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Early serum tumor marker dynamics predict progression-free and overall survival in single PD-1/PD-L1 inhibitor treated advanced NSCLC—A retrospective cohort study☆,☆☆

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ABSTRACT

Objectives: To evaluate serum tumor markers (STM) as biomarkers for treatment monitoring and prognosis in advanced non-small cell lung cancer (NSCLC) treated with single-agent PD-1/PD-L1-directed immune checkpoint inhibitor (ICI) therapy.

Materials and Methods: Carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), cytokeratin-19 fragments (CYFRA 21-1) and neuron specific enolase (NSE) were routinely measured at NSCLC diagnosis, initially elevated markers were used for follow-up. Leading STM change between ICI initiation and first subsequent restaging as well as corresponding computed tomography evaluations according to response evaluation criteria in solid tumors (RECIST) were retrospectively analyzed regarding progression-free (PFS) and overall survival (OS). In uni- and multivariate stepwise Cox-regression analyses, STM and RECIST response were analyzed for their impact on PFS and OS together with other known prognostic patient and tumor characteristics.

Results: Among 84 patients (61% men, mean age 68 years), median PFS was significantly ($p < 0.001$) longer, when STM decreased (11 M (7,19) N = 37) than in case of increases (< 2-fold: 6 M (3,8) N = 31; ≥2-fold: 2 M (1,2) N = 16). Patients with initial STM decrease had longer ($p < 0.001$) median OS (not reached) than with STM increase (< 2-fold: 14 M (12,26); ≥2-fold: 4 M (3,7)). Patients with stable or progressive disease by RECIST and concomitant STM decrease had longer ($p < 0.001$) PFS and OS (8 M (4,14) and 18 M (10,n.e.) N = 24) than upon STM increase (PFS: 2 M (2,4); OS: 10 M (6,13) N = 42). Significant impact on PFS was shown for STM response ($p < 0.001$), RECIST response ($p = 0.003$) and PD-L1 status ($p = 0.003$). For OS, STM response ($p < 0.001$), presence of cerebral metastases ($p = 0.036$) and therapy line ≥3 ($p = 0.001$) were identified.

Conclusion: Decreasing leading STM at first restaging predict longer PFS and OS and identify patients with favorable outcomes among initial radiological non-responders in ICI treated NSCLC patients.

1. Introduction

Immune checkpoint inhibitor (ICI) therapy, especially programmed death-ligand 1 (PD-L1) or programmed cell death protein 1 (PD-1) inhibition, has revolutionized the therapeutic landscape in advanced non-small cell lung cancer (NSCLC). Still, only a minority benefits from such therapies and individual patient response is hard to foresee. Extensive research effort is currently being made to identify predictive biomarkers. Immunohistochemical staining for PD-L1 status has been used to predict response to ICI therapies from the first clinical trials on, but also PD-L1 negative patients may benefit and a positive status does not guarantee a therapeutic effect [1,2]. Also, more elaborate composite biomarkers like the Teff score reflecting effector T-cell gene expression have been proposed [3]. More recently, tumor mutational burden

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(TMB) measured in tumor tissue or peripheral blood has been introduced, identifying a partly different ICI-responsive patient collective. However, due to high cost and current lack of a consensus on methods, TMB is not yet widely available in clinical practice [1,2,4,5]. Other reported predictors of response to ICI in NSCLC include Eastern Cooperative Oncology Group (ECOG) performance status, routine laboratory parameters like lactate dehydrogenase and peripheral blood cell counts [6,7]. Recent research results suggest, that rather than single biomarkers, composite approaches may be the future of response prediction to ICI therapies: A currently proposed model relies on the genetic tumor profile reflected by TMB as well as on the immune phenotype, referring to the presence and reactivity of T-cells within the tumor. TMB is usually high in the context of increased neoantigen-formation, whereas immunosuppressant tumor mechanisms like PD-L1 expression or indoleamine 2,3-dioxygenase (IDO) may be surrogate parameters of enhanced T-cell inflammation [4].

Notably, some authors have reported on a prognostic relevance of baseline serum tumor marker (STM) concentrations like carcinoembryonic antigen (CEA) or cytokeratin-19 fragments (CYFRA 21-1) in retrospective study settings in ICI treated patients [7,8]. More recently, Dal Bello et al. reported, that the also the dynamics of STM, especially decreases in CYFRA 21-1 and CEA under treatment with nivolumab might a predictor of therapy efficacy [9,10]. Analyzing STM is not routinely recommended in either diagnosis or follow-up of NSCLC [1]. However, besides the aforementioned studies on ICI treated patients, there is evidence that tumor markers like CEA, CA19-9 and CYFRA 21-1 can be used in treatment monitoring and longitudinal follow-up in patients undergoing chemotherapy or target therapy [11,12]. Ideal biomarkers should be easily obtainable by peripheral blood draw, inexpensive to analyze and allow serial measurement for longitudinal observation [2], all of which applies to STM. We have used STM as additional longitudinal biomarkers of disease activity in NSCLC for several years and have experienced, that STM provide clinically relevant information especially when radiological examination results leave uncertainty. In ICI therapies, we have seen distinct and especially sustained tumor responses, when STM had decreased upon first restaging [13]. Based on such observations, this analysis aimed to examine whether STM response at first restaging had implications on overall- (OS) and progression-free survival (PFS) compared to computed tomography (CT) evaluation in patients with advanced NSCLC treated with PD-1/PD-L1 directed ICI therapies.

2. Methods

This study was conducted in accordance with the guidelines for the REporting of tumor MARKer studies (REMARK) [14]. We retrospectively screened all patients having received PD-1/PD-L1-directed ICI therapy for advanced (all stage IV; stage III if not otherwise treatable, e.g. by radiotherapy) NSCLC at the lung cancer unit of the Kepler University Hospital in Linz, Austria. Patients were included in this analysis, if they had received more than one cycle of nivolumab, pembrolizumab or atezolizumab and had undergone a CT restaging afterwards. We excluded patients in clinical trials, on ICI/chemotherapy or ICI/ICI combinations and patients, who received ICI in named patient use programs for thoracic malignancies other than NSCLC.

Patients were retrospectively followed from ICI therapy initiation to death or censored at the date of last verified contact before the data cut at the end of July 2018. The time of disease progression was retrospectively defined, considering notes by the treating physicians and imaging. ICI treatment beyond radiological progression was conducted in selected cases with significant clinical benefit. Restaging was routinely conducted after every four cycles of nivolumab or three cycles of pembrolizumab or atezolizumab, equaling an interval of 8 or 9 weeks. Examinations could be preponed due to increasing symptoms suggesting disease progression. Radiological response was routinely assessed by a CT scan of the chest and the upper abdomen using iodinated contrast medium unless contraindicated and additional imaging like cerebral magnetic resonance tomography if necessary, according to the clinician's judgement. For this study, radiological response was reevaluated by an expert thoracic radiologist and graded by response evaluation criteria in solid tumors (RECIST) for first response (CR-complete remission, PR-partial remission, SD-stable disease, PD-progressive disease) at the first restaging as well as for the best response [15]. The same analyses were also conducted for immunotherapy adapted criteria (iRECIST) [16]. Routine blood sampling at primary lung cancer staging at our center includes analysis of CEA, CYFRA 21-1, carbohydrate antigen 19-9 (CA19-9) and neuron specific enolase (NSE). STM are not analyzed in hemolyzed blood samples according to the established standard laboratory proceedings. All STM initially elevated above the upper limit of normal are then used as longitudinal parameters, being analyzed at every restaging together with imaging. For the reported cohort, STM analyses were conducted using a cobas e 801 immunoassay module (Roche Diagnostics, Rotkreuz, Switzerland) and the corresponding ElectroChemiLuminescence-ImmunoAssay (ECLIA) kits acquired from Roche. Upper limits of normal were 3.4 ng/ml for CEA, 3.3 ng/mL for CYFRA 21-1, 27U/mL for CA 19-9, and 16.3 ng/mL for NSE. We defined a maximum of 14 days between STM analysis and the corresponding CT scan acceptable for inclusion. If more than one STM was available upon ICI initiation, the "leading" marker with the highest elevation in relation to the upper limit of normal was selected. If none of the analyzed STM was elevated at ICI therapy initiation, the leading STM was determined as the marker with the highest value in relation to the upper limit of normal. STM response was expressed as fraction of restaging versus baseline concentration. To estimate the prognostic yield of both baseline STM and STM dynamics, we conducted receiver operating characteristic curve (ROC) analyses for progression/survival and for survival for every single STM as well as for the model of a leading STM. Sensitivity and specificity analyses were calculated for baseline STM ($<$ upper limit of normal, $\geq$upper limit of normal, $\geq$2-fold, $\geq$4-fold, $\geq$8-fold) as well as for STM dynamics (STM decrease with respect to baseline STM level, STM increase $<$ 1.25-fold, $\geq$1.25 fold, $\geq$2-fold), the predictive power of the respective STM model was estimated by the area under the ROC curve.

Kaplan-Meier-analyses for PFS and OS were conducted according to the relative change in the leading STM (decrease, increase $<$ 2-fold, increase$\geq$2-fold) as well as to radiological change using RECIST criteria (CR/PR, SD, PD). Results were expressed as median in months (M) and 95% confidence interval unless otherwise specified. The resulting survival curves were compared statistically using the log rank test, whereas a p-value $<$ 0.05 was regarded statistically significant. Evaluation of predictive factors for progression/death and death was conducted applying univariate and step-wise multivariate Cox-regression analyses. Variables analyzed in these models were age in groups ($<$ 70, $\geq$70 years), sex, smoking status ($<$, $\geq$5 pack years), histological subtype (adeno-, squamous-cell carcinoma), presence of brain metastases, palliative therapy line (1/2, $\geq$3), TNM stage (III, IV) [17], ECOG (0, 1/2) and presence of a targetable mutation. Co-morbidities were assessed by the Charlson Comorbidity Index (CCI) [18], whereas a cutoff value $\geq$3 was determined for separation of patients with little versus extensive burden of comorbidity [19–21]. Additionally, PD-L1 status (positive, negative) assessed with a 22C3 assay for Autostainer Link 48 by Dako (Agilent Technologies, Santa Clara, CA), STM response (decrease, increase $<$ 2-fold and $\geq$2-fold) and RECIST response (CR/PR, SD/PD) were included.

3. Results

Eighty-four patients with ICI treatment initiation between August 2015 and May 2018 met the predefined requirements to be included in the analysis. Baseline patient and tumor characteristics are shown in Table 1. Mean STM baseline values and STM dynamics are depicted in

Table 1
Baseline patient and tumor characteristics. Figures are given as N (%) unless otherwise specified. SD = standard deviation, ECOG = Eastern Cooperative Oncology Group, BMI = body mass index, ICI = Immune checkpoint inhibitor, py = pack years, py = pack years, TKI = tyrosine kinase inhibitor, BSC = best supportive care, CRP = C-reactive protein, LDH = lactate dehydrogenase, TNM = TNM Classification of Malignant Tumours, EGFR = epidermal growth factor receptor, ROS1 = Proto-oncogene tyrosine-protein kinase ROS, PD-L1 = Programmed death-ligand 1, STM = serum tumor marker, CEA = Carcinoembryonic antigen, CYFRA 21-1 = cytokeratin-19 fragments, NSE = neuron specific enolase, CA19-9 = carbohydrate antigen 19-9.

Baseline Patient and Tumor Characteristics (N = 84)	
Age (years, mean (SD))	68 (9.9)
Age range (years)	46-90
ECOG Performance Status	
0	29 (35)
1	54 (64)
2	1 (1)
BMI (kg/m^2, mean (SD))	24.9 (4.4)
Male Sex	51 (61)
ICI Substance	
Nivolumab	33 (39)
Pembrolizumab	38 (45)
Atezolizumab	13 (16)
Smoking status	
Never/≤5py	12 (14)
> 5py	72 (86)
Total py (mean (SD))	45 (29)
Therapy line (palliative)	
1	32 (38)
2	34 (41)
3 or higher	18 (21)
Previous therapy (n = 52)	
Platinum-based doublet chemotherapy	37 (71)
Docetaxel	7 (13)
TKI	8 (15)
Subsequent therapy	
Docetaxel	13 (15)
TKI	12 (14)
other	10 (12)
none/BSC	49 (58)
Charlson Comorbidity Index ≥3	13 (15)
Laboratory parameters (mean (SD))	
Lymphocyte count (G/L)	1.6 (1.9)
CRP (mg/dL)	2.8 (3.8)
LDH (U/L)	264.5 (298.9)
Histology	
Adenocarcinoma	47 (56)
Squamous cell carcinoma	37 (44)
TNM stage	
III	13 (15)
IV	71 (85)
Cerebral metastases	17 (20)
Targetable Mutation	
EGFR	4 (5)
ROS1	2 (2)
PD-L1 status	
not available	8 (10)
positive	49 (58)
negative	27 (32)
PD-L1 expression	
not available	8 (10)
< 1%	27 (32)
1-50%	29 (35)
≥50%	19 (23)
positive, not quantifiable	1 (1)
Leading STM	
CEA	44 (53)
CYFRA 21-1	32 (38)
NSE	1 (1)
CA 19-9	7 (8)
STM concentrations (mean (SD))	
CEA (ng/mL)	99.9 (491.8)
CYFRA 21-1 (ng/mL)	11.9 (13.4)
NSE (ng/mL	16.3 (9.5)
CA 19-9 (U/mL)	96.2 (167.4)

Table 2
Figures are given as median in months (95% confidence interval) or N (%), p is for comparison of survival curves by log rank test, n.r. means the median has not been reached. n.e. = not evaluable, PFS = progression-free survival, OS = overall survival, STM = serum tumor markers, RECIST = response evaluation criteria in solid tumors, CR = complete remission, PR = partial remission, SD = stable disease, PD = progressive disease.

Median PFS and OS according to STM and RECIST first response (N = 84)			
	N (%)	PFS (months)	OS (months)
STM response		**p < 0.001**	**p < 0.001**
Decrease	37 (44)	11 (7,19)	n.r.
Increase (< 2x)	31 (37)	6 (3,8)	14 (12,26)
Increase (≥2x)	16 (19)	2 (1,2)	4 (3,7)
RECIST response		**p < 0.001**	**p < 0.001**
CR/PR	18 (21)	12 (6,n.e.)	n.r.
SD	31 (37)	8 (5,11)	18 (12,n.e.)
PD	35 (42)	2 (2,3)	9 (4,12)

Tables 1 and 2. Most frequently identified leading STM were CEA (53%) and CYFRA 21-1 (38%). CA19-9 was leading STM in 8% of cases, NSE in one patient (1%). This individual also displayed an elevated CYFRA 21-1 level at baseline decreasing in parallel with NSE upon the first restaging. In eight cases, no STM analyzed was elevated at the time of ICI initiation. Among those, in three patients with a leading STM of CEA and no other available STM, CEA increased within the normal range in 2 cases (best radiological response for both: PD) and decreased in one case (best radiological response: SD). Of 5 patients with non-elevated CYFRA 21-1 as leading STM, there were four consecutive increases above the upper limit of normal (best response: 1 SD, 1 PR, 2 PD) and one decrease (best response: PD). Two of those cases had concomitant CEA dynamics also within the normal range. ROC analyses revealed the best predictive power of baseline STM levels for both death and progression/death for CYFRA 21-1 (AUC death: 68.9%, progression/death: 71.8%) and for CA19-9 (AUC: 64.7% and 67.6%), followed by CEA (AUC: 54.6% and 51.1%), and NSE (AUC: 53.2% and 44.1%). In terms of STM dynamics, CEA (AUC death: 67.7%, progression/death: 64.7%) and CYFRA 21-1 (AUC: 60.4% and 58.1%) were superior predictors as compared to CA19-9 (AUC: 53.9% and 48.1%). Concerning NSE, the number of measurements was too small for a valid ROC analysis of STM dynamics. The model of a leading STM surpassed the power of single STM analyses for STM dynamics (AUC death 70.3%, progression/death: 72.3%) but not for baseline STM (AUC: 60.4% and 57.2%). (Supplementary Tables 1–3, supplementary Figures 1–4)

During the observational period, disease progression was evident in 63 (75%) patients, while 38 (45%) died. Overall median PFS was 6 M (4,7), median OS was 14 M (11,26). At first restaging, STM had decreased in 37 (44%), increased by < 2-fold in 31 (37%) and by ≥2-fold in 16 (19%) patients. Applying RECIST criteria, CR or PR was present in 18 (21%), SD in 31 (37%) and PD in 35 (42%) individuals. Best response was CR/PR in 25 (30%), SD in 25 (30%) and PD in 34 (40%). All response analyses were also calculated using iRECIST criteria, however with no relevant difference in results. Nineteen out of 37 (51%) patients with STM decrease reached a radiological best response of PR/CR. Among individuals with STM more than doubled, one out of 16 (6%) had a radiological best response of PR. One out of 35 individuals (3%) with initial PD according to RECIST later reached a best response of PR, suggesting the only case of radiologically evident pseudo-progression in the collective.

Both median PFS and OS significantly ($p < 0.001$) differed between the specified STM and RECIST response categories (Table 2, Fig. 1). When separating individuals with radiological responses (PR/CR) from stable or progressive disease (SD/PD), both groups showed a significantly ($p < 0.001$) better median PFS and OS, when STM concurrently decreased. In several well-responding subgroups (CR/PR + STM decrease, CR/PR + STM decrease and increase), median PFS and

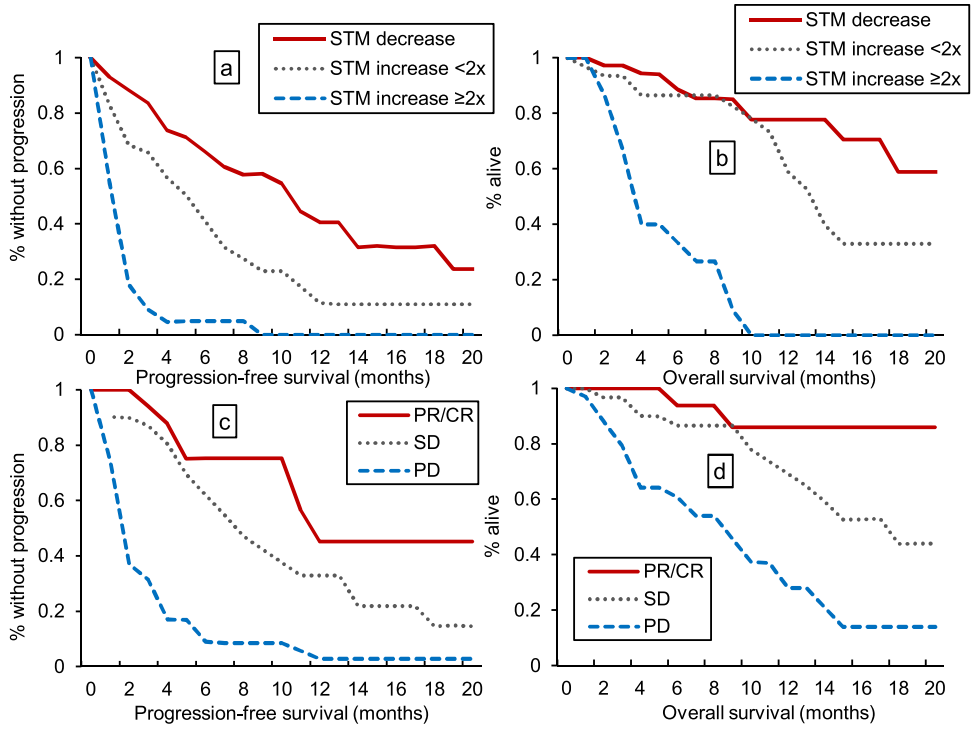

Fig. 1. Kaplan-Meier curves for progression-free and overall survival according to STM dynamics (a,b) and RECIST (c,d). STM = serum tumor markers, PR = partial remission, CR = complete remission, SD = stable disease, PD = progressive disease.

Table 3
Median PFS and OS according to STM and RECIST response categories. Figures are given as median in months (95% conficence interval) or N (%), n.r. means the median has not been reached. n.e. = not avaluable, PFS = progression-free survival, OS = overall survival, STM = serum tumor markers, RECIST = response evaluation criteria in solid tumors, CR = complete remission, PR = partial remission, SD = stable disease, PD = progressive disease.

REC IST	IST STM	CR/PR PFS	OS	SD/PD PFS	OS
Decrease N = 35 (42)		**n.r.** N = 13 (15)	**n.r.**	**8** (4,14) N = 24 (29)	**18 (10,n.e.)**
Increase N = 47 (56)		**6 (3,n.e.)** N = 5 (6) N = 18 (21)	**n.r.**	**2 (2,4)** N = 42 (50) N = 66 (79)	**10** (6,13)

OS could not be calculated, as the number of patients having progressed or died was too small. (Table 3, Fig. 2)

Univariate and stepwise multivariate Cox-regression analyses for variables influencing PFS and OS are depicted in Table 4. Multivariate analyses revealed a significant effect on PFS (hazard ratio (HR) for progression/death) for leading STM response ($p < 0.001$), RECIST response ($p = 0.003$) and PD-L1 status ($p = 0.003$). Analogously for OS (HR for death), STM response ($p < 0.001$), presence of cerebral metastases ($p = 0.036$) and therapy line ≥ 3 ($p = 0.001$) prove significant.

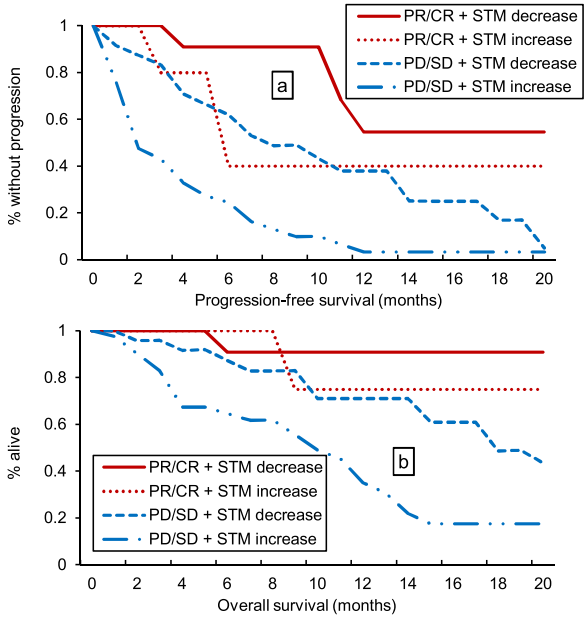

Fig. 2. Kaplan-Meier curves of progression-free (a) and overall survival (b) according to RECIST (PR/CR vs. SD/PD) and STM response (decrease vs. increase). STM = serum tumor markers, PR = partial remission, CR = complete remission, SD = stable disease, PD = progressive disease.

4. Discussion

This analysis suggests that the early change of the leading STM out of a panel of CEA, CYFRA21-1 and CA19-9 is predictive of PFS and OS in single-agent PD-1/PD-L1-directed ICI treated NSCLC. NSE was only found to be the leading STM in one patient and its baseline concentrations did not provide meaningful predictive power. The combination of STM response with RECIST assessment of restaging CT scans showed consistent results, especially in the clinically highly relevant group of patients with initial radiological SD or PD, where a concomitant STM decrease predicted significantly superior PFS and OS as compared to patients with initial STM increase.

There is a clinical need for additional biomarkers of disease activity and prediction of prognosis next to imaging in ICI treated advanced NSCLC patients. On the one hand, radiological responses may be delayed and phenomena like pseudo-progression upon ICI therapy do exist [1,22]. Also, selected patients may benefit from ICI treatment beyond progression [23]. On the other hand, timely determination of progressive disease is essential, as no time should be lost to ineffective

Table 4
Uni- and stepwise multivariate analyses for progression/death and death. Figures are given as hazard ratio (95% confidence interval). PFS = progression-free survival, OS = overall survival, HR = hazard ratio, CI = Confidence Interval, STM = serum tumor marker, PD-L1 = Programmed Death-Ligand 1, RECIST = response evaluation criteria in solid tumors, SD = stable disease, CR = complete remission, PR = partial remission, PD = progressive disease, ECOG = Eastern Cooperative Oncology Group, BMI = body mass index, CRP = C-reactive protein, LDH = lactate dehydrogenase.

Variable	PFS (HR for progression/death)				OS (HR for death)			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age (≥70 vs, < 70 years)	1.178 (0.712 – 1.949)	0.523			1.168 (0.614 – 2.222)	0.636		
Sex (male vs. female)	**2.061 (1.187 – 3.577)**	**0.010**			1.502 (0.761-2.965)	0.241		
Smoking status (≥5py vs. < 5py)	1.067 (0.505 – 2.256)	0.857			1.363 (0.524 – 3.548)	0.526		
STM dynamics (vs. STM decrease)		**< 0.001**		**< 0.001**		**< 0.001**		**< 0.001**
STM icrease < doubled	**2.741 (1.493 – 5.030)**	**0.001**	1.826	0.076	2.182 (0.948-5.023)	0.067	1.576	0.305
STM increase ≥ doubled	**14.448 (6.281 – 33.234)**	**< 0.001**	**9.075**	**< 0.001**	**16.464 (6.271-43.226)**	**< 0.001**	**21.123**	**< 0.001**
Histological subtype (Squamous-cell vs. Adenocarcinoma)	1.207 (0.729 – 1.997)	0.465			1.015 (0.526 – 1.958)	0.965		
PD-L1 status (negative vs. positive)	**2.286 (1.296 – 4.032)**	**0.004**	2.432	**0.003**	1.885 (0.933 – 3.807)	0.077		
Radiological dynamics (RECIST first response)		**< 0.001**		**0.003**		**< 0.001**		
SD (vs. CR/PR)	1.915 (0.813 – 4.512)	0.137	1.677	0.252	3.037 (0.677-13.615)	0.147		
PD (vs. CR/PR)	**6.915 (3.024 – 15.813)**	**< 0.001**	**4.139**	**0.002**	**10.195 (2.394 – 43.420)**	**0.002**		
Cerebral Metastases (yes vs. no)	0.997 (0.540 – 1.842)	0.993			**0.692 (0.288 – 1.661)**	**0.41**	**2.870**	**0.036**
Therapy line ≥3 (vs. 1/2)	**2.189 (1.227—3.905)**	**0.008**			**2.292 (1.514 – 5.613)**	**0.001**	**3.409**	**0.001**
Tumor stage (IV vs. III)	1.406 (0.666 – 2.969	0.372			1.406 (0.666 – 2.969	0.372		
ECOG (0 vs. 1/2)	1.267 (0.752 – 2.136)	0.374			0.866 (0.426 – 1.757)	0.689		
Targetable genetic alteration (no vs. yes)	1.710 (0.678 – 4.311)	0.256			1.991 (0.603 – 6.568)	0.258		
BMI (kg/m^2)		0.604				0.655		
≥20 < 25 (vs. < 20)	1.143 (0.531-2.461)	0.733			0.618 (0.235 – 1.628)	0.33		
≥25 < 30 (vs. < 20)	0.768 (0.344 – 1.711)	0.518			0.565 (0.211 – 1.514)	0.256		
≥30 (vs. < 20)	1.137 (0.461 – 2.804)	0.780			0.512 (0.155 – 0.1688)	0.271		
Charlson index ≥3 (vs. < 3)	1.238 (0.609 – 2.518)	0.349			1.505 (0.532 – 4.254)	0.441		
Absolute lymphocyte count (G/L)		0.106				0.146		
< 1 (vs. ≥ 2)	1.018 (0.501 – 2.066)	0.961			1.972 (0.764 – 5.087)	0.160		
< 2 (vs. ≥ 2)	0.585 (0.304 – 1.127)	0.109			1.036 (0.407 – 2.635)	0.941		
CRP (mg/dL)≥0.5 (vs. < 0.5)	1.356 (0.720 – 2.551)	0.346			0.956 (0.434 – 2.102)	0.910		
LDH (U/L) ≥248 (vs. < 248)	0.990 (0.532 – 1.845)	0.975			1.086 (0.505 – 2.336)	0.832		

therapies, being neither beneficial nor cost-effective in most patients. In that context, our observation that patients with leading STM more than doubled (19%) have distinctly inferior PFS and OS resembles recent observations on hyper-progressive disease upon PD-1/PD-L1 inhibitor therapy as reported by Ferrara et al. in approximately 14% of patients [24]. Recently published data by Dal Bello et al. show, that CEA and CYFRA 21-1 change in parallel with radiological tumor response in nivolumab treated NSCLC patients, while NSE did not provide similar results. A decrease in CEA and CYFRA 21-1 of ≥20% was associated with radiologically assessed disease control rate, PFS and OS [9,10]. The similarity of the published results support the findings presented in this paper. Our reported cohort contains a slightly higher number of patients, also treated with other PD-1/PD-L1 inhibitors, e.g. pembrolizumab and atezolizumab. Also, we used CA19-9 as an additional STM and proposed the model of a leading STM out of an initially assessed STM panel. We believe, that STM elevated at the time of diagnosis are more likely to be associated with tumor activity, while values within or near the normal range may often be unspecific. NSE it is being analyzed in our routine STM panel upon primary lung cancer diagnosis with special regard to neuroendocrine tumors, where data suggests a higher value than for NSCLC [25]. Like also suggested by Dal Bello et al. [10], we do not regard NSE as an adequate tumor marker for the monitoring of NSCLC under ICI therapy. In our presented dataset, only one patient had NSE as a leading STM (with concomitantly elevated CYFRA 21-1).

Our study has several limitations, mainly its retrospective design and its limited patient number. Nevertheless, the distinct differences between the subgroups identified by STM dynamics strongly suggest a significant prognostic impact of these biomarkers. A general limitation of STM measurement is that none of the tested markers is specific to a certain cancer entity. Also, various chronic or acute conditions apart from malignancies may influence STM concentrations [26,27]. Another limitation may be, that although a panel of four STM was initially assessed, the leading STM was not elevated in a considerable fraction of patients (n = 8; 9.5%). Whether the predictive value of the leading STM differed in those cases cannot be comprehensively assessed due to the low absolute number of cases. Obviously, STM change can only be measured retrospectively after a patient has already been treated with ICI therapy, while biomarkers like PD-L1 status or TMB allow an a priori prognosis. Previously published data [7,8], and our presented ROC analyses show that baseline STM may provide some prognostic information. A limitation of the reported ROC analysis however is, that it only accounts for progression/death or death, respectively, not considering the time to the event.

At present, we do not suggest, that baseline STM should be used as a priori predictive biomarkers to guide therapy decisions. Current treatment guidelines depict clear indications for all available therapeutic options, so there is no clinical need for another baseline biomarker now. Future research may however provide further knowledge on molecular profiling of the tumor genotype and the immunological status [4], where perhaps baseline STM may play a role as one factor in panel of predictive biomarkers. At the moment however, rather than for baseline biomarkers, there is a need for additional dynamic parameters to be measured longitudinally throughout therapy that can supplement radiological re-staging examinations in unclear situations. Clearly, we do not suggest the application of STM analyses over the undisputed gold standard, which remains CT imaging. As shown by our data, initial radiological tumor regression is a sufficiently robust predictor or favorable patient outcomes with only little (but present) additional prognostic benefit of a concurrent STM decrease. The true highlight of our reported findings is the significantly improved PFS and OS that comes with STM decrease in the group of patients with initial SD or PD, as these are the very cases, where the treating physician needs additional biomarker information. Whether STM analyses can ultimately help clinicians overcome the challenges facing indeterminate initial radiological response to ICI therapy, needs to be elucidated in future larger-scale prospective study settings.

We conclude that STM, especially CEA, CYFRA 21-1 and CA19-9 should be used for treatment monitoring in ICI treated NSCLC patients. Particularly in patients with indeterminate radiological response or progressive disease upon initial restaging, STM could aid the decision whether to continue ICI treatment or to switch the therapeutic regimen.

Conflicts of interest statement

DL: received travel/accommodation funding from Roche and Merck Sharp & Dohme

AH: received travel/accommodation funding from Roche

EB: received speakers fees from and served as consultant/advisor to Roche, Merck Sharp & Dohme, Bristol-Myers Squibb; received travel/accommodation funding from Roche, Merck Sharp & Dohme

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Appendix A. Supplementary data

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