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TUMOR BURDEN AS ASSESSED BY 18 F FDG PET SCAN AS PREDICTIVE BIOMARKER FOR IMMUNE CHECKPOINT BLOCKERS IN ADVANCED NSCLC – A MULTICENTRIC STUDY.

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Abstract

Introduction

Only a proportion of patients with advanced NSCLC benefit from Immune checkpoint blockers (ICBs). No biomarker is validated to choose between ICBs monotherapy or in combination with chemotherapy (Chemo-ICB) when PD-L1 expression is above 50%. The aim of the present study is to validate the biomarker validity of total Metabolic Tumor Volume (tMTV) as assessed by 2-deoxy-2-[18F]fluoro-d-glucose positron emission tomography ([18F]FDG-PET)

Material and methods

This is a multicentric retrospective study. Patients with advanced NSCLC treated with ICBs, chemotherapy plus ICBs and chemotherapy were enrolled in 12 institutions from 4 countries. Inclusion criteria was a positive PET scan performed within 42 days from treatment start. TMTV was analyzed at each center based on a 42% SUVmax threshold. High tMTV was defined ad tMTV>median

Results

493 patients were included, 163 treated with ICBs alone, 236 with chemo-ICBs and 94 with CT. No correlation was found between PD-L1 expression and tMTV. Median PFS for patients with high tMTV (100.1 cm3) was 3.26 months (95% CI 1.94–6.38) vs 14.70 (95% CI 11.51–22.59) for those with low tMTV (p=0.0005). Similarly median OS for pts with high tMTV was 11.4 months (95% CI 8.42 – 19.1) vs 33.1 months for those with low tMTV (95% CI 22.59 –

NA), p .00067. In chemo-ICBs treated patients no correlation was found for OS (p = 0.11) and a borderline correlation was found for PFS (p=0.059).

Patients with high tMTV and PD-L1 ≥ 50% had a better PFS when treated with combination of chemotherapy and ICBs respect to ICBs alone, with 3.26 months (95% CI 1.94 – 5.79) for ICBs vs 11.94 (95% CI 5.75 – NA) for Chemo ICBs (p = 0.043).

Conclusion

tMTV is predictive of ICBs benefit, not to CT benefit. tMTV can help to select the best upfront strategy in patients with high tMTV.

Introduction

Immune-checkpoint blockers (ICBs) have revolutionized the treatment of many types of cancers, particularly advanced-stage cancers. Nonetheless, despite a proportion of patients having dramatic and long-lasting disease regression in response to ICBs, most do not benefit from these therapies, and some might even experience the detrimental phenomenon of hyperprogressive disease[1,2]. While the PD-L1 tumor proportion score as assessed by immunohistochemistry, is currently one of the most commonly used biomarkers for predicting the outcome of anti-PD-1 / PD-L1 agents[3], other tumor specific markers have been explored, such as high microsatellite instability (MSI-H)[4,5] and tumor mutation burden (TMB). However none of these potential biomarkers can be considered the "ultimate biomarker", as responses are seen in biomarker-negative patients, while a large number of biomarker-positive patients unfortunately do not respond despite their positivity[6,7]. Moreover, the assessment of some of these biomarkers (such as TMB and neoantigen load) requires complex, expensive and time-consuming analysis, rendering them unsuitable for use in routine clinical practice in most cancer centers worldwide. In addition, TMB has failed to show predictive value for overall survival (OS) across different tumor types[8–11]. Other possible biomarker candidates include lactate dehydrogenase (LDH), number of metastatic sites, circulating free tumor DNA (ctDNA), circulating tumor cells (CTC), type of circulating white blood cells and their respective ratios.

Many of these proposed biomarkers correlate with tumor burden, and increasing clinical and preclinical evidence supports the negative impact of tumor burden on the immune response to cancer. Tumor burden can be assessed by many tools, including computed tomography (CT) scans, 18-FDG positron emission tomography (PET), and liquid biopsy methods such as ctDNA or CTCs, and serum markers such as LDH or tumor markers. In addition, this parameter

can be relatively easy to assess (at least when based on imaging) and might have important clinical implication, especially for advanced, PD-L1 positive ≥ 50% NSCLC, where physicians are facing the challenging choice between ICBs alone or in combination with chemotherapy. Both strategies, in fact, proven to be superior to chemotherapy in patients with high PD-L1 expression in phase 3 trials[12–14], but no data are available for the head-to-head comparison of the two. A recent FDA pooled analysis showed only marginal benefit in PFS and no benefit in OS in the PD-L1 high trial population, thus limiting the recommendation coming from the evidences to a shared decision making [15].

Today, no consensus has been reached on the several candidate biomarkers, each one having pros and cons in terms of reliability, availability and generalizability.

MTV has been described to correlate with outcome from ICBs in different cancer, such as melanoma[16,17] and NSCLC[18], but most of the evidences come from small monocentric studies, often without a comparable control group to discriminate between a merely prognostic role vs a predictive one.

Scope of this study is to analyse the impact of tMTV on advanced NSCLC patients treated with ICBs alone, ICBs in combination with chemotherapy and chemotherapy alone ad explore if chemotherapy added to ICBs may mitigate its detrimental effect.

Methods

Patients

Patients were retrospectively identified in each institution who received first line treatment for advanced NSCLC with either Immune Checkpoint Inhibitors monotherapy (ICBs), combination of platinum based chemotherapy and ICBs (chemo-ICBs) or chemotherapy alone (chemo, the latter provided by Gustave Roussy institute).

To be included, patients should have received a 18-F fluorodesossiglucose Positron Emission Tomography (PET) scan within 42 days from treatment initiation.

PET scan analysis

FDG PET/CT images has been acquired 55-75 min post-injection with patient in supine position with preferably arms up and breathing normally. FDG PET/ CT images using respiratory gated protocols were allowed provided that the local analysis would be performed using all the time the same acquisition. Subject should has been fasting for at least 6 hours prior to FDG PET/CT scan.

There was no restriction in medication intake except in the case of anti-diabetic drugs which must be have been stopped on the day of PET/CT examination and injected 18F-FDG activity should be optimised for body weight, following EANM guidelines [19].

The serum blood glucose should have been ≤ 200 mg/dL (11.1 mmol/L) at the time of FDG administration. In case of hyperglycaemia (>200 mg/dl or >11.mmol/L) the patient has been excluded of the analysis.

If contrast enhanced CT as part of the FDG PET/CT examination was performed; the FDG PET attenuation correction images need to be reconstructed using a low/ultralow dose CT.

Statistical analysis

Primary endpoints of the study were progression-free survival (PFS) and overall survival (OS) after first line treatment initiation. Predictors of PFS and OS were analysed using univariate and multivariable Cox models. PFS and OS curves were estimated using the Kaplan-Meier method. Comparisons between subgroups were performed using logrank testing. Fisher's exact probability test and Pearson Chi-square test were used for comparisons between 2 dichotomous categorical variables. Comparisons of 2 continuous variables were performed using the Mann-Whitney U test.

Statistical analysis were performed with RStudio software (v 2022.07.2+576).

Results

Baseline characteristics

493 patients were retrospectively enrolled at 12 centers across 4 countries. Of them, 163 were treated with ICBs alone, 236 with combination of chemotherapy and immunotherapy and 94 with chemotherapy alone.

Median tMTV in the whole cohort was 100.1 cm3. There was no difference in terms of tMTV distribution among treatment groups (p=0.4264), as well as no difference in terms of other prognostic biomarker apart for age, with patients who received ICBs alone being slightly older than the others. PD-L1 was always ≥ 50% for patients treated with ICBs alone, 22.5% of patients in Chemio-IO group had PD-L1 ≥ 50% while most of the control group had no PD-L1 available.

Table 1 – Clinico-pathological characteristics of the whole cohort.

		Treatment		
		ICBs	Chemotherapy + ICBs	Chemotherapy
Total		163	236	94
Sex	Male	110 (67 %)	167 (70 %)	58 (62 %)
	Female	53 (32 %)	68 (28 %)	36 (38 %)
Age (mean)		70	64	62
Histological type	Adenocarcinoma	116 (71 %)	175 (74 %)	66 (70 %)

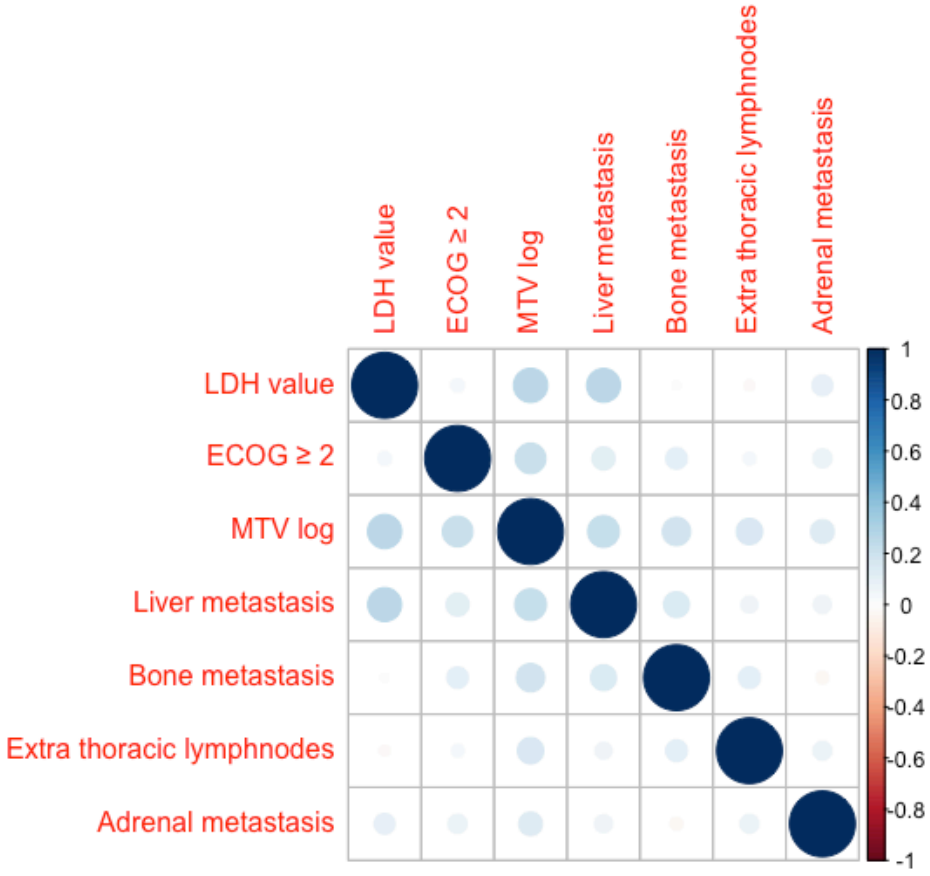
	Squamous Cell Carcinoma	32 (19 %)	40 (16 %)	14 (15 %)
	Undifferentiated/other NSCLC	15 (10 %)	21 (9 %)	14 (15 %)
Bone metastasis	No	95 (58 %)	127 (54 %)	49 (52 %)
	Yes	68 (41 %)	109 (46 %)	45 (48 %)
Liver Metastasis	No	138 (84 %)	206 (87 %)	73 (78 %)
	Yes	25 (15 %)	30 (12 %)	21 (22 %)
Brain metastasis	No	131 (80 %)	180 (76 %)	75 (80 %)
	Yes	32 (20%)	56 (24%)	19 (20%)
LDH levels	< ULN	83 (50 %)	115 (49 %)	58 (62 %)
	> ULN	45 (27 %)	86 (36 %)	29 (31 %)
ECOG PS	0 – 1	128 (79 %)	191 (81 %)	77 (82 %)
	≥ 2	34 (21 %)	39 (17 %)	17 (18 %)
	NA	1 (<1%)	6 (2 %)	0
PD-L1	0 %	0	92 (39 %)	13 (14 %)
	1 – 49 %	0	80 (34 %)	5 (5 %)
	≥ 50 %	163 (100 %)	50 (21 %)	2 (2 %)
	NA	0	14 (6 %)	74 (79 %)

Platinum Based Chemo	Cisplatin	-	65 (28 %)	57 (61 %)
	Carboplatin	-	171 (72 %)	37 (39 %)
totaltMTV (median)		94.4	103.03	97.28

Median follow up was 27 months for ICBs, 16 months for combination chemo-ICBs and 79 months for chemotherapy alone.

Higher tMTV (after log transformation for normalization) was correlated with LDH blood levels ($r = 0.266$, $p < 0.001$), with ECOG PS of 2 or more ($r = 0.212$, $p < 0.001$),
with the presence of liver metastasis ($r = 0.222$, $p < 0.001$), bone metastasis (r 0.181, $p < 0.001$), extrathoracic nodal metastasis ($r = 0.155$, $p = 0.001$), with adrenal metastasis ($r = 0.126$, $p = 0.009$), figure 1.

Figure 1 – correlation between totaltMTV and clinical characteristics of patients included in the study



Progression free survival

Median PFS for patients treated with ICBs alone was 8.35 months (95% CI 6 – 14.7). Patients were divided according to median and quartiles for tMTV in the whole cohort. Median PFS for patients with tMTV > median was 3.26 months (95% CI 1.94 – 6.38) vs 14.70 (95% CI 11.51 – 22.59) for those with tMTV < median (p = 0.0005, figure 2a). To investigate the impact of different cutoff of tMTV on PFS, we divided ICBs group in quartiles according to tMTV. Median PFS in the first quartile was 14.01 (95% CI 11.77 - 31.00) vs 15.92 for those in the 2nd quartile (95% CI 8.02 - 27.59), 4.83 in the 3rd quartile (95% CI 3.00 – 18.12) and 1.91 in 4th quartile (95% CI 1.32 - 5.79), p < 0.001.

When adjusted for other prognostic factors, tMTV remained significantly associated with PFS in the multivariate analysis, along with LDH value above ULN (Table 2).

Median PFS for patients treated with ICBs in combination with chemotherapy was 7.63 months (95% CI 7 – 10.01). Patients were divided according to median and quartiles for tMTV in the whole cohort. Median PFS for patients with tMTV > median was 6.54 months (95% CI 5.49 – 7.83) vs 10.13 (95% CI 8.12 – 14.1) for those with tMTV < median (p = 0.059), figure 2b. Median PFS for patients in the first quartile was 14.04 months (95% CI 10.8 – NA) vs 7.07 for those in the 2nd quartile (95% CI 6.28 – 10.06), 7.4 in the 3rd quartile (95% CI 5.95 – 12.30) and 5.52 in 4th quartile (95% CI 4.11 – 9.31), p = 0.017 .

When adjusted for other prognostic biomarker, tMTV resulted correlated with PFS, along with PD-L1 expression, bone and liver metastasis, LDH and squamous histology (table 3).

No correlation was found fortMTV in the chemotherapy control group in terms of PFS (p = 0.16), figure 2c and table 4.

Table 2 – Univariate and multivariate analysis for PFS in ICBs treated patients.

Variables	Univariate				Multivariate			
	HR	95.0% CI for Exp(B)		p	HR	95.0% CI for HR		p
		Lower	Upper			Lower	Upper	
Liver Mets	1.938	1.213	3.095	0.006	1.075	0.596	1.941	0.809
Sex	1.131	0.771	1.659	0.53	1.135	0.732	1.76	0.57
Histo	1.347	0.876	2.071	0.175	1.502	0.901	2.504	0.119
Bone Mets	1.379	1.007	1.89	0.045	1.355	0.918	2.001	0.126
ECOG PS 2	1.67	1.067	2.614	0.025	1.446	0.862	2.427	0.162
LDH > ULN	1.73	1.145	2.613	0.009	1.643	1.086	2.487	0.019
Log tMTV	1.515	1.235	1.858	< 0.001	1.34	1.056	1.702	0.016

Table 3 - Univariate and multivariate analysis for PFS in chemo-ICBs treated patients.

Variables	Univariate				Multivariate			
	HR	95.0% CI for HR		p	HR	95.0% CI for HR		p
		Lower	Upper			Lower	Upper	
Sex	0.964	0.681	1.363	0.834	1.12	0.754	1.662	0.574
LiverM	1.72	1.094	2.706	0.019	1.712	0.991	2.958	0.054
squamo	1.497	0.99	2.265	0.056	1.804	1.121	2.905	0.015
BoneM	1.642	1.199	2.249	0.002	1.509	1.044	2.18	0.029
ECOG PS 2	1.311	0.881	1.952	0.182	1.48	0.937	2.339	0.093

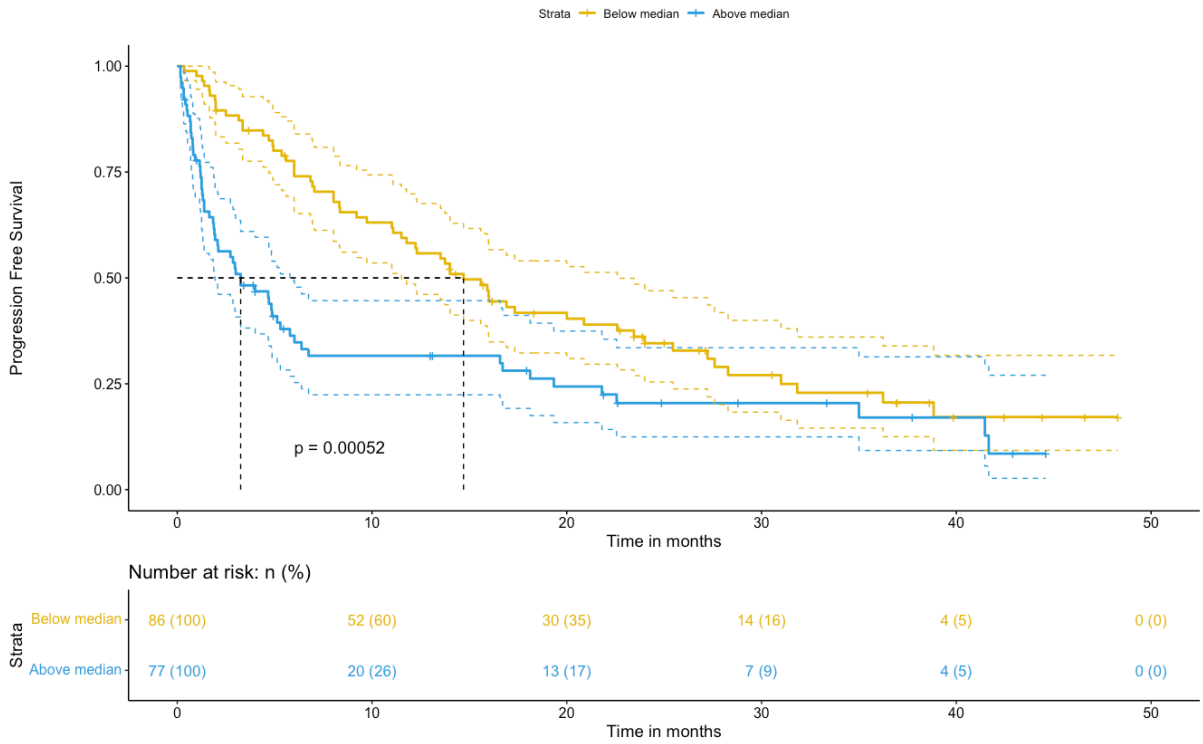
LDH_H	0.767	0.537	1.096	0.146	0.666	0.449	0.987	0.043
logMTV	1.281	1.112	1.477	0.001	1.24	1.044	1.471	0.014
PDL1_H	0.623	0.419	0.928	0.02	0.604	0.385	0.949	0.029

Table 4 - Univariate and multivariate analysis for PFS in chemotherapy treated patients.

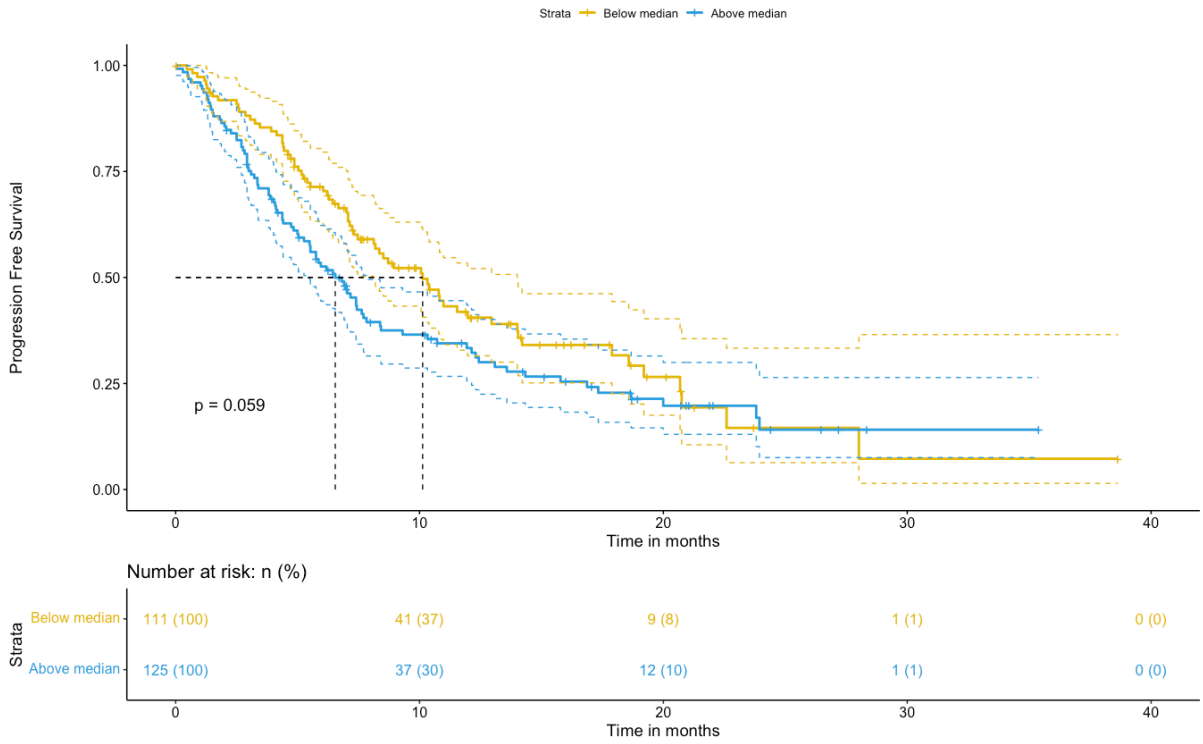
Variables	Univariate				Multivariate			
	HR	95.0% CI for HR		p	HR	95.0% CI for HR		p
		Lower	Upper			Lower	Upper	
Sex	1.292	0.835	2	0.249	1.549	0.936	2.561	0.089
LiverM	1.324	0.801	2.187	0.274	0.807	0.443	1.471	0.484
LDH_H	1.756	1.079	2.858	0.023	1.995	1.205	3.302	0.007
ECOG2	1.836	1.06	3.179	0.03	1.784	0.962	3.307	0.066
squamo	1.069	0.589	1.94	0.827	2.143	1.056	4.349	0.035
BoneM	0.907	0.596	1.382	0.651	0.618	0.38	1.004	0.052
logMTV	1.276	1.011	1.609	0.04	1.063	0.83	1.362	0.629

Figure 2 – Kaplan Meyer curves for Progression free survival according to total Metabolic Tumor Volume above or below median in ICBs (a), chemo-ICBs (b) or chemotherapy (c) groups.

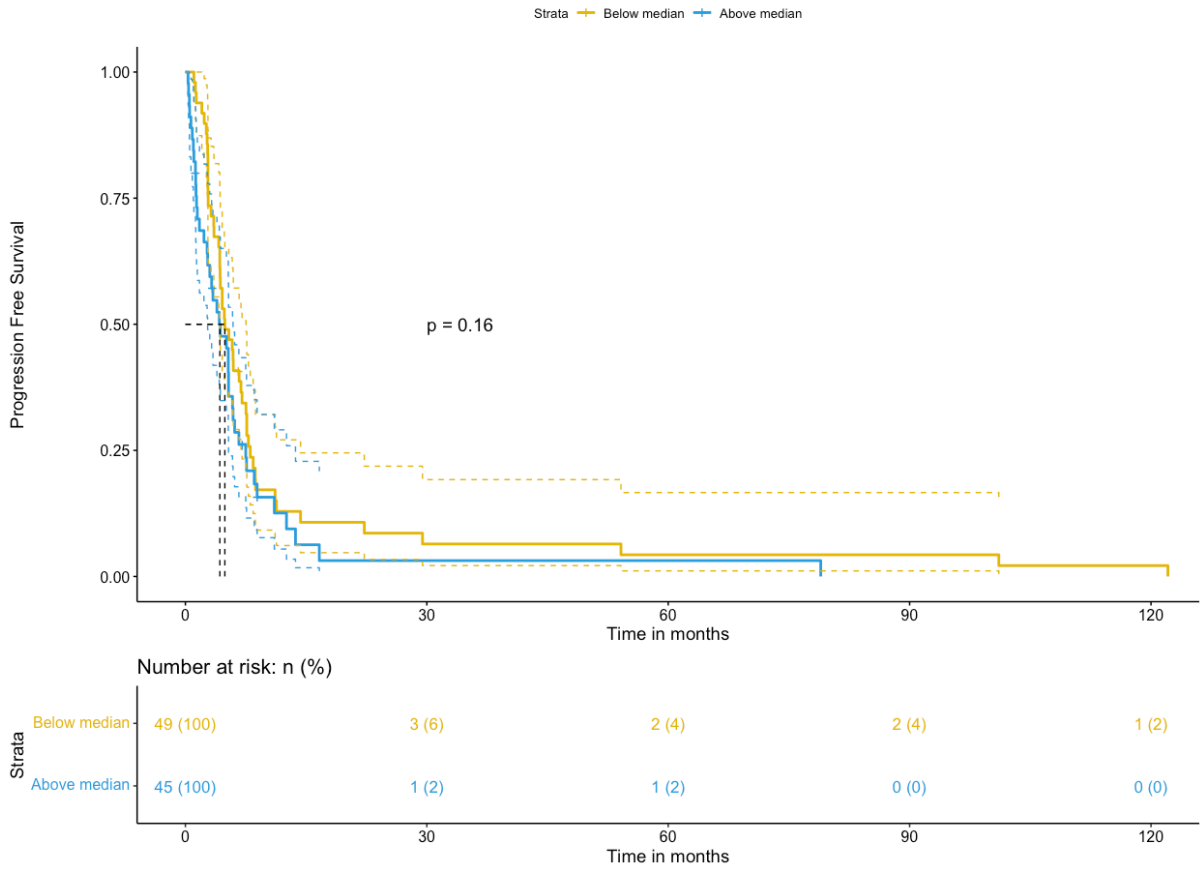
a



b



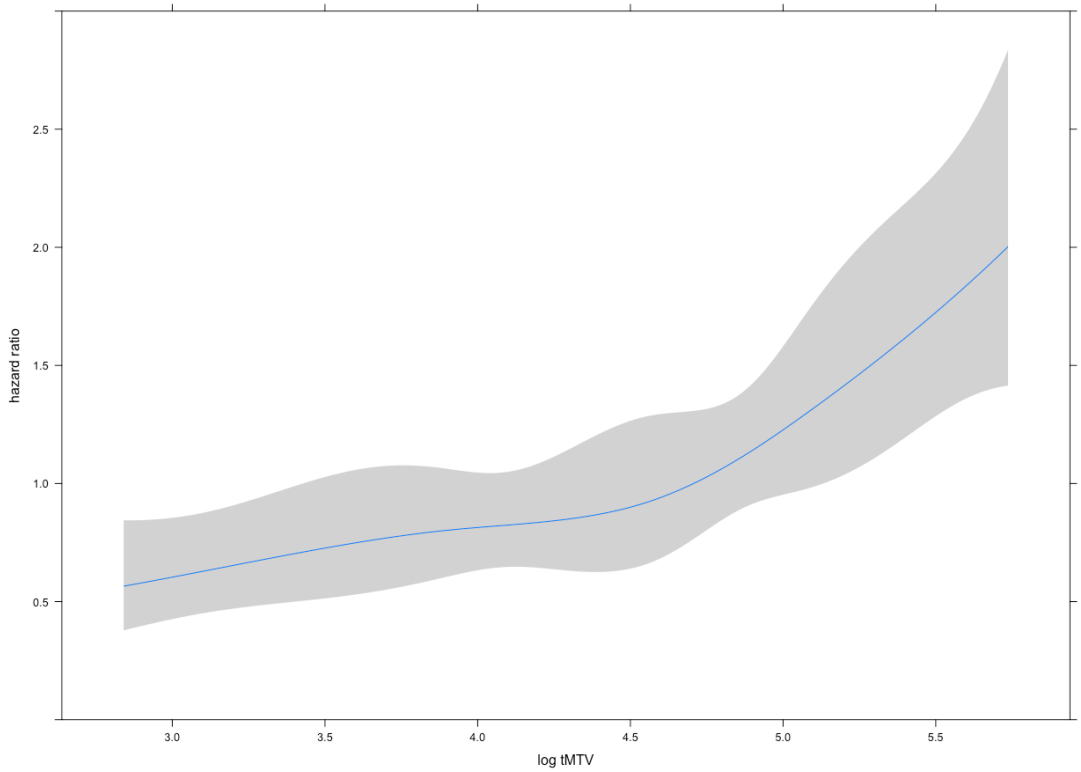
c


Strata
Below median
Above median
Progression Free Survival
1.00
0.75
0.50
0.25
0.00
p = 0.16
0
30
60
90
120
Time in months
Number at risk: n (%)
Strata
Below median
Above median
49 (100)
3 (6)
2 (4)
2 (4)
1 (2)
45 (100)
1 (2)
1 (2)
0 (0)
0 (0)
0
30
60
90
120
Time in months

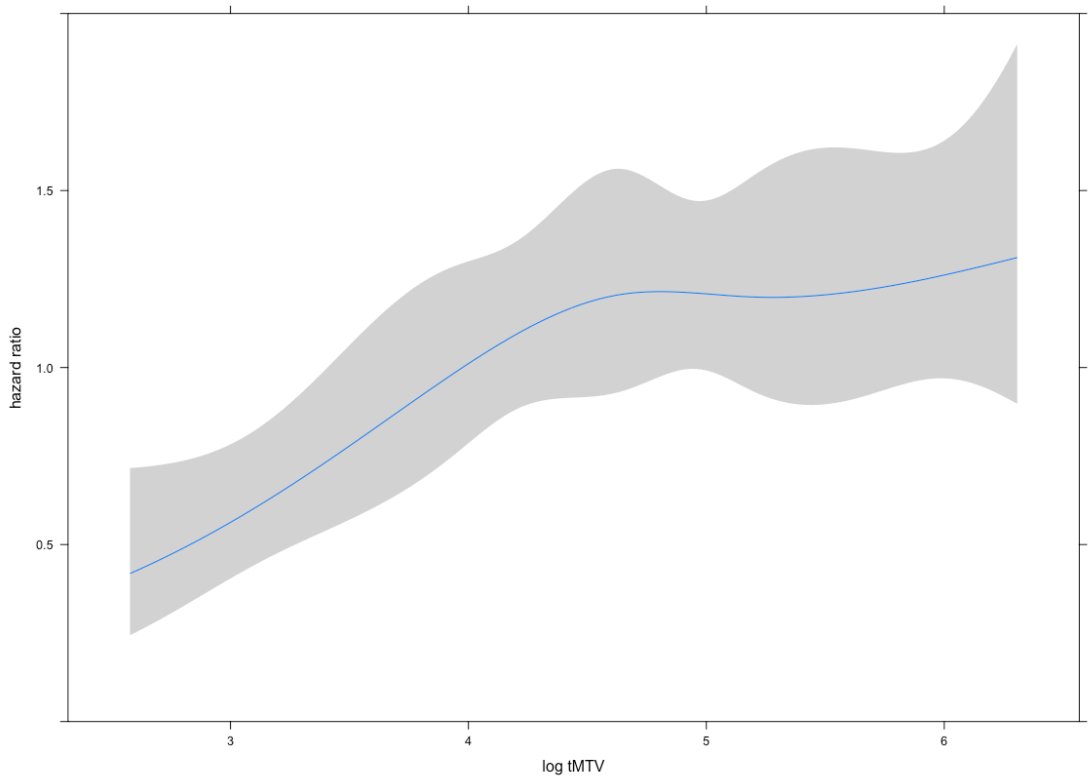
To better understand the relationship between tMTV and PFS for ICBs alone and in combination, we used a restricted cubic splines approach to explore how different cutoff may affect the outcome of different treatments. The effect of tMTV log transformed (figure 3) appeared to differ between ICBs alone and in combination, with the former having a flat shape under the value of 4.5 and then having a sustained growth. The latter, on the other side, showed a rapid increase of the HR at the beginning, and then stabilized for higher volume.

Figure 3 – Restricted cubic splines for PFS in ICBs (a) and combination of chemotherapy and ICBs (b)

a



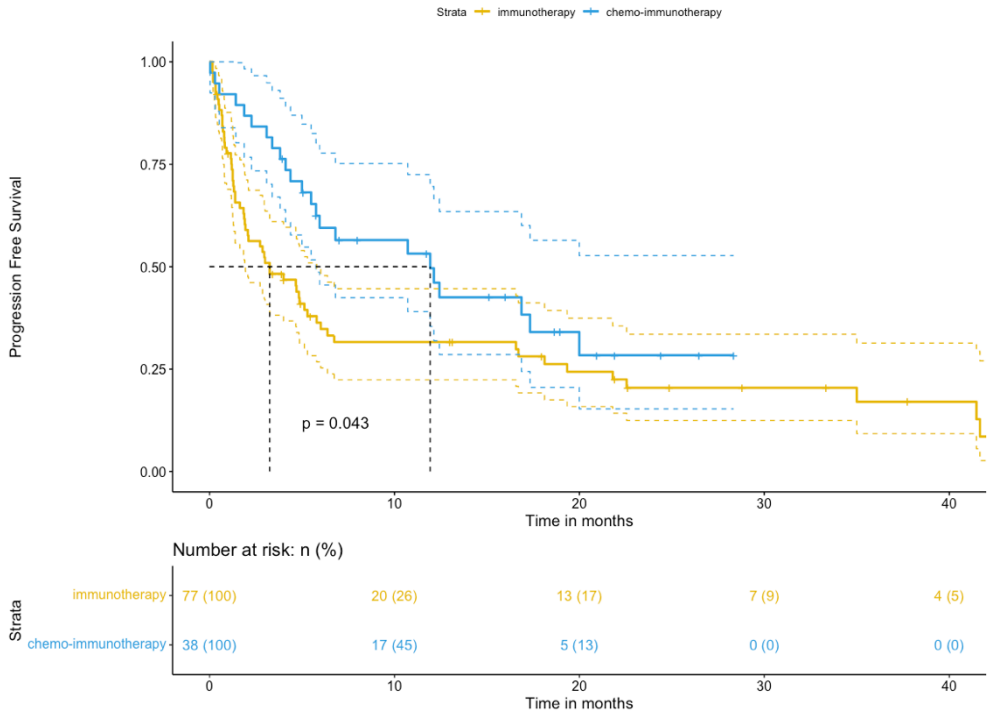
b



We then investigated the outcome of ICBs alone vs combination of chemo-ICBs in patients according totMTV.

In patients withtMTV > median and PD-L1 ≥ 50%, median PFS was 3.26 (95% CI 1.94 – 5.79) for ICBs vs 11.94 (95% CI 5.75 – NA) for Chemo ICBs (p = 0.043, figure 4).

Figure 4 – Median PFS for patients with tMTV > median and PD-L1 ≥ 50%

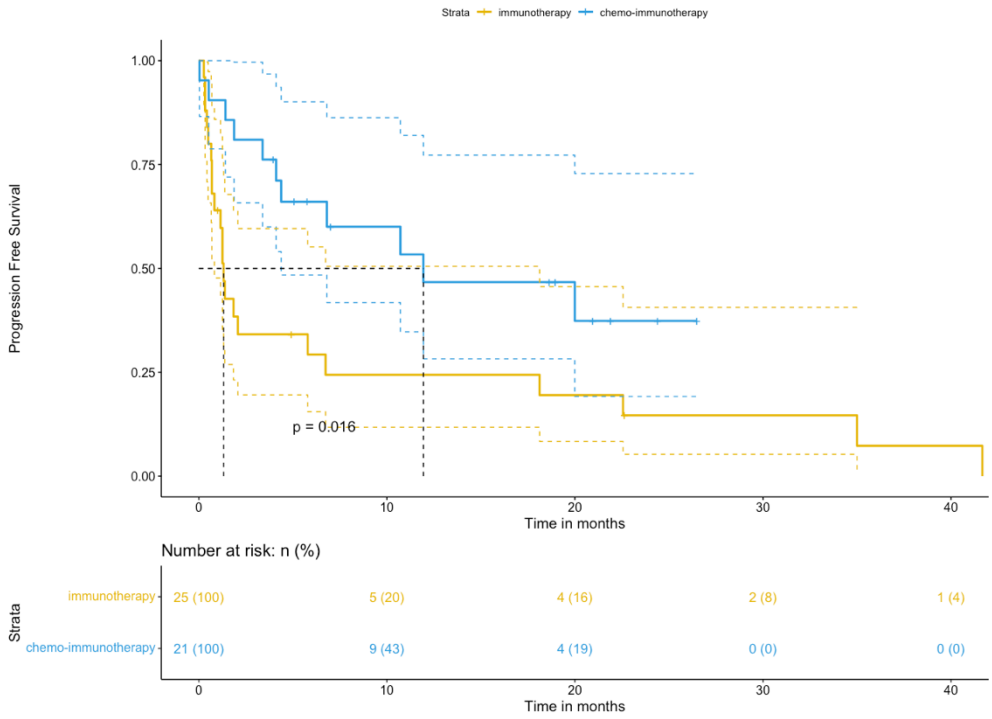


No difference was seen in those with tMTV < median (p = 0.78).

In order to explore the additive effect of LDH on the tumor burden, we included LDH in the algorithm.

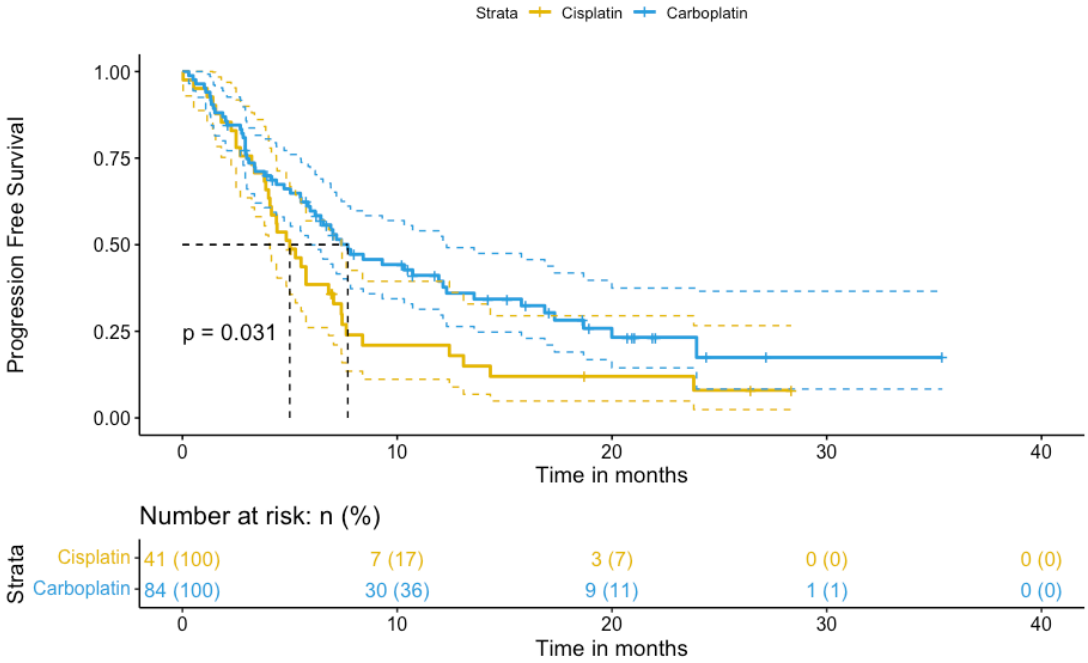
We found out that those patients with PD-L1 ≥ 50% and with tMTV > median and LDH > ULN, chemo-ICBs was superior to ICBs, with a median PFS of 11.94 months (95% CI 4.37 – NA) vs 1.32 (95% CI 0.82 – 18.1), p = 0.016 (figure 5).

Figure 5 – Kaplan Meyer curve for ICBs and chemotherapy plus ICBs in patients with PD-L1 ≥ 50%, tMTV > median and LDH > ULN.



Finally, when comparing carboplatin to cisplatin in chemo-ICBs cohort, we found that in those with tMTV high, carboplatin seemed superior to cisplatin, with a median PFS of 7.7 months (95% CI 5.95 – 12.3) vs 5 months (95% CI 4.08 – 7.4), p = 0.031 (figure 6).

Figure 6 – Kaplan Meyer curve for PFS in high tMTV patients treated with chemo-ICBs according to cisplatin vs carboplatin containing regime.



Overall Survival

Median OS for patients treated with ICBs alone was 19.1 months (95% CI 16.2 – 29.6). Patients with tMTV > median had a median OS of 11.4 months (95% CI 8.42 – 19.1) vs 33.1 months for those with tMTV < median (95% CI 22.59 – NA), p .00067 (figure 7a). To investigate the impact of different cutoff of tMTV on OS, we divided ICBs group in quartiles according to tMTV. Median OS for patients in the first quartile was 33.1 months (95% CI 22.6 – NA) vs 29.2 for those in the 2nd quartile (95% CI 16.2 – NA), 18.3 in the 3rd quartile (95% CI 10.1 – NA) and 7.6 in 4th quartile (95% CI 2.4 – 17.6), p = 0.00062.

When adjusted for other prognostic factors, tMTV remained associated with OS, along with ECOG PS 2, while LDH above ULN was borderline correlated (Table 5).

Median OS for patients treated with Chemo-ICBs was 16.9 (95% CI 13.1– 25.9). Patients with tMTV > median had a median OS of 14.5 months (95% CI 11.3 – NA) vs 18.3 months for those with tMTV < median (95% CI 15.3 – NA), p = 0.12 (figure 6b). Median OS for patients in the first quartile was 25.9 (95% CI 15.26 - NA) vs 15.3 for those in the 2nd quartile (95% CI 10.6 – NA), 16.5 in the 3rd quartile (95% CI 11.81 – NA) and 12.6 in 4th quartile (95% CI 7.37 – NA), p 0.37.

When adjusted for other prognostic biomarker,tMTV resulted not correlated with OS in a multivariate model (table 6).

Median OS in the chemotherapy treated cohort was 9.31 months (95% CI 6.91 – 11.7). Patients with tMTV above median has a median OS of 7.04 (95% CI 4.93 – 11.7) vs 10.75 months for those with tMTV below median (95% CI 6.97 – 15.3), p = 0.022 (figure 7c).

The multivariate model showed no correlation in terms of OS in chemotherapy group (table 7).

Table 5 Univariate and multivariate model for Overall Survival in patients treated with ICBs monotherapy.

Variables	Univariate				Multivariate			
	HR	95.0% CI for HR		p	HR	95.0% CI for HR		p
		Lower	Upper			Lower	Upper	
Sex (F vs M)	0.902	0.57	1.426	0.659	0.995	0.59	1.676	0.984
Liver Metastasis	1.672	0.983	2.842	0.058	0.872	0.46	1.65	0.673
Squamous vs non squamous	1.622	1.007	2.611	0.047	1.976	1.108	3.523	0.021
Bone metastasis	1.483	1.034	2.128	0.032	1.698	1.093	2.639	0.019
ECOG PS	2.417	1.49	3.919	<0.000	2.114	1.162	3.847	0.014
LDH levels above ULN	1.708	1.054	2.77	0.03	1.631	0.994	2.677	0.053
logMTV	1.677	1.313	2.143	<0.001	1.488	1.098	2.017	0.01

Table 6 - Univariate and multivariate model for Overall Survival in patients treated with ICBs in combination with chemotherapy.

Variables	Univariate	Multivariate

	HR	95.0% CI for HR		p	HR	95.0% CI for HR		p
		Lower	Upper			Lower	Upper	
Sex (F vs M)	0.705	0.451	1.104	0.127	0.718	0.435	1.184	0.194
Liver Metastasis	1.952	1.175	3.245	0.01	1.681	0.935	3.023	0.083
Squamous vs non squamous	1.679	1.06	2.66	0.027	1.979	1.174	3.335	0.01
Bone metastasis	1.676	1.145	2.453	0.008	1.481	0.965	2.274	0.073
ECOG PS	1.442	0.907	2.292	0.122	1.551	0.915	2.629	0.103
LDH levels above ULN	0.871	0.567	1.338	0.528	0.817	0.519	1.287	0.383
logMTV	1.236	1.037	1.472	0.018	1.103	0.902	1.349	0.338
PD-L1 ≥ 50%	0.667	0.413	1.077	0.097	0.652	0.376	1.129	0.127

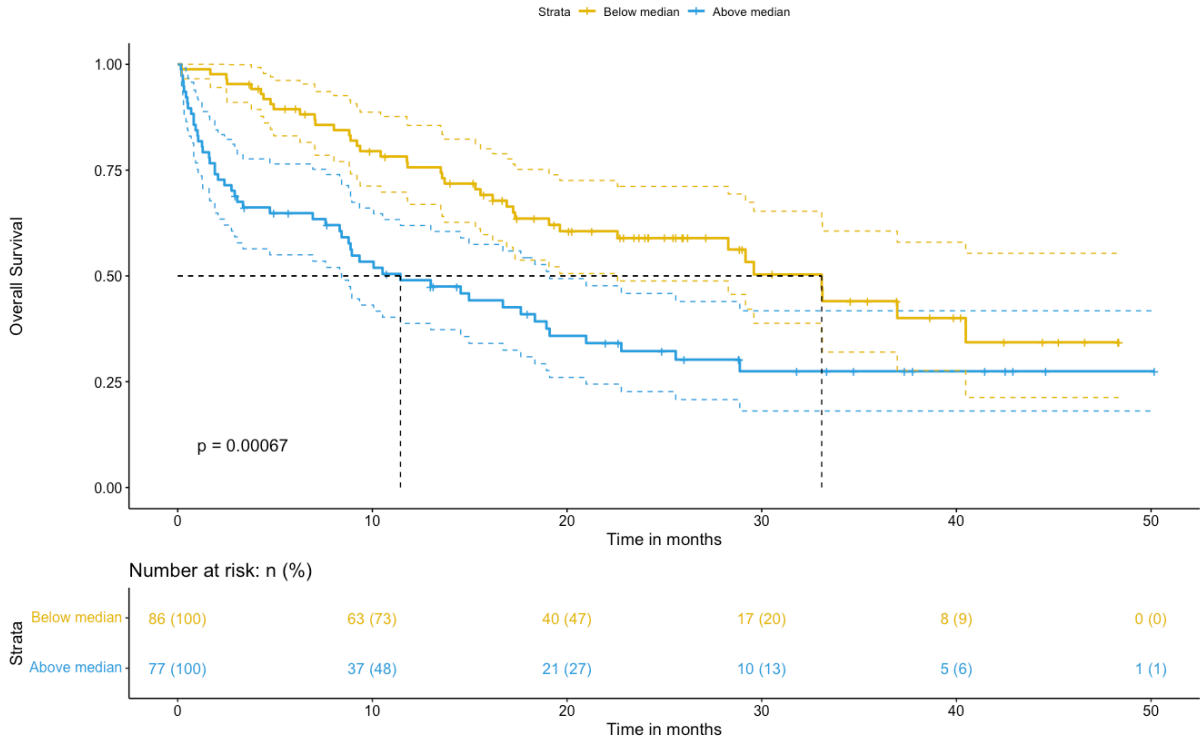
Table 7 - Univariate and multivariate model for Overall Survival in patients treated with chemotherapy.

Variables	Univariate			Multivariate		
	HR	95.0% CI for HR	p	HR	95.0% CI for HR	p

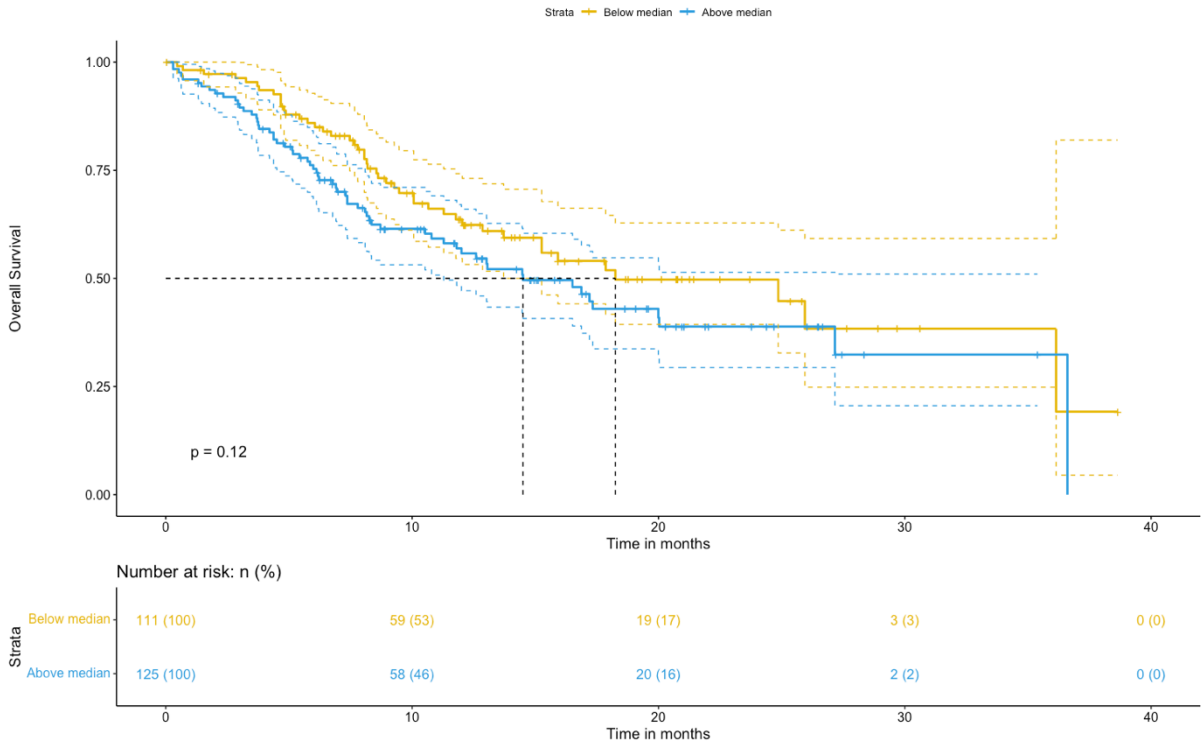
		Lower	Upper			Lower	Upper	
Sex (Female vs Male)	0.905	0.586	1.398	0.653	1.013	0.608	1.686	0.96
Liver Metastasis	1.538	0.929	2.544	0.094	1.102	0.611	1.987	0.748
Squamous vs non squamous	1.347	0.729	2.488	0.341	1.993	1.02	3.892	0.044
Bone metastasis	0.911	0.595	1.393	0.666	0.67	0.417	1.075	0.097
ECOG PS	1.982	1.124	3.496	0.018	2.068	1.099	3.891	0.024
LDH levels above ULN	1.834	1.133	2.969	0.014	1.93	1.185	3.144	0.008
logMTV	1.38	1.088	1.75	0.008	1.176	0.91	1.519	0.214

Figure 7 - Kaplan Meyer curves for Overall Survival according to total Metabolic Tumor Volume above or below median in ICBs (a), chemo-ICBs (b) or chemotherapy (c) groups.

a



b



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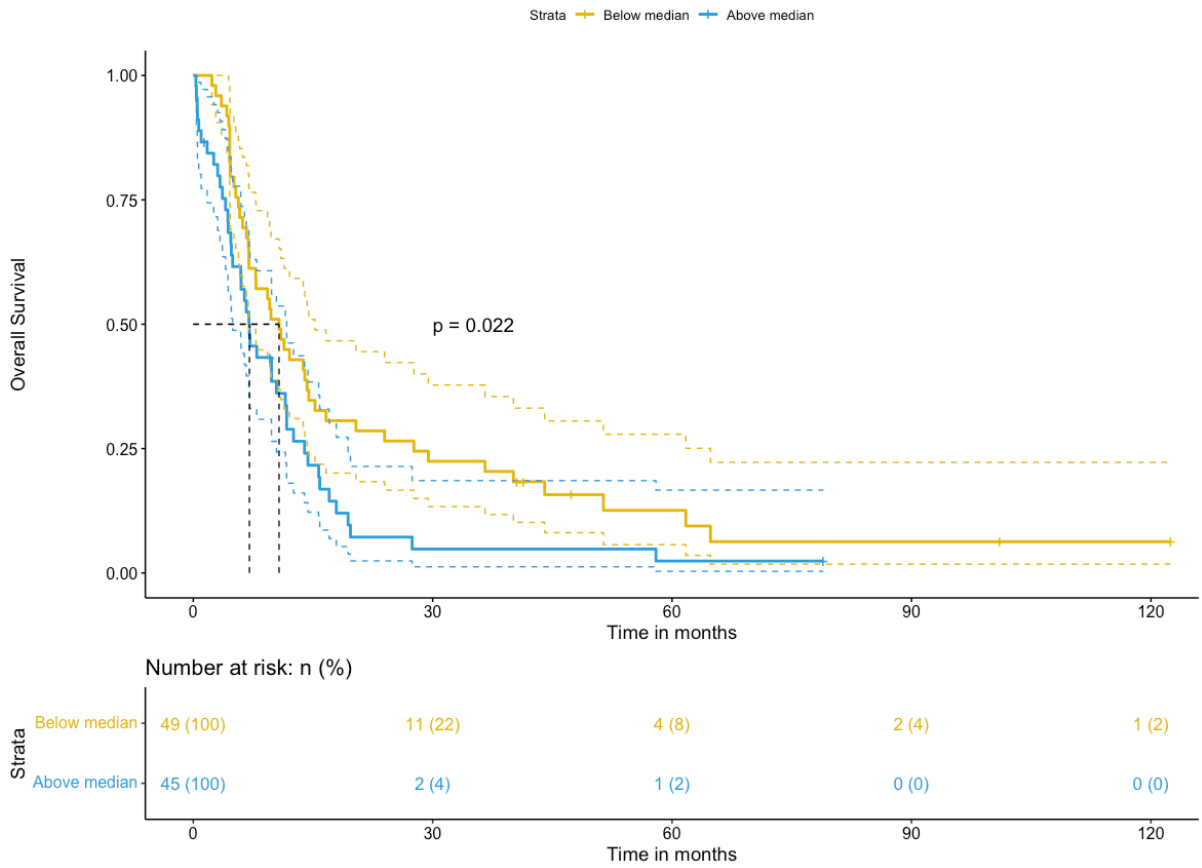
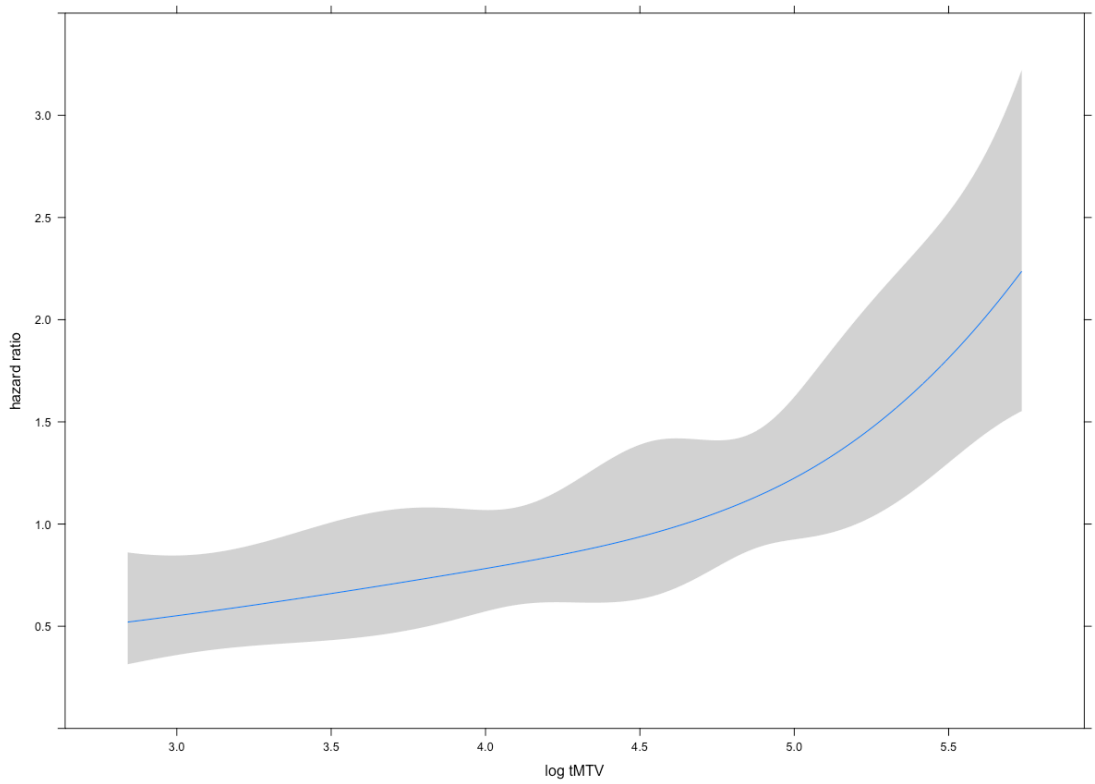


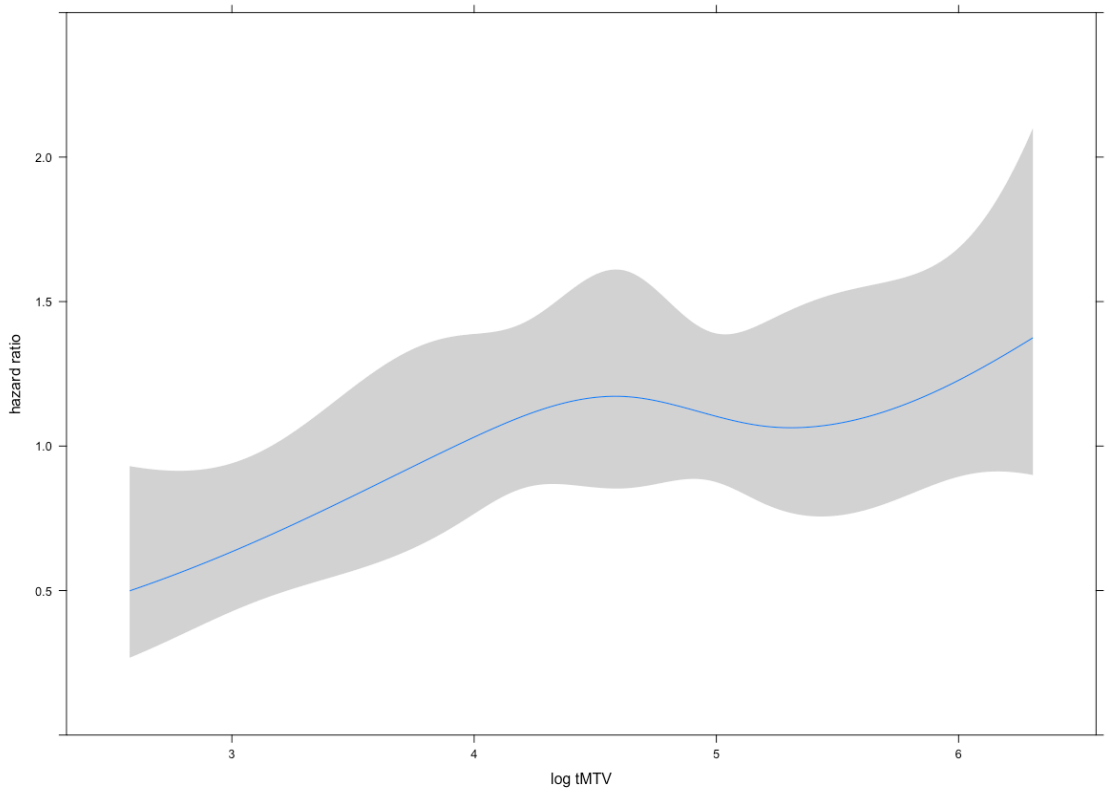
To better understand the relationship between tMTV and OS for ICBs alone and in combination, we used a restricted cubic splines approach to explore how different cutoff may affect the outcome of different treatments. The effect of tMTV log transformed (figure 8) appeared to differ between ICBs alone and in combination, with the former having a flat shape under the value of 4.5 and then having a sustained growth. The latter, on the other side, showed a rapid increase of the HR at the beginning, and then stabilized for higher volume.

Figure 7 – Restricted cubic splines plots for Overall Survival in patients treated with ICBs alone (a) and in combination with chemotherapy (b).

a



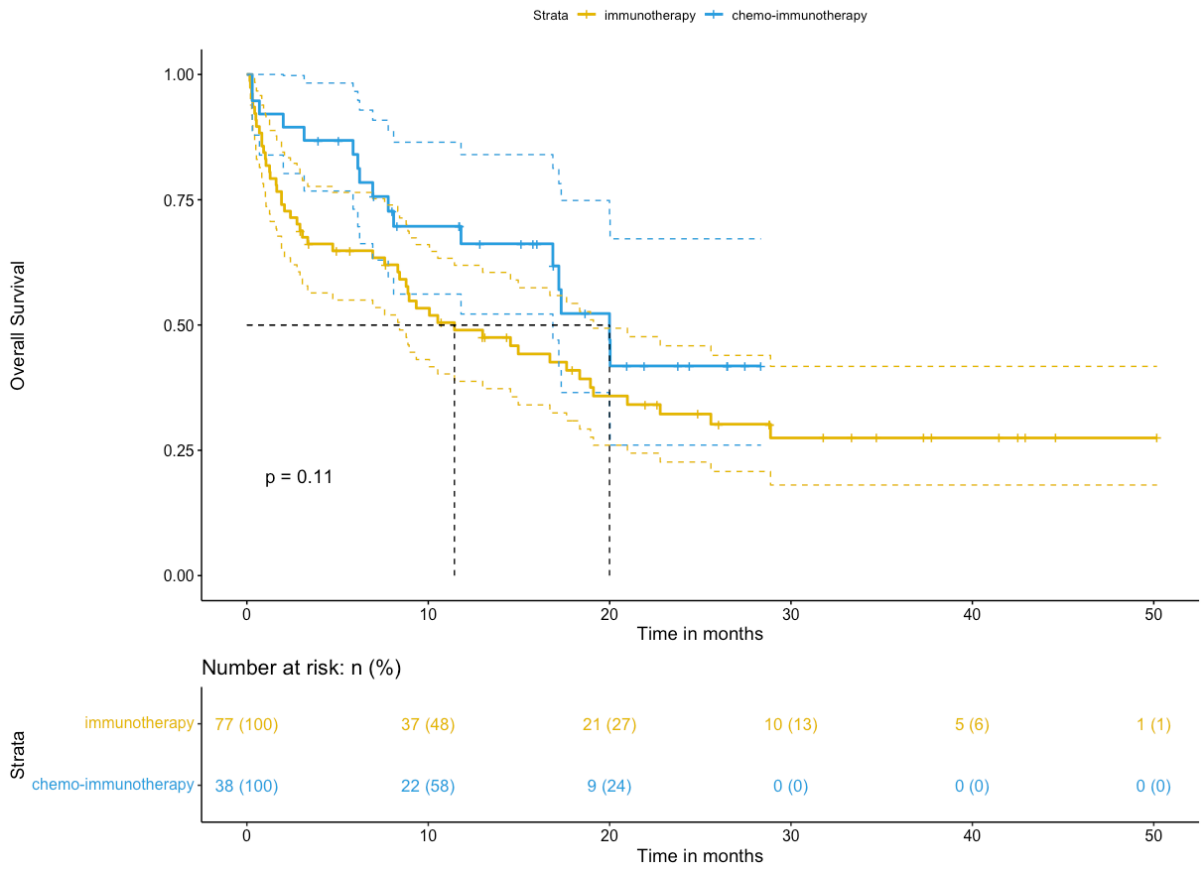
b



We then investigated the OS results of ICBs alone vs combination of chemo-ICBs in patients with PD-L1 ≥ 50 % according to tMTV above or below the median.

Patients treated with ICBs alone had a median OS of 11.4 months (95% CI 8.42 – 19.1) vs 20.0 months (95% CI 16.87 – NA) for the combination, p = 0.11 (figure 8).

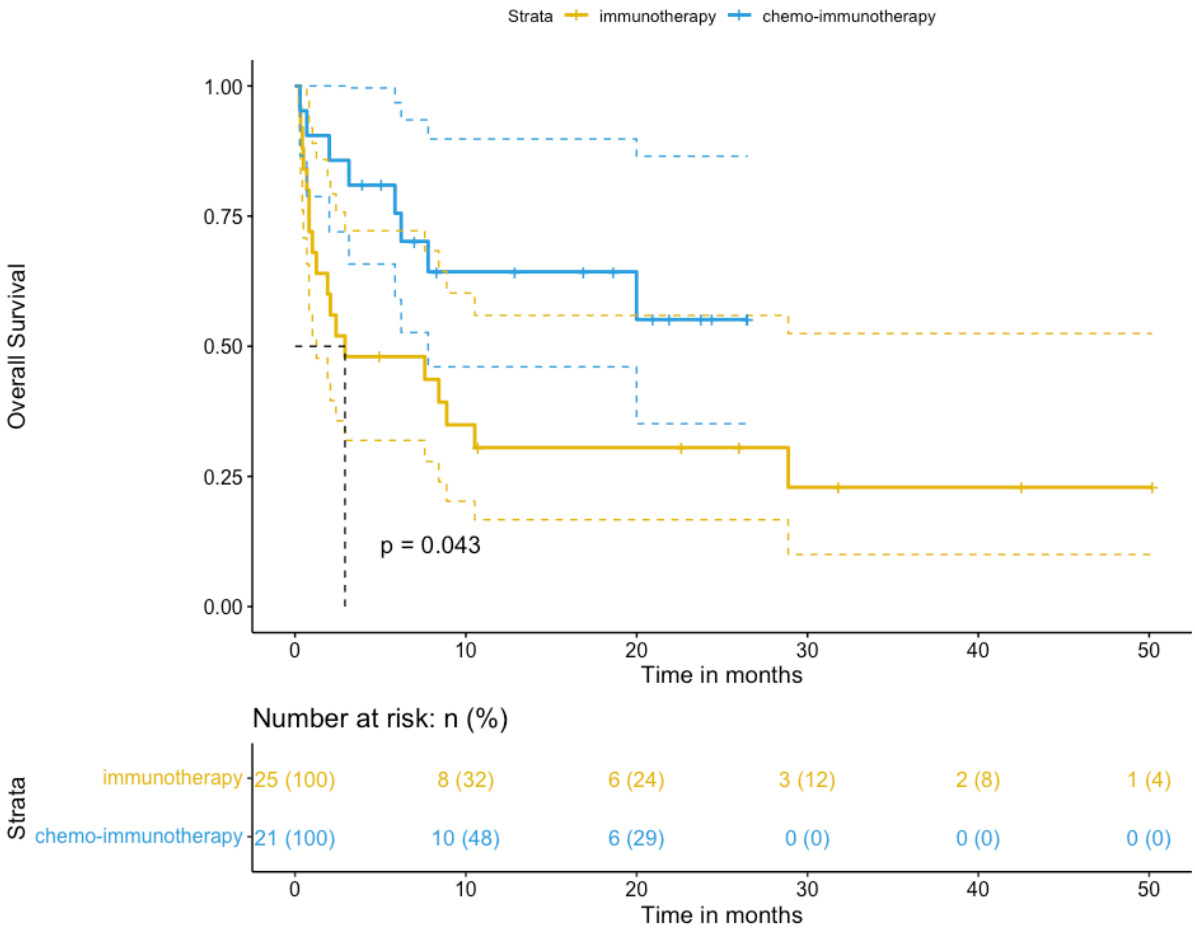
Figure 8 – Kaplan Meyer curve for Overall Survival in patients with PD-L1 ≥ 50% and tMTV > median.



When LDH and tMTV were combined, patients with the two factors had an median OS not reached for the combination of chemotherapy and ICBs (95% CI 7.79 – NA) vs 2.93 (95% CI 1.25 – NA) for ICBs alone, p = 0.043 (figure 9)

Finally, when comparing carboplatin to cisplatin no difference was seen for OS, independently from tMTV above (p = 0.82) or below the median (p = 0.4).

Figure 9 – Kaplan Meyer Curves for Overall Survival in patients with PD-L1 ≥ 50 % and tMTV above the median, LDH above ULN.



Discussion

This study was designed to evaluate the different prognostic value of total metabolic tumor volume in NSCLC according to the treatment administered, with the aim to understand how tMTV may be useful to discriminate between patients having good chances to benefit from ICBs alone and those who may need an escalating approach.

Our findings indicate thattMTV retains a strong, incremental and detrimental effect on the efficacy of ICBs when administered alone, both in terms of overall survival and progression free survival.

This is in line with previous evidences coming from smaller studies in different type of cancer, from NSCLC to melanoma to other malignancies[20].

All these studies, anyway, were focused on the prognostic effect with no or insufficient control group, and so it was not possible to estimate if an intensification strategy would allow to mitigate the effect of tMTV with the level of evidences provided.

Our study, conversely, showed that tMTV retains a marginal effect on PFS and no effect on OS when chemotherapy is added to ICBs. Moreover, comparing the two strategies in patients with PD-L1 $\geq$ 50% and high tMTV, an advantage in PFS and a numerically better median OS was seen, particularly in the first months of treatment where the negative phenomenon of hyper-progression may be experienced by ICBs treated patients, often leading to an early death. Of note, previous studies suggested that patients with high tMTV are at higher risk of experiencing HPD under ICBs monotherapy[21].

Different biological hypothesis may be done to explain the poor prognosis linked to this. Cancer cell has an highly inefficient anaerobic metabolism even in presence of oxygen, known as aerobic glycolysis (also termed the Warburg effect[22]). It drives rapid consumption of glucose and other nutrients, whereas waste products, i.e. lactate is released and accumulate in the tumor

micro environment (TME), contributing to local extracellular acidification. As tumors grow in size, oxygen perfusion can also become limited, creating regions of hypoxia. Under hypoxic conditions, anaerobic glycolysis also leads to accumulation of waste products as lactate. Together, these metabolic characteristics of cancer cells generate a nutrient-deficient, waste product-replete, acidic, hypoxic and generally immunosuppressive microenvironment[23]. Recent evidences shows also that, while lactate within the TME impairs the effector function of tumor infiltrating CD8 effector T cells[24], Treg cell suppressive function is maintained. Watson and colleagues found that Treg cells utilize lactate within the TCA cycle and generate phosphoenolpyruvate, a critical intermediate that can fuel intra-tumoral Treg cell proliferation in vivo. They are thus able to utilize 'alternative' metabolites present in the TME to maintain their suppressive identity, supporting the notion that tumors avoid immune destruction not only by depriving effector T-cells of essential nutrients, but also by metabolically supporting regulatory T cells[25].

The better efficacy of chemo ICBs combination in high tumor burden patients is also consistent with data about lower efficacy of ICBs when ctDNA plasma levels are higher, e.g. when tumor burden is bigger[26,27]. More recently, data has been presented on composite tumor fraction, a liquid biopsy-derived parameter that reflects the amount of ctDNA in the blood[28]. Authors has shown that patients with higher cTF (above the 10% threshold) may obtain a benefit from adding chemotherapy to ICBs [29].

This is in line with analysis coming from different tumors, such as BRAF mutant melanoma, where a sequence of induction treatment with BRAF/MEK inhibitors followed by immunotherapy by nivolumab plus ipilimumab showed interesting results int patients with high LDH and high number of metastasis.

Regarding LDH, an interesting finding of our study is the independency of prognostic effect of LDH and tMTV. LDH has been traditionally considered a surrogate marker of tumor burden,

albeit recent data suggest that its role may go well beyond this surrogacy[30]. Our data go in the same direction, suggesting that, despite a correlation between the two is apparent, this correlation is mild and the detrimental effect of LDH on ICBs efficacy is independent from tMTV. Further study should unveil the biological basis of this relationship.

Data about the platinum-containing compound in the chemo-ICBs cohort may be quite surprising, with carboplating being better than cisplatin in patients with high tMTV. Traditionally, cisplatin based regimens were shown to be associated with an higher response rate respect to carboplatin based ones[31]. Anyway, evidences from Checkmate 816 neoadjuvant study showed that carboplatin based chemotherapy in association with ICBs was associated with an higher proportion of pathological responses and with a more pronounced effect on disease free survival respect to cisplatin based[32]. This may be related to the concept of immunogenic cell death (ICD), that happens when cancer cell death culminate with adaptive immune responses that are executed by cytotoxic T lymphocytes and elicit immunological memory[33]. This has been described to be differently induced by the different platin compound, with cisplatin being less proficient inducer of ICD[34]. Therefore, our results could be interpreted in light of these evidences, suggesting that a combination of carboplatin based chemotherapy may give deeper responses in those patients with high disease burden that need a shrinkage in order to maximize the benefit from ICBs. Data on overall survival are negative for the distinction between carboplatin and cisplatin but this may also be due to the low number of events.

Among the strength of our study, the relatively high number of patients and centers included in the analysis allows a better generalizability of the findings.

In fact, albeit tMTV seemed to be the most robust volume estimation among PET derived parameters in terms of inter and intra operator reproducibility, and less dependent respect to

Total Lesion Glycolysis to time from radio-glucose administration and image capture, still concerns remained about the heterogeneity of the measures between different centers, software and machines.

Previous studies on tMTV as biomarker for ICBs used several different methods of tMTV calculation, based sometimes on a fixed absolute threshold of SUV(e.g. all voxel with SUV > 3), sometimes on a fixed relative threshold (a percentage of SUVmax of each lesion) or on the background (e.g. liver + 1 or 2 standard deviation) reduced again the reproducibility.

We chose a fixed relative threshold of SUVmax for each lesion, that allowed a simple and easy assessment and is as observer independent as possible, albeit it may lead to an underestimation in heterogeneous tumor and an overestimation in low signal to noise lesion (low tumor uptake and/or high background)[35].

Among the weakness, apart for the potential underestimation of tMTV in heterogeneous tumor due to the segmentation method, the retrospective nature may have led to some missing data. Moreover, as PET scan is not always performed routinely at diagnosis, it could have led to a potential bias in center selection. Anyway, the relatively large sample size is a way to overcome this limitation. The number of patients with PD-L1 $\geq$ 50% who received combination of chemotherapy and immunotherapy is limited (50 patients) but it is, at the best of our knowledge, the largest number reported.

Conclusion and perspectives

Our data confirm that metabolic tumor volume, a surrogate biomarker of tumor burden, is highly prognostic in patients treated with immune checkpoint blockers monotherapy, while its prognostic value is mitigated by the addition of chemotherapy.

In the case of PD-L1 high patients, a combination approach that includes chemotherapy along with ICBs may be a better choice when tMTV is high.

In perspective, several other questions are needed to be answered to fully understand the relationship between tumor burden, measured by tMTV or other techniques, and the immune system.

The first one is the dynamic process that lays behind it. Conceptually, in fact, ICBs act on the immune system that, differently from other treatment, is already there when tumor start its grow. So, when a small tumor is detected, we do not know whether it is small because it has been detected earlier or because the immune system is already active, preventing it from growing further, two biological situations that may be quite different in terms of ICBs efficacy. To explore this potential dynamic biomarker linked to tumor burden, further studies are needed using imaging (for example with tumor growth rate analysis) or other blood based biomarkers such as liquid biopsy parameters.

Secondly, the heterogeneity of measurement could be overcome by an artificial intelligence approach, that could be more reproducible and allow to establish a cutoff to optimize the biomarker's predictive value. Moreover, such an approach could be also used to derive the same measurement by other more diffused technique, such as CT scans.

Finally, the relationship between tumor burden measured by imaging and other blood biomarkers such as LDH and ctDNA are still to be unveiled, potentially driving to a better

stratification and a deeper understanding of the biological bases of the relationship between metabolism, tumor size and shedding.

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