Clinical Impact of High Throughput Sequencing on Liquid Biopsy in Advanced Solid Cancer

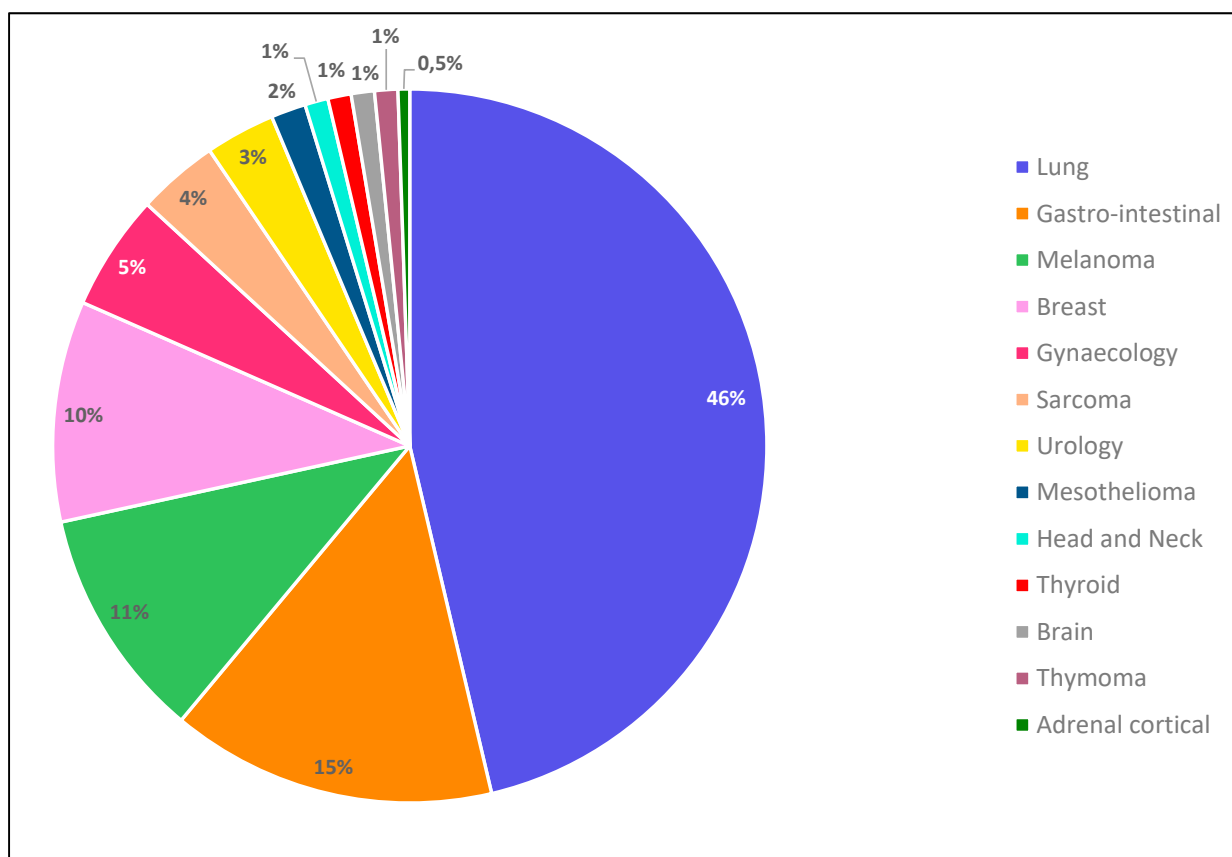## SUPPLEMENTARY MATERIALS

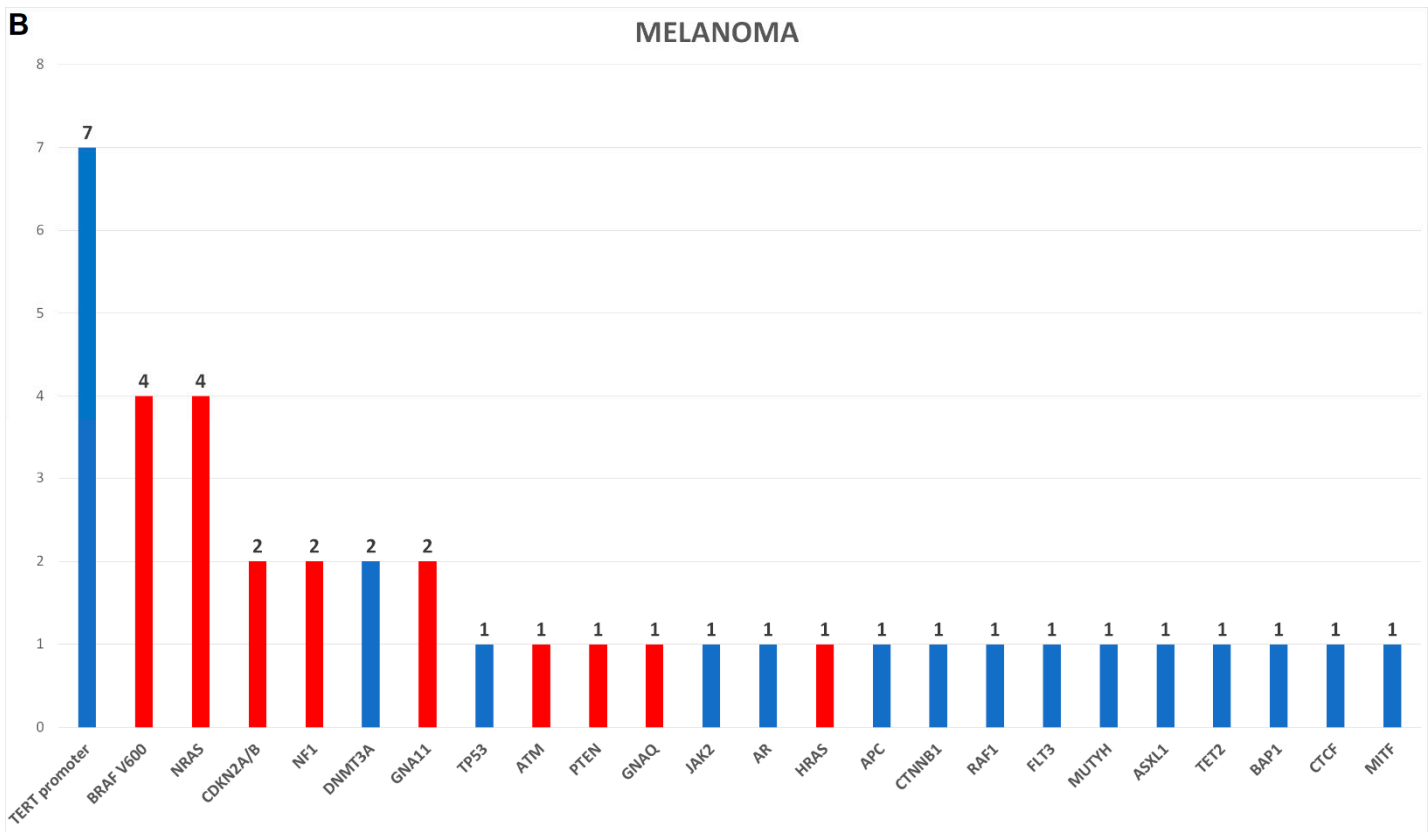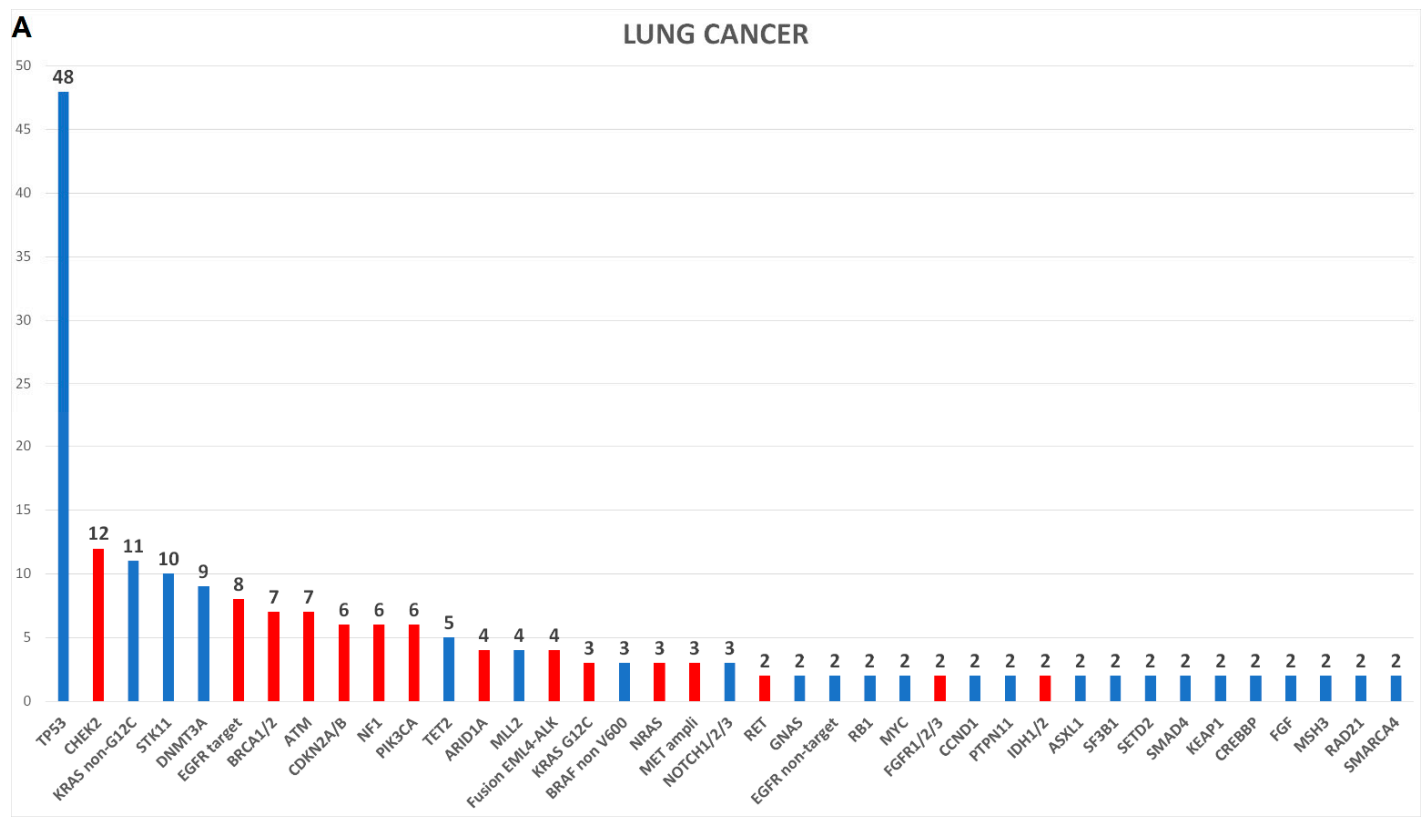**Figure S1:** Proportions of tumor types.

Gastro-intestinal tumors include: pancreas (6%), colorectal (4%), stomach (3%), oeosophagus (1%), and cholangiocarcinoma (1%). Gynaecological tumors include: endometrial (3%), ovarian (2%), and cervix (0.5%). Urological tumors include: prostate (2%), kidney (1%), and urinary tractus (0.5%).

Table S1: Targeted molecular alterations and molecularly matched therapy used.

Altered gene	N (%) (n = 37)	Specific alteration	VAF (%)	Cancer type	Targeted therapy used
EGFR	5 (14%)	T790M	18.9%	Lung	osimertinib
		T790M	0.17%	Lung	osimertinib
		Deletion *exon 19*	20.2%	Lung	osimertinib
		Splice site *2947-2A>C*	6.8%	Lung	afatinib
		Amplification	Detected	Lung	osimertinib
ATM	3 (8%)	R3008C	0.15%	Lung	olaparib
		R3008H	-	Colorectal	olaparib + ceralasertib
		Deletion *exons 39-46*	22%	Melanoma	olaparib + dabrafenib + trametinib
BRAF	3 (8%)	K601N	5.7%	Lung	dabrafenib + trametinib
		G466R	2.1%	Urinary tractus	dabrafenib + trametinib
		V600E	9.3%	Melanoma	dabrafenib + trametinib
MET	3 (8%)	Amplification	Detected	Lung	crizotinib
		Amplification	Detected	Lung	crizotinib
		Splice site *exon 14* *(3028+3_3028+12delATATTTCAGT)*	0.38%	Lung	capmatinib
PTEN	3 (8%)	G127*	30.7%	Melanoma	everolimus
		PTEN loss	Detected	Breast	everolimus + exemestane
		K183fs*16	11.2%	Breast	everolimus
PIK3CA	3 (8%)	E545K	1.1%	Lung	everolimus
		E542K	15.7%	Breast	olaparib + fulvestrant + gosereline
		E545K	1.5%	Breast	everoliumus + exemestane
CHEK2	2 (5%)	L96*	-	Lung	olaparib + ceralasertib
		R523fs*43	0.24%	Stomach	rucaparib + atezolizumab
TMB	2 (5%)	19 mut/Mb	-	Pancreas	pembrolizumab
		95 mut/Mb	-	Stomach	nivolumab
PALB2	1 (2%)	S779	49.7%	Lung	olaparib
BRCA2	1 (2%)	R1738fs*2	50.9%	Prostate	olaparib
GNAQ	1 (2%)	Q209L	4.7%	Melanoma	trametinib

Gene	N (%)	Variant	VAF	Cancer	Drug
GNA11	1 (2%)	*Q209L*	0.52%	Melanoma	trametinib
HRAS	1 (2%)	*Q61R*	22.3%	Lung	tipifarnib
CDKN2A/B	1 (2%)	*p16INK4a W15**	6.8%	Melanoma	abemaciclib
MTOR	1 (2%)	*T1977R*	11.9%	Lung	everolimus
RET	1 (2%)	*V804M*	48.8%	Lung	selpercatinib
ERBB2	1 (2%)	Amplification	Detected	Breast	trastuzumab-emtansine
ESR1	1 (2%)	*Y537N*	-	Breast	fulvestrant
TSC1	1 (2%)	*Q280** *L627fs*22*	8.8% 1.2%	Breast	everolimus + exemestane
ROS1-FGD	1 (2%)	Fusion (non-canonical)	4.1%	Lung	lorlatinib
EML4-ALK	1 (2%)	Fusion (variant 1)	1.1%	Lung	Alectinib

TMB: Tumor mutational burden; VAF: Variant allelic frequency
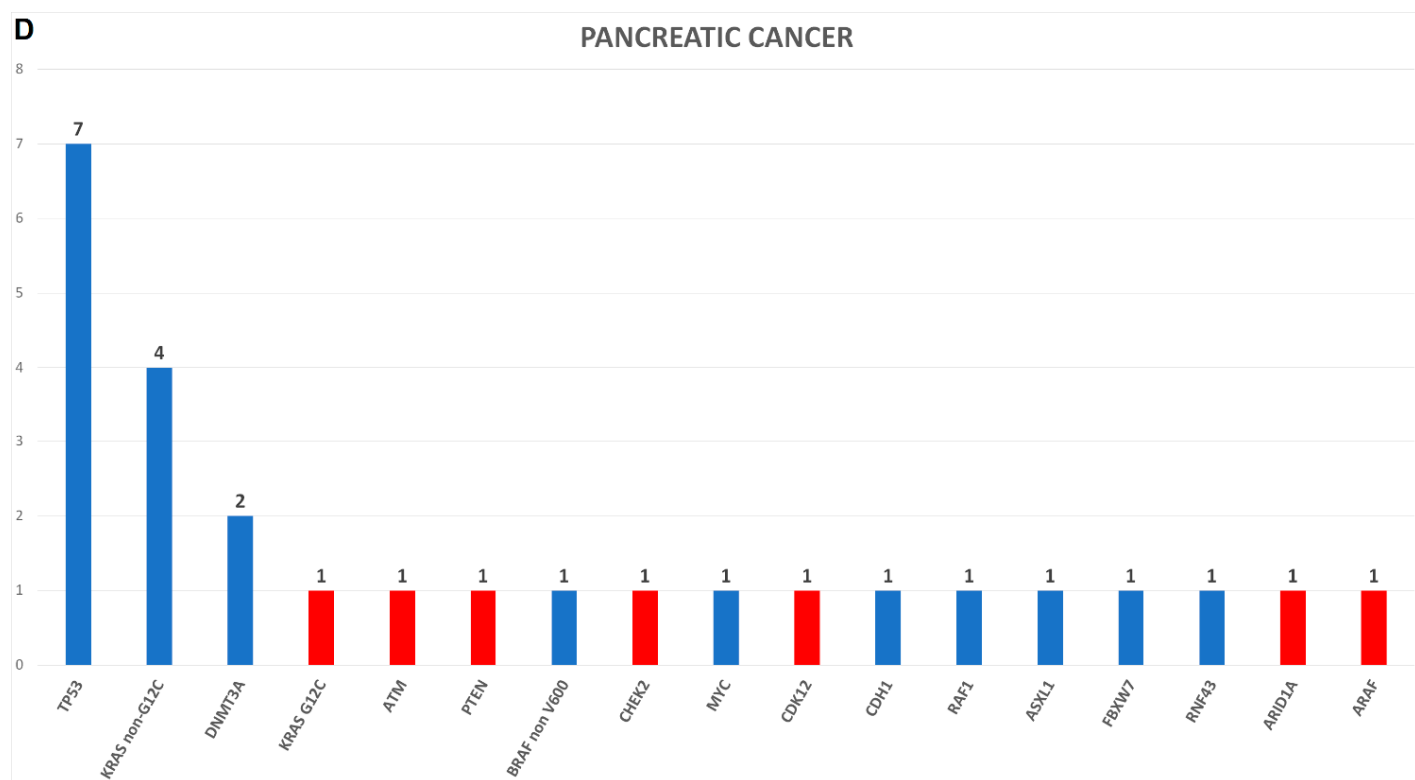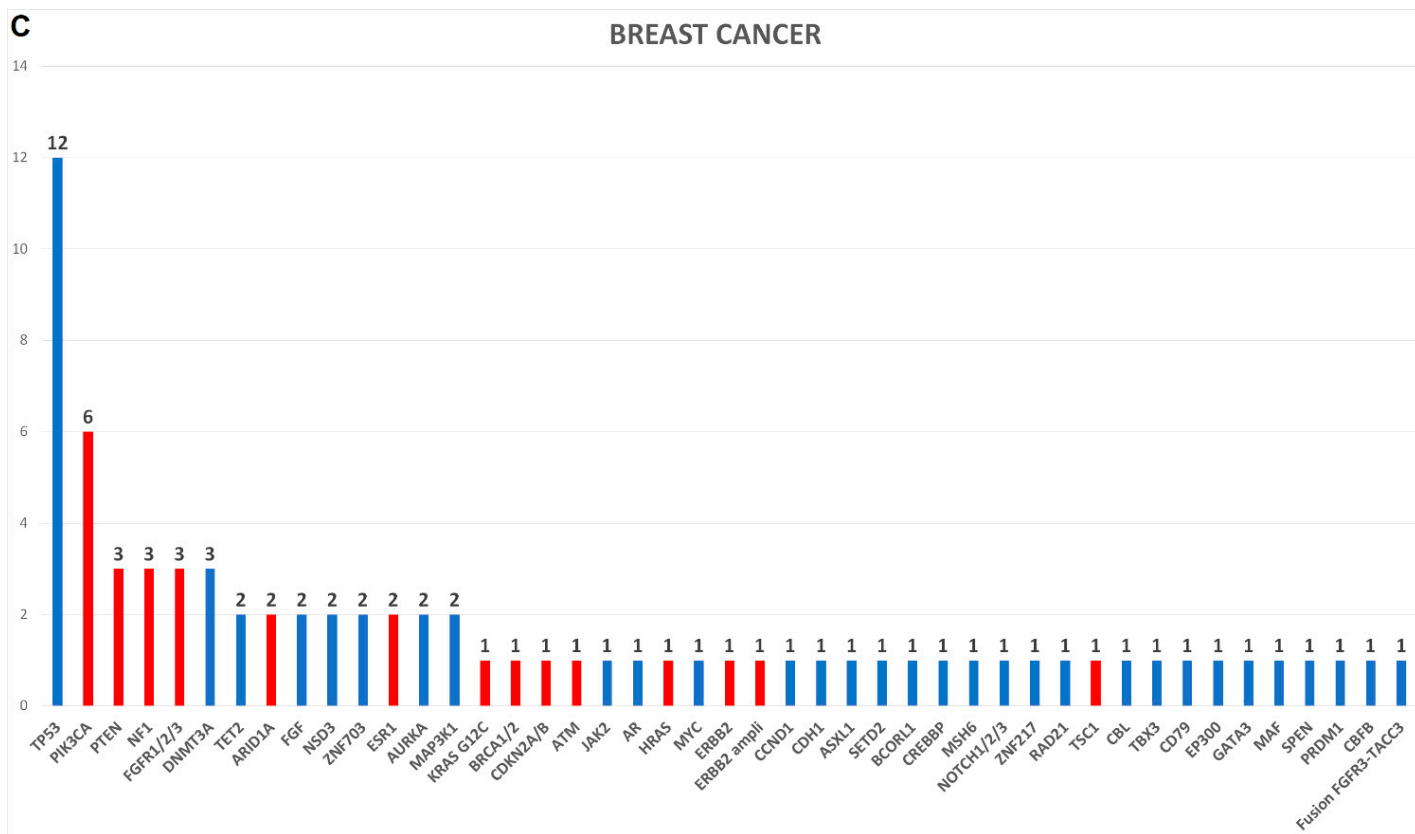
A LUNG CANCER

B MELANOMA

Figure S2: Molecular alterations according to the 4 most common tumor types: **(A)** Lung cancer (39 most frequent), **(B)** Breast cancer, **(C)** Melanoma, and **(D)** Pancreatic cancer.

Actionable molecular alterations are colored in red. Non-actionable molecular alterations are colored in blue.
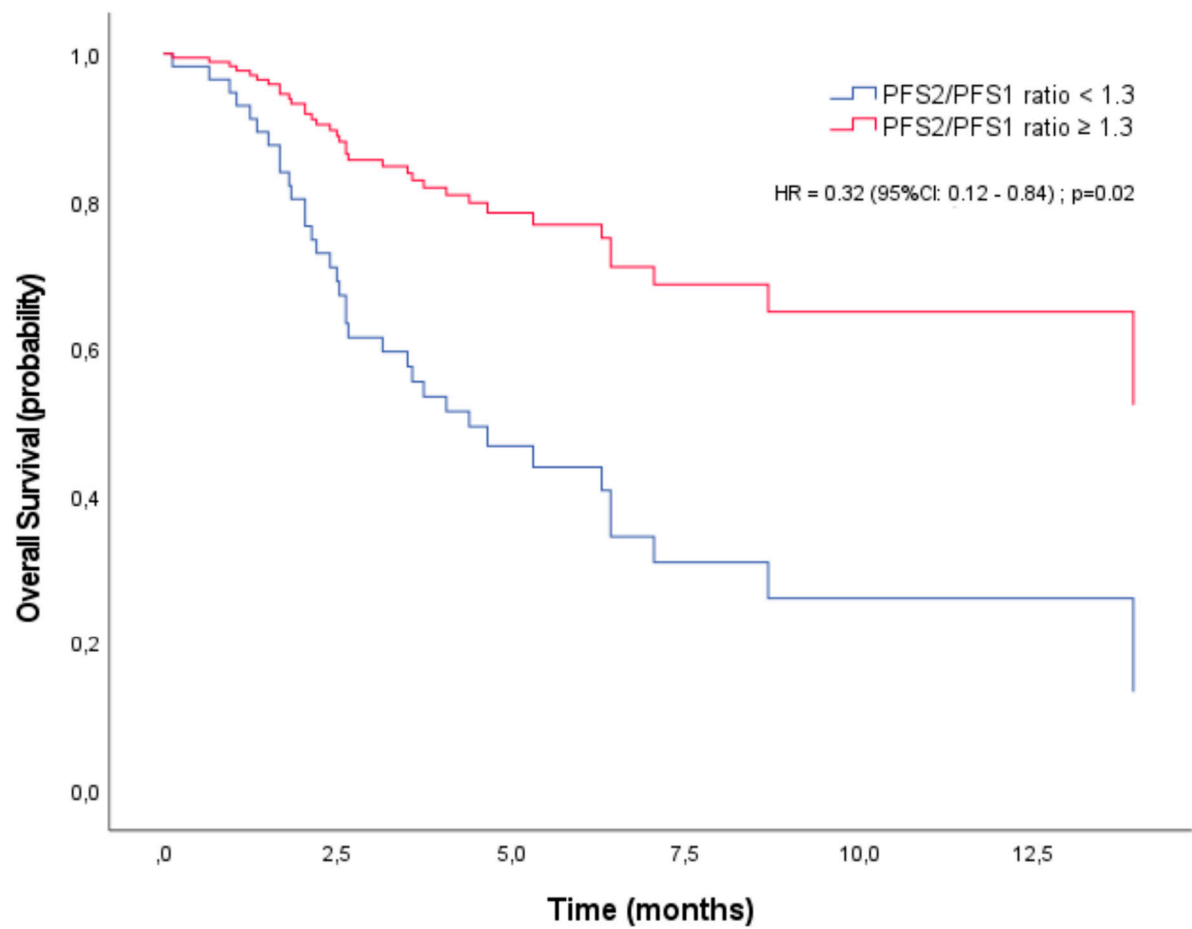
Figure S4: Kaplan-Meier curves of overall survival (OS) according to PFS2/PFS1 ratio.

Table S2: Efficacy parameters in the cohort of patients with level 1-2 AMA (according to OncoKB database [34]) (*n=50*).

Efficacy	Level 1-2 AMA + MMT *n=23*	Level 1-2 AMA + non-MMT *n=27*	*p*-Value*
PFS2/PFS1			
Median (range)	0.63 (0 – 11.9)	0.81 (0 – 7.2)	-
Ratio ≥ 1.3 (%)	4/21 (19%)	7/26 (27%)	0.73
Missing	2	1	-
PFS2			0.87
Median (95%CI)	2.7 (0.7 – 4.7)	2.8 (2.3 – 3.2)	
OS			0.60
Median (95%CI)	4.7 (1.4 – 7.9)	7.2 (0.0 – 14.5)	
Tumor response			
Complete response	1 (4%)	0	-
Partial response	4 (17%)	3 (11%)	-
Stable disease	4 (17%)	7 (26%)	-
Progressive disease	9 (39%)	11 (41%)	-
ORR	5 (22%)	3 (11%)	0.43
Disease control	9 (39%)	10 (37%)	1.00
Not available	5 (22%)	6 (22%)	-

AMA: actionable molecular alteration; MMT: molecularly matched therapy; ORR: overall response rate; OS: overall survival; PFS: progression-free survival

* *p*-Value is provided for comparison between groups: "Level 1-2 AMA + MMT" *vs* "Level 1-2 AMA + non-MMT"