

Radiomics predicts survival of patients with advanced non-small cell lung cancer undergoing PD-1 blockade using Nivolumab

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Abstract. Immune checkpoint blockade is an emerging anticancer strategy, and Nivolumab is a human mAb to PD-1 that is used in the treatment of a number of different malignancies, including non-small cell lung cancer (NSCLC), kidney cancer, urothelial carcinoma and melanoma. Although the use of Nivolumab prolongs survival in a number of patients, this treatment is hampered by high cost. Therefore, the identification of predictive markers of response to treatment in patients is required. In this context, PD-1/PDL1 blockade antitumor effects occur through the reactivation of a pre-existing immune response, and the efficacy of these effects is strictly associated with the presence of necrosis, hypoxia and inflammation at the tumour sites. It has been indicated that these events can be evaluated by specific assessments using a computed tomography (CT) texture analysis (TA) or radiomics. Therefore, a retrospective study was performed, which aimed to evaluate the potential use of this analysis in the identification of patients with NSCLC who may benefit from Nivolumab treatment. A retrospective analysis was performed of 59 patients with metastatic NSCLC who received Nivolumab treatment

between January 2015 and July 2017 at Siena University Hospital (35 patients, training dataset), Catanzaro University Hospital and Reggio Calabria Grand Metropolitan Hospital, Italy (24 patients, validation dataset). Pre- and post-contrast CT sequences were used to contour the gross tumour volume (GTV) of the target lesions prior to Nivolumab treatment. The impact of variations on contouring was analysed using two delineations, which were performed on each patient, and the TA parameters were tested for reliability using the Intraclass Coefficient Correlation method (ICC). All analyses for the current study were performed using LifeX Software[®]. Imaging, clinical and pathological parameters were correlated with progression free survival and overall survival (OS) using Kaplan Meier analysis. An external validation testing was performed for the TA Score using the validation dataset. A total of 59 patients were included in the analysis of the present study. The reliability ICC analysis of 14 TA parameters indicated a highly reproducibility (ICC >0.70, single measure) in 12 (85%) pre-contrast and 13 (93%) post-contrast exams. A specific cut-off was detected for each of the following parameters: volume (score 1 >36 ml), histogram entropy (score 1 > 1.30), compacity (score 1 <3), gray level co-occurrence matrix (GLCM)-entropy (score 1 >1.80), GLCM-Dissimilarity (score 1 >5) and GLCM-Correlation (score 1 <0.54). The global texture score allowed the classification of two subgroups of Low (Score 0-1; 36 patients; 61%) and High Risk patients (Score >1; 23 patients; 39%) that respectively, showed a median OS of 26 (mean +/- SD: 18 +/- 1.98 months; 95% CI 14-21 months) and 5 months (mean +/- SD: 6 +/- 0.99 months; 95% CI: 4-8 months; P=0.002). The current study indicated that TA parameters can identify patients that will benefit from PD-1 blockage by defining the radiological settings that are

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potentially suggestive of an active immune response. These results require further confirmation in prospective trials.

Introduction

Non-small cell lung cancer (NSCLC) represents the most common malignancy and the leading cause of cancer death (1,2). Systemic therapy with platinum doublets with or without Radiation Therapy is the standard front-line treatment for patients in advanced stage of disease (stage IIIB-IV). Patients with driver mutations/rearrangements require molecular targeted specific drugs against EGFR or EML-ALK (3-5) while first line Pembrolizumab an immune checkpoint inhibitor (ICI) can be offered to patients whose tumors have PD-1 ligand-1 (PDL1) expression >50%. Recently, the clinical development of ICI mAbs has offered new treatment opportunities for NSCLC patients in different settings. In fact, salvage therapy with mAbs to programmed cell death receptor-1 (PD-1) and PDL1 has improved the survival of many of these patients by rescuing pre-existing tumor-specific cytotoxic-T-cells (CTLs) in the tumor sites (6-12). CTLs are the ultimate immune-surveillance system effectors able to recognize and kill tumour cells. PD-1/PDL1 axis is a terminal inflammatory-mediated immune-checkpoint able to protect tumour target cells by delivering an inhibitory signal to tumour-specific CTLs expressing the PD-1 receptor in the tumour site. Experimental evidence have shown that PD-1/PDL1 blockade with specific mAbs restores the antitumor activity of these tumour-antigen specific CTLs with consequent therapeutic effects in cancer patients. Nivolumab, in particular is a human immune-globulin to PD-1 approved in the treatment of a number of different malignancies including NSCLC, kidney cancer, urothelial carcinoma, and melanoma. Although its use yields a clear benefit and a prolonged survival in a number of patients, this treatment is hampered by high costs, which makes eagerly needed the identification of predictive markers of response, that so far are still undefined and PDL1 analysis has not a clear role in the second line setting. Additionally, its mechanism of action, so different by the conventional cytotoxic treatments or radio/chemotherapy, makes very difficult the monitoring of the patients with the conventional radiology as well as the prevention of possible side effects. In order to identify possible predictive markers of response, we took in consideration that PD-1/PDL1 blockade antitumor effects occur throughout the reactivation of a pre-existing immune response, whose efficacy is strictly related to the presence of necrosis, hypoxia, and inflammation in the tumour sites. We considered that these biological events could be potentially evaluated by specific imaging assessments with a computed tomography (CT) texture analysis (TA) or radiomics.

Radiomics, in particular, is the extraction of quantitative imaging features from medical images. These quantitative values can be used to develop models for cancer diagnosis, patient prognosis, or relative tumor heterogeneity that can then guide clinical decisions (13-15).

This process can be conceptually similar to the current application of tumor staging or genetic and biomolecular information derived from tumor biopsy specimens for clinical decision-making.

TA is performed by means of a computer quantification of both gray-level intensity, position of pixels, and its use is being investigated in several fields (16-23).

In recent years, numerous studies have examined the potential clinical utility of radiomics features calculated from computed tomography (CT) images of NSCLC, correlated with tumor histology (24-26), staging (27), patient prognosis (19,28-30) and genetic mutations (31-33).

On these bases, we have designed a retrospective study to evaluate the potential use of CT TA to predict the OS of pre-treated advanced NSCLC patients treated with PD-1/PDL1 ICI Nivolumab.

Patients and methods

Patient series. We performed a retrospective analysis of pretreated advanced NSCLC cancer patients treated with Nivolumab between January 2015 and July 2017 at the Radiation Oncology Unit of Siena University Hospital, at the Medical Oncology and Translational Oncology Units of Catanzaro University Hospital and at Reggio Calabria Grand Metropolitan Hospital in Italy.

In our analysis all patients with a complete setting of diagnostic histological samples and a clinical-radiological pre-treatment staging, including a total body CT scan with and without contrast, and an intact (i.e., not previously treated with surgery or radiotherapy) evaluable target lesion in the lung were included.

Ethics approval. All the patients gave written consent to anonymous use of their examinations for research scope. A study notification was submitted to local ethical committee as established by national laws. The University Hospital of Siena Institutional Review Board at the pilot center authorized the retrospective analysis of the data. All procedures were undertaken in compliance with the ethical statements of the Helsinki Declaration (2008) of the World Medical Association.

Nivolumab therapy. All the patients were staged as IIIB/IV and progressed after systemic therapy with cis-platinum doublets. All the patients received intravenous nivolumab (3 mg/kg in 60 min) on biweekly bases. Treatment was given until progression, unacceptable toxicity or death.

Computed tomography imaging. All CTs at Siena University Hospital were performed using a 64-detector row CT scanner (Discovery 750 HD; GE Healthcare, Milwaukee, WI, USA).

In all patients, CT of the chest was performed with a spiral technique in tail-cranial direction (from the bases of the lungs to a plane cutting through the upper thoracic outlet, with the patient lying supine). Enhanced CT scans were obtained in the portal venous phase (delay 65-80 sec) with an intravenous injection of 2 ml/kg of non-ionic contrast material after a bolus injection of non-ionic contrast material (Iopamidolo 370 mgI/ml; Iopamiro, Bracco Diagnostics, Italy or Iopromide 370 mgI/ml, Ultravist 370 mgI/ml; Bayer HealthCare Pharmaceuticals, Italy), followed by 30 ml of saline solution using a peristaltic semiautomated power injector (3-4 ml/sec flow rate; SIAS 757, Bologna, Italy) with an 18-gauge needle in the antecubital vein. The following technical parameters were

used: slice thickness 2.5 mm, beam pitch 1.375/0.937, reconstruction interval 0.8 mm, 120-140 kVp and 250-500 mA. An automatic current modulation tube was used to minimize radiation exposure. Standard reconstruction algorithm was used. Patients were instructed not to breathe during helical imaging in order to avoid motion artefacts.

Follow-up. A CT-scan was repeated, in order to assess the response to immunotherapy, every 3 months or in any case showing clinical signs suggesting progressive disease (PD). General examinations with recording of toxicity, blood cell counts and chemistry and serum markers levels were evaluated every two weeks.

Feature extraction and TA. The gross tumour volume (GTV) was contoured by a radiation oncologist (VN) and confirmed by three expert radiologists (EA, MAM, VR) on pre-contrast and post-contrast sequences (Fig. 1). The impact of variations on contouring was analysed performing two delineation on each patient, and the TA parameters were evaluated for reliability with Intraclass Coefficient Correlation method (ICC). All the analyses for work have been accomplished with LifeX Software[®] (34). TA parameters were limited to features of gray-level co-occurrence matrix (GLCM), sphericity and indices from the gray-level histogram, to avoid an excessive number of parameters and overfitting of models.

Preselection of variables. The reliability analysis performed with ICC showed that the TA parameters that resulted significantly reproducible (ICC>0.70, single measure) were, respectively, 12 out of 14 for the post-contrast CT (85%), 13 out of 14 (93%) for the pre-contrast CT (Table I).

End-points and statistical analysis. In order to perform a statistical correlation among the reliable TA parameters and outcome, we used the software *X-Tile* (35) to calculate for each TA parameter a cut-off value that could be significant at survival analysis (Kaplan Meier analysis), on the overall population, after normalization of the parameters for the training and validation population.

For each significant cut-off, we assigned the score 1 for the subgroup with the worse prognosis and 0 for the subgroup with the better prognosis.

We then summed the scores to obtain a global texture score that was finally partitioned into two subgroups. The clinical variables of the two subgroups (sex, age, histology) were compared with Chi-square test, in order to exclude biases due to clinical factors not taken into account for the comparison of the above-mentioned subsets.

To validate our performance model, our cohort of patients were separated into two partitions, with the patients treated in our Unit (35 patients) as the training dataset and the patients treated in other Institutions (24 patients) as the validation set.

A survival analysis of progression free survival (PFS) and overall survival (OS) with Kaplan-Meier method was used in the two subgroups to test the texture score.

PFS was calculated from the date of the patient's beginning of immunotherapy to date of CT examination showing progression of disease, or censored to last follow-up visit. Conversely, OS was calculated from the date of the patient's beginning of

immunotherapy to the death of patients or censored to last follow-up visit.

All the statistical analysis was conducted with SPSS software v.23.0.

Results

Patients' features. This is a retrospective analysis performed on an unmasked sample of fifty-nine consecutive patients with advanced NSCLC cancer who had progressed standard frontline chemotherapy and had received salvage therapy with Nivolumab, between January 2015 and July 2017. We have enrolled 48 (81%) males and 11 (19%) females with a median age of 69 years (mean \pm SD=67.4 \pm 9.7 years, range 42-86 years). Thirty-six patients (61%) were diagnosed as adenocarcinoma, 18 (30.5%) as squamous cell carcinoma and five as undefined NSCLC histology. Forty-five patients were in stage IV (76%), whereas 14 patients were in stage IIIB (24%). The description of parameters in the two datasets is presented in Table II.

Median follow-up was 12 months and, on the overall, these patients presented a median PFS of 9 months (mean \pm SD=11 months \pm 1.3 months, 95% CI 8.3-13.7 months), and a median OS of 13 months (mean \pm SD=14.5 months \pm 1.6 months, 95% CI 11.1-17.4 months), with 32 (54%) patients still alive at the last follow-up examination (Fig. 1).

Median cycles of Nivolumab were 10 for the whole population (mean 14, range 2-52 cycles). In our series, survival analysis did not find any correlation with gender (male: Median OS 13 months, mean 14 \pm 1.8 months, 95% CI 10-18 months, vs. female: Median OS 7 months, mean 10 \pm 2.7 months, 95% CI 5-15 months, P-value: 0.58), with age (Cox-Regression P-value: 0.44), stage (IIIB: Median OS 15 months, mean 14 \pm 2.4 months, 95% CI 10-19 months, vs. IV: Median OS 13 months, mean 12 \pm 1.7 months, 95% CI 9-15 months, P-value: 0.60) and with histology (squamous cell: Median OS 15 months, mean 14 \pm 2.7 months, 95% CI 9-20 months, vs. adenocarcinoma: Median OS 13 months, mean 12 \pm 1.6 months, 95% CI 9-15 months, P-value 0.88).

Survival analysis and texture score. In order to calculate a reproducible cut-off for the targeted TA parameters in CT images, we used the *X-Tile* Software as described in the Methods section.

A valid parameter cut-off could be selected in the whole population for: 1) volume (score 1 > 36 ml); 2) histogram entropy (score 1 > 1.30), 3) compacity (score 1 < 3.4), GLCM-entropy (score 1 > 1.8), 5) GLCM-dissimilarity (score 1 > 5.6) GLCM-correlation (score 1<0.5). These cut-off values were applied to both training and validation set.

In our analysis, we have restricted the testing of texture parameters describing morphological, histogram and GLCM features, as these parameters have been successfully used and correlated in lung cancer imaging (26), to avoid the risk of overfitting our model using too many parameters (36).

The global texture score, as described in the Methods section, allowed to classify the patients into two cohorts (low risk: Score 0-1, and high risk: Score >1).

The clinical parameters of the two cohorts were then tested with Chi-square analysis, that demonstrated no statistical differences of the clinical variables in the two subsets, as follows: Age (P=0.282), sex (P=0.843), histology (P=0.107), stage (P=0.834) (Table II).

Table I. Reliability analysis of TA parameters.

TA Parameter	Enhanced CT ICC (single measure)	CT ICC (single measure)
Volume (ml)	0.998 ^a	0.996 ^a
Volume	0.996 ^a	0.994 ^a
Skewness	0.519	0.829 ^a
Kurtosis	0.386	0.616
Entropy	0.879 ^a	0.968 ^a
Energy	0.808 ^a	0.841 ^a
Sphericity	0.985 ^a	0.888 ^a
Compacity	0.994 ^a	0.989 ^a
GLCM-homogeneity	0.862 ^a	0.963 ^a
GLCM-energy	0.787 ^a	0.917 ^a
GLCM-contrast	0.935 ^a	0.986 ^a
GLCM-correlation	0.707 ^a	0.847 ^a
GLCM-entropy	0.863 ^a	0.973 ^a
GLCM-dissimilarity	0.919 ^a	0.994 ^a

^aParameters that are reliable (ICC >0.7); ICC, Intraclass Coefficient Correlation; TA, texture analysis; GLCM, gray level co-occurrence matrix.

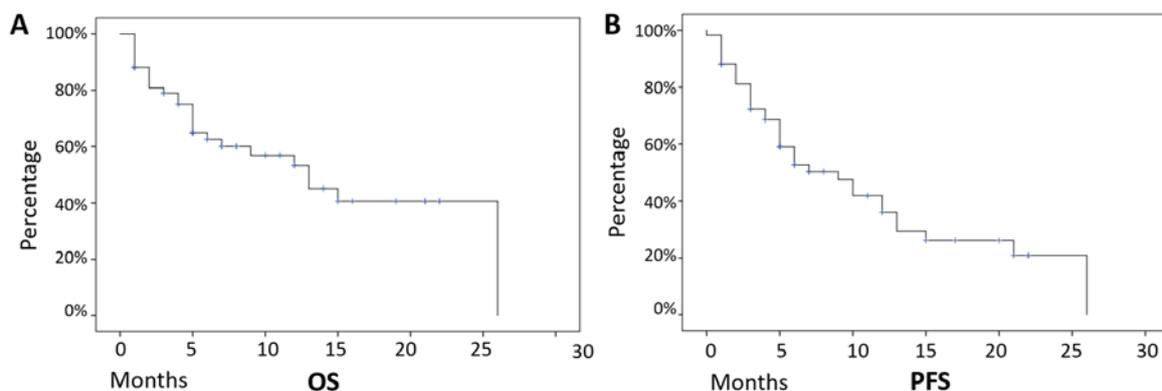


Figure 1. OS and PFS of the whole patient dataset. Median follow-up was equal to 12 months and these patients presented (A) a median PFS of 9 months, and (B) a median OS of 13 months, with 32 (54%) patients still alive at the last follow-up examination. OS, overall survival; PFS, progression free survival; SD, standard deviation.

PFS resulted significant for both the subsets TRAINING SUBSET (*low risk* median PFS 10 months, mean 10.9 +/- 1.8 months, 95% CI 7-14 months, vs. *high risk* median PFS 3 months, mean 4.7 +/- 1.1 months, 95% CI 2-6 months, P-value: 0.04) and VALIDATION SUBSET (*low risk* median PFS 15 months, mean 15.5 +/- 2.9 months, 95% CI 9-21 months, vs. *high risk* median PFS 6 months, mean 6 +/- 0.9 months, 95% CI 4-8 months, P-value: 0.04; Fig. 2).

OS, also, resulted significant in both subsets TRAINING SUBSET (*low risk* median OS: Not reached, mean 14.4 +/- 2 months, 95% CI 10-18 months, vs. *high risk* median OS: 5 months, mean 6.2 +/- 1.6 months, 95% CI 3-9 months, P-value: 0.02) and VALIDATION SUBSET (*low risk* median OS: 26 months, mean 19 +/- 3 months, 95% CI 13-25 months, vs. *high risk* median OS 6 months, mean 6.1 +/- 0.9 months, 95% CI 4-8 months, P-value 0.03; Fig. 2).

The global texture score correlated with patients' survival allowed to classify patients included into two cohorts at low (Score 0-1, 36 patients, 61%) and high risk (Score > 1,

23 patients, 39%) of recurrence, respectively, presenting a median PFS of 12 months (mean 13 +/- 1.7 months, 95% CI 9-16 months), vs. a median PFS of 5 months (mean 5.4 +/- 0.7 months, 95% CI 4-7 months), with a P-value of 0.01, and a median OS of 26 months (mean +/- SD: 17.5 +/- 1.98 months, 95% CI 14-21 months) and 5 months (mean +/- SD: 6 +/- 0.99 months, 95% CI: 4-8 months), with a P-value <0.01, in the low and high risk groups, respectively (Fig. 2).

Number of cycles of Nivolumab, also, were different among subgroups (P=0.026), with low risk subset showing a median of 12 cycles (mean 18.5 cycles, range 2-52 cycles) and high risk subset showing a median of 10 cycles (mean 8.8 cycles, range 2-20 cycles).

Discussion

The recent clinical development of immune-biological drugs such as PD-1/PDL1 inhibitor mAbs for the treatment of common malignancies has clearly risen an extraordinary

Table II. Patient characteristics.

Parameter	Training dataset	Validation dataset	Whole dataset
Sex			
Males	29 (83%)	19 (79%)	48 (81%)
Females	6 (17%)	5 (21%)	11 (19%)
Age			
Median	71	65	69
Mean +/- s.d.	68 +/- 8	67 +/- 11	67 +/- 10
Stage			
IIIB	8 (23%)	6 (25%)	14 (24%)
IV	27 (77%)	18 (75%)	45 (76%)
Histology			
Adenocarcinoma	23 (66%)	13 (54%)	36 (61%)
Squamous cell	7 (20%)	11 (46%)	18 (30.5%)
Not specified	5 (14%)		5 (8.5%)
PFS			
Progression	22 (63%)	15 (62%)	37 (62%)
No progression	13 (37%)	9 (38%)	22 (38%)
OS			
Dead	15 (43%)	12 (50%)	27 (45%)
Alive	20 (57%)	12 (50%)	32 (55%)

PFS, progression free survival; OS, overall survival.

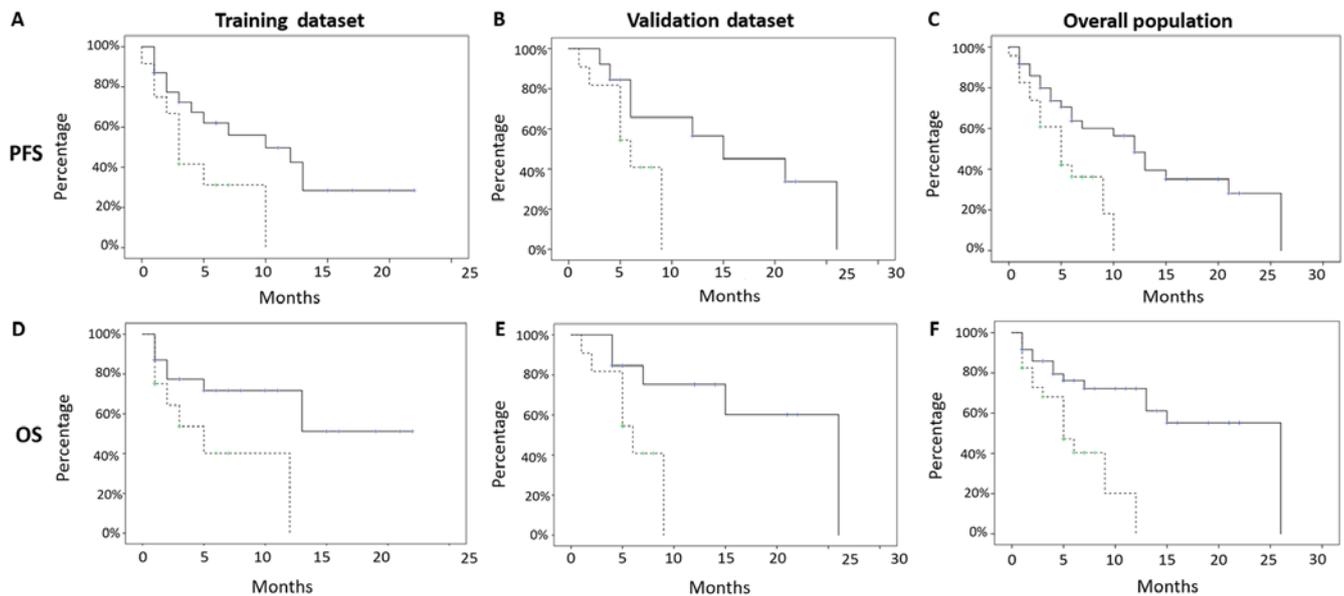


Figure 2. Analysis of survival (OS, PFS) with texture score of the different subsets. PFS was significant for both subsets. (A) TRAINING SUBSET: Low risk PFS: Median PFS 10 months; mean 10.9 +/- 1.8 months; 95% CI 7-14 months; vs. high risk median PFS 3 months; P=0.044. (B) VALIDATION SUBSET: Low risk median PFS 15 months; vs. high risk median PFS 6 months; P=0.048. OS was significant for both subsets. (D) TRAINING SUBSET: Low risk median OS: Not reached; vs. high risk median OS: 5 months; P=0.023. (E) VALIDATION SUBSET: Low risk median OS: 26 months; vs. high risk median OS 6 months; P-value 0.032. The global texture score, which correlated with patients' survival, allowed the classification of these patients into two cohorts at low (Score 0-1; 36 patients; 61%) and high risk (Score >1; 23 patients; 39%). (C): WHOLE COHORT: low risk median PFS of 12 months, vs. high risk median PFS 5 months (P=0.009). (F) WHOLE COHORT: low risk median OS 26 vs high risk median OS 5 months (P=0.002).

challenge in terms of cost, adverse events and patients' monitoring. So far, no clear biomarkers are presently able to select patients who may benefit by treatment with Nivolumab in

NSCLC, although some interesting preliminary results (37). Additionally, these drugs act by turning the immune-balance between tumour and immune system in favour of the latter,

thus providing a clinical benefit and prolonging patients' survival even in the presence of an apparent radiological progression. This fact makes still more difficult the monitoring of these patients with conventional imaging techniques. In fact, the latter have been designed to evaluate the effects of classical cytotoxic/cytoreductive strategies, as well as chemo-radiotherapy, aimed to provide serial comparisons of tumour volume and number of lesions. In this context, functional imaging techniques, such as diffusion-weighted magnetic resonance imaging (MRI), perfusion methods, positron emission tomography (PET), and radiomics, which are also able to define more qualitative analysis of targeted tumour lesions, are under investigation for providing useful information for these patients (38,39). These techniques are of interest in the search for surrogate biomarkers to potentially define and monitor tumour specific biological processes, including tumour cellularity, growth, necrosis, tumour-associated inflammation and angiogenesis. All these biological events can strictly reflect either tumour progression or responsiveness to the new generation of biological drugs including inhibitors of PD-1/PDL1 immune-checkpoint axis alike Nivolumab, Pembrolizumab, or Atezolizumab mAbs (monoclonal antibodies) (40-44).

In our retrospective multi-institutional analysis, we have restricted the testing of texture parameters to NSCLC patients undergone to salvage treatment with Nivolumab. We have described morphological, histogram and GLCM matrix features defining parameters which have been subsequently correlated in lung cancer imaging, and in recent years with immunotherapy in NSCLC (26,36,45-49). Interestingly, within our sample of NSCLC patients, subjected to Nivolumab treatment, we defined morphological features able to identify a cohort of patients with a very poor prognosis. These patients before the beginning of the immunological treatment had a larger volume of the primary lung lesions associated to a lower compactness, measured as compacity of volume. These combined parameters reflect larger tumour cell burdens paralleled by central hypoxia, necrosis, and invasion beyond the tumour and poor response to cytotoxic treatments. These events additionally define a disease with a very unbalanced antitumour immune response (19,50,51).

In our analysis, a higher histogram entropy was associated with worse outcome. Several reports suggest that this parameter reflects the aggressiveness of the disease as associated to a rapid growth and invasiveness (27,30,52).

The GLCM-entropy, specifically measures the randomness of gray-level voxel pairs, and has been significantly associated, as above, with a worse prognosis in other works (19,27,30).

The GLCM-dissimilarity, on the other hand, measures the variation of gray-level voxel pairs, and can reflect intra-tumour heterogeneity, whereas the GLCM-correlation measures the linear dependence of gray-level in GLCM, and it has been described an important feature in previous studies (19).

These radiomics parameters including higher GLCM-entropy and -dissimilarity and lower GLCM-correlations were strictly associated with a worse prognosis. These TA parameters describe a poor immunogenic tumour pattern weakly responsive to PD-1/PDL1 blockade. However, it has to be considered that our results need to undergo critical consideration for many methodological and technical refinements.

In particular, our study is retrospective, and the correlations between textural parameters and clinical outcome requires additional investigations in order to better understand the pathological bases of the TA parameters.

Moreover, the arbitrary choice of the cut-off can lead to significant bias even if we have not unbalanced the subgroups (35) and we have validated our model with an external validation cohort. Conversely, in our series, survival analysis did not show any correlation with gender and age.

In this regard, we believe that the low number of patients analyzed, as well as the low ratio of females (19%) and the low standard deviation of the age (mean age 67.4 years \pm 9.7 years) can easily justify these discrepancies with the literature (53). At the same time, the specific subset of patients analyzed (NSCLC patients in progression after first line chemotherapy) could also justify the non-significance of clinical variables such as sex and age.

In conclusion, the predictive value of our TA parameters seems to deserve consideration as a potential and inexpensive biomarker to select NSCLC patients who may benefit by Nivolumab treatment and deserves further development in prospective Randomized trial.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors have substantially contributed to the conception of the study. VN, PTi, PP, CB, AR, SFC, PC, VB, RG, GC, CT, CG, PTas, PTag, SC, RC, AL, MC, AG, MAM and LP have acquired the data, performed the analysis and interpretation of the data and they have contributed to the drafting and revision of the different versions of the manuscript. All authors have given final approval of the version to be published and agree to be accountable for all the aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Ethics approval and consent to participate

All patients gave written consent for anonymous use of their examinations for research scope. All procedures were undertaken in compliance with the ethical statements of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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