Supplementary

Table S1 List of genes of the 733-gene panel

ABL1	*CDX2*	*FGFR4*	*MLH1*	*PTEN*	*VEGFA*	*JMJD1C*	*TRIM37*	*BCL11A*	*EZR*	*TBL1XR1*	*PLXNB1*	*LIG1*	*RNF168*	*POLD3*
ACVR2A	*CHD2*	*FH*	*MLLT3*	*PTK6*	*VHL*	*LMO1*	*TSHR*	*BCL11B*	*FAT4*	*TCF7L2*	*SPRED1*	*LIG3*	*RNF4*	*POLD4*
AFF3	*CHEK1*	*FHIT*	*MPL*	*PTPRD*	*NSD3*	*LZTR1*	*UROD*	*BCORL1*	*FUBP1*	*TCL1A*	*ERF*	*LIG4*	*RNF8*	*POLE2*
AKT1	*CHEK2*	*FLCN*	*MRE11A*	*RAC1*	*ZNF479*	*MAX*	*WAS*	*BIRC3*	*FUS*	*TET1*	*RPS6KA3*	*MAD2L2*	*RPA1*	*POLE4*
AKT2	*CHIC2*	*FLT1*	*MSH2*	*RAD50*	*ZNRF3*	*MEN1*	*WRN*	*BRD4*	*GAS7*	*TFE3*	*GSK3B*	*MBD4*	*RPA2*	*PPP4R1*
AKT3	*CIC*	*FLT3*	*MSH3*	*RAD51*	*ABCB11*	*MTAP*	*WT1*	*CACNA1D*	*H3F3A*	*TNFAIP3*	*NOTCH3*	*MDC1*	*RPA3*	*PPP4R3A*
ALK	*CIITA*	*FLT4*	*MSH6*	*RAD51C*	*APOBEC3B*	*MUTYH*	*XPA*	*CALR*	*HIF1A*	*USP8*	*NOTCH4*	*MGMT*	*RPA4*	*PPP4R3B*
ANK1	*CRBN*	*FOXA1*	*MTOR*	*RAF1*	*AXIN2*	*NBN*	*XPC*	*CAMTA1*	*HIP1*	*WIF1*	*ALKBH2*	*MLH3*	*RRM2B*	*PPP4R4*
APC	*CRLF2*	*FRS2*	*MYC*	*RARA*	*BARD1*	*NHP2*	*XRCC2*	*CANT1*	*HNRNPA2B1*	*XPO1*	*ALKBH3*	*MMS19*	*SETMAR*	*RAD9B*
AR	*CRNKL1*	*G6PD*	*MYCN*	*RB1*	*BMPR1A*	*NME1*	*HOXB13*	*CARD11*	*HOXA11*	*ZFHX3*	*APEX1*	*MNAT1*	*SEM1*	*RBX1*
ARAF	*CRTC3*	*GATA3*	*MYD88*	*RET*	*BUB1B*	*NOP10*	*BCL2L1*	*KNL1*	*IL6ST*	*ACVR1B*	*APEX2*	*MPG*	*SHPRH*	*RFC1*
AREG	*CSF1R*	*GLI2*	*NF1*	*RGS7*	*CDC73*	*NTHL1*	*BCL6*	*CASP8*	*KDM6A*	*ARID1B*	*CENPS*	*MSH4*	*SMUG1*	*RFC2*
ARHGAP5	*CSF3R*	*GNA11*	*NF2*	*RICTOR*	*CDKN1C*	*PHOX2B*	*CDK8*	*CBFA2T3*	*KEAP1*	*DNMT1*	*APLF*	*MUS81*	*SPO11*	*RFC3*
ARID1A	*CTNNB1*	*GNAQ*	*NFE2L2*	*RNF43*	*CEBPA*	*PMS1*	*FOXP1*	*CBFB*	*KLF4*	*FOXL2*	*APTX*	*NEIL1*	*TDG*	*RFC4*
ARNT	*CTNND2*	*GNAS*	*NFIB*	*ROS1*	*COL7A1*	*POLH*	*GRIN2A*	*CBLB*	*LCK*	*GATA1*	*ATRIP*	*NEIL2*	*TDP1*	*TELO2*
ASXL1	*CUL3*	*HDAC2*	*NKX2-1*	*RPTOR*	*CTR9*	*POLQ*	*IKBKE*	*CCDC6*	*LEF1*	*HIST1H3B*	*FAAP100*	*NEIL3*	*TDP2*	*TIMELESS*
ATM	*CYSLTR2*	*HEY1*	*NOTCH1*	*RUNX1*	*CXCR4*	*POT1*	*MEF2B*	*CCNB1IP1*	*LIFR*	*KDM5C*	*FAAP24*	*NHEJ1*	*TOP3A*	*TMEM189*
ATR	*DDR2*	*HGF*	*NOTCH2*	*SDC4*	*CYLD*	*PRDM9*	*NFKBIA*	*CD79A*	*MAPK1*	*MAP3K1*	*FAAP20*	*NUDT1*	*TOP3B*	*WDR48*
AURKA	*DICER1*	*HOOK3*	*NPM1*	*SDHC*	*DDB2*	*PRF1*	*PIK3CD*	*CD79B*	*MED12*	*KMT2C*	*MPLKIP*	*NABP2*	*TOPBP1*	*GFI1*
AXL	*DNMT3A*	*HRAS*	*NRAS*	*SERPINB3*	*DIS3L2*	*PRKAR1A*	*SRC*	*CDH11*	*NAB2*	*NCOR1*	*CCNH*	*OGG1*	*TP53BP1*	*CYP17A1*
B2M	*DPYD*	*IDH1*	*NRG1*	*SETD2*	*DKC1*	*PRSS1*	*BTG1*	*CHD4*	*NCOR2*	*PHF6*	*CDK7*	*PARP1*	*TREX1*	*ELF3*
BAP1	*EGFR*	*IDH2*	*NTRK1*	*SF3B1*	*DOCK8*	*PTPN11*	*DIS3*	*CLIP1*	*NDRG1*	*PPP2R1A*	*CETN2*	*PARP2*	*TREX2*	*SGK1*
BAZ1A	*EPHA2*	*IGF1R*	*NTRK2*	*SH2B3*	*DROSHA*	*PTPN13*	*EED*	*CLTCL1*	*NONO*	*PRDM1*	*CHAF1A*	*PARP3*	*UBE2A*	*GSTT1*
BCL2	*EPHA3*	*IGF2*	*NTRK3*	*SLC29A1*	*ELANE*	*RAD51B*	*GNA13*	*CNBP*	*PAX3*	*SOCS1*	*CLK2*	*PCNA*	*UBE2B*	*AEN*
BCOR	*ERBB2*	*IL7R*	*PAK1*	*SMAD4*	*EPCAM*	*RAD51D*	*NT5C2*	*CNOT3*	*PAX7*	*SOX9*	*DCLRE1A*	*PNKP*	*UBE2N*	*CCNO*
BLM	*ERBB3*	*INPP4B*	*PALB2*	*SMARCA1*	*ERCC3*	*RECQL*	*PPP2R2A*	*CREB3L1*	*PAX8*	*TRAF7*	*DCLRE1B*	*POLB*	*UBE2T*	*CENPX*
BMP5	*ERBB4*	*ITGAV*	*PAX5*	*SMARCA4*	*ERCC5*	*RECQL4*	*NSD2*	*CREB3L2*	*PER1*	*IKZF1*	*DCLRE1C*	*POLI*	*UBE2V2*	*CUL4A*
BRAF	*ERCC1*	*JAK1*	*PBRM1*	*SMARCB1*	*ETV6*	*RFWD3*	*EPHA7*	*CREBBP*	*PICALM*	*MYCL*	*DDB1*	*POLK*	*UNG*	*CUL5*
BRCA1	*ERCC2*	*JAK2*	*PDCD1LG2*	*SMO*	*EXT1*	*RHBDF2*	*GLI1*	*CRTC1*	*PIM1*	*NCOA3*	*DMC1*	*POLL*	*USP1*	*DNTT*
BRCA2	*ERCC4*	*JAK3*	*PDGFB*	*SRGAP3*	*EXT2*	*SBDS*	*MYB*	*CTCF*	*POU2AF1*	*CDK2*	*DUT*	*POLM*	*XAB2*	*ELOA*
BRIP1	*ERCC6*	*JUN*	*PDGFRA*	*SRSF2*	*FAH*	*SDHA*	*NRG3*	*CUX1*	*POU5F1*	*LATS1*	*EME1*	*POLN*	*XRCC1*	*HUS1B*
BTK	*EREG*	*KCNJ5*	*PDGFRB*	*STAG2*	*FANCD2*	*SDHAF2*	*NUP93*	*DAXX*	*PPP6C*	*LATS2*	*EME2*	*PRKDC*	*XRCC3*	*PER2*
CARS	*ESR1*	*KDR*	*PDPK1*	*STK11*	*FANCE*	*SDHB*	*PTK2*	*DDIT3*	*PRDM16*	*YAP1*	*ENDOV*	*PRPF19*	*XRCC4*	*PER3*
CBL	*EWSR1*	*KIT*	*PIK3CA*	*SUZ12*	*FANCF*	*SDHD*	*RXRA*	*DDX10*	*PREX2*	*TEAD2*	*ERCC8*	*RAD1*	*XRCC5*	*MSH5*
CCND1	*EZH2*	*KMT2A*	*PIK3CB*	*SYK*	*FANCI*	*SERPINA1*	*SMARCA2*	*DDX3X*	*PRKACA*	*MGA*	*EXO1*	*RAD18*	*XRCC6*	*PARP4*
CCND2	*FAM135B*	*KMT2D*	*PIK3R1*	*TBX3*	*FANCL*	*SETBP1*	*TYK2*	*DDX5*	*PTPRT*	*HES1*	*FAN1*	*RAD23A*	*ABRAXAS1*	*POLE3*
CCND3	*FAM47C*	*KRAS*	*PIK3R2*	*TCF3*	*FANCM*	*SH2D1A*	*ZNF750*	*DDX6*	*QKI*	*KDM5A*	*FANCB*	*RAD23B*	*FRK*	*PPP4R2*
CCNE1	*FANCA*	*LASP1*	*PLCG2*	*TERT*	*FAS*	*SHOC2*	*ABI1*	*DNM2*	*RAD21*	*SPEN*	*GEN1*	*RAD52*	*BIRC5*	*SLX1A*
CD274	*FANCC*	*LMNA*	*PML*	*TET2*	*FEN1*	*SLC25A13*	*ACKR3*	*EBF1*	*RANBP2*	*THBS2*	*GTF2H1*	*RAD54B*	*EMSY*	*RAD54L2*
CDH1	*FANCG*	*LRP1B*	*PMS2*	*TMEM127*	*GALNT12*	*SLX4*	*ACSL3*	*EIF3E*	*RAP1GDS1*	*CUL1*	*GTF2H3*	*RAD54L*	*CRKL*	*RFC5*

Table S1 *(Continued)*

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Table S1 *(Continued)*

CDH10	*FAT1*	*MAP2K1*	*POLD1*	*TMPRSS2*	*GATA2*	*SOS1*	*ACVR1*	*EIF4A2*	*RBM10*	*HDAC1*	*GTF2H4*	*RAD9A*	*EPHB1*	*HMGA2*
CDK12	*FBXW7*	*MAP2K2*	*POLE*	*TOP2A*	*GBA*	*SPOP*	*AFF4*	*ELF4*	*RHOA*	*MLST8*	*GTF2H5*	*RBBP8*	*GLI3*	*TSPAN31*
CDK4	*FES*	*MAP2K4*	*POLG*	*TP53*	*GJB2*	*SPRTN*	*AMER1*	*ELK4*	*RHOH*	*PIK3R3*	*H2AFX*	*RDM1*	*IRS2*	*MYOD1*
CDK6	*FGF19*	*MCL1*	*PPARG*	*TPMT*	*GPC3*	*SRY*	*ARID2*	*ELL*	*RNF213*	*RHEB*	*HELQ*	*RECQL5*	*RUNX1T1*	*CHD1*
CDKN1A	*FGF3*	*MDM2*	*PPM1D*	*TSC1*	*GREM1*	*STAT3*	*ATP1A1*	*EP300*	*SFPQ*	*RPS6KB1*	*HFM1*	*REV1*	*SLIT2*	*ZBTB16*
CDKN1B	*FGF4*	*MDM4*	*PRCC*	*TSC2*	*HFE*	*SUFU*	*ATP2B3*	*EPAS1*	*SLC34A2*	*GRB2*	*HLTF*	*REV3L*	*SOX2*	*PCDH9*
CDKN2A	*FGFR1*	*MECOM*	*PRKCH*	*U2AF1*	*HMBS*	*TGFBR1*	*ATRX*	*EPS15*	*SLC45A3*	*RIT1*	*HMGB1*	*RIF1*	*SPTA1*	*PLXNA1*
CDKN2B	*FGFR2*	*MET*	*PSIP1*	*UGT1A1*	*HNF1A*	*TGFBR2*	*AXIN1*	*ERC1*	*SMAD2*	*RASA1*	*HUS1*	*RMI1*	*ZNF217*	
CDKN2C	*FGFR3*	*MITF*	*PTCH1*	*USP6*	*ITK*	*TP63*	*BCL10*	*ETNK1*	*SMAD3*	*ERRFI1*	*UVSSA*	*RMI2*	*ZNF703*	

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Table S2 Clinicophysiological and molecular characteristics of each patient

Patient #	Gender	Age	Site of metastases	Gene variation	TMB (Muts/Mb)	PD-L1 expression
1	Male	58	None	*TP53, EGFR, MYC*	8.4	Negative
2	Male	58	LN, liver, spleen	*TP53, CDK6, CHEK2*	6	Positive
3	Female	58	LN, ovary	*ARID1A, CCND1, CDKN1B, ERBB3, FGFR2, FRS2, TP53*	10.1	Negative
4	Female	50	Ovary	*PIK3CA*	5.0	Negative
5	Female	41	Ovary	*ARID1A, TP53*	3.9	Negative
6	Male	58	Bladder, ureter	*CDH1, EGFR, ERBB3, NTRK1, POLD1, PREX2, TP53*	9.2	Negative
7	Female	35	Pelvic cavity	*CDH1, SMAD4, TP53*	3.2	Negative
8	Male	68	LN	*LRP1B, AR, GRM3, JAK3, MAP3K1, RICTOR, ROS1, TP53*	6.3	Positive
9*	Male	67	Liver, para-abdominal aorta, liver stomach space	*Unknown*	Unknown	Unknown
10	Male	69	LN	*ACVR2A, APC, ARID1A, CTCF, MSH3, RNF43, SETD2, CCND1, FGF19, FGF4, FGF3, CDK8*	24.02	Negative
11	Female	40	LN	*NA*	2.23	Negative
12	Male	59	LN, liver	*TP53*	16.7	Negative
13	Female	34	Meninges	*CCNE1, CDH1, DOT1L, PRKAR1A, TP53, BUB1B, PRSS1*	5.3	Negative
14	Male	52	Peritoneum, pelvic cavity	*TP53*	7.8	Positive
15*	Male	60	LN, liver	*Unknown*	Unknown	Unknown
16	Female	55	Liver	*TP53, IL7R, RICTOR, PREX2, MYC, PTK2, KMT2A, CDK8, FLT3, FLT1, IRS2, CCNE1, SRC, AURKA, GNAS, PTK6, AR, BTK, BCORL1*	5.03	Negative
17	Male	56	Liver, peritoneum	*TP53, MET*	2.13	Negative
18*	Female	53	Liver, LN, peritoneum	*Unknown*	Unknown	Negative
19	Female	57	LN, anastomotic, porta, mesentery	*TP53, LATS1, ERBB4, PTPRO, CTNNA2, APC, HIST1H3B, PLCB1, MLL3*	7.68	Negative
20	Female	32	Bone	*ARID1A, KRAS, RNF43, CDH1, RHOA, KMT2A, KMT2B, PIK3C3, STAG2*	8.2	Negative
21*	Female	56	Peritoneum	*Unknown*	Unknown	Negative
22	Male	46	Liver	*ERBB2, TP53, BAP1, BCORL1, PHF6, RARA, SMARCE1, TOP2A, XIAP*	6.3	Negative
23	Male	61	Lung	*TP53, CDK4, MDM2*	4.96	Negative
24	Male	48	Peritoneum	*NA*	4.5	Negative
25*	Male	73	LN, peritoneum	*Unknown*	Unknown	Positive
26	Female	57	LN, liver	*TP53, RICTOR, ERBB2, RARA, TOP2A, STAT3, RNF43*	11.17	Positive
27	Male	64	Liver	*KRAS, BLK, CCND2, FGF23, FGF6, GATA4, RAD51P1*	11.5	Positive
28	Male	67	Abdominal cavity	*CDK4, ERBB3, MDM2, BRAF, CDK2, FRS2, GRM3, HMGA2, NAB2, STAT6, TSPAN31*	2.1	Negative
29	Male	63	Liver	*ERBB2, TP53, RARA*	6.6	Negative
30	Female	68	Abdominal aorta, LN	*ERBB2, TP53, APC, GNA13, MYC, SOX9, GATA3, TET2*	15	Positive

*, patient without gene variation and TMB results. LN, lymph node; TMB, tumor mutation burden; LN, lymph node.

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Table S3 The regimens, duration of therapy, response data and adverse events

Patient #	Lines of therapy	Anti-PD-1	Anti-angiogenesis	Chemotherapy	Duration of therapy (cycles)	Clinical response	Pathological response	PFS (months)	OS (months)	Adverse events
1	First-line	Pembrolizumab	Regorafenib	XELOX	3	PR	MPR	24	NR	Anaphylaxis
2	First-line	Pembrolizumab	Apatinib	Paclitaxel	5	PR	MPR	18	NR	Leucopenia
3	First-line	Pembrolizumab	Lenvatinib	XELOX	6	PR	pCR	16	NR	Febrile neutropenia
4	First-line	Tislelizumab	Lenvatinib	Paclitaxel + capecitabine	1	SD	Non-MPR	14	NR	Anaphylaxis
5	Third-line	Tislelizumab	Apatinib	XELOX	8	PR	Non-MPR	18	NR	Leucopenia
6	First-line	Pembrolizumab	Regorafenib	XELOX	8	PR	MPR	24	NR	Rash
7	First-line	Pembrolizumab	Regorafenib	XELOX	7	PR	MPR	24	24	Leucopenia
8	First-line	Pembrolizumab	Lenvatinib	Capecitabine	5	PR	MPR	12	NR	Anaphylaxis
9	First-line	Tislelizumab	Anlotinib	Oxaliplatin	5	CR	MPR	26	NR	Vomiting
10	First-line	Pembrolizumab	Lenvatinib	Oxaliplatin	5	PR	MPR	28	NR	Anorexia
11	First-line	Pembrolizumab	Lenvatinib	Oxaliplatin	5	PR	MPR	12	NR	Anemia
12	First-line	Toripalimab	Regorafenib	XELOX	8	PR	/	40	NR	Rash
13	First-line	Camrelizumab	Apatinib	Paclitaxel	1	PD	/	5	5	Leucopenia
14	Second-line	Penpulimab	Anlotinib	Paclitaxel	3	PD	/	10	12	Nausea
15	First-line	Pembrolizumab	Regorafenib	Paclitaxel + S-1	6	PR	/	12	14	Leucopenia
16	First-line	Tislelizumab	Lenvatinib	SOX	8	CR	/	36	NR	Vomiting
17	First-line	Sintilimab	Lenvatinib	XELOX	4	PR	/	10	12	Leucopenia
18	First-line	Camrelizumab	Apatinib	SOX	8	PR	/	16	NR	Anorexia
19	First-line	Sintilimab	Lenvatinib	XELOX	3	CR	/	16	NR	Asthenia
20	First-line	Tislelizumab	Lenvatinib	XELOX	3	SD	/	12	16	Anemia
21	First-line	Sintilimab	Apatinib	Lipusu + Tegafur	12	PD	/	8	10	Nausea
22	First-line	Sintilimab	Lenvatinib	XELOX	3	PR	/	14	NR	Leucopenia
23	First-line	Sintilimab	Lenvatinib	XELOX	3	SD	/	14	18	Nausea
24	First-line	Camrelizumab	Apatinib	TS	8	PR	/	36	NR	Vomiting
25	First-line	Sintilimab	Apatinib	Lipusu + Tegafur	3	PR	/	10	NR	Asthenia
26	First-line	Tislelizumab	Lenvatinib	XELOX	3	PR	/	14	NR	Nausea
27	First-line	Sintilimab	Lenvatinib	Irinotecan	3	PR	/	16	NR	Asthenia
28	First-line	Sintilimab	Lenvatinib	Oxaliplatin + capecitabine	3	PR	/	18	NR	Anemia
29	First-line	Tislelizumab	Apatinib	Lipusu + capecitabine	14	PD	/	12	12	Nausea
30	First-line	Pembrolizumab	Lenvatinib	Lipusu + tegafur	15	PR	/	14	NR	Vomiting

PFS, progression-free survival; OS, overall survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; MPR, major pathological response; pCR, pathological complete response; NR, not recorded.

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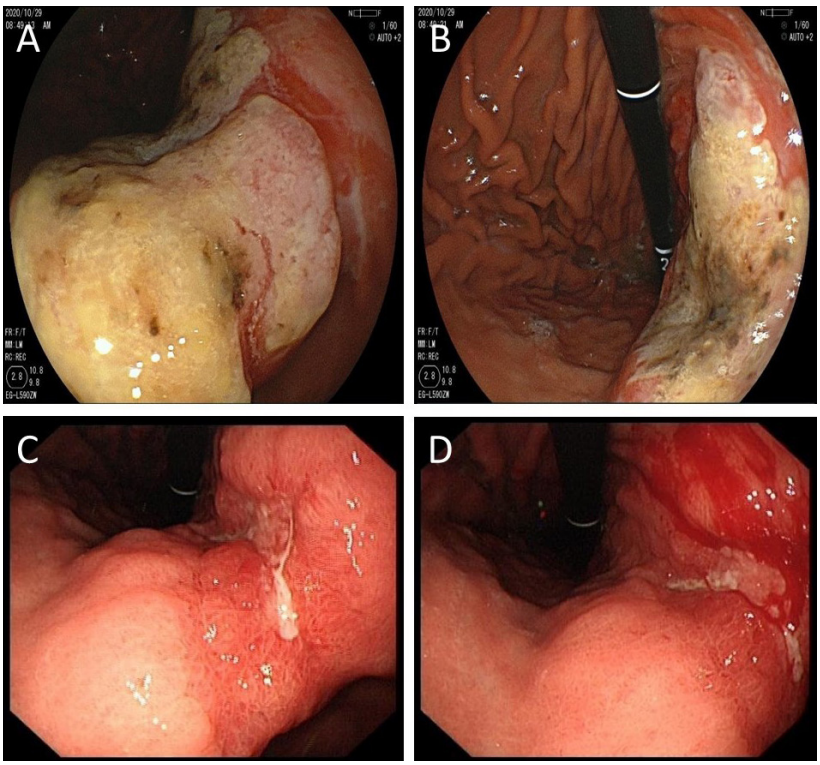

Figure S1 Gastroscopy findings of patient 1 before and after treatment of combination regimens. (A,B) Lesions in the lesser curvature of the stomach with surface erosion could be observed before treatment; (C,D) lesions disappear largely after 3 cycles of treatment.

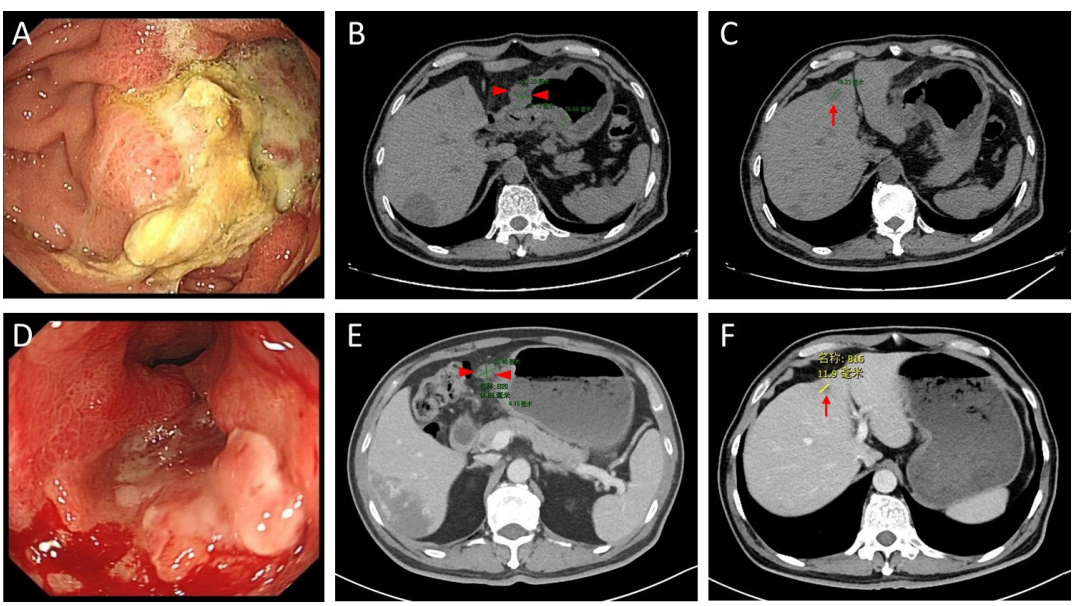

Figure S2 Gastroscopy and CT images of patient 2 before and after treatment of combination regimens. Cancerous ulcers in the body of the stomach (A), a 22.28 mm ×22.12 mm perigastric lymph node metastasis (red arrowheads), thickened gastric wall (B) and liver metastases (red arrow) (C) could be observed before treatment. After 5 cycles of treatment, cancerous ulcers disappeared largely (D), the perigastric lymph node metastasis shrank to 20.48 mm ×14.66 mm (E), and one case of liver metastasis shrank (F). CT, computed tomography.

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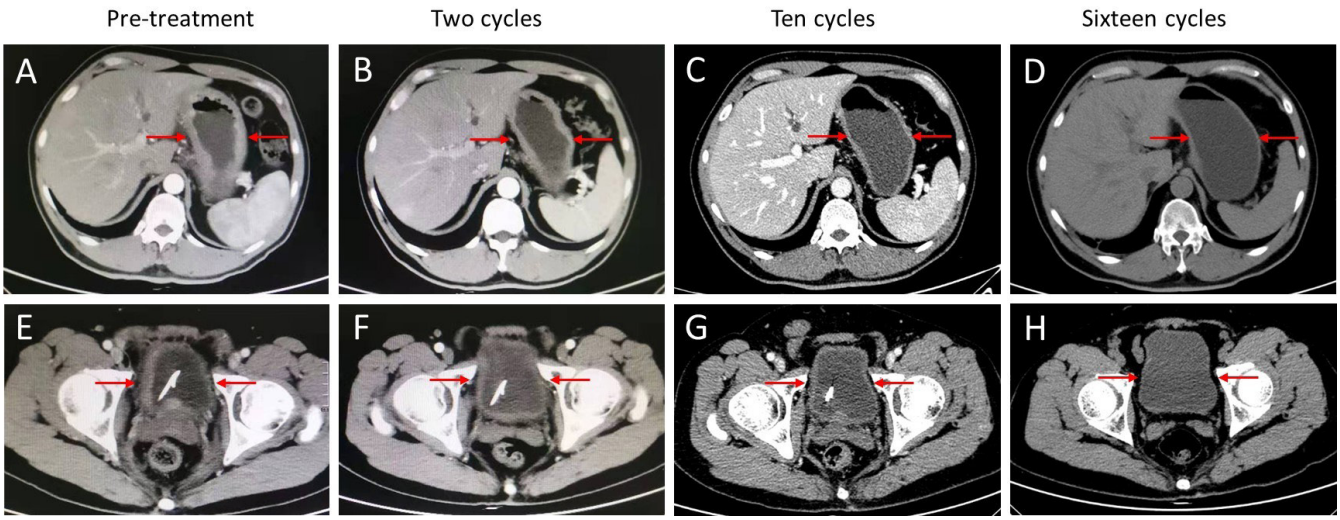

Figure S3 CT findings of patient 6 before and after treatment of combination regimens. Aberrant thickened stomach wall marked by red arrow (A) and bladder wall marked by red arrow (E) could be observed. After combination treatment, the thickened stomach wall (B-D) and bladder wall (F-H) gradually became thinner. CT, computed tomography.

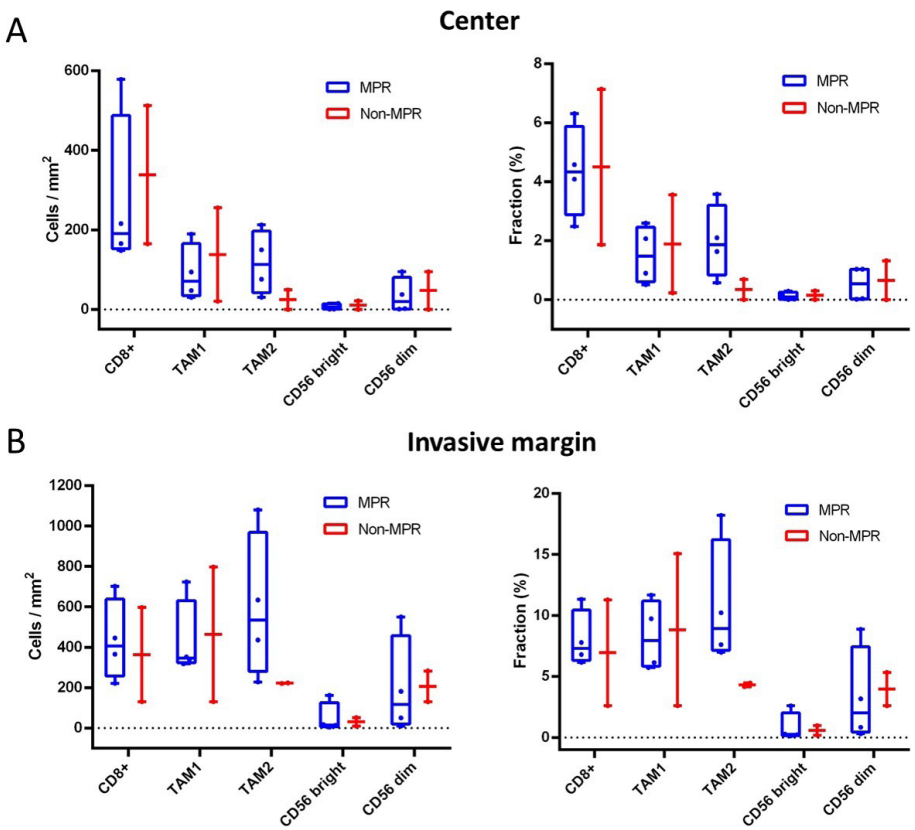

Figure S4 Immune cells in center or invasive margin between MPR and non-MPR. (A) Comparison of immune cells in tumor center between MPR and non-MPR; (B) Comparisons of immune cells in invasive margin between MPR and non-MPR. MPR, major pathological response.

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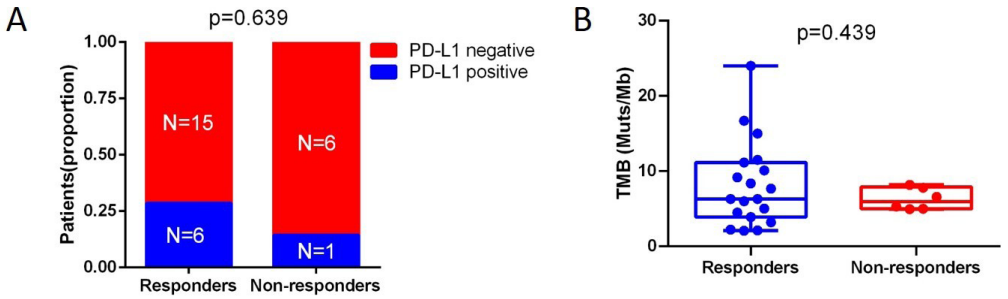

Figure S5 PD-L1 expression and TMB were not associated with the efficacy of combination therapy. (A) Dichotomized association between response to combination treatment and PD-L1 expression was analyzed (N=28, p=0.639); (B) poor correlation between response to combination treatment and TMB level was found (N=25, t=0.787, p=0.439). PD-L1, programmed death ligand 1; TMB, tumor mutation burden.

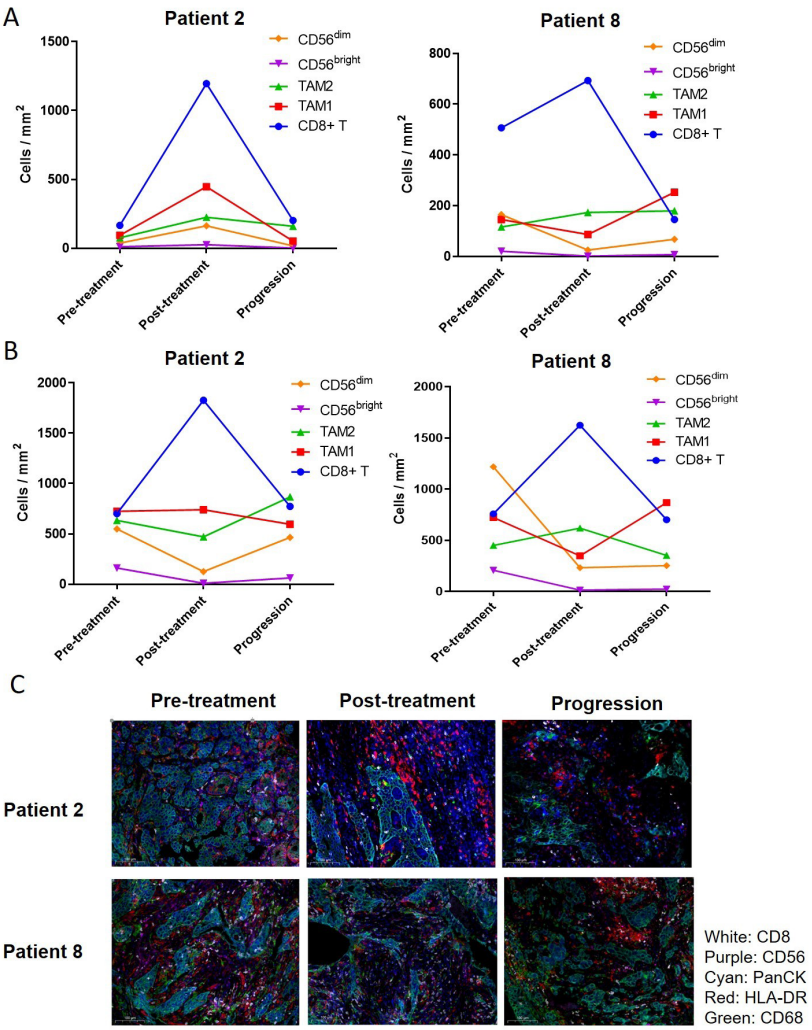

Figure S6 Variation of immune cells of two patients at three time points (pre-treatment, post-treatment, progression). The abundance of immune cells of two patients at three time points in center (A) and invasive margin (B). (C) The images of mIF of two patients at three time points (300× magnification). mIF, multiplex immunofluorescence.

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