in this article are available upon request to the corresponding author.

REFERENCES

1. Shitara K, Fleitas T, Kawakami H, et al. Pan-Asian adapted ESMO Clinical Practice Guidelines for the diagnosis, treatment and follow-up of patients with gastric cancer. *ESMO Open*. 2024;9(2):102226.
2. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229-263.

3. Pectasides E, Stachler MD, Derks S, et al. Genomic heterogeneity as a barrier to precision medicine in gastroesophageal adenocarcinoma. *Cancer Discov.* 2018;8(1):37-48.
4. Alsina M, Arrazubi V, Diez M, Tabernero J. Current developments in gastric cancer: from molecular profiling to treatment strategy. *Nat Rev Gastroenterol Hepatol*. 2023;20(3):155-170.
5. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand*. 1965;64:31-49.
6. Nagtegaal ID, Odze RD, Klimstra D, et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology.* 2020;76(2):182-188.
7. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513(7517):202-209.
8. Cristescu R, Lee J, Nebozhyn M, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med*. 2015;21(5):449-456.
9. Thrift AP, Wenker TN, El-Serag HB. Global burden of gastric cancer: epidemiological trends, risk factors, screening and prevention. *Nat Rev Clin Oncol*. 2023;20(5):338-349.
10. Pinto MP, Córdova-Delgado M, Retamal IN, et al. Molecular stratification of Chilean gastric cancer patients with potential clinical applicability. *Cancers (Basel)*. 2020;12(7):1863.
11. van Schooten TS, Derks S, Jiménez-Martí E, et al. The LEGACY study: a European and Latin American consortium to identify risk factors and molecular phenotypes in gastric cancer to improve prevention strategies and personalized clinical decision making globally. *BMC Cancer.* 2022;22:646.
12. Lee KW, Van Cutsem E, Bang YJ, et al. Association of tumor mutational burden with efficacy of pembrolizumab ± chemotherapy as first-line therapy for gastric cancer in the phase III KEYNOTE-062 study. *Clin Cancer Res*. 2022;28(16):3489-3498.
13. Deng N, Goh LK, Wang H, et al. A comprehensive survey of genomic alterations in gastric cancer reveals systematic patterns of molecular exclusivity and co-occurrence among distinct therapeutic targets. *Gut*. 2012;61(5):673-684.
14. The Cancer Genome Atlas Research Network. Integrated genomic characterization of oesophageal carcinoma. *Nature*. 2017;541(7636):169-175.
15. Ye XS, Yu C, Aggarwal A, Reinhard C. Genomic alterations and molecular subtypes of gastric cancers in Asians. *Chin J Cancer.* 2016;35:42.
16. Petrelli F, Berenato R, Turati L, et al. Prognostic value of diffuse versus intestinal histotype in patients with gastric cancer: a systematic review and meta-analysis. *J Gastrointest Oncol*. 2017;8(1):148-163.
17. Kim ST, Cristescu R, Bass AJ, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med*. 2018;24(9):1449-1458.
18. Janjigian YY, Sanchez-Vega F, Jonsson P, et al. Genetic predictors of response to systemic therapy in esophagogastric cancer. *Cancer Discov.* 2018;8(1):49-58.
19. Diaz LA Jr, Shiu KK, Kim TW, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomized, open-label, phase 3 study. *Lancet Oncol*. 2022;23(5):659-670.
20. André T, Tougeron D, Piessen G, et al. Neoadjuvant nivolumab plus ipilimumab and adjuvant nivolumab in localized deficient mismatch repair/microsatellite instability-high gastric or esophagogastric junction adenocarcinoma: The GERCOR NEONIPIGA phase II study. *J Clin Oncol*. 2023;41(2):255-265.
21. Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomized, open-label, phase 3 trial. *Lancet*. 2021;398(10294):27-40.
22. Rha SY, Oh DY, Yañez P, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for HER2-negative advanced gastric cancer (KEYNOTE-859): a randomized, double-blind, phase 3 trial. *Lancet Oncol*. 2023;24(11):1181-1195.
23. Fukuoka S, Hara H, Takahashi N, et al. Regorafenib plus nivolumab in patients with advanced gastric or colorectal cancer: an open-label, dose-escalation, and dose-expansion phase Ib trial (REGONIVO, EPOC1603). *J Clin Oncol*. 2020;38(18):2053-2061.
24. Yanez PE, Ben-Aharon I, Rojas C, et al. First-line lenvatinib plus pembrolizumab plus chemotherapy versus chemotherapy in advanced/metastatic gastroesophageal adenocarcinoma (LEAP-015): safety run-in results. *J Clin Oncol*. 2023;41(suppl 4):411.
25. Pavlakis N, Shitara K, Sjoquist KM, et al. INTEGRATE IIb: a randomized phase III open label study of regorafenib + nivolumab versus standard chemotherapy in refractory advanced gastroesophageal cancer (AGOC). *J Clin Oncol*. 2022;40(suppl 4):TPS366.
26. Lei M, Janjigian YY, Ajani J, et al. Abstract CT023: Nivolumab (NIVO) plus chemotherapy (chemo) vs chemo as first-line (1L) treatment for advanced gastric cancer/gastroesophageal junction cancer/esophageal adenocarcinoma (GC/GEJC/EAC): CheckMate 649 biomarker analyses. *Cancer Res*. 2022;82(suppl 12):CT023.
27. Pietrantonio F, Fucà G, Morano F, et al. Biomarkers of primary resistance to trastuzumab in HER2-positive metastatic gastric cancer patients: the AMNESIA case-control study. *Clin Cancer Res*. 2018;24(5):1082-1089.
28. Pietrantonio F, Manca P, Bellomo SE, et al. HER2 copy number and resistance mechanisms in patients with HER2-positive advanced gastric cancer receiving initial trastuzumab-based therapy in JACOB trial. *Clin Cancer Res*. 2023;29(3):571-580.
29. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010;376(9742):687-697. Erratum in: *Lancet*. 2010;376(9749):1302.
30. Shitara K, Bang YJ, Iwasa S, et al. Trastuzumab deruxtecan in previously treated HER2-positive gastric cancer. *N Engl J Med*. 2020;382(25):2419-2430.
31. Van Cutsem E, di Bartolomeo M, Smyth E, et al. Trastuzumab deruxtecan in patients in the USA and Europe with HER2-positive advanced gastric or gastroesophageal junction cancer with disease progression on or after a trastuzumab-containing regimen (DESTINY-Gastric02): primary and updated analyses from a single-arm, phase 2 study. *Lancet Oncol*. 2023;24(7):744-756.
32. Kang Y, Shah MA, Shitara K, et al. First-line (1L) zolbetuximab + chemotherapy in patients (pts) with claudin 18.2 (CLDN18.2) +, HER2-, locally advanced (LA) unresectable or metastatic gastric or gastroesophageal junction (mG/GEJ) adenocarcinoma: a pooled final analysis of SPOTLIGHT + GLOW. *Ann Oncol*. 2024;35(suppl 2):S878-S912.
33. Lordick F, Carneiro F, Cascinu S, et al. Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2022;33(10):1005-1020.
34. Bono P, Minchom A, Shetty S, et al. LBA38 Bexmarilimab, a novel macrophage re-programmer shows promising anti-tumour activity in phase I/II trial in several last line solid tumour types. *Ann Oncol*. 2021;32:S1313.
35. DeNardo DG, Galkin A, Dupont J, et al. GB1275, a first-in-class CD11b modulator: rationale for immunotherapeutic combinations in solid tumors. *J Immunother Cancer.* 2021;9(8):e003005. Erratum in: *J Immunother Cancer.* 2021;9(11):e003005corr1.
36. Wilke H, Muro K, Van Cutsem E, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol*. 2014;15(11):1224-1235.
37. Van Cutsem E, de Haas S, Kang YK, et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a biomarker evaluation from the AVAGAST randomized phase III trial. *J Clin Oncol*. 2012;30(17):2119-2127.
38. Fuchs CS, Shitara K, Di Bartolomeo M, et al. Ramucirumab with cisplatin and fluoropyrimidine as first-line therapy in patients with

metastatic gastric or junctional adenocarcinoma (RAINFALL): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2019;20(3):420-435. Erratum in: *Lancet Oncol*. 2019;20(5):e242.
39. Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2024;35(7):588-606.
40. Labiano I, Huerta AE, Arrazubi V, et al. State of the Art: ctDNA in upper gastrointestinal malignancies. *Cancers (Basel)*. 2023;15(5):1379.
41. Janjigian YY, Al-Batran S, Wainberg ZA, et al. Pathological complete response (pCR) to 5-fluorouracil, leucovorin, oxaliplatin and docetaxel (FLOT) with or without durvalumab (D) in resectable gastric and gastroesophageal junction cancer (GC/GEJC): subgroup analysis by region from the phase 3, randomized, double-blind MATTERHORN study. *J Clin Oncol*. 2024;42:LBA246.
42. Sahasrabudhe R, Lott P, Bohorquez M, et al. Germline mutations in PALB2, BRCA1, and RAD51C, which regulate DNA recombination repair, in patients with gastric cancer. *Gastroenterology*. 2017;152(5):983-986.e6.
43. Bang YJ, Xu RH, Chin K, et al. Olaparib in combination with paclitaxel in patients with advanced gastric cancer who have progressed following first-line therapy (GOLD): a double-blind, randomized, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2017;18(12):1637-1651.
44. Shitara K, Shah MA, Lordick F, et al. Zolbetuximab in gastric or gastroesophageal junction adenocarcinoma. *N Engl J Med*. 2024;391(12):1159-1162.
45. Wainberg ZA, Kang YK, Lee KW, et al. Bemarituzumab as first-line treatment for locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma: final analysis of the randomized phase 2 FIGHT trial. *Gastric Cancer*. 2024;27(3):558-570.