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Tumor glycolytic profiling through ^{18}F-FDG PET/CT predicts immune checkpoint inhibitor efficacy in advanced NSCLC

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Abstract

Background: A significant proportion of patients with non-small-cell lung cancer (NSCLC) do not respond to immune checkpoint inhibitors (ICIs). Since metabolic reprogramming with increased glycolysis is a hallmark of cancer and is involved in immune evasion, we used ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography (^{18}F-FDG PET/CT) to evaluate the baseline glycolytic parameters of patients with advanced NSCLC submitted to ICIs, and assessed their predictive value.

Methods: ^{18}F-FDG PET/CT results in the 3 months before ICIs treatment were included. Maximum standardized uptake values, whole metabolic tumor volume (wMTV), and whole-body total lesion glycolysis (wTLG) were evaluated. Cutoff values for high or low glycolytic categories were determined using receiver-operating characteristic curves. Progression-free survival (PFS) and overall survival (OS) were evaluated. Patients with a complete response and a matching group with resistance to ICIs underwent immunohistochemistry analysis. An unsupervised k-means clustering model integrating programmed cell death ligand 1 (PD-L1) expression, glycolytic parameters, and ICIs therapy was performed.

Results: In all, 98 patients were included. Lower baseline ^{18}F-FDG PET/CT parameters were associated with responses to ICIs. Patients with low wMTV or wTLG had improved PFS and OS. High wTLG, strong tumor expression of glucose transporter-1, and lack of responses were significantly associated. Patients with low glycolytic parameters benefited from ICIs, regardless of chemotherapy. Conversely, those with high parameters benefited from the addition of chemotherapy. Patients with higher wTLG and lower PD-L1 were associated with progression and worse survival to ICIs monotherapy.

Conclusions: Glycolytic metabolic profiles established through baseline ^{18}F-FDG PET/CT are useful biomarkers for evaluating ICI therapy in advanced NSCLC.

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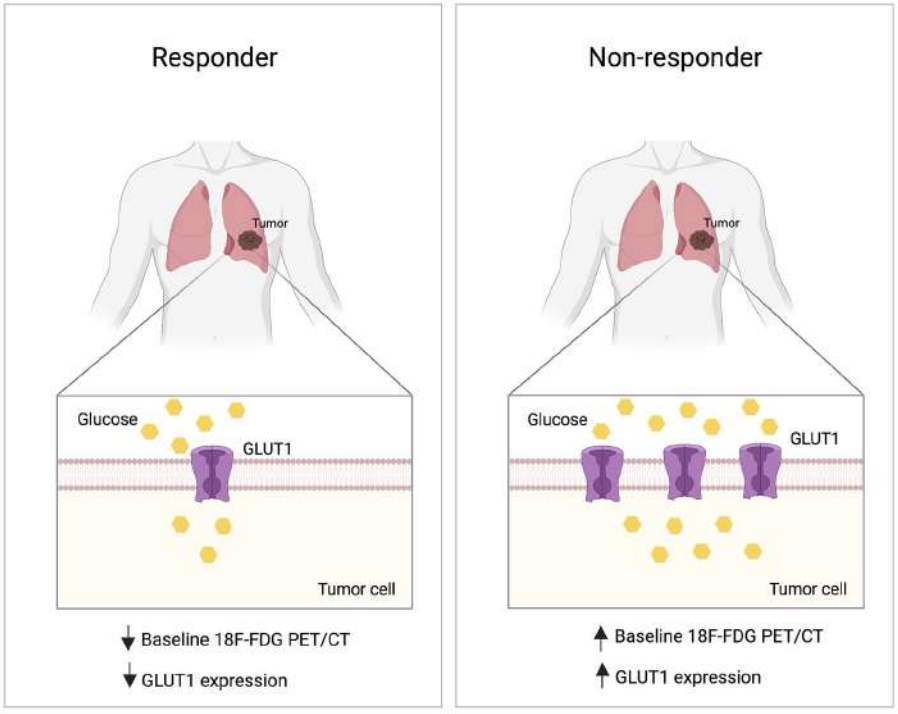

Keywords: biomarkers, ^{18}F-FDG PET/CT, glycolysis, immunotherapy, non-small-cell lung cancer, Warburg effect

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Introduction

The restoration of antitumor immune responses with immune checkpoint inhibitors (ICIs) is critical in treating metastatic non-small-cell lung cancer (NSCLC), but efficacy can vary significantly.[1] Although predictive biomarkers such as the programmed cell death ligand 1 (PD-L1) score and tumor mutation burden (TMB) have been used to tailor first-line treatment, a significant proportion of patients remain primarily refractory to ICIs by mechanisms not fully understood.[2] Thus, research addressing novel predictive biomarkers is essential to optimize patient selection and treatment effectiveness.

Tumor metabolic reprogramming with aerobic glycolysis is a hallmark of cancer.[3] Tumor cells increase the glucose uptake rate for glycolysis, even in the presence of oxygen, in a metabolic process known as the Warburg effect.[4] The overexpression of glucose transporters (GLUT) and glycolytic enzymes can provide tumor cells energy and carbon intermediates that foster tumor growth.[5] Moreover, recent studies demonstrate that the high glucose consumption by tumor cells contributes to immune evasion. Cancer cells can, therefore, restrict glucose availability in the tumor microenvironment[6] and thereby restrain T cells from attaining antitumoral states, which are dependent on glycolysis.[7,8] This metabolic competition in the tumor microenvironment is a driver of local immunosuppression and cancer progression.

Tumor glycolysis uptake imaging is routinely used for NSCLC staging: ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography (^{18}F-FDG PET/CT) is a non-invasive exam that can indirectly indicate tumor glycolysis[9] and thus be used to predict immune response. Since the link between tumor glycolytic rate and resistance to ICIs has not yet been fully elucidated, we aimed to comprehensively address the role of tumor glycolysis assessed through a pretreatment ^{18}F-FDG PET/CT as a predictive biomarker in respect of ICIs treatment in patients with advanced NSCLC.

Materials and methods

Patients and outcomes

We conducted a retrospective single-center study, including patients with metastatic NSCLC for whom a baseline ^{18}F-FDG PET/CT was performed up to 3 months before ICIs initiation. Patients with an epidermal growth factor receptor or anaplastic lymphoma kinase alteration being treated with tyrosine kinase inhibitors were excluded. Clinical data retrieval, ^{18}F-FDG PET/CT imaging, and immunohistochemical (IHC) analyses were performed independently. The best response under ICIs was determined according to Response Evaluation Criteria in Solid Tumors (RECIST 1.1).[10] Progression-free survival (PFS) was defined as the time from ICIs initiation to disease progression or death. Overall survival (OS) was calculated from the time ICIs treatment started until death from any cause.

^{18}F-FDG PET/CT exams

Baseline ^{18}F-FDG PET/CT was obtained 60 min after intravenous administration of 8–12 mCi (296–444 MBq) of the radiopharmaceutical ^{18}F-fluorodeoxyglucose after a 6-h fast and with a blood glucose $<$200 mg/dl. Studies were performed using the Discovery MI series (Ge Healthcare, Waukesha, WN, USA) and Siemens Biograph 16 (Siemens Healthineers, Knoxville, TN, USA) PET/CT scanners. Quantitative parameters of glycolytic uptake were reviewed and obtained through the PET VCAR software on the Advanced Workstation – AW platform (GE Healthcare). The maximum FDG glycolytic value of all lesions related to cancer disease was measured as the maximum standardized uptake value (SUVmax) normalized by body mass. The volume of the tumor lesions was measured following a semiautomatic protocol by a trained observer (a nuclear medicine physician with 15 years of experience in PET imaging and whole-body quantification), who was blinded to patient disease and any clinical data. The protocol comprised the following steps: first, the tumor lesions were visually identified and selected to receive a volume of interest (VOI), which was manually outlined on the images. After the insertion, the limits of the automatically drawn VOI and the lesion coverage were checked before acceptance, and adjusted when necessary, such as in lesions with heterogeneous uptake. The VOI had a pre-specified threshold corresponding to the volume of the lesion that includes a metabolic activity of at least 41% of the maximum SUV value in each lesion, thereby defining the metabolic tumor volume (MTV). The whole MTV (wMTV) was obtained by the sum of the MTV in all lesions considered related to cancer.[11] Each lesion's total lesion glycolysis (TLG) was calculated by multiplying its MTV by the mean SUV, and the whole-body TLG (wTLG) was obtained as the sum of all the TLG values.[12] Each VOI accepted (containing the lesion values of SUVmax, MTV, and TLG) was stored, displayed, ranked, and summed by the software, providing the whole-body values for each patient. The observer was blinded to patient disease and any clinical data.

IHC analysis

All patients who achieved a complete radiological response were selected for IHC analysis, with a matching number of cases with primary resistance to ICIs. All tumor biopsies were obtained prior to ICIs treatment. Sequential histological tumor sections of 4–5 µm thick were obtained from a representative formalin-fixed, paraffin-embedded tumor block and used for IHC analysis. All IHC slides were evaluated by two pathologists blinded to the patients' clinical outcomes. Only slides with good staining quality were analyzed. IHC staining was performed to determine PD-L1 expression using the tumor proportion score (TPS) and to determine GLUT-1 expression in tumor cells, in addition to CD8, CD4, FOXP3, and PD-1 expression in immune cells. The following primary antibodies were used: 22C3 Dako/Agilent or SP263 VENTANA/Roche for PD-L1 TPS, GLUT-1 (Polyclonal rabbit, 1:100 Cell Marque, Rocklin, CA, USA), anti-CD8 (C8/144B, ready to use-Dako/Agilent, Glostrup, Denmark), anti-CD4 (4B12, ready to use, Agilent/Dako, Glostrup, Denmark), FOXP3 (EP340, 1:50, Cell Marque, Rocklin, CA, USA), and PD-1 (NAT105, 1:100, Cell Marque, Rocklin, CA, USA). The chromogen detection system used was a DAB kit (Sigma Diagnostics, St Louis, MO, USA), and slides were counterstained with hematoxylin. All reactions were performed with a positive control slide for the selected primary antibody. The positive number of tumor-associated immune cells for GLUT-1, CD8, CD4, FOXP3, and PD-1 were quantified and recorded as percentages of positive cells per total tumor and stroma area. Tumor infiltrating lymphocytes (TILs) were evaluated as the percentages of mononuclear inflammatory cells divided by the total tumor-stromal area. GLUT-1 positivity was assessed regarding both

percentages of positive tumor cells and intensity of cytoplasmatic staining of tumor cells and graded into 1+ (weak), 2+ (moderate), or 3+ (strong) using a non-digital morphological semiquantitative scoring system based on the pathologist's assessment of the intensity of staining under the microscope. Regarding the PD-L1 TPS expression, the same assessment was performed for both anti-PD-L1 antibodies (22C3 and SP263) as the percentage of any positivity in tumor cells divided by the total number of tumor cells.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 24.0 (2016) (IBM Corp, Armonk, NY, USA) and graphical representations through GraphPad Prism (GraphPad Prism, version 9). The study sample size was estimated with 80% of power to detect a 20% difference in the proportion of response rates between the subgroups defined by biomarkers (high or low glycolytic tumor burden) who underwent treatment with ICIs. A p value of 0.05 or less was considered significant. Categorical variables were presented as numbers (percentages) and continuous as mean or medians. Statistical significance was determined using a chi-squared or Fisher's exact test for categorical variables and Student's t-test or Mann–Whitney U test for continuous variables.

The distribution of each ^{18}F-FDG PET/CT parameter according to the best response was represented graphically by box plots and compared through the Kruskal–Wallis test and *post hoc* Dunn's test for multiple comparisons. For each glycolytic parameter, a receiver-operating characteristic (ROC) curve was plotted to calculate the area under the curve (AUC), and optimal cutoff values for the determination of responder status were estimated through Youden's index calculation. The diagnostic accuracy can be graded as follows: excellent, 0.9–1.0; good, 0.8–0.9; fair, 0.7–0.8; poor, 0.6–0.7; and failure, 0.5–0.6. Subgroups were then defined as either high or low glycolytic tumor burden. The Kaplan–Meier methodology was used to estimate event-time distributions. Log-rank tests were used to test for differences in event-time distributions, and Cox proportional hazards models were fitted to obtain estimates of hazard ratios (HRs) in the univariate and multivariable analyses, which included the subgroups according to ^{18}F-FDG PET/CT parameters, the PD-L1 TPS, and the tumor burden according to RECIST 1.1 criteria for the measurable disease.

To assess whether patients could be divided into different groups according to PD-L1 status and glycolysis, unsupervised k-means clustering was performed using the two-dimensional datasets of PD-L1 and each of the glycolytic markers (wTLG, SUVmax, and wMTV). Only data from patients with known PD-L1 were used, and patients were stratified by treatment received (ICI monotherapy or ICIs and chemotherapy). The optimal number of clusters was pragmatically defined by the silhouette coefficient from 2 to 11 clusters. Differences in continuous variables between groups were assessed by the Mann–Whitney U test, while the t-test was used for categorical variables. p Values were adjusted for the comparison between clusters using the false discovery rate Benjamini–Hochberg method. The false discovery rate was controlled at the level of 0.05.

Results

Patient characteristics

In this study, 107 patients with NSCLC diagnosed between 2014 and 2020 were included. Adenocarcinoma was present in 80.3% ($n=86$), followed by squamous cell lung cancer in 8.4% ($n=9$). Most patients were metastatic, except three who had locally advanced NSCLC staged as IIIC. All of them underwent immunotherapy with ICIs (anti-PD1 or anti-PD-L1), either as a monotherapy (52.3%, $n=56$) or combined with chemotherapy (47.7%, $n=51$). Nine of the 107 patients were excluded since the ^{18}F-FDG PET/CT scans were not performed institutionally, thus precluding imaging analysis. Other patient characteristics are described in Supplemental Table 1.

Baseline NSCLC glycolytic profiles distinguish clinical responses to ICIs

Patients who did not respond to ICIs presented significantly higher pre-treatment glycolytic parameters (SUVmax, wMTV, and wTLG) (Figure 1(a)–(c)). Representative images of ^{18}F-FDG PET/CT uptake showed that non-responders presented higher glucose uptake as compared to responders (Figure 1(d)). Patients who achieved complete response (CR) or partial response according to RECIST 1.1. had lower ^{18}F-FDG PET/CT values (SUVmax, wMTV, and wTLG) in comparison to those with progressive disease (Figure 1(e)–(g)). Taken together, these data reveal distinct glycolytic metabolic patterns associated with ICIs responses in NSCLC patients.

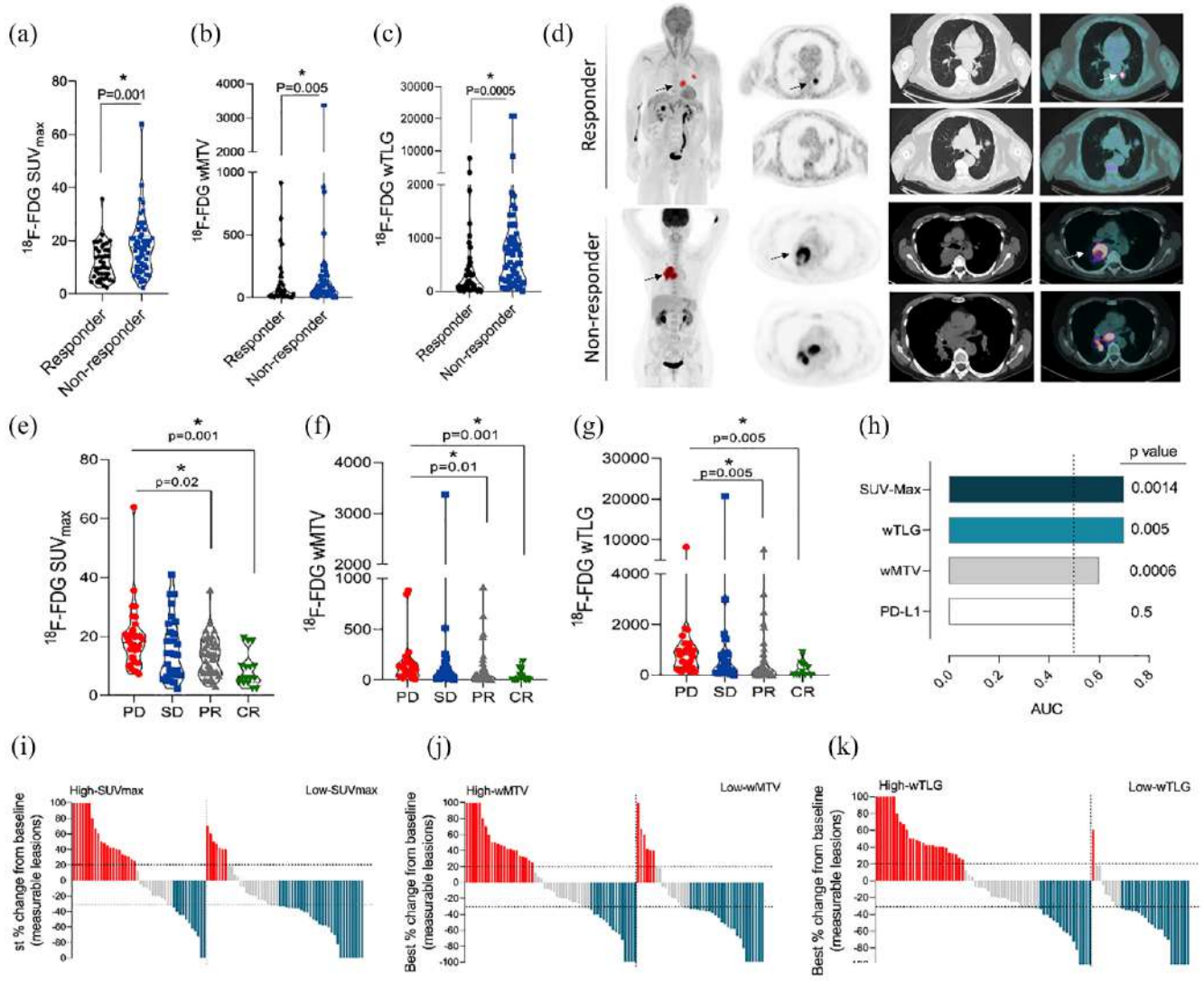

Figure 1. Higher baseline PET NSCLC glycolytic profiles predict worse clinical response to ICIs. Patients' baseline PET glycolytic values concerning (a) SUVmax, (b), wMTV, and (c) wTLG demonstrated higher quantitative distributions for patients characterized as non-responders (SD or PD) than responders (PR or CR). In (d), the ^{18}F-FDG PET/CT images represent a non-responder and a responder patient with a similar tumor burden but distinct ^{18}F-FDG uptake (high: arrows; low: arrowheads). Patients with RECIST 1.1. PD, SD, PR, or CR according to (e) SUVmax, (f) wMTV, and (g) wTLG. In (h), AUC from ROC curves prediction models for PET glycolytic parameters (SUVmax, wMTV, and wTLG) and PD-L1 score (<50% or ⩾50%). The delta tumor variation was evaluated as the difference between RECIST 1.1. tumor measurement in baseline and after ICIs best response. Patients were stratified into low and high metabolism according to (i) SUVmax, (j) wMTV, and (k) wTLG.*$p<0.05$, the Mann–Whitney test was used in (a)–(c) and the Kruskal–Wallis test with *post hoc* Dunn's test for multiple comparison in (e)–(g).

CR, complete response; 18F-FDG PET/CT, 18F-fluorodeoxyglucose positron emission tomography-computed tomography; ICIs, immune checkpoint inhibitors; NSCLC, non-small-cell lung cancer; PD, progressive disease; PD-L1, programmed cell death ligand 1; PR, partial response; RECIST 1.1., response evaluation criteria in solid Tumors; SD, stable disease; SUVmax, maximum standardized uptake value; wMTV, whole metabolic tumor volume; wTLG, whole-body total lesion glycolysis.

Low versus *high baseline NSCLC glycolytic profile and oncologic outcome*

Objective response rate

ROC curves analyses evaluating the accuracy of ^{18}F-FDG PET/CT parameters to predict ICIs responses revealed AUCs of 0.70 for SUVmax ($p=0.0014$), 0.67 for wMTV ($p=0.005$), and 0.70 for wTLG ($p=0.0006$). The diagnostic accuracy of AUC in ROC curves can be graded as fair for SUVmax and wTLG (AUC: 0.7–0.8), and poor for wMTV (AUC: 0.6–0.7). Of note, in these samples, ^{18}F-FDG PET/CT parameters achieved higher C-statistics than PD-L1 scores

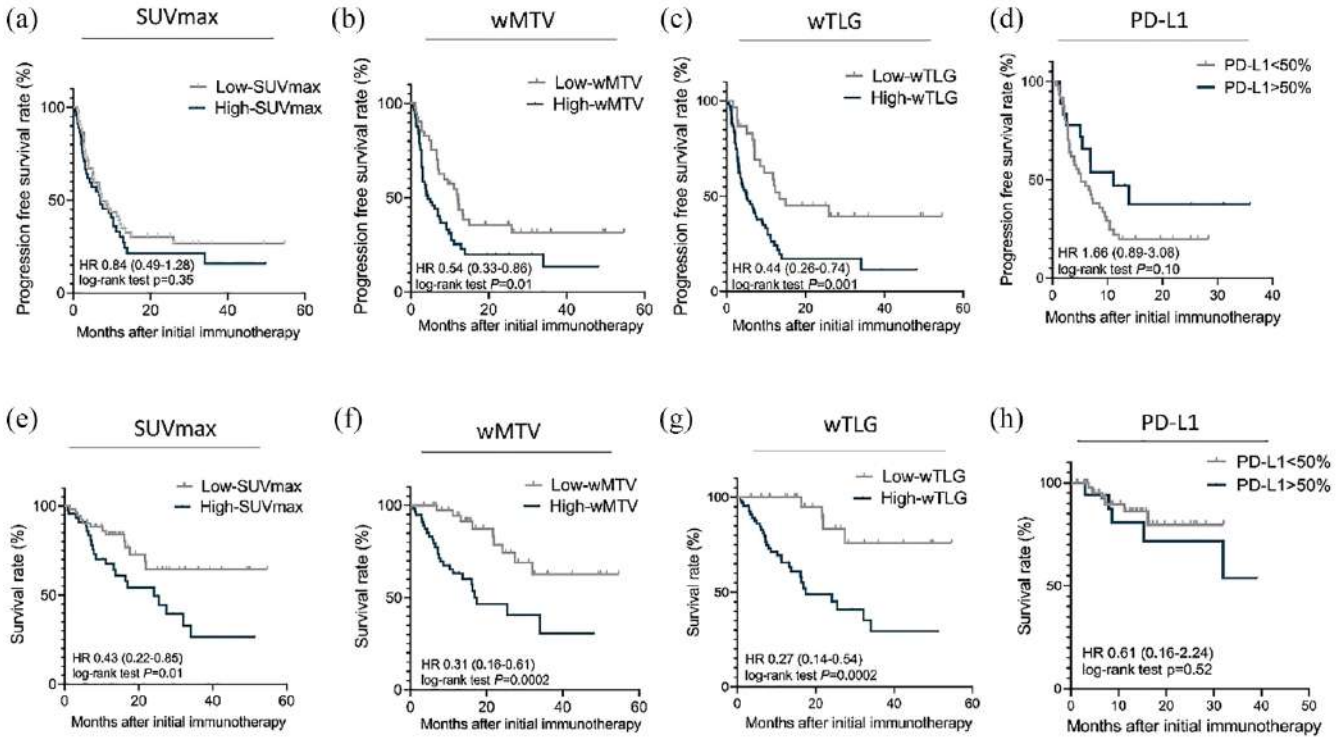

Figure 2. High baseline PET NSCLC glycolytic profiles predict worse PFS and OS to ICIs. Kaplan–Meier survival curves concerning PFS for (a) SUVmax, (b) wMTV, (c) wTLG, and (d) PD-L1 demonstrate significantly worse PFS for patients with high wMTV (⩾43.05 cm^3) and wTLG (⩾146.9 g/ml cm^3) than those with low baseline glycolytic profiles. Kaplan–Meier survival curves concerning OS (e) SUVmax, (f) wMTV, (g) wTLG, and (h) PD-L1 demonstrate significant worse OS for patients with high SUVmax (⩾14.3), wMTV (⩾43.05 cm^3), and wTLG (⩾146.9 g/ml cm^3) than for those with low baseline glycolytic profiles. High *versus* low PD-L1 expression did not distinguish different PFS or OS in the study population.
ICIs, immune checkpoint inhibitors; NSCLC, non-small-cell lung cancer; OS, overall survival; PD, progressive disease; PD-L1, programmed cell death ligand 1; PET, positron emission tomography; PFS, progression-free survival; SUVmax, maximum standardized uptake value; wMTV, whole metabolic tumor volume; wTLG, whole-body total lesion glycolysis.

(Figure 1(h) and Supplemental Figure 1). Through ROC curves analysis, we identified optimal cutoff values for discrimination according to responder status: 14.3 for SUVmax (sensitivity 60.4% and specificity 71.1%), 43.05 cm^3 for wMTV (sensitivity 73.6% and specificity 60.0%), and 146.9 g/ml cm^3 for wTLG (sensitivity 86.8% and specificity 51.1%). Patients were then classified into subgroups of either high (>cutoffs) or low (⩽cutoffs) glycolytic tumor burden. Responses occurred more frequently in patients with low glycolytic parameters (Figure 1(i)–(k)). Remarkably, 97% of patients (29/30) with low wTLG (<146 g cm^3/ml) did not have a RECIST 1.1. progression after ICIs initiation.

Survival

Kaplan–Meier survival analyses were undertaken to compare high and low glycolytic tumor burden subgroups. Baseline characteristics among subgroups are described in Supplemental Table 2. Although no differences were observed concerning SUVmax [HR: 0.84, 95.0% confidence interval [CI]: 0.49–1.28, $p = 0.35$), patients with low wMTV (HR: 0.54, 95.0% CI: 0.33–0.86, $p = 0.01$), or wTLG (HR: 0.44, 95.0% CI: 0.26–0.74, $p = 0.001$) showed longer PFS (Figure 2(a)–(c)). However, no significant difference was found in PFS according to PD-L1 expression (Figure 2(d)). Among all low glycolytic subgroups, there was a significant improvement in OS in comparison to patients with high metabolic activity evaluated by SUVmax (HR: 0.43, 95.0% CI: 0.22–0.85, $p = 0.01$), wMTV (HR: 0.31, 95.0% CI: 0.16–0.61, $p = 0.0002$), or wTLG (HR: 0.27, 95.0% CI: 0.14–0.54, $p = 0.0002$) (Figure 2(e)–(g)). No statistically significant difference was found in respect of OS according to high (⩾50%) or low (<50%) PD-L1 tumor

proportional score (TPS) expression (Figure 2(h)). Multivariable analysis with Cox proportional hazards models, including each of the glycolytic subgroups according to ^{18}F-FDG PET/CT parameters, PD-L1 TPS, and baseline tumor burden of each patient according to the RECIST 1.1. criteria, demonstrated an independent statistically significant impact for progression with the biomarker wTLG (HR: 5.29, 95.0% CI 1.72–16.25, $p = 0.004$) (Supplemental Table 3).

GLUT-1 tumor-cell expression correlates with a different clinical and IHC pattern of immune responses

All patients with strong GLUT-1 expression (at least 20% of tumor cells stained for the marker) did not respond to ICIs (Figure 3(a)–(c)) and were correlated with a high wTLG. Moreover, it was noted that in these non-responder patients with a high GLUT-1 expression, there was a trend to present fewer immune CD8+ and more FOXP3+ and PD-1+ cells. However, a larger dataset is needed to power this analysis to make any statistically significant conclusions. Representative images of complete responders or non-responders in respect of each marker's IHC staining are shown in Figure 3(d)–(i). No statistical difference was found between glycolytic status, genetic alterations, and histological subtypes (Supplemental Figure 2).

Baseline glycolytic profile stratified by treatment

ROC curves analyses evaluating the accuracy of ^{18}F-FDG PET/CT parameters to predict responses revealed higher AUCs for ICIs in monotherapy than for combined with chemotherapy. Of note, tumors in patients with low wMTV and wTLG were more likely to respond to treatment than progress following ICIs monotherapy, whereas those in patients with high glycolytic baseline ^{18}F-FDG PET/CT parameters predominantly progressed. Regarding patients with a high wTLG, response rates were significantly higher in the presence of chemotherapy combination (Supplemental Figure 3). Moreover, PFS and OS analyses among patients with low glycolytic parameters demonstrated similar efficacy patterns irrespective of chemotherapy combination. However, significant improvements in PFS were noticed in patients with high glycolytic parameters who received ICIs combined with chemotherapy (Figure 4).

Unsupervised clustering according to PD-L1 TPS and baseline glycolytic profile

A k-means unsupervised clustering model integrating PD-L1 TPS, with baseline glycolytic parameters was used to evaluate their relation to patient outcomes (Supplemental Figure 4). Among patients submitted to ICI monotherapy, a clear cluster of non-responders was formed at higher wTLG values (Figure 5(a)). Moreover, the unsupervised clustering of such patients based on wTLG and PD-L1 values unveiled two distinct patterns of distributions: patients with lower wTLG and a broader range of PD-L1 expression (cluster 0), and patients with higher wTLG and predominantly low PD-L1 TPS (cluster 1) (Figure 5(b)–(d)). Notably, the clinical efficacy of ICIs was significantly different between clusters in respect of objective response rates, PFS and OS (Figure 5(e)–(g)). This analysis suggests that patients with a low PD-L1 TPS may still derive clinical benefits from ICI monotherapy in low wTLG settings.

Discussion

Based on the correlations between baseline glycolytic profile and outcomes (objective response rate, PFS, and OS), this study suggests that tumor glycolysis assessed through a baseline ^{18}F-FDG PET/CT scan may have a role in the prediction of ICI effectiveness in the treatment of NSCLC. Accordingly, patients with high glycolytic tumor volumes may be less likely to benefit from ICI treatment. Thus, ^{18}F-FDG PET/CT, a non-invasive and routinely performed evaluation, could provide crucial predictive information and perhaps help guide future clinical decision-making regarding the association between immunotherapy and chemotherapy in certain NSCLC cases. Furthermore, this study expands the body of evidence supporting the hypothesis that metabolic reprogramming constitutes a cancer hallmark that mediates immune evasion, thus contributing to the translation of preclinical knowledge into clinical practice.[13]

The prominent glucose competition within highly glycolytic tumor volumes hinders CD8+ lymphocytes from engaging in glycolysis, consequently reducing interferon gamma production.[7] This interaction establishes interstitial metabolic conditions in which oxidative phosphorylation-addicted immune cells, such as FOXP3-high CD4+ T-cell lymphocytes, are the most likely to resist.[14] Moreover, such a nutrient-deprived

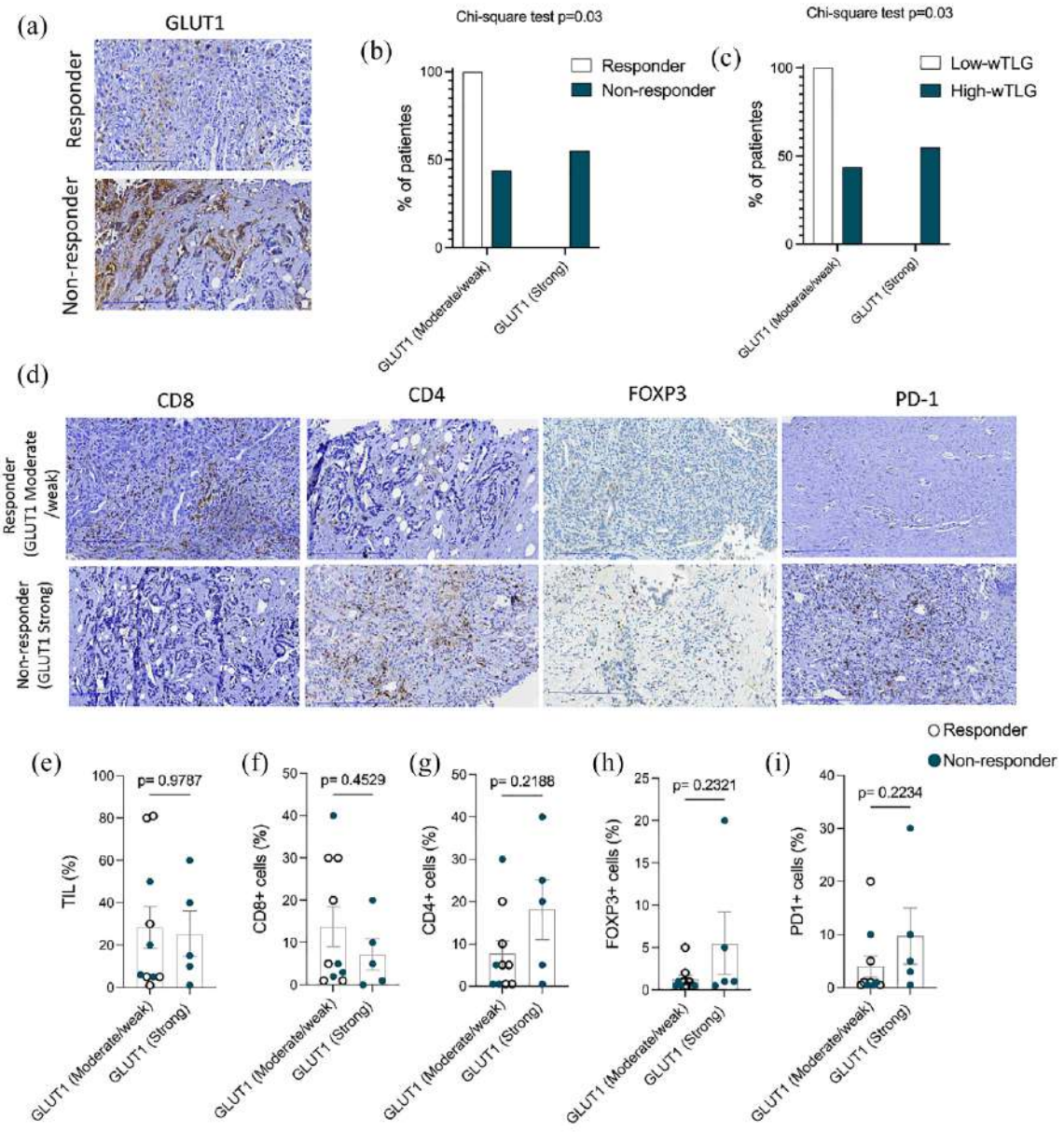

Figure 3. Strong GLUT-1 expression in NSCLC tumor cells correlates with a worse clinical response and a cold-like immune infiltrate. IHC GLUT-1 expression in a responder *versus* non-responder patient (a) and its correlation with objective responses (b) and with ^{18}F-FDG PET/CT wTLG (c) demonstrates that all patients with strong (3+) GLUT-1 tumor-cell expression did not respond to ICIs and had a high wTLG. In (d), representative images for a responder and non-responder patient for each of the IHC immune markers, which are then compared according to GLUT-1 intensity score [strong (3+) *versus* moderate (2+)/weak (1+)], (e) TIL, (f) CD8+, (g) CD4+, (h) FOXP3, and (i) PD-1. CD8+ immune cells are numerically lower in patients with strong GLUT-1 tumor-cell expression, whereas CD4+, FOXP3+, and PD-1+ immune cells are numerically higher.
^{18}F-FDG PET/CT, ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography; GLUT-1, glucose transporter 1; ICIs, immune checkpoint inhibitors; NSCLC, non-small-cell lung cancer; PD-L1, programmed cell death ligand 1; TIL, tumor infiltrating lymphocyte; wTLG, whole-body total lesion glycolysis.

tumor microenvironment drives T cells to functional states of hyporesponsiveness despite appropriate antigenic recognition, a process in which T cells accumulate co-inhibitory molecules such as PD-1.[15] After conducting an outcome-blinded IHC analysis among cases with opposite outcomes in respect of ICIs treatment (CR *versus* progression), it was found that all patients with a

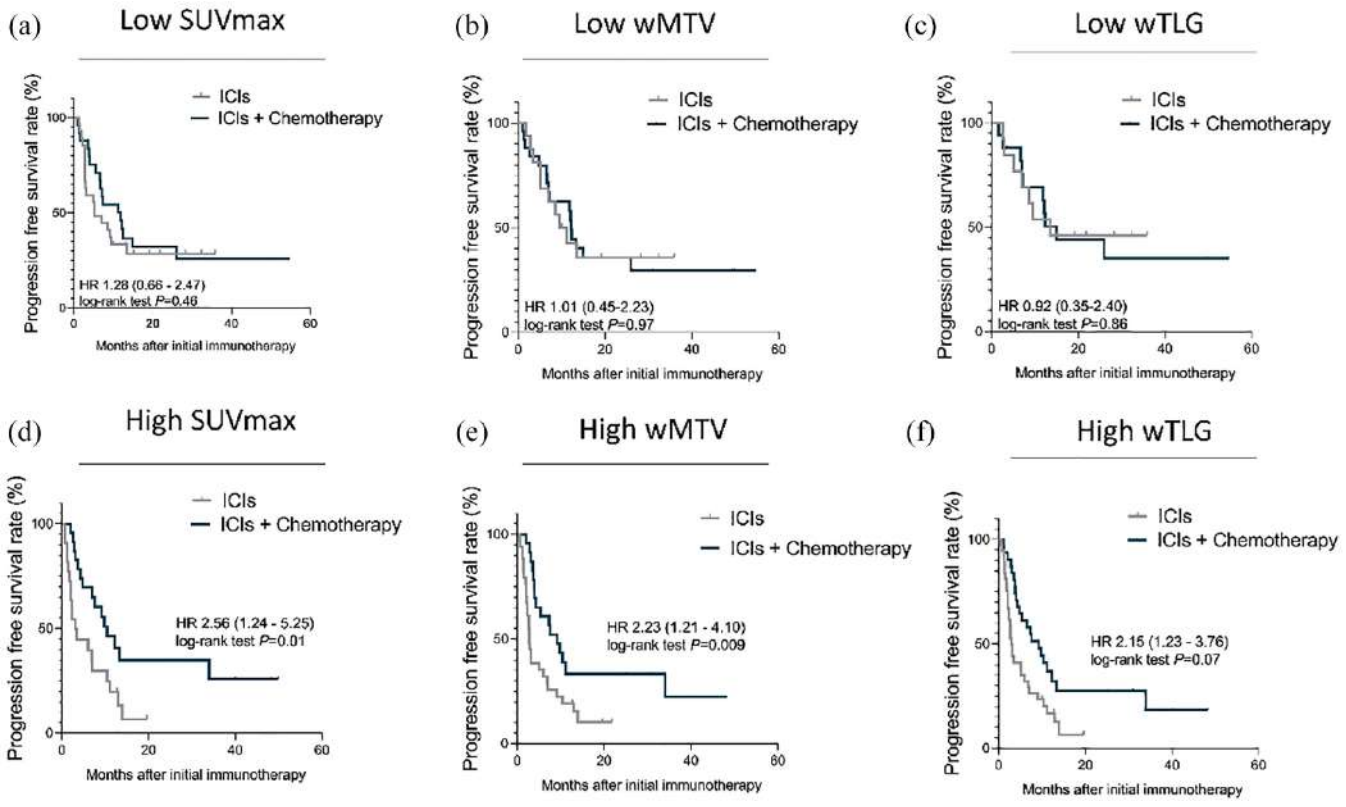

Figure 4. NSCLC baseline glycolytic profile as a predictive biomarker for ICI monotherapy or in combination with chemotherapy: PFS analysis. Kaplan–Meier survival curves concerning PFS for low glycolytic parameters: (a) SUVmax < 14.3, (b) wMTV $< 43.05\,cm^3$, and (c) wTLG $< 146.9\,g/ml\,cm^3$ do not demonstrate a significant difference in respect of either ICIs monotherapy or combined with chemotherapy. Kaplan–Meier survival curves concerning PFS for high glycolytic parameters: (d) SUVmax $\geqslant 14.3$, (e) wMTV $\geqslant 43.05\,cm^3$, and (f) wTLG $\geqslant 146.9\,g/ml\,cm^3$ demonstrate a significant difference favoring ICIs combined with chemotherapy.
ICI, immune checkpoint inhibitor; NSCLC, non-small-cell lung cancer; SUVmax, maximum standardized uptake value; wMTV, whole metabolic tumor volume; wTLG, whole-body total lesion glycolysis.

strong GLUT-1 IHC expression had progressed. Moreover, fewer immune CD8+ and more FOXP3+ and PD-1+ cells were noticed in patients with strong GLUT-1 expression. Although such an analysis is compromised by the lack of statistical power to encounter significant differences, it supports the hypothesis that tumor glycolysis alters anti-tumoral immune responses.

It is worth considering that independent cytotoxic effects of chemotherapies might mitigate the PET parameters' accuracy in predicting responses to ICIs, as revealed by higher AUCs for ICIs in monotherapy than for combined with chemotherapy. In a hypothetical instance, a highly glycolytic NSCLC that would be truly ICI-alone resistant may still respond to ICIs plus chemotherapy solely due to the independent cytotoxic effects of chemotherapies. Thus, although the accuracy of PET parameters to predict the lack of response to ICIs alone in such a highly glycolytic scenario is accurate, the independent chemotherapy effects that elicit a tumor response would eventually cause a decline in the AUCs of PET parameters that predict the effects of combined therapy. It is also noteworthy that NSCLC tumors that feature high glycolysis have, simultaneously, a theoretically increased sensitivity to chemotherapies owing to high mitotic rates, along with limited sensitivity to ICIs, as supported by this study. Therefore, we interpreted with caution what was found with respect to the ROC curves and further analyzed the clinical effects each approach (alone or chemo-combined) had according to each different set of high or low tumor glycolysis. Accordingly, it was noted that patients receiving ICIs as a monotherapy had higher objective response rates if they had a lower baseline glycolytic status. Moreover, although patients predominantly received ICI monotherapy in the second-line or subsequent settings (as opposed to ICIs plus chemotherapy, which was

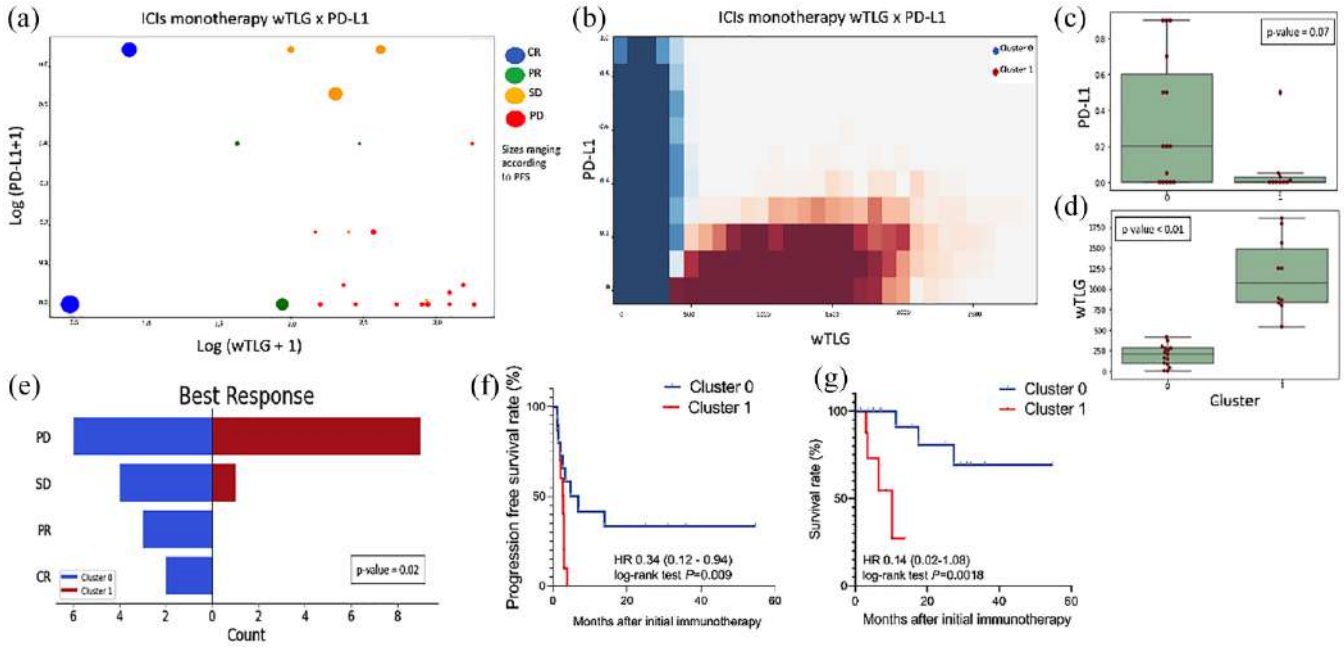

Figure 5. The combination of PD-L TPS and baseline 18F-FDG PET/CT glycolytic profile may predict ICI outcomes. (a) A k-means unsupervised clustering model integrating PD-L1 TPS with baseline ^{18}F-FDG PET/CT glycolytic parameters demonstrates that a cluster of patients whose best response was PD was formed at higher wTLG values; (b) there were two distinct patterns of distributions: cluster 0 and 1, which differed in respect of (c) PD-L1 TPS and (d) wTLG. Patients in Cluster 1 did not respond to ICIs (e) and had worse PFS (f) and OS (g). Mann-Whitney U test was used to compare statistical significance between clusters, and log-rank test to compare Kaplan-Meier survival curves.

^{18}F-FDG PET/CT, ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography; ICI, immune checkpoint inhibitor; PD, progressive disease; PD-L1, programmed cell death ligand 1; RECIST 1.1., response evaluation criteria in solid tumors; TPS, tumor proportional score; wTLG, whole-body total lesion glycolysis.

predominantly used in the first-line setting), unexpectedly, no differences between the PFS curves of patients treated with either approach were seen if they had a low baseline glycolytic status. These findings suggest that in scenarios featuring low tumor glycolytic activities, immune response restoration through ICIs is highly efficacious. Conversely, high glycolytic subgroups were less sensitive to ICIs as a monotherapy, as indicated by significantly worse radiological responses and PFS curves. Taken together, this strengthens the hypothesis that ICI monotherapy is less effective in patients with high glycolytic profiles and that they would benefit from associated chemotherapy. Furthermore, it is reasonable to speculate that novel ICIs-combinations that specifically tackle tumor glycolysis in the specific scenario of high glycolysis – by means of baseline ^{18}F-FDG PET/CT parameters – might enhance the effectiveness of immunotherapies.[16]

To date, for metastatic NSCLC patients eligible for ICIs, the PD-L1 TPS has been paramount in guiding first-line treatment, whether through monotherapy or chemotherapy combinations, although less than 50% of patients are expected to achieve an objective response using such a biomarker to guide the use of ICIs.[17–22] Likewise, PD-L1 TPS in our cohort demonstrated a low accuracy in predicting responses. However, after combining PD-L1 TPS and baseline ^{18}F-FDG PET/CT parameters, it could be seen that patients with a low PD-L1 TPS and a high wTLG did not achieve objective responses with ICIs in monotherapy and rapidly progressed following this approach, whereas those with a low wTLG had clinical benefits regardless of the PD-L1 TPS. This finding could potentially contribute to clinical practices in respect of patients with advanced NSCLC that have less than 50% of PD-L1 TPS, for whom the benefit with ICIs in monotherapy over chemotherapy is controversial, according to clinical trials.[18,21,22] Indeed, the multimodal integration of radiology and pathology has increasingly been seen as a promising predictive tool for guiding immunotherapies in NSCLC.[23]

Due to its retrospective and single-center nature, this study has some limitations that might bias the

results and conclusions. The lack of an independent validation cohort further evaluating the ^{18}F-FDG PET/CT cutoffs for high or low glycolytic profiles may limit the drawing of definitive conclusions. However, it should be noted that a recent prospective study evaluating baseline PET glycolytic parameters as predictive biomarkers for the treatment with ICIs using ROC curves analysis obtained a similar MTV cutoff value (36.5 cm^3) to that found in this study (43.05 cm^3), and prospectively validated such a quantitative biomarker as accurate.[24] Moreover, although research conducted so far has reported different wMTV cutoff values,[24–32] it is notable that such emerging studies have consistently underscored the fact that ^{18}F-FDG PET/CT quantitative parameters are robust prognostic tools that can help to predict the efficacy of ICIs. Furthermore, the complex interplay between the immune system, tumor cells, and the microenvironment makes obtaining reliable predictive biomarkers a challenge, as demonstrated by the lack of accuracy of current markers such as PD-L1 and TMB in the NSCLC scenario. Thus, efforts to identify novel predictive biomarkers for ICIs remain highly desirable. Future prospective studies could validate standardized cutoff values that would support more effective treatment strategies.

In summary, glycolytic tumor burden assessed through baseline ^{18}F-FDG PET/CT scan in patients with metastatic NSCLC predicts a different profile of responses and survivals to ICIs. This metabolic characterization could leverage the benefits of immunotherapy and assist clinical decision-making. ICIs monotherapies can be highly efficacious in tumors with low metabolic glycolytic burden, but chemotherapy combinations may be beneficial for patients with high glycolytic profiles, particularly a high wTLG. The findings of this study add to the growing body of evidence supporting the hypothesis that baseline ^{18}F-FDG PET/CT parameters can be helpful predictive biomarkers for ICI treatment in patients with NSCLC, and warrants their incorporation in prospective clinical trials that address new approaches to immunotherapy.

Declarations

Ethics approval and consent to participate

This study was conducted according to the Declaration of Helsinki and approved by the responsible ethical review board (reference #3.814.604). Immunohistochemical analyses were performed after receiving the patients' informed consent.

Consent for publication

Not required.

Author contribution(s)

Saulo Brito Silva: Conceptualization; Formal analysis; Investigation; Methodology; Writing – original draft; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Data are available upon reasonable request. Deidentified participant data are available upon reasonable request to Gilberto de Castro Jr (gilberto.cjunior@hsl.org.br) and Saulo Brito Silva (sbrito@hcrp.usp.br). Reuse can be permitted in the setting of a discussed research collaboration.

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Supplemental material

Supplemental material for this article is available online.

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