

Supplemental Figure Legends

Supplemental Figure 1.

Study design. (a) Sample collection schedule. Blood sample collection was scheduled at baseline (just before initiation of nivolumab treatment) and immediately before the third administration (6 weeks after the first dose).

(b) CONSORT diagram of 31 patients enrolled and samples analyzed.

bTMB, blood tumor mutation burden, maxVAF; maximum variant allele frequency

Supplemental Figure 2

(a) Venn diagram shows the distribution of cases of dMMR, HER2-positive, EBV-positive, and PD-L1 CPS ≥ 1 or ≥ 5 . b) Comparison of PD-L1 CPS between EBV-positive vs. negative cases. EBV, Epstein-Barr virus; dMMR, mismatch repair-deficient; HER2, human epidermal growth factor receptor 2; PD-L1 CPS, programmed death ligand 1 combined positive score;

Supplemental Figure 3

The clinical outcome of gastric cancer patients who received nivolumab monotherapy in the overall population, and its association with various PD-L1 CPS cut-offs. Kaplan–Meier estimates of progression-free survival (PFS, a) and overall survival (OS, b) of patients stratified by PD-L1 CPS ≥ 5 vs. < 5 . Kaplan–Meier estimates of PFS (c), and OS (d) of patients stratified by PD-L1 CPS ≥ 10 vs. < 10 . Hazard ratio and 95% confidence interval for PFS (e) and OS (f) in patient subgroups defined by PD-L1 CPS cut-off values of 1, 5, and 10. PD-L1 CPS, programmed death ligand 1 combined positive score;

Supplemental Figure 4

The clinical outcome of gastric cancer patients who received nivolumab monotherapy in the overall population, and its association with EBV or HER2 positivity. Kaplan–Meier estimates of progression-free survival (PFS, a) and overall survival (OS, b) of EBV-positive vs. negative patients. PSF (c), and OS (d) of HER2-positive vs. HER2-negative patients. EBV, Epstein-Barr virus; HER2, human epidermal growth factor receptor 2;

Supplemental Figure 5

(a-n) Kaplan–Meier estimates of progression-free survival (PFS) and overall survival (OS) of patients stratified by alteration of *TP53* (a, b), *CCNE1* (c,d), *ERBB2* (e,f), *PIK3CA* (g,h), *EGFR* (i, j), *CDK4* (k, l), *KRAS* (m,n). (o-p) Kaplan–Meier estimates of PFS (o) and OS (p) of patients with or without copy number alteration (CNA).

Supplemental Figure 6

The clinical outcome of MSI-H not detected gastric cancer patients who received nivolumab monotherapy, and its association with various cut-offs of bTMB. Kaplan–Meier estimates of progression-free survival (PFS, a) and overall survival (OS, b) of patients stratified by bTMB ≥ 4 vs. < 4 . PSF (c), and OS (d) of patients stratified by bTMB ≥ 6 vs. < 6 . bTMB, blood tumor mutation burden, MSI-H, microsatellite instability-high;

Supplemental Figure 7

The association between maxVAF dynamics and clinical outcome of gastric cancer patients treated with nivolumab monotherapy.

(a) Association between tumor response and changes of maxVAF between pre- vs. 6 weeks after the first dose (on-treatment) samples. (b) Correlation between maxVAF change from the baseline and degree of tumor shrinkage. (c) Comparison of DCR between cases with decreased Δ maxVAF vs. non-decreased Δ maxVAF. Kaplan–Meier estimates of progression-free survival (PFS, d) and overall survival (OS, e) of patients with decreased- vs. non-decreased- Δ maxVAF. maxVAF; maximum variant allele frequency

Supplementary Table 1.

The association between tissue- and liquid- based biomarkers

(a) The concordance between HER2 status on tissue and ERBB2 CNA status on liquid.

(b) The association between PIK3CA alteration and EBV status.

(c) The concordance between MMR status and MSI status.

EBV, Epstein-Barr virus; HER2, human epidermal growth factor receptor 2; MMR, mismatch repair

a)		HER2 IHC/ISH status	
		Positive	Negative
ERBB2 CNA	Amplification	1	0
	Aneuploid	3	3
	No	1	23

b)		EBER-ISH	
		Positive	Negative
PIK3CA alteration (+)		2	4
PIK3CA alteration (-)		1	23

**p* value: 0.0936

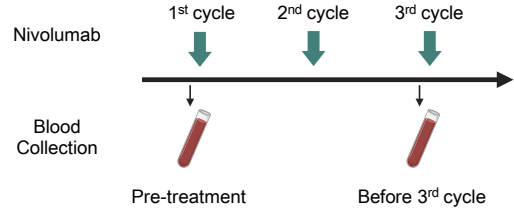
c)		MMR status		
		dMMR	pMMR	NA
MSI-H		3	0	0
MSI-H not detected		0	27	1

*MSI-dMMR concordance: 3/3 (100%)

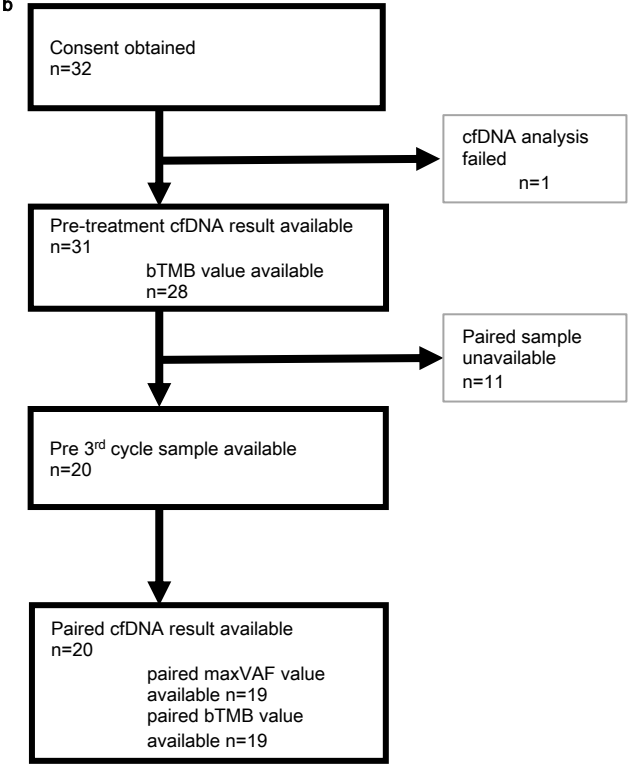
Abbreviations: CNA, copy number alteration; MMR, mismatch repair-deficient; EBER-ISH, Epstein-Barr virus (EBV)-encoded RNA in-situ hybridization; ERBB2, epidermal growth factor receptor 2; HER2, human epidermal growth factor receptor 2; MSI-H, microsatellite instability-high; MMR, mismatch repair; NA, not available; PD-L1 CPS, programmed death ligand 1 combined positive score; *PIK3CA*, *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha*; MMR, mismatch repair-proficient.

Supplemental Figure 1

a

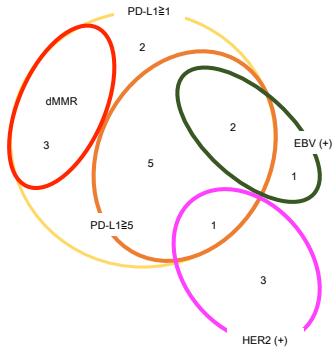


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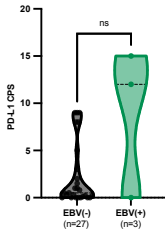


Supplemental Figure 2

a

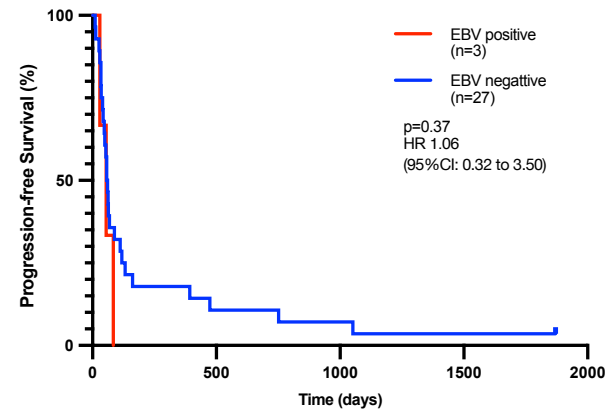


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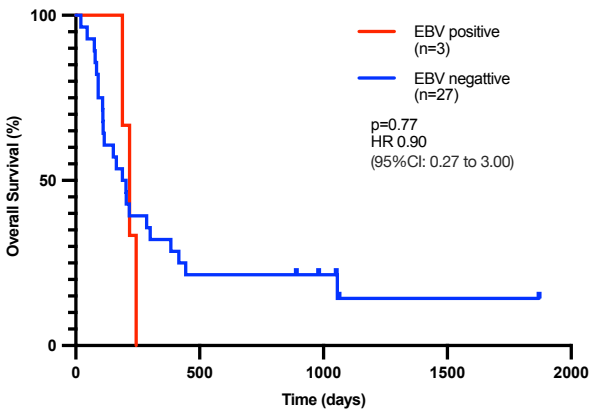


Supplemental Figure 4

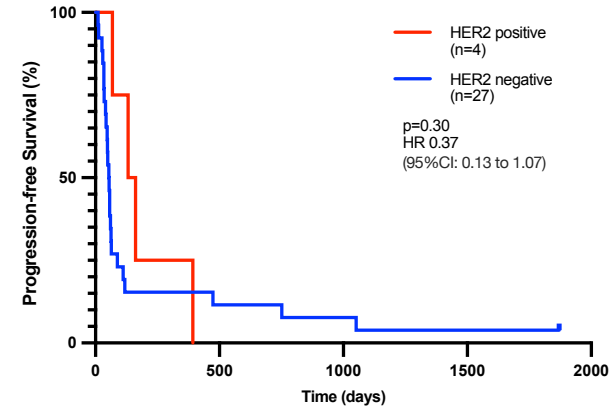
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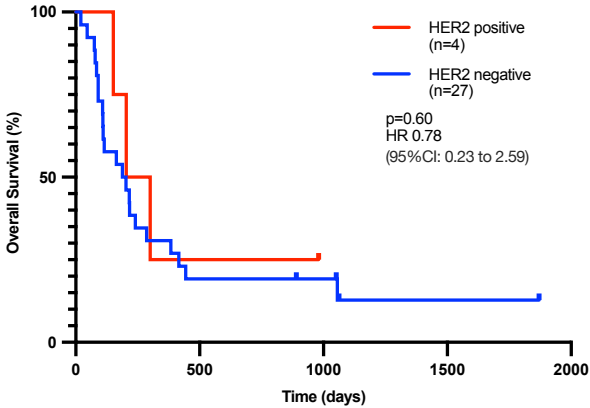
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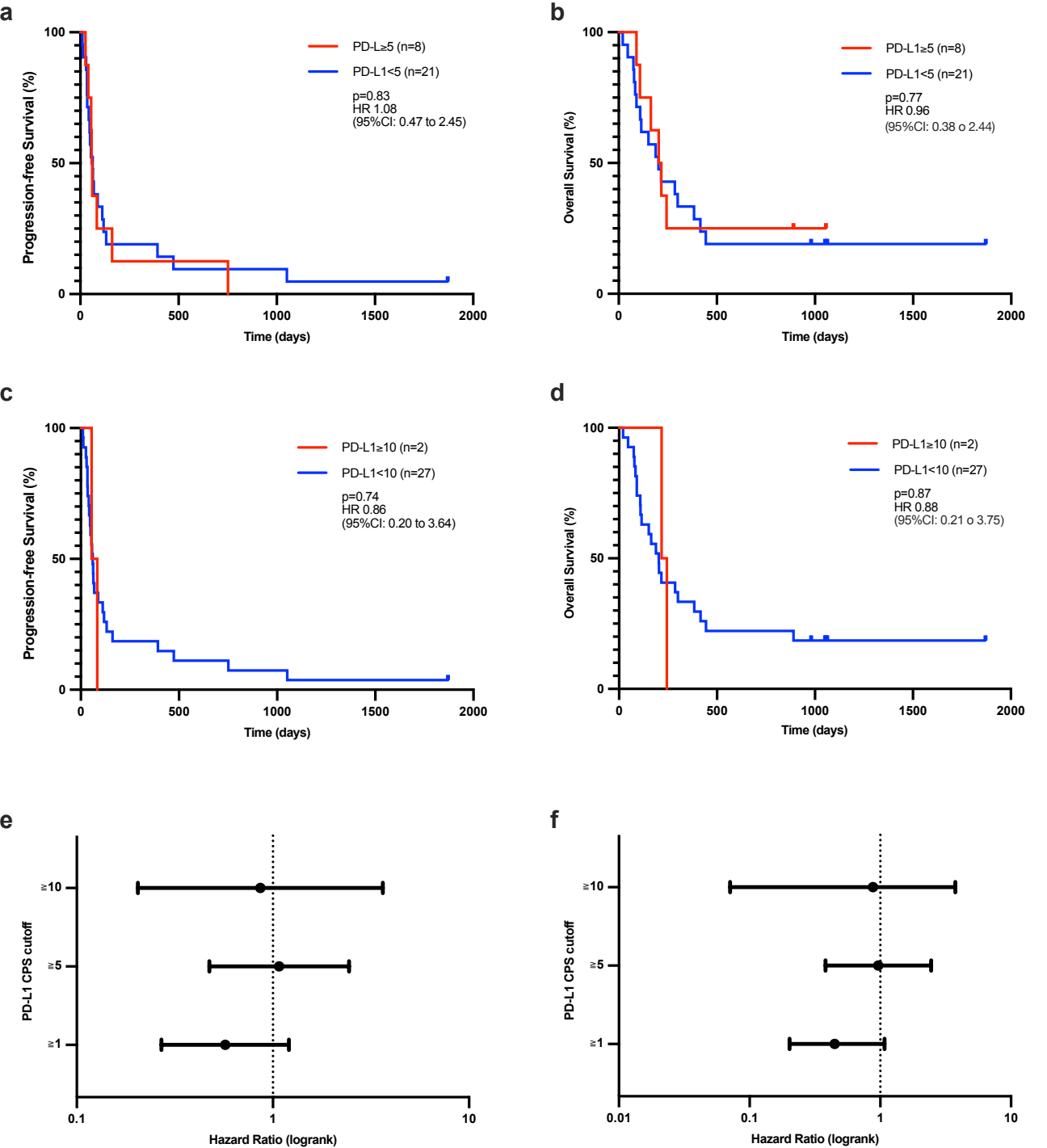
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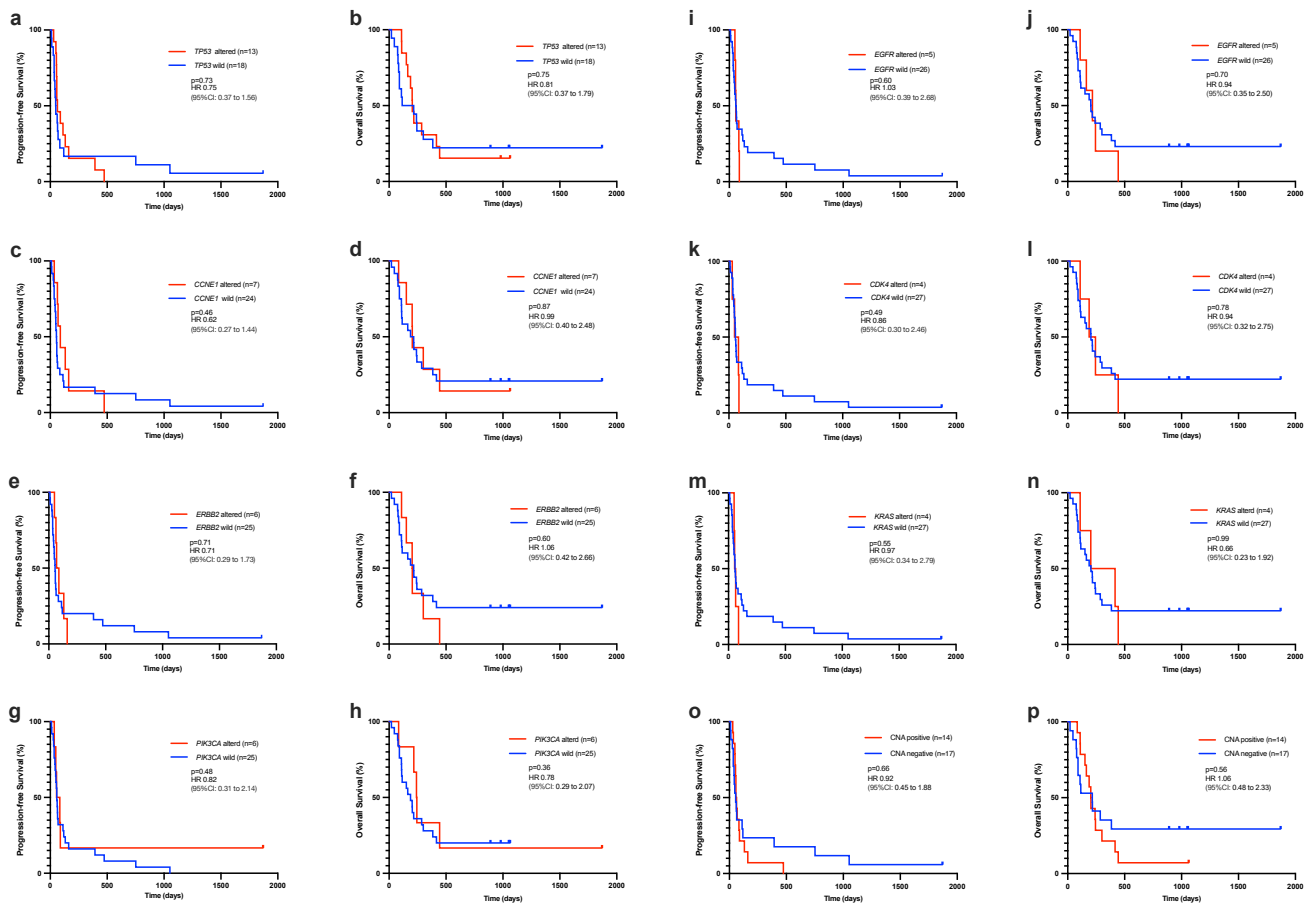
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Supplemental Figure 3

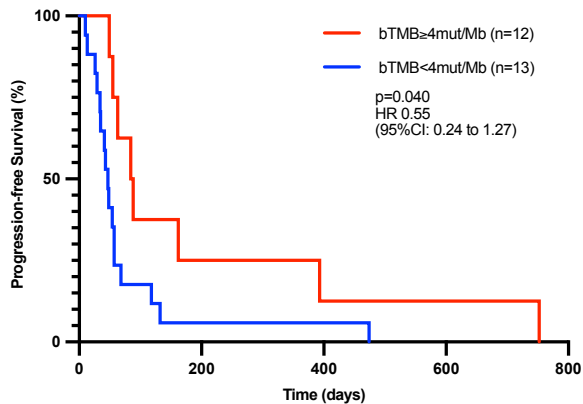


Supplemental Figure 5

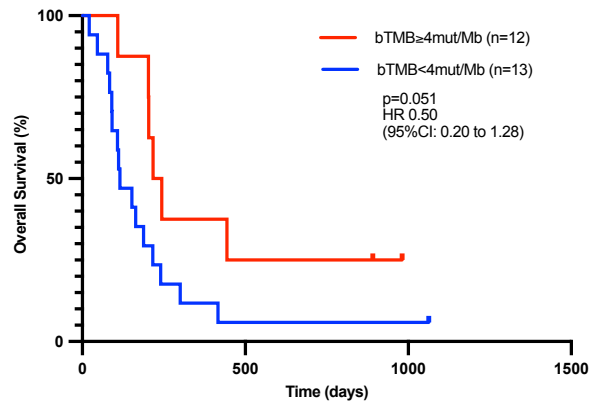


Supplemental Figure 6

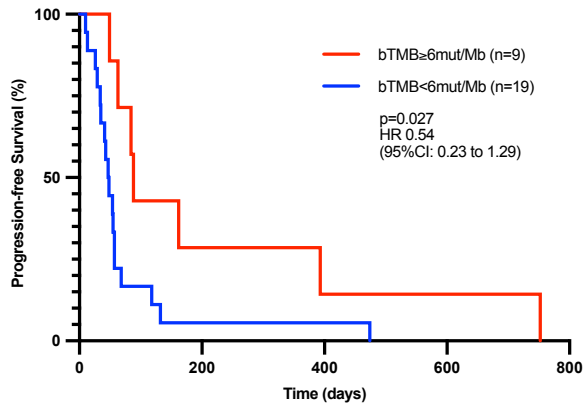
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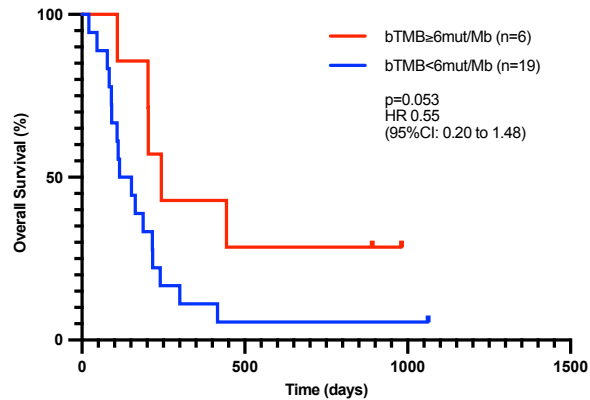
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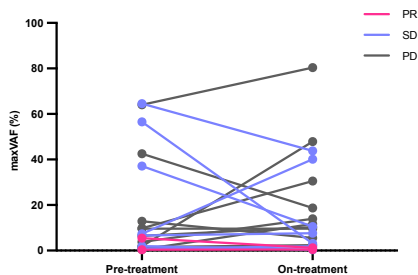


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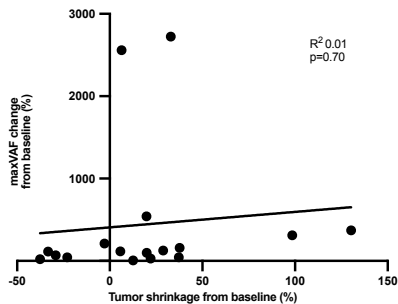


Supplemental Figure 7

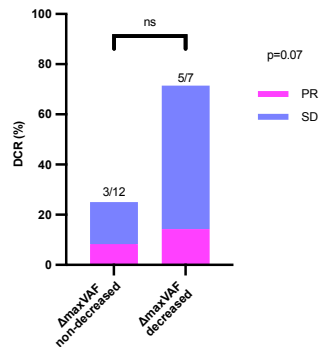
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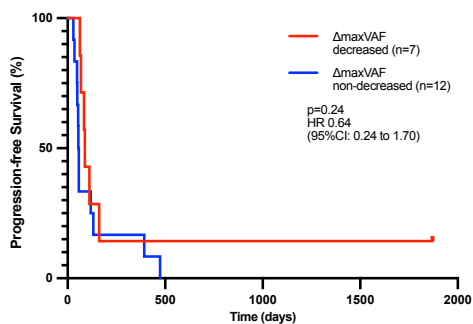
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