Online-Only Supplement

Supplement to: Y. Bai, et al. **Efficacy and potential predictive biomarkers of immunotherapy in Epstein-Barr Virus-associated gastric cancer**

Supplemental Methods

DNA preparation and next-generation sequencing (NGS)

DNA extraction and NGS analysis were conducted by 3D Medicines, Inc., a College of American Pathologists (CAP)-accredited and Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. Genomic DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissues using the ReliaPrep™ FFPE gDNA Miniprep System (Promega) and quantified using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific) following the manufacturer's instructions. DNA extracts (30-200 ng) were sheared to 250 bp fragments using an S220 Focused-ultrasonicator (Covaris). Libraries were prepared using the KAPA Hyper Prep Kit (KAPA Biosystems) following the manufacturer's protocol. Indexed libraries were subjected to hybridize with probes targeting the Epstein-Barr virus (EBV) genes and a customized 733-gene panel (3D Medicines). The captured libraries were subsequently loaded onto a NovaSeq 6000 platform (Illumina) for 100 bp paired-end sequencing with a mean sequencing depth of 1000×. Raw data of paired samples (an FFPE sample and its paired white blood sample) were mapped to the reference human genome hg19 using the Burrows-Wheeler Aligner (v0.7.12). PCR duplicate reads were removed, and sequence metrics were collected using Picard (v1.130) and SAMtools (v1.1.19), respectively. Variant calling was performed only in the targeted regions. Somatic single nucleotide variants (SNVs) were detected using an in-house developed R package to execute a variant detection model based on the binomial test. Local realignment was performed to detect insertions and deletions (indels). Variants were then filtered by their unique supporting read depth, strand bias, base quality as previously described.[1] All variants were then filtered using an automated false positive filtering pipeline to ensure sensitivity and specificity at an allele frequency (AF) of ≥ 1%. Single-nucleotide polymorphism (SNPs) and indels were annotated by ANNOVAR

against the following databases: dbSNP (v138), 1000Genome and ESP6500 (population frequency > 0.015). Only missense, stopgain, frameshift, and non-frameshift indel mutations were kept.

EBV and tumor mutational burden (TMB) detection by NGS

A detailed description of the algorithm for EBV score calculation was provided in the Results section. Any sample with an EBV score of ≥ 0.05695 was considered EBV-positive. TMB was defined as the number of somatic SNVs and indels in examined coding regions. All SNVs and indels in the coding regions of targeted genes, including missense, silent, stopgain, stoploss, in-frame, and frameshift mutations, were considered.

EBV detection by EBER-ISH and qPCR

EBER-ISH was performed as previously reported.[2] To examine the correlation between EBV score and viral copy number, the EBV BamHI-W region (GenBank accession number V01555.2) of each DNA sample was subjected to probe-based qPCR using the QuantiNova Probe PCR Kit (QIAGEN). A standard curve was generated using synthetic BamHI-W fragments as templates (GENEWIZ). The EBV copy number for each sample was determined in reference to the standard curve.

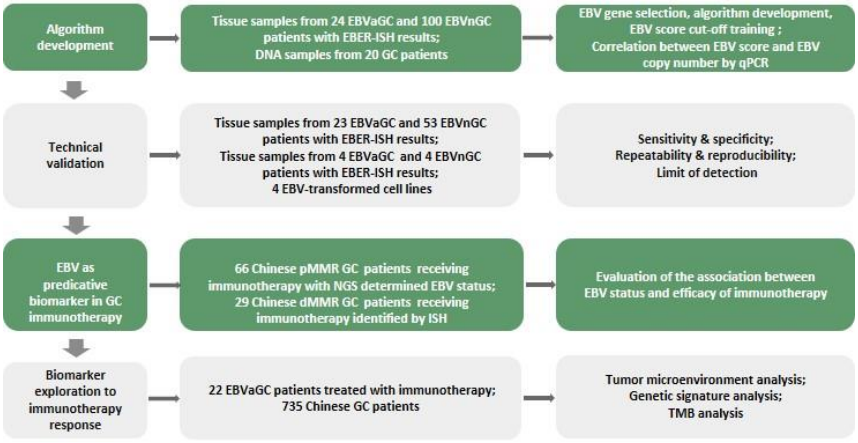
PD-L1, MMR, and HRE2 expression by IHC

IHC detection of PD-L1, MMR, and HRE2 expression was conducted according to the standard procedure.[2] Briefly, FFPE tissue sections were subjected to assessment of PD-L1 expression using the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies). PD-L1 expression was defined using a combined positive score (CPS) by dividing the number of PD-L1-stained cells (tumor cells, lymphocytes, macrophages) with the total number of viable tumor cells and multiplying by 100. Any sample with a CPS of ≥ 1 was considered positive for PD-L1 expression. MMR expression in FFPE tissue sections was evaluated by MMR IHC panel comprised of four primary antibodies, anti-hMLH1, anti-hMSH2, anti-hMSH6, and anti-hPMS2 according to the manufacturer's protocol. Results were ultimately presented as dMMR (one or more than one of four MMR proteins missing) and pMMR (all four proteins present). For IHC staining for HER2, the extent of overall staining or membranous staining was recorded. All antibodies employed in this study were listed in Supplementary Table 5. Equivocal and uninterpretable cases required repeated staining of the proteins needed.

Multiplex immunofluorescence

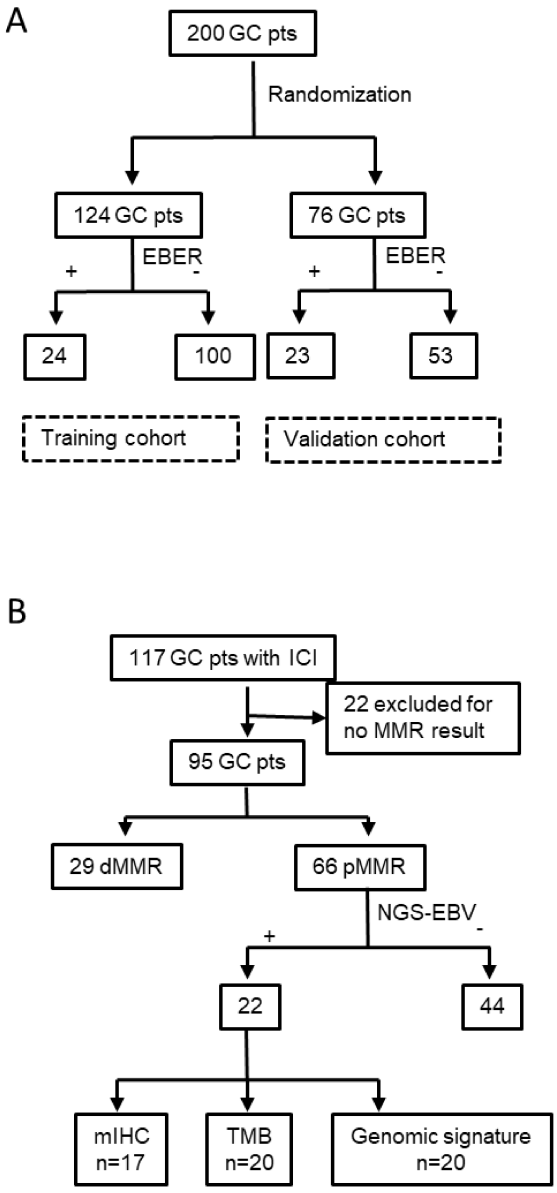
Multiplex immunofluorescence staining was carried out using the PANO 7-plex IHC kit (Panovue). FFPE tissue slides were incubated with specific primary antibodies, followed by interaction with horseradish peroxidase-conjugated secondary antibody and tyramide signal amplification. Each panel included different immune cell markers along with PanCK (Cocktail) (Supplemental Table 5). The slides were heat-treated after each round of amplification. Cell nuclei acids were counterstained with 4', 6-diamidino-2-phenylindole (DAPI, SIGMA-ALDRICH). Multiplex stained slides were scanned, and all scans for each slide were then merged to determine the relative localization of the proteins. The quantity of each marker was expressed as the number of stained cells per square millimeter and as the percentage of positively stained cells in all nucleated cells, representing each kind of immune cell or immune structures calculated from the combination of different markers.

Supplementary Figure 1. The flowchart of the study



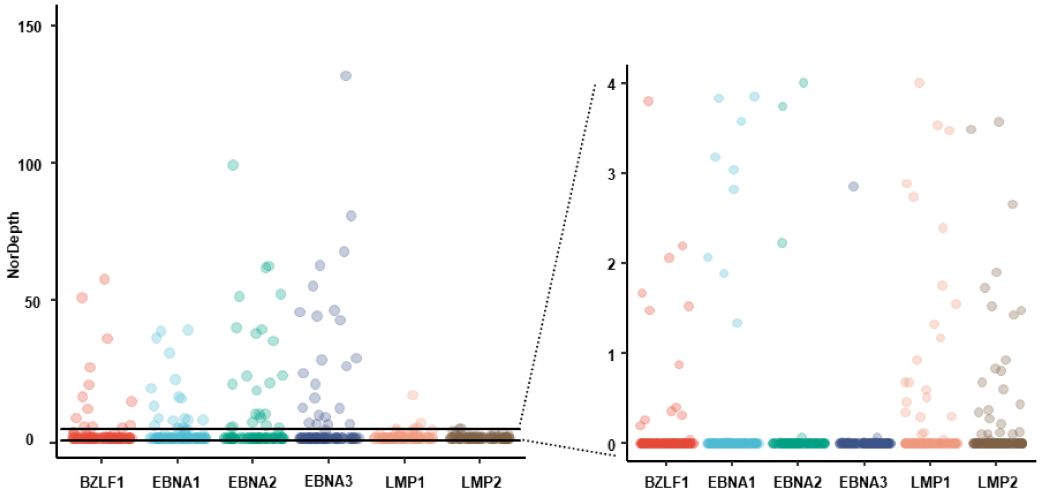
Abbreviations: EBV: Epstein-Barr virus; GC: gastric cancer; EBVaGC: Epstein-Barr virus-associated gastric cancer; EBVnGC: Epstein-Barr virus-negative gastric cancer; EBER: EBV-encoded RNA; ISH: in situ hybridization; pMMR: DNA mismatch repair proficient; dMMR: mismatch repair deficient; qPCR: quantitative polymerase chain reaction; NGS: next-generation sequencing; TCGA: The Cancer Genome Atlas; TMB: tumor mutation burden.

Supplementary Figure 2. Consort patient flow diagram



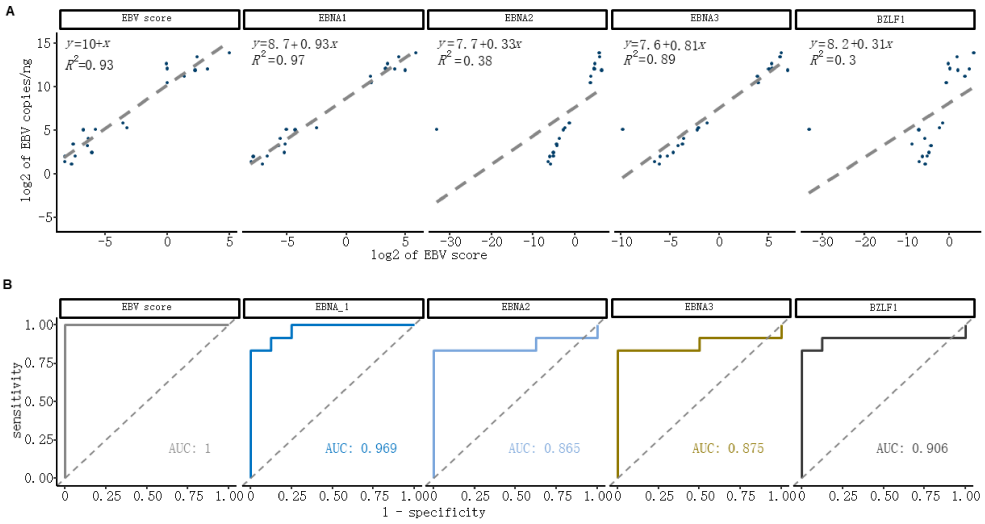
Abbreviations: EBV: Epstein-Barr virus; GC: gastric cancer; EBER: EBV-encoded RNA; pMMR: DNA mismatch repair proficient; dMMR: mismatch repair deficient; NGS: next-generation sequencing; TMB: tumor mutation burden; mIHC: multiplex immunohistochemistry; CNV: copy number variation; pts: patients.

Supplementary Figure 3. The NorDepth of 6 EBV genes in 124 EBVaGC tissue samples



X-axis represents different genes, and Y-axis represents the NorDepth. The right panel shows the amplification from black dotted lines-marked regions in the left panel.
Abbreviations: EBV: Epstein-Barr virus; Epstein-Barr virus-associated gastric cancer.

Supplementary Figure 4. The linear correlations between EBV score and EBV copy number, and the AUC values for EBV positivity prediction in GC

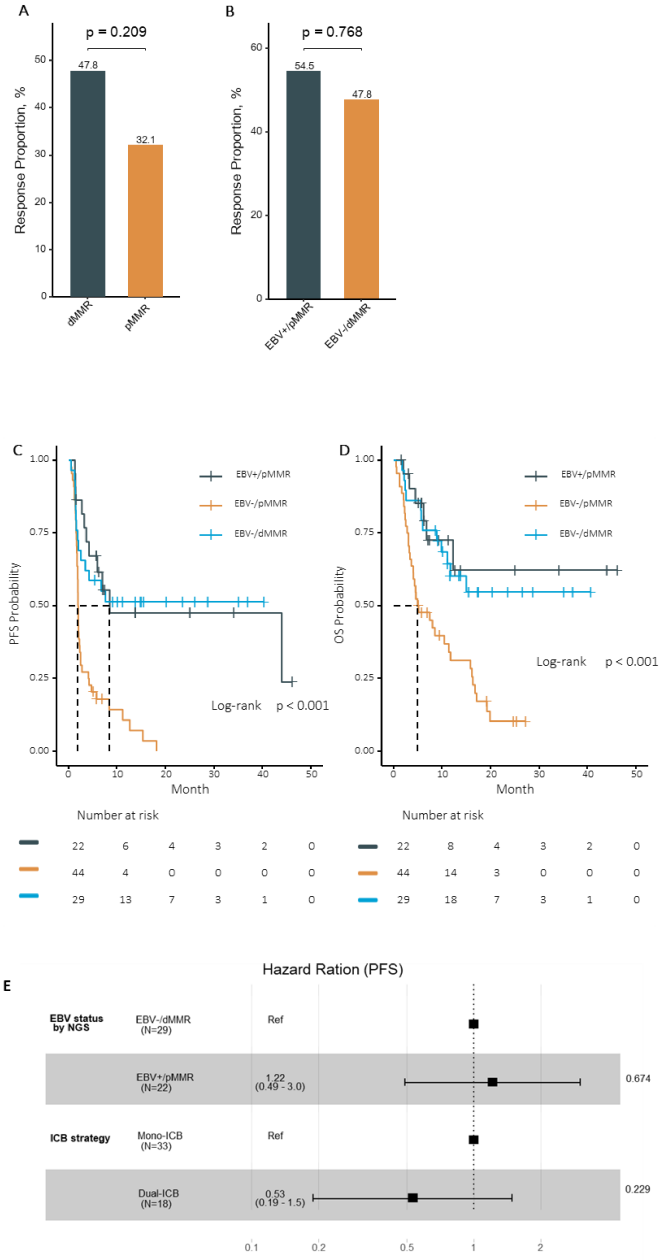


A. The linear correlations between the NGS algorithm-developed EBV score and the EBV copy number for the individual EBV gene or the combination of four EBV genes in 20 tumor tissue samples from GC patients. B. The ROC curve of the individual gene- and four-gene combination- EBV score for predicting EBV status in 124 GC tissue samples where EBV

status was previously identified by EBER-ISH.

Abbreviations: GC: gastric carcinoma; NGS: next-generation sequencing; EBV: Epstein-Barr virus; EBER: Epstein-Barr virus-encoded small RNA; ISH: in situ hybridization; ROC: receiver operator characteristic; AUC: an area under the curve.

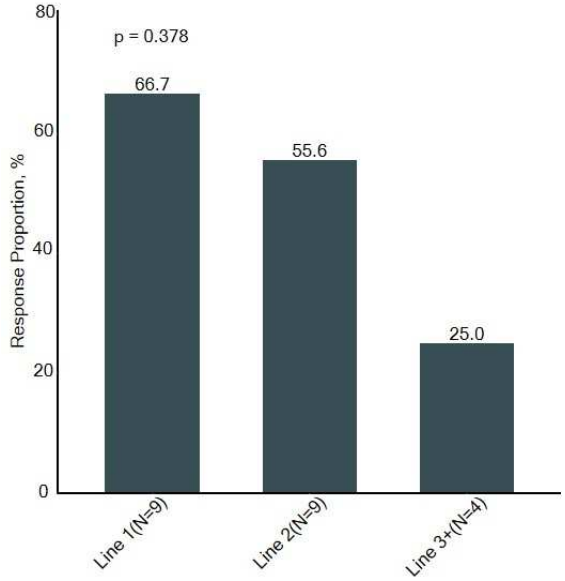
Supplementary Figure 5. Survival analysis of the GC patients with EBV+/pMMR, EBV-/pMMR, or EBV-/dMMR receiving ICB therapy



A. The percentage of responders and non-responders in dMMR and pMMR patients. B. The

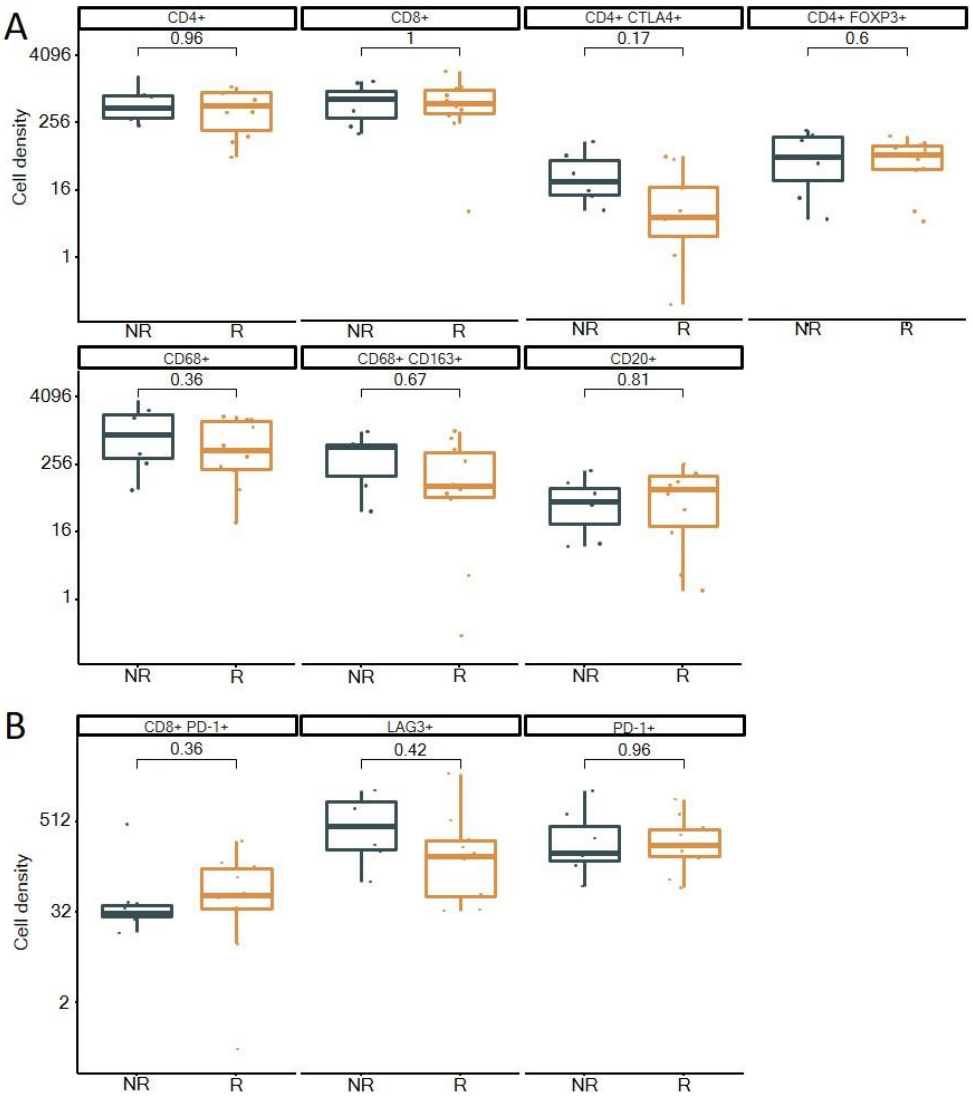
percentage of responders and non-responders in EBV-/dMMR and EBV+/pMMR patients. C-D. Kaplan-Meier curves of the PFS (C) and OS (D) of the patients with EBV+/pMMR, EBV-/pMMR, or EBV-/dMMR receiving ICB therapy. E. The multivariate Cox analysis of the EBV status and ICB strategy against PFS in the EBV-/dMMR and EBV+/pMMR patients.
Abbreviations: EBV: Epstein-Barr virus; pMMR: DNA mismatch repair proficient; dMMR: mismatch repair deficient; OS: overall survival; PFS: progression-free survival; Mono-ICB: anti-PD1/L1 mono-therapy; Dual-ICB: combination anti-CTLA-4 plus anti-PD-1/L1 therapy.

Supplementary Figure 6. The efficacy of ICB as different treatment lines in EBVaGC patients



The ORRs to first-line, second-line, and at least third-line ICB therapy in EBVaGC patients.
Abbreviations: ORR: the objective response rate; ICB: immune checkpoint blockade; EBVaGC: Epstein-Barr virus-associated gastric cancer.

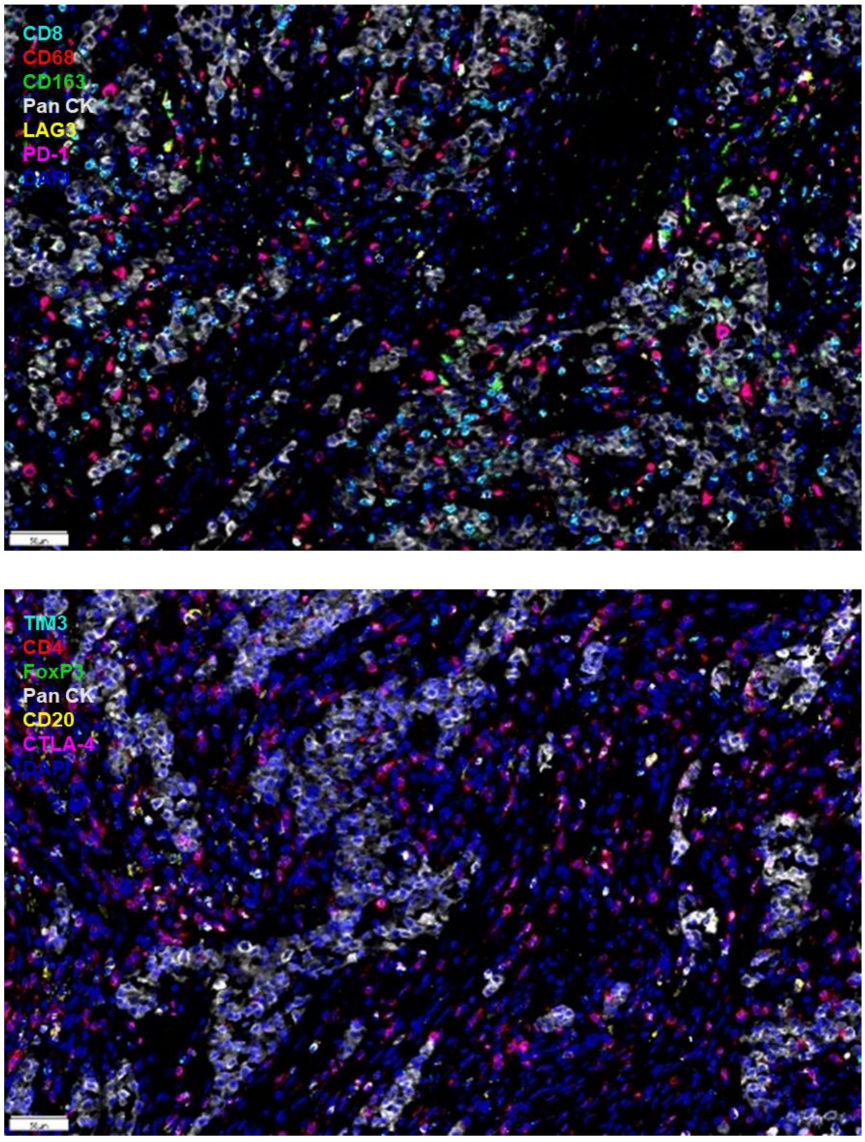
Supplementary Figure 7. The density of multiple lymphocyte subgroups and the proposed ICB biomarker-expressed cells in the ICB response and non-response group in EBVaGC



A. Multiple lymphocyte subgroups including helper T cells, cytotoxic T cells, regulatory T cells, macrophages, M1 type macrophages, and B lymphocytes. B. The proposed ICB biomarker-expressed cells including $CD8^{+}PD\text{-}1^{+}$, $LAG3^{+}$, and $PD\text{-}1^{+}$ cells in the ICB response and non-response group.

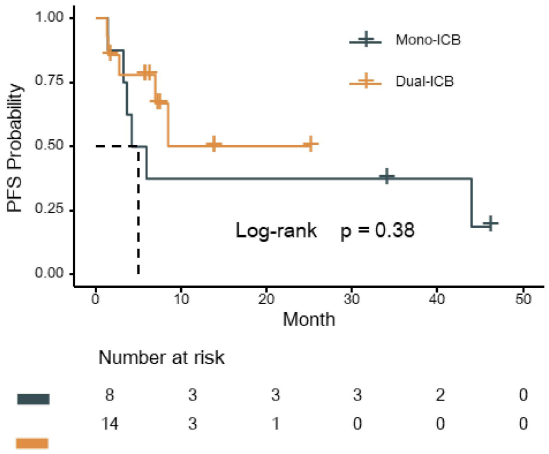
Abbreviations: ICB: immune checkpoint blockade; EBVaGC: Epstein-Barr virus-associated gastric cancer; R: responder; NR: non-responder.

Supplementary Figure 8. Representative images of tumor-infiltrating immune cells in EBVaGC patients.



The images were obtained from an EBVaGC patient via multiplex immunofluorescence.
Abbreviations: EBVaGC: Epstein-Barr virus-associated gastric cancer.

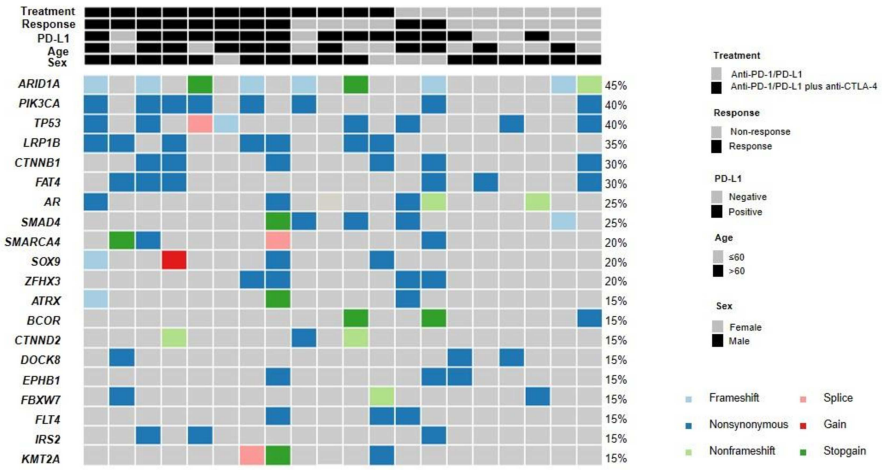
Supplementary Figure 9. Progression-free survival analysis of EBVaGC patients with mono-ICB or dual-ICB therapy



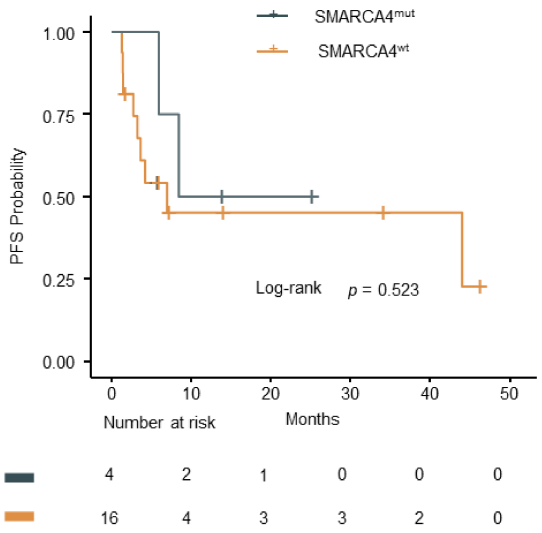
Kaplan-Meier curves of the PFS of the EBVaGC patients receiving mono- or dual-ICB therapy.

Abbreviations: EBVaGC: Epstein-Barr virus-associated gastric cancer; ICB: immune checkpoint blockade; PFS: progression-free survival; Mono-ICB: anti-PD1/L1 mono-therapy; Dual-ICB: combination anti-CTLA-4 plus anti-PD-1/L1 therapy.

Supplementary Figure 10. Genomic landscape of alterations in EBVaGC immunotherapy cohort



Supplementary Figure 11. Progression-free survival analysis of the EBVaGC patients with SMARCA4 mutation or wild-type following ICB



Kaplan-Meier curves of the PFS of the EBVaGC patients with *SMARCA4* mutation or wild-type following ICB.

Abbreviations: EBVaGC: Epstein-Barr virus-associated gastric cancer; ICB: immune checkpoint blockade; PFS: progression-free survival.

Supplementary Table 1. The exact ICB drugs for 95 advanced or metastatic GC patients

Group	Immunotherapy Type	Exact therapy		
		anti	drug	number
dMMR (n=29)	Mono (n=25)	PD-1 (n=16)	zimberelimab	1
			tislelizumab	6
			sintilimab	1
			pembrolizumab	3
			nivolumab	2
			JS001	2
			CS1003	1
		PD-L1 (n=9)	envafolimab	6
			CS1001	1
			atezolizumab	2
	Dual (n=4)	PD-1+CTLA-4 (n=3)	nivolumab+ ipilimumab	3
		PD-L1+CTLA-4 (n=1)	durvalumab+ tremelimumab	1
EBV+/pMMR (n=22)	Mono (n=8)	PD-1 (n=5)	zimberelimab	2
			tislelizumab	1
			pembrolizumab	1
			JS001	1
		PD-L1 (n=3)	MSB2311	1
			CS1001	1
			atezolizumab	1
	Dual (n=14)	PD-1+CTLA-4 (n=14)	sintilimab +IBI310	13
			nivolumab+ ipilimumab	1
EBV-/pMMR (n=44)	Mono (n=43)	PD-1 (n=35)	zimberelimab	12
			tislelizumab	8
			sintilimab	1
			pembrolizumab	6
			LZM009	1
			JS001	2
			CS1003	5
		PD-L1 (n=8)	envafolimab	2
			durvalumab	1
			CS1001	1
			atezolizumab	4
	Dual (n=1)	PD-1+CTLA-4 (n=1)	sintilimab +IBI310	1

Supplementary Table 2. List of the genes in the 3DMed 733-gene panel

Gene list
ABCB11 ABI1 ABL1 ABRAXAS1 ACKR3 ACSL3 ACVR1 ACVR1B ACVR2A AEN AFF3 AFF4 AKT1 AKT2 AKT3 ALK ALKBH2 ALKBH3 AMER1 ANK1 APC APEX1 APEX2 APLF APOBEC3B APTX AR ARAF AREG ARHGAP5 ARID1A ARID1B ARID2 ARNT ASXL1 ATM ATP1A1 ATP2B3 ATR ATRIP ATRX AURKA AXIN1 AXIN2 AXL B2M BAP1 BARD1 BAZ1A BCL10 BCL11A BCL11B BCL2 BCL2L1 BCL2L11 BCL6 BCOR BCORL1 BCR BIRC3 BIRC5 BLM BMP5 BMPR1A BRAF BRCA1 BRCA2 BRD4 BRIP1 BTG1 BTK BUB1B CACNA1D CALR CAMTA1 CANT1 CARD11 CARS CASP8 CBFA2T3 CBFB CBL CBLB CCDC6 CCNB1IP1 CCND1 CCND2 CCND3 CCNE1 CCNH CCNO CD274 CD74 CD79A CD79B CDC73 CDH1 CDH10 CDH11 CDK12 CDK2 CDK4 CDK6 CDK7 CDK8 CDKN1A CDKN1B CDKN1C CDKN2A CDKN2B CDKN2C CDX2 CEBPA CENPS CENPX CETN2 CHAF1A CHD1 CHD2 CHD4 CHEK1 CHEK2 CHIC2 CIC CIITA CLIP1 CLK2 CLTCL1 CNBP CNOT3 COL7A1 CRBN CREB3L1 CREB3L2 CREBBP CRKL CRLF2 CRNKL1 CRTC1 CRTC3 CSF1R CSF3R CTCF CTNNB1 CTNND2 CTR9 CUL1 CUL3 CUL4A CUL5 CUX1 CXCR4 CYLD CYP17A1 CYP2C19 CYP2D6 CYSLTR2 DAXX DCLRE1A DCLRE1B DCLRE1C DDB1 DDB2 DDIT3 DDR2 DDX10 DDX3X DDX5 DDX6 DICER1 DIS3 DIS3L2 DKC1 DMC1 DNM2 DNMT1 DNMT3A DNTT DOCK8 DPYD DROSHA DUT EBF1 EED EGFR EIF3E EIF4A2 ELANE ELF3 ELF4 ELK4 ELL ELOA EME1 EME2 EMSY ENDOV EP300 EPAS1 EPCAM EPHA2 EPHA3 EPHA7 EPHB1 EPS15 ERBB2 ERBB3 ERBB4 ERC1 ERCC1 ERCC2 ERCC3 ERCC4 ERCC5 ERCC6 ERCC8 EREG ERF ERRFI1 ESR1 ETNK1 ETV1 ETV4 ETV5 ETV6 EWSR1 EXO1 EXT1 EXT2 EZH2 EZR FAAP100 FAAP20 FAAP24 FAH FAM135B FAM47C FAN1 FANCA FANCB FANCC FANCD2 FANCE FANCF FANCG FANCI FANCL FANCM FAS FAT1 FAT4 FBXW7 FEN1 FES FGF19 FGF3 FGF4 FGFR1 FGFR2 FGFR3 FGFR4 FH FHIT FLCN FLT1 FLT3 FLT4 FOXA1 FOXL2 FOXP1 FRK FRS2 FUBP1 FUS G6PD GALNT12 GAS7 GATA1 GATA2 GATA3 GBA GEN1 GFI1 GJB2 GLI1 GLI2 GLI3 GNA11 GNA13 GNAQ GNAS GPC3 GRB2 GREM1 GRIN2A GSK3B GSTT1 GTF2H1 GTF2H3 GTF2H4 GTF2H5 H2AFX H3F3A HDAC1 HDAC2 HELQ HES1 HEY1 HFE HFM1 HGF HIF1A HIP1 HIST1H3B HLTF HMBS HMGA2 HMGB1 HNF1A HNRNPA2B1 HOOK3 HOXA11 HOXB13 HRAS HUS1 HUS1B IDH1 IDH2 IGF1R IGF2 IKBKE IKZF1 IL6ST IL7R INPP4B IRS2 ITGAV ITK JAK1 JAK2 JAK3 JMJD1C JUN KCNJ5 KDM5A KDM5C KDM6A KDR KEAP1 KIT KLF4 KMT2A KMT2C KMT2D KNL1 KRAS LASP1 LATS1 LATS2 LCK LEF1 LIFR LIG1 LIG3 LIG4 LMNA LMO1 LRP1B LZTR1 MAD2L2 MAP2K1 MAP2K2 MAP2K4 MAP3K1 MAPK1 MAX MBD4 MCL1 MDC1 MDM2 MDM4 MECOM MED12 MEF2B MEN1 MET MGA MGMT MITF MLH1 MLH3 MLLT3 MLST8 MMS19 MNAT1 MPG MPL MPLKIP MRE11 MSH2 MSH3 MSH4 MSH5 MSH6 MTAP MTOR MUS81 MUTYH MYB MYC MYCL MYCN MYD88 MYOD1 NAB2 NABP2 NBN NCOA3 NCOR1 NCOR2 NDRG1 NEIL1 NEIL2 NEIL3 NF1 NF2 NFE2L2 NFIB NFKBIA NHEJ1 NHP2 NKX2-1 NME1 NONO NOP10 NOTCH1 NOTCH2 NOTCH3 NOTCH4 NPM1 NR4A3 NRAS NRG1 NRG3 NSD2 NSD3 NT5C2 NTHL1 NTRK1 NTRK2 NTRK3 NUDT1 NUP93 NUTM1 OGG1 PAK1 PALB2 PARP1 PARP2 PARP3 PARP4 PAX3 PAX5 PAX7 PAX8 PBRM1 PCDH9 PCNA PDCD1LG2 PDGFB PDGFRA PDGFRB PDPK1 PER1 PER2 PER3 PHF6 PHOX2B PICALM PIK3CA PIK3CB PIK3CD PIK3R1 PIK3R2 PIK3R3 PIM1 PLCG2 PLXNA1 PLXNB1 PML PMS1 PMS2 PNKP POLB POLD1 POLD3 POLD4 POLE POLE2 POLE3 POLE4 POLG POLH POLI POLK POLL POLM POLN POLQ POT1 POU2AF1 POU5F1 PPARG PPM1D PPP2R1A PPP2R2A PPP4R1 PPP4R2 PPP4R3A PPP4R3B PPP4R4 PPP6C PRCC PRDM1 PRDM16 PRDM9 PREX2 PRF1 PRKACA PRKAR1A PRKCH PRKDC PRPF19 PRSS1 PSIP1 PTCH1 PTEN PTK2 PTK6 PTPN11 PTPN13 PTPRD PTPRT QKI RAC1 RAD1 RAD18 RAD21 RAD23A RAD23B RAD50 RAD51 RAD51B RAD51C RAD51D RAD52 RAD54B RAD54L RAD54L2 RAD9A RAD9B RAF1 RANBP2 RAP1GDS1 RARA RASA1 RB1 RBBP8 RBM10 RBX1 RDM1 RECQL RECQL4 RECQL5 RET REV1 REV3L RFC1 RFC2 RFC3 RFC4 RFC5 RFWD3 RGS7 RHBDF2 RHEB RHOA RHOH RICTOR RIF1 RIT1 RMI1 RMI2 RNF168 RNF213 RNF4 RNF43 RNF8 ROS1 RPA1 RPA2 RPA3 RPA4 RPS6KA3 RPS6KB1 RPTOR RRM2B RUNX1 RUNX1T1 RXRA SBDS SDC4 SDHA SDHAF2 SDHB SDHC SDHD SEM1 SERPINA1 SERPINB3 SETBP1 SETD2 SETMAR SF3B1 SFPQ SGK1 SH2B3 SH2D1A SHOC2 SHPRH SLC25A13 SLC29A1 SLC34A2 SLC45A3 SLIT2 SLX1A SLX4 SMAD2 SMAD3 SMAD4 SMARCA1 SMARCA2 SMARCA4 SMARCB1 SMO SMUG1 SOCS1 SOS1 SOX2 SOX9 SPEN SPO11 SPOP SPRED1 SPRTN SPTA1 SRC SRGAP3 SRSF2 SRY SS18 STAG2 STAT3 STK11 SUFU SUZ12 SYK TBL1XR1 TBX3 TCF3 TCF7L2 TCL1A TDG TDP1 TDP2 TEAD2 TELO2 TERT TET1 TET2 TFE3 TGFBR1 TGFBR2 THBS2 TIMELESS TMEM127 TMEM189 TMPRSS2 TNFAIP3 TOP2A TOP3A TOP3B TOPBP1 TP53 TP53BP1 TP63 TPMT TRAF7 TREX1 TREX2

TRIM37 TSC1 TSC2 TSHR TSPAN31 TYK2 U2AF1 UBE2A UBE2B UBE2N UBE2T UBE2V2 UGT1A1 UNG UROD USP1 USP6 USP8 UVSSA VEGFA VHL WAS WDR48 WIF1 WRN WT1 XAB2 XPA XPC XPO1 XRCC1 XRCC2 XRCC3 XRCC4 XRCC5 XRCC6 YAP1 YWHAE ZBTB16 ZFHX3 ZNF217 ZNF479 ZNF703 ZNF750 ZNRF3

Supplementary Table 3. The sensitivity and specificity of NGS-based EBV detection in 76 gastric adenocarcinoma patients

		EBER-ISH		Total
		+	-	
NGS-EBV	+	22	0	22
	-	1	53	54
Total		23	53	76

	Value	95% CI (%)
Sensitivity	95.65%	77.3 - 99.8
Specificity	100%	93.2 - 100
PPV	100%	85.1 - 100
NPV	98.15%	90.9 99.9
Concordance	98.68%	92.9 - 99.9

Abbreviations: EBER: Epstein-Barr virus (EBV)-encoded small RNA; ISH: in situ hybridization; NGS: next generation sequencing; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval.

Supplementary Table 4. Repeatability and reproducibility test of EBV score detection

Sample ID	EBV-Batch 1	Replicate 1		Replicate 2		Replicate 3		Replicate 4		EBV score	
		Score	Status	Score	Status	Score	Status	Score	Status	Average	Concordance
tMSIbz-055	Neg	0.00	Neg	0.00	Neg	0.00	Neg	0.00	Neg	**0.001**	**100%**
tMSIbz-133	Neg	0.00	Neg	0.00	Neg	0.00	Neg	0.00	Neg	**0.001**	**100%**
tEBVbz-21	Neg	0.03	Neg	0.05	Neg	0.04	Neg	0.04	Neg	**0.038**	**100%**
tMSIbz-146	Neg	0.25	Neg	0.20	Neg	0.23	Neg	0.26	Neg	**0.234**	**100%**
tEBVbz-7	Pos	2.12	Pos	1.90	Pos	2.24	Pos	2.29	Pos	**2.138**	**100%**
tEBVbz-35	Pos	10.11	Pos	10.32	Pos	10.25	Pos	9.73	Pos	**10.102**	**100%**
tEBVbz-28	Pos	29.10	Pos	29.35	Pos	29.56	Pos	29.38	Pos	**29.348**	**100%**
tEBVbz-27	Pos	91.07	Pos	89.02	Pos	87.41	Pos	89.98	Pos	**89.370**	**100%**

Sample ID	EBV-Batch 2	Replicate 1		Replicate 2		Replicate 3		Replicate 4		EBV score	
		Score	Status	Score	Status	Score	Status	Score	Status	Average	Concordance
tMSIbz-055	Neg	0.01	Neg	0.00	Neg	0.00	Neg	0.00	Neg	**0.00**	**100%**
tMSIbz-133	Neg	0.00	Neg	0.00	Neg	0.02	Neg	0.00	Neg	**0.01**	**100%**
tEBVbz-21	Neg	0.03	Neg	0.02	Neg	0.03	Neg	0.04	Neg	**0.03**	**100%**
tMSIbz-146	Neg	0.07	Neg	0.08	Neg	0.06	Neg	0.07	Neg	**0.07**	**100%**
tEBVbz-7	Pos	2.03	Pos	2.47	Pos	2.28	Pos	2.32	Pos	**2.27**	**100%**
tEBVbz-35	Pos	10.46	Pos	10.62	Pos	11.14	Pos	10.74	Pos	**10.74**	**100%**
tEBVbz-28	Pos	28.41	Pos	29.57	Pos	28.98	Pos	31.42	Pos	**29.59**	**100%**
tEBVbz-27	Pos	88.85	Pos	86.99	Pos	86.84	Pos	88.40	Pos	**87.77**	**100%**

Abbreviations: Neg: negative; Pos: positive.

Supplementary Table 5. Univariate and multivariate Cox regression against OS

Covariate	Univariate cox		Multivariate cox	
	HR	P value	HR	P value
Gender	0.77 [0.39-1.51]	0.451		
Age	0.97 [0.94-0.99]	0.005	0.96 [0.93-0.98]	0.002
ICB strategy	0.18 [0.04-0.74]	0.018	0.24 [0.05-1.26]	0.091
Prior systemic therapy	7.86 [1.08-57.2]	0.042	2.7 [0.34-21.36]	0.347
Her2 status	0.92 [0.46-1.83]	0.809		
PD_L1 status	0.62 [0.33-1.16]	0.135		
EBV status	0.28 [0.12-0.66]	0.004	0.6 [0.22-1.63]	0.317

Abbreviations: PFS: progression-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval.

Supplementary Table 6. Antibodies used in mIHC and IHC

Name	Clone	Company	Usage
PD1	EH33	Cell Signaling Technology	mIHC
CD8	C8/144B	Cell Signaling Technology	mIHC
PanCK	C11	Cell Signaling Technology	mIHC
CTLA4	SP355	Abcam	mIHC
CD4	BP6028	Biolynx	mIHC
FoxP3	259D	BioLegend	mIHC
CD68	KP1	ZSGB-BIO	mIHC
HLADR	EPR3692	Abcam	mIHC
CD20	EP459Y	Abcam	mIHC
LAG3	D2G4O™	Cell Signaling Technology	mIHC
TIM3	D5D5R™	Cell Signaling Technology	mIHC
Her-2	4B5	Roche (ULTRA)	IHC
MLH1	GM002	Genetech	IHC
MSH2	RED2	Genetech	IHC
MSH6	EP49	Genetech	IHC
PMS2	EP51	Genetech	IHC

Reference

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