

Detection of PD-L1 mRNA expression in circulating tumor cells (CTCs) from patients with metastatic NSCLC, HNSCC and melanoma using a novel highly sensitive commercially available CE-IVD kit

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ABSTRACT

Introduction: Oncolipsy PD-L1 kit, an RT-qPCR assay for biomarker mRNA expression in CTCs (Strati et al, Ann Oncol, 2017), is a commercially available CE-IVD molecular diagnostic product (Pharmassist, Greece). In this study, we report it's application in size-based enriched CTCs from patients with metastatic NSCLC, HNSCC and melanoma, before and during PD-1/PD-L1 blockade therapies.

Patients and Methods: In total 95 patients were enrolled in the study: 69 patients with metastatic NSCLC, 19 patients with metastatic HNSCC and 7patients with metastatic melanoma. 40 PB samples from HD were used as a control group. 10 ml of PB was collected at baseline (V0), two months after immunotherapy (V1) and at the time of disease progression (PD) or 18 months after initiation of immunotherapy (V2). A sample was considered as CTC-positive when cDNAs were positive for CK-8, CK-18 or CK-19 expression.

Results: In CTC-positive samples, overexpression of PD-L1 was detected in 15/58(25.8%) patients at V0, in 6/34(17.6%) patients at V1 and in 3/11(27.3%) at V2. Patients with elevated expression of PD-L1 at V1 in respect to V0 and detectable CTCs had longer PFS (24.9mo vs. 12.9mo, p=0.033) and longer OS (33.2mo vs. 24.6mo, p = 0.071) after initiation of immunotherapy. Patients with elevated PD-L1 at V1 compared to V0 benefited from immunotherapy, with a longer DFS (17.8mo vs. 13.4mo) and OS (29.4mo vs.26.9mo).

Conclusions: The commercially available Oncolipsy PD-L1 kit can be used for the quantification of PD-L1 transcripts in CTCs. Expression of PD-L1 in size-based enriched CTCs can predict response to PD-1/PD-L1 blockade therapy.

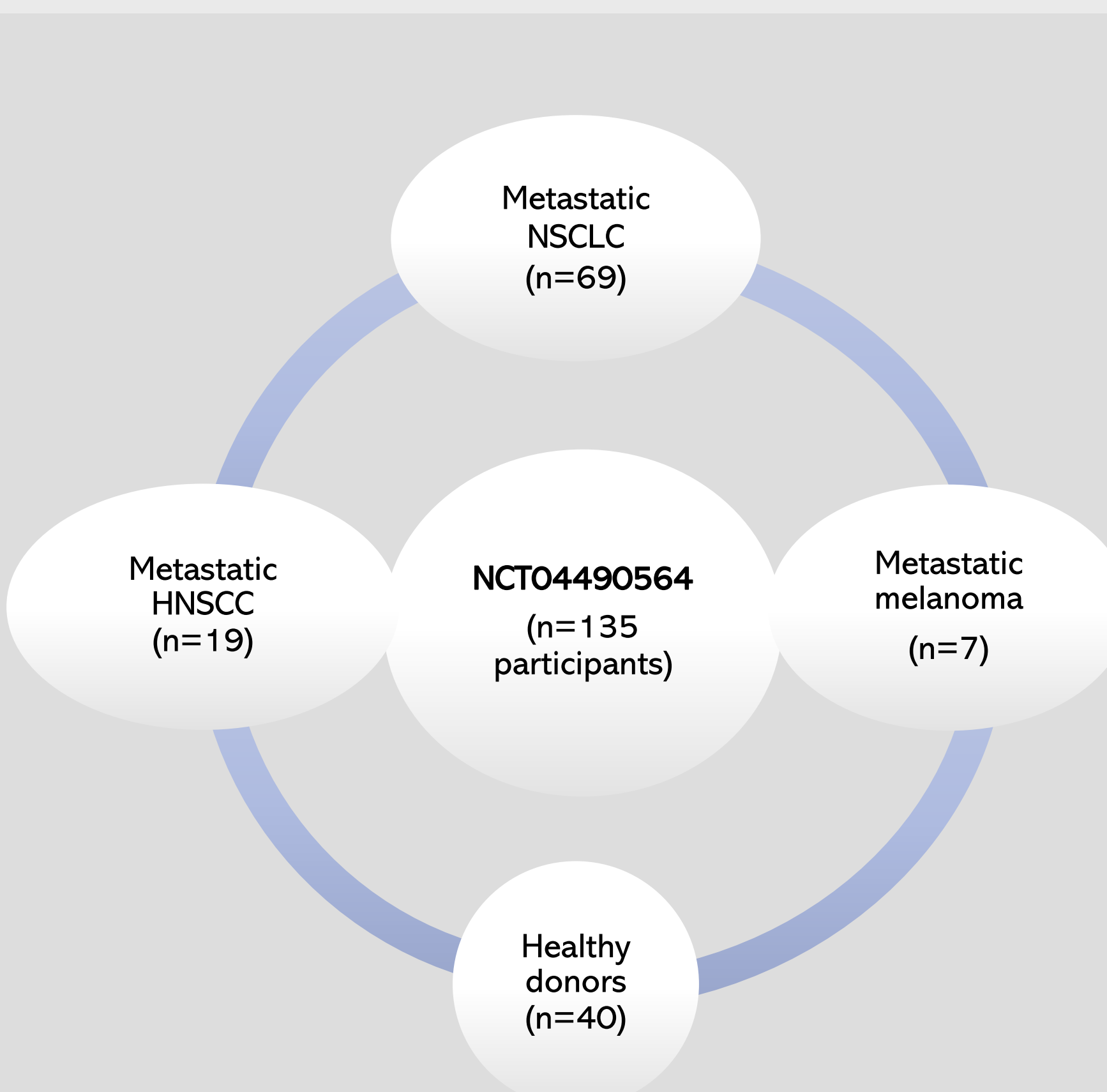
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INTRODUCTION

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METHODS AND MATERIALS



CTC isolation



Oncolipsy PD-L1 kit

Table 1. Overall reproducibility of the Oncolipsy PD-L1 kit using three quality controls: QC1, QC2, QC3

Reproducibility of Oncolipsy PD-L1 kit, QC1				
Marker	LOT	Mean (Cq value)	SD	CV _{Cq}
PD-L1	A	35.70	0.71	0.52
	B	36.31	0.71	0.52
	C	37.24	0.72	0.53
	Between-LOT	36.39	0.95	0.74
B2M	A	35.71	0.63	0.46
	B	35.54	1.1	0.89
	C	34.98	1.5	1.4
	Between-LOT	35.4	1.2	0.95
Reproducibility of Oncolipsy PD-L1 kit, QC2				
Marker	LOT	Mean (Cq value)	SD	CV _{Cq}
PD-L1	A	31.71	0.22	0.15
	B	31.14	0.15	0.10
	C	31.66	0.26	0.18
	Between-LOT	31.50	0.34	0.24
B2M	A	31.54	0.27	0.19
	B	32.53	0.28	0.20
	C	31.15	0.46	0.33
	Between-LOT	31.74	0.68	0.50
Reproducibility of Oncolipsy PD-L1 kit, QC3				
Marker	LOT	Mean (Cq value)	SD	CV _{Cq}
PD-L1	A	24.62	0.29	0.20
	B	23.96	0.090	0.062
	C	24.75	0.40	0.069
	Between-LOT	24.4	0.45	0.32
B2M	A	24.41	0.13	0.091
	B	25.47	0.14	0.098
	C	24.24	0.16	0.11
	Between-LOT	24.71	0.56	0.40

RESULTS

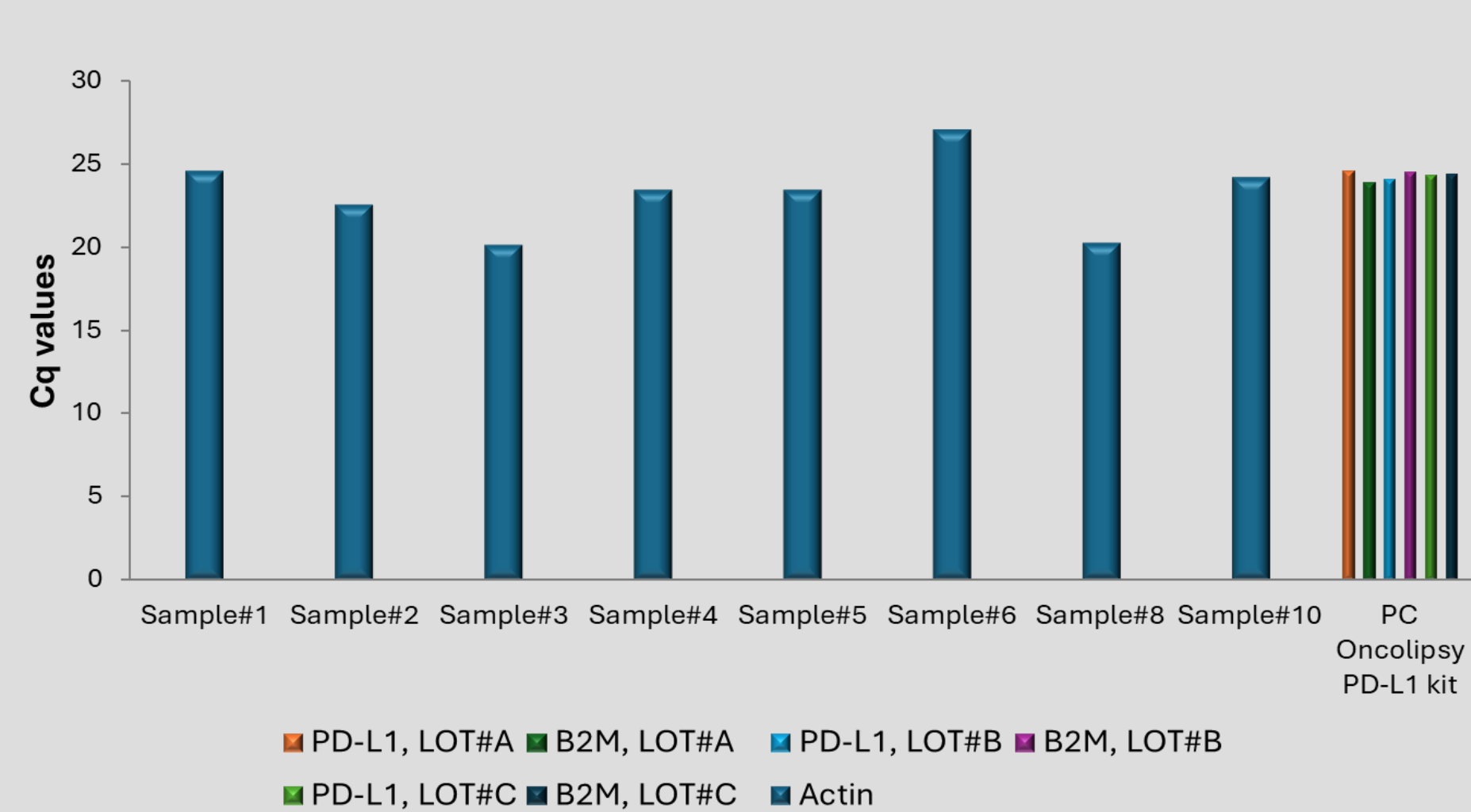


Figure 1. Analytical specificity for the three batches using gDNAs

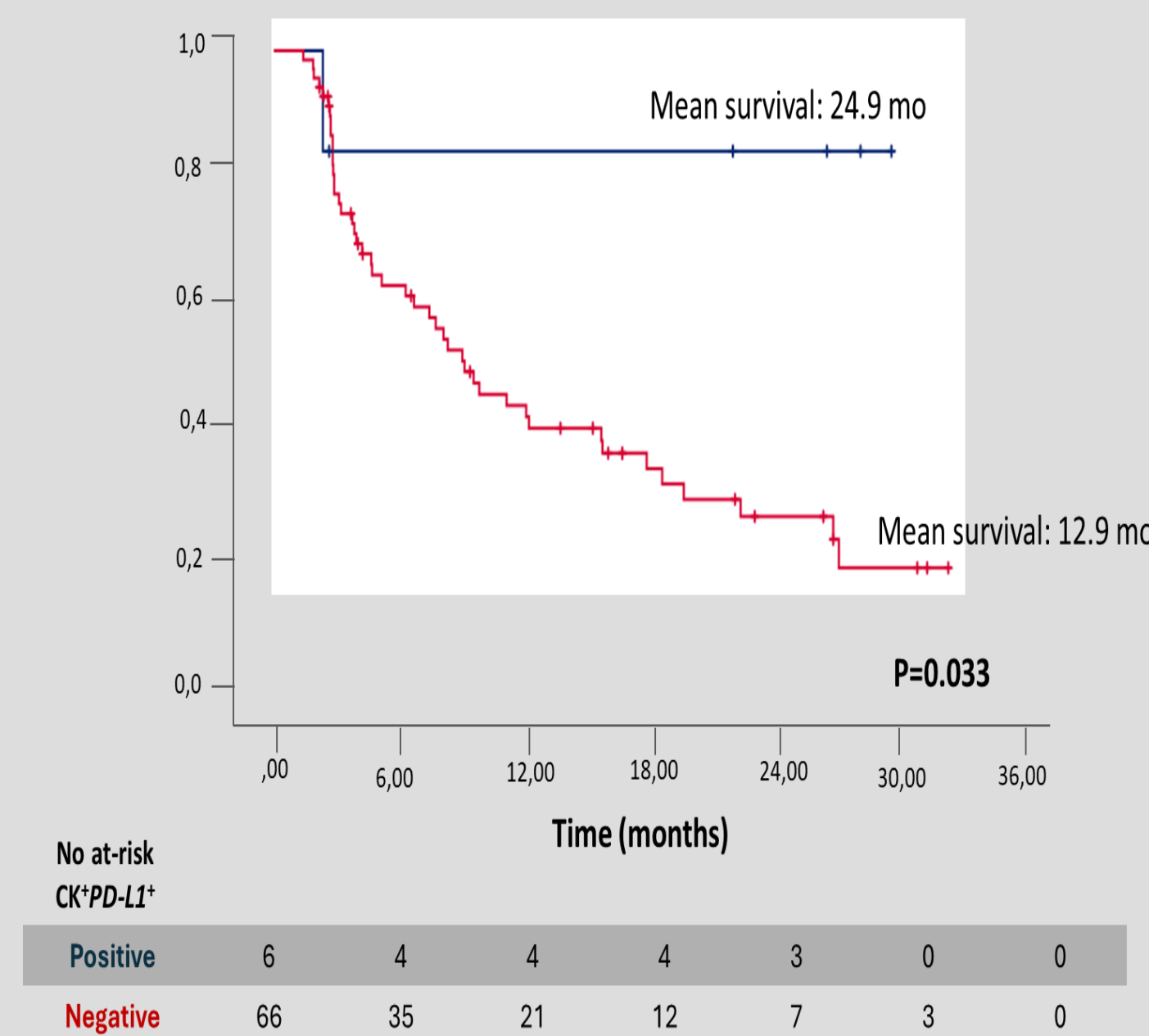


Figure 3A. Kaplan–Meier estimates of PFS for patients with metastatic cancer expressing CK+/PD-L1 (increase/+) at the time of V1 therapy

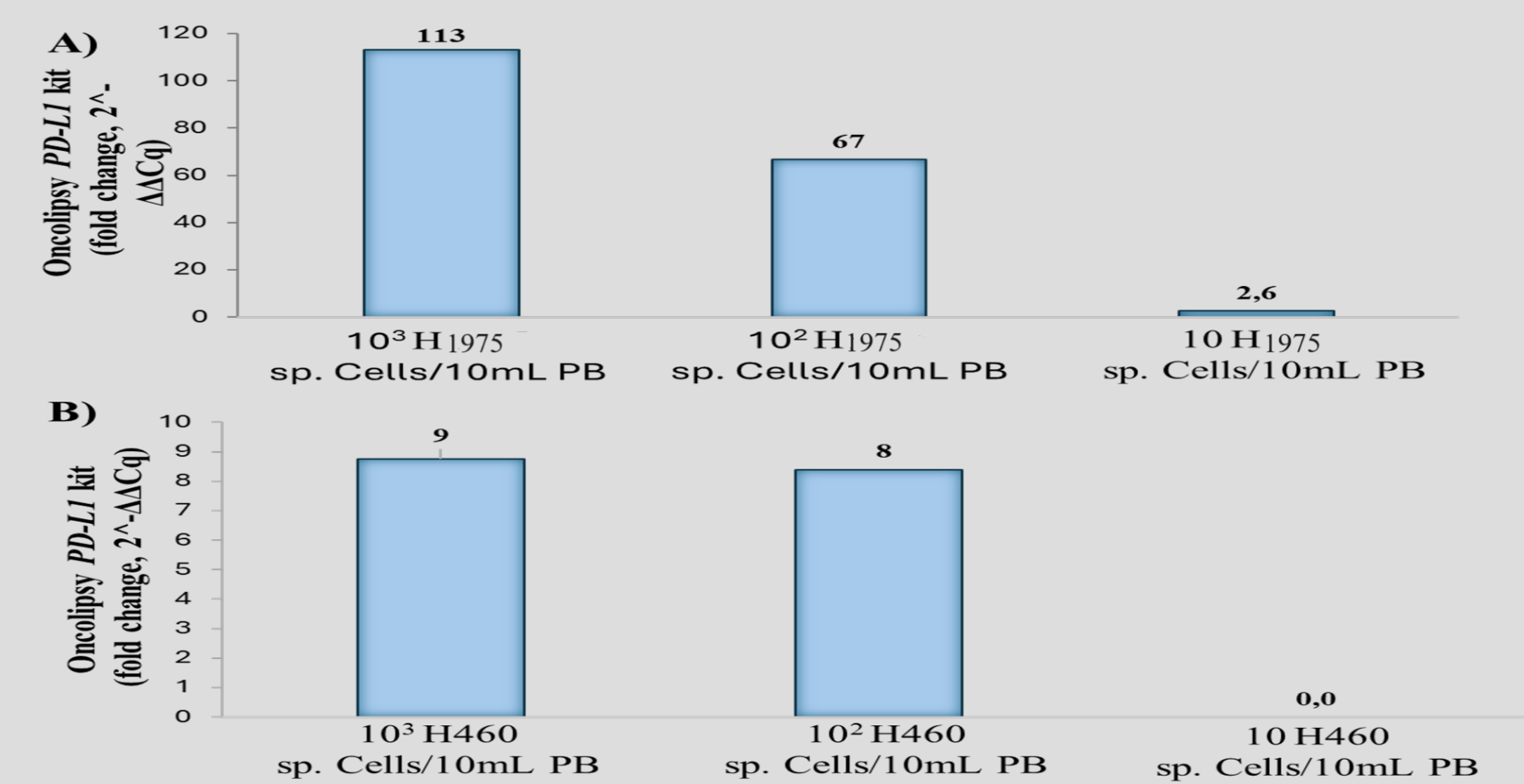


Figure 2. Fold change in PD-L1 expression after spiking of A) H1975 and B) H460 cells in 10mL PB.

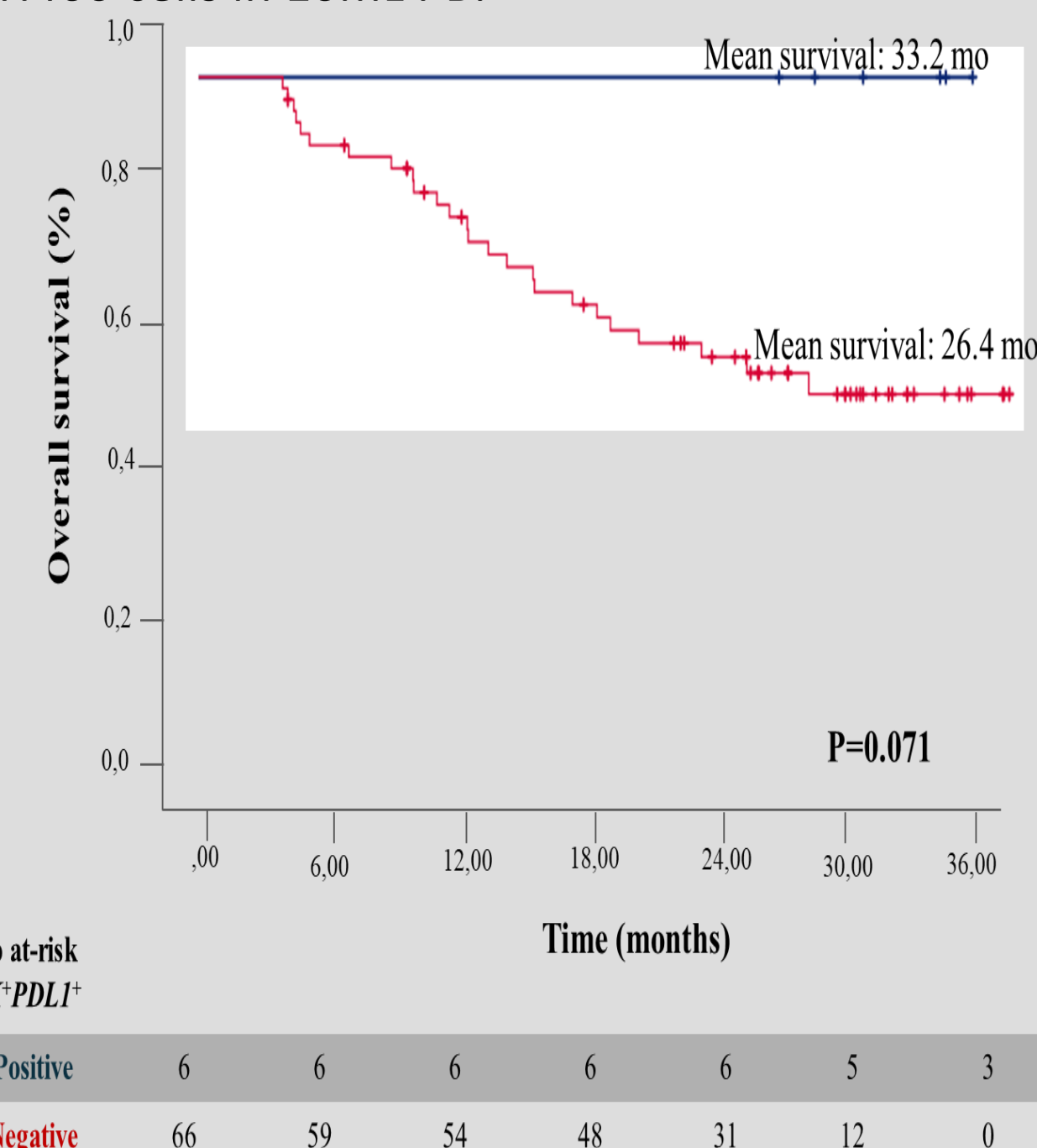


Figure 3B. Kaplan–Meier estimates of OS for patients with metastatic cancer expressing CK+/PD-L1 (increase/+) at the time of V1 therapy

CONCLUSIONS

- The commercially available Oncolipsy PD-L1 kit can be used for the quantification of PD-L1 transcripts in CTCs.
- Expression of PD-L1 in size-based enriched CTCs can predict response to PD-1/PD-L1 blockade therapy.

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- Strati A., et. al. Ann Oncol. 2017 ;28(8):1923-1933.
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