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CORRELATION BETWEEN B7-H4 EXPRESSION AND SURVIVAL OF NON-SMALL CELL LUNG CANCER PATIENTS TREATED WITH NIVOLUMAB

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SUMMARY

INTRODUCTION

OVERVIEW ON NON-SMALL CELL LUNG CANCER

Lung cancer represents a major healthcare issue, as it is the third most frequently diagnosed malignancy, and the first cause of cancer-related deaths in the United States. The high mortality of lung cancer is at least partially related to the fact that this cancer is often diagnosed in advanced stage, when only palliative treatments are available [1]. Lung cancer is divided into two distinct entities based on histology: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), the former being the most represented (roughly 80-90% of cases); additionally, NSCLC is divided into two further sub-groups: non-squamous NSCLC and squamous NSCLC. The rationale for a distinction among different histologic sub-groups is that the therapeutic approach may be significantly different on the basis of histology, as explained later [2].

With regards to the therapeutic management of NSCLC, the optimal approach is based on the disease stage according to the International Association for the Study of Lung Cancer (IASLC) TNM staging system [3]. Early stage NSCLC is typically managed with loco-regional approaches, represented in first place by radical surgery (mostly lobectomy, but including more extensive procedures such as pneumonectomy or more limited techniques such as segmentectomy), which is expected to include a proper hilar and mediastinal lymphadenectomy. After surgery, a pathological report showing tumor size > 4 cm or lymph nodal involvement identifies those patients for whom adjuvant chemotherapy (4 cycles of a platinum-based combination) is associated with a reduction of recurrence risk and is therefore indicated [4]; additionally, adjuvant mediastinal radiation is usually offered to patients with mediastinal lymph nodal involvement (pN2) as this approach is described to improve local control and survival in this specific

patients' sub-group [5]. Locally advanced NSCLC (including large tumors or pre-operative evidence of mediastinal lymph nodal involvement) represents a heterogeneous and challenging setting, in which multi-disciplinary assessment is advised for each patient, as some cases might be eligible for radical surgery after neoadjuvant chemotherapy, while the standard upfront approach for non-resectable, locally advanced NSCLC is represented by concurrent or sequential chemo-radiation. Superior sulcus tumors are a specific and uncommon entity that might be managed through a multimodality approach including chemo-radiation and surgery, provided that the tumor is potentially resectable in first place [6,7]. With regards to advanced or recurrent disease not amenable to loco-regional treatments, the approach of choice is systemic therapy, represented by chemotherapy, targeted agents, and immune checkpoint inhibitors (the latter will be discussed in the following sections).

Chemotherapy has been for many years the corner-stone for the treatment of advanced NSCLC, and the most widely employed first-line regimens include a combination of a platinum-derivate (cisplatin or carboplatin) and a third-generation compound, while second-line and further line regimens usually involve the administration of a single-agent such as docetaxel (the standard second-line chemotherapy until recently), vinorelbine, or gemcitabine [8]. Notably, histology drives the choice of the employed chemotherapeutic regimens and, based on efficacy as well as safety data, some combinations have been limited to specific histologic sub-groups (e.g.: regimens including a platinum-derivate and pemetrexed with subsequent maintenance with single-agent pemetrexed are limited to non-squamous histology) [9]. A noteworthy addition to chemotherapy (also limited to non-squamous NSCLC) is represented by angiogenesis-disrupting agents; such agents include bevacizumab, which inhibits the vascular endothelial growth factor (VEGF) and has been employed in addition to platinum-based

combinations with benefit in terms of efficacy, and nintedanib, which is a VEGF-inhibitor as well as a multi-target tyrosine kinase inhibitor (TKI) and has been added to docetaxel resulting in survival advantage [10].

Targeted agents are compounds directed against specific molecules that are the product of genic alterations. The most clinically relevant molecular alterations identified so far are the activating mutations of the epidermal growth factor receptor (*EGFR*) gene, the rearrangements of the anaplastic lymphoma kinase (ALK) gene and rearrangements of the c-ros oncogene 1 (ROS1) gene. These aberrations are typically observed in non-squamous histology and are relatively uncommon in the Caucasian population; indeed, *EGFR* mutations account for 10-15% of patients, while *ALK* and *ROS1* rearrangements account for approximately 5% and 1% of patients, respectively [11]. From a therapeutic perspective, antineoplastic agents directed against these mutated targets have generally achieved improved outcomes compared to standard chemotherapy. With regards to EGFR inhibitors, several TKIs are currently available in clinical practice, ranging from first-generation TKIs (erlotinib and gefitinib) to second-generation inhibitors (afatinib and dacomitinib) and to third-generation inhibitors, currently represented by osimertinib; while the original indication for this drug was limited to the treatment of patients whose tumor had progressed during treatment with earlier-generation EGFR inhibitors by developing an acquired *EGFR* mutation in exon 20, known as T790M, osimertinib has recently been approved for the upfront treatment of patients harboring activating EGFR mutations, with a significant impact on the therapeutic algorithm of this specific population [12]. With regards to *ALK* rearrangements, several inhibitors have become available in clinical practice, their forerunner being represented by crizotinib, which achieved improved outcomes over first and second-line chemotherapy in populations of patients with *ALK*-rearranged NSCLC. Currently, the new ALK-inhibitor alectinib

has achieved improved progression-free survival (PFS) over crizotinib in first-line, thus becoming the new standard for treatment-naïve *ALK*-rearranged NSCLC. Finally, with regards to advanced NSCLC harboring *ROS1* rearrangements, crizotinib represents the current agent of choice for first-line treatment [13].

THE ROLE OF IMMUNE CHECKPOINT INHIBITORS IN NSCLC

The notion that neoplastic cells progressively acquire the ability to escape the surveillance of cells belonging to the immune system is well established; indeed, immuno-editing is known as the dynamic process leading to the development of immune-resistant neoplastic clones and is represented by three phases: 1) elimination (when innate and adaptive immune response engage tumor cells); 2) equilibrium (when non-immunogenic tumor cells have been selected and start growing); 3) escape (when tumor cells grow uncontrolled by the immune system, potentially developing cancer) [14]. The knowledge of this mechanism has encouraged studies with the aim of improving anti-neoplastic response from immune cells, and has led to various experimental therapeutic approaches, such as cancer vaccines.

Immune checkpoints are crucial mechanisms of self-tolerance, developed to prevent autoimmune reactions. The most widely known actors of immune checkpoints are represented by cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which is expressed on activated T-cells and exerts its inhibitory function by binding CD-80 and CD-86 on the surface of antigen-presenting cells, and by the axis involving the programmed death protein 1 (PD-1) expressed on T-cells, and its ligand (PD-L1), expressed on tumor cells. Since the pathways involving CTLA-4 and PD-1/PD-L1 are both associated with decreased T-cell activity, it has been postulated that the blockade of such signals could disrupt

immune tolerance, with subsequent antineoplastic effects. Furthermore, it has been observed that the expression of PD-L1, which is infrequent in normal tissue, might be up-regulated on tumor cell surface and hence play a relevant role in immune escape mechanisms; this notion has led to extensive investigation designed to define the role of PD-L1 expression as a clinically meaningful biomarker, as reported below. [15].

The first clinical model for immune checkpoint inhibition is represented by CTLA-4 blockade in metastatic melanoma, a malignancy well known for its immunogenicity, through the use of ipilimumab, a fully human IgG monoclonal antibody directed against CTLA-4 [16]. Subsequently, different agents designed to disrupt the PD-1/PD-L1 axis were developed in the same setting (metastatic melanoma), achieving superior outcomes even over ipilimumab, and hence becoming a standard of care for the management of metastatic melanoma [17].

While lung cancer had not previously been considered an immunogenic neoplasm, subsequent pre-clinical experiences suggested the contrary. While the studies involving immuno-modulation in NSCLC in the form of cancer vaccines led to underwhelming results [18], the first trial involving the CTLA-4 inhibitor ipilimumab in association with platinum-based chemotherapy as first-line for advanced NSCLC resulted in a significant advantage in terms of PFS over chemotherapy alone [19]. Subsequently, nivolumab, a fully human monoclonal antibody directed against PD-1, was compared to docetaxel as therapy for patients who had previously been treated with platinum-based chemotherapy for advanced NSCLC in two distinct randomized, phase III trials: Checkmate 017 (squamous histology), and Checkmate 057 (non-squamous histology). Both trials showed a statistically significant advantage in terms of median overall survival (OS) for nivolumab over docetaxel (9.2 vs. 6.0 months in Checkmate 017 and 12.2 vs. 9.4 months in Checkmate 057); furthermore, the survival advantage was maintained

after a 3-year follow-up, with a 3-year OS rate of 17% with nivolumab as compared to 8% with docetaxel in the pooled populations from both trials. [20 , 21 , 22]. Another PD-1 inhibitor, pembrolizumab, was compared with docetaxel in the same setting (pre-treated advanced NSCLC) in the randomized, phase III Keynote 010 trial; notably, two different doses of pembrolizumab were evaluated (2 mg/Kg and 10 mg/Kg), and a significant advantage in terms of OS was observed with both doses of pembrolizumab as compared with docetaxel [23]. Notably, while a positive expression of PD-L1 on tumor cell membrane assessed by immunohistochemistry (IHC) was not required for enrolment in Checkmate 017 or Checkmate 057 (and the role of TPS was evaluated post-hoc), a tumor proportion score (TPS) of at least 1% was required in order to be enrolled in Keynote 010. The expression of PD-L1 was not prognostic or predictive of benefit from nivolumab in Checkmate 017 (squamous histology), while a positive association between positive PD-L1 expression (cut-offs 5% and 10%) and OS was observed in Checkmate 057 (non-squamous histology). In Keynote 010, while the trial population was enriched for tumors expressing PD-L1, the investigators observed that higher expression levels were predictive of improved survival benefit, as the hazard ratio (HR) for death with pembrolizumab vs. docetaxel was 0.53 in the sub-group with PD-L1 ≥ 50% and 0.76 for PD-L1 expression 1%-49% [24]. Finally, atezolizumab is another monoclonal antibody designed to disrupt the PD-1/PD-L1 axis, with the difference that its target is PD-L1. This agent was compared to docetaxel in a similar setting (pre-treated advanced NSCLC), and achieved significantly longer survival over chemotherapy (median OS: 13.8 vs. 9.6 months), and the benefit was observed irrespective of histology and PD-L1 status (patients who were negative for PD-L1 expression were eligible) [25]. An updated analysis after 2 years of follow-up was consistent

with the primary analysis of clinical benefit, and a tolerable safety profile was observed in spite of prolonged exposition to the agent [26].

Based on the results achieved in pre-treated NSCLC, immune checkpoint inhibitors were the subjects of several first-line trials, involving them both as single-agents and as part of combination regimens. Checkmate 026 compared single-agent nivolumab with platinum-based chemotherapy in a population of patients affected by treatment-naïve advanced NSCLC with positive PD-L1 expression; the trial failed to show an advantage in terms of outcomes with nivolumab (median PFS and OS with nivolumab and chemotherapy were 4.2 vs. 5.9 months, and 14.4 vs. 13.2 months, respectively), and was hence considered negative [27]. The phase III, randomized Keynote 024 trial was designed to compare pembrolizumab and first-line platinum-based chemotherapy in a population of NSCLC patients selected for high expression of PD-L1 (≥ 50%), on the basis of previous clinical data with pembrolizumab. Contrarily to what observed in Checkmate 026, in this trial, the PD-1 inhibitor achieved a statistically significant advantage in terms of PFS (10.3 vs. 6.0 months) and OS (30.0 vs. 14.2 months) over chemotherapy in this selected patients' population; therefore, pembrolizumab was approved as single-agent in treatment-naïve patients affected by advanced NSCLC with high PD-L1 expression [28,29].

In addition to single-agent regimens, immune checkpoint inhibitors have been employed also as part of combination strategies, including multiple checkpoint inhibitors or associations of immunotherapy and chemotherapy. Checkmate 227 was a randomized, phase III trial designed to compare ipilimumab plus nivolumab versus platinum-based chemotherapy; notably, while the patients were not selected for PD-L1 expression, the enrolled patients were stratified also on the base of tumor mutational burden (TMB), as previous studies suggested that TMB is associated with immunogenicity; hence, tumor harboring high TMB (at least 10

mutations / megabase) could be more likely to respond to immunotherapy. The checkpoint inhibitors combination achieved longer PFS over chemotherapy (7.2 vs. 5.5 months) in patients with high TMB, irrespective of PD-L1 expression [30]. However, subsequent OS data showed that also patients with low TMB achieved superior outcomes with ipilimumab-nivolumab compared to chemotherapy, thus questioning the role of TMB as a potential biomarker of benefit from immunotherapy [31]. A different phase III trial, Keynote 189, compared pembrolizumab versus placebo in combination with platinum-pemetrexed regimen in non-squamous advanced NSCLC. The combination of chemotherapy plus pembrolizumab achieved superior outcomes in terms of both PFS and OS over chemotherapy plus placebo (the median PFS was 8.8 vs. 4.9 months, while the median OS was not reached in the combination arm and it was 11.3 months in the placebo arm). Notably, the survival advantage was observed regardless of the level of PD-L1 expression [32]. A similar trial, Keynote 407, compared pembrolizumab versus placebo in combination with chemotherapy based on carboplatin plus paclitaxel/nab-paclitaxel in a population of patients affected by squamous advanced NSCLC. In this study, the addition of pembrolizumab resulted in significantly longer PFS and OS (6.4 vs. 4.8 months and 15.9 vs. 11.3 months, respectively); similarly to Keynote 189, the survival advantage was consistent regardless of the expression of PD-L1 [33]. Finally, several trials explored the role of the addition of atezolizumab to different chemotherapy regimens, such as carboplatin plus paclitaxel, carboplatin plus nab-paclitaxel, or platinum plus pemetrexed. The most relevant of such trials is IMpower 150, which explored the effect of the addition of atezolizumab to carboplatin-paclitaxel +/- bevacizumab. In this study, the addition of atezolizumab to a regimen containing bevacizumab plus carboplatin-paclitaxel resulted in longer PFS (8.3 vs. 6.8 months) and OS (19.2 vs. 14.7 months), and the benefit was consistent

regardless of PD-L1 expression; notably, the benefit was also observed in patients with liver metastases or with molecular alterations involving *EGFR* or *ALK*, which are typically clinical factors for poor response to immune checkpoint inhibition [34].

NOVEL PREDICTIVE BIOMARKERS FOR IMMUNOTHERAPY IN PREVIOUSLY TREATED NSCLC

As reported, the expression of PD-L1 alone has a limited role in selecting which patients should receive immune checkpoint inhibitors or chemotherapy in second or subsequent lines of treatment for NSCLC. In first place, it has been observed that different assays yield discordant results; additionally, PD-L1 has been recognized to have a heterogeneous expression, associated to variable and not completely understood immune mechanisms [35,36,37]. Thus, identifying other potential biomarkers would result in improved patient selection for treatment with immune checkpoint inhibitors.

According to preclinical studies, the cross-talk during immune response to cancer is not limited to PD-1/PD-L1 axis, and involves different molecules, either acting as co-stimulators or as immune checkpoints. For instance, PD-L2 binds PD-1 with inhibitory function, similarly to PD-L1; however, while PD-L1 is expressed in many cell lines, PD-L2 has a more restricted expression pattern, limited to dendritic cells, macrophages, and mast cells. Furthermore, PD-L2 might be associated with immune tolerance to normal respiratory cells [38,39]. Other molecules, such as B7-H3 and B7-H4, have been acknowledged as potential regulators of immunity and as prognostic factors in solid tumors [40]. B7-H3 has a controversial role in T cell response [41,42,43,44], and its expression on tumor cells is reportedly associated with poor prognosis in NSCLC [45,46]. B7-H4 is a trans-membrane protein suggested to inhibit the activation and the clonal

expansion of $CD4^+$ and $CD8^+$ cells, as well as the production of immune-promoting cytokines; notably, the receptor of B7-H4 on immune cells has not been identified yet [47,48]. High expression of B7-H4 has been associated with poor prognosis in different solid tumors, including NSCLC [49,50,51,52].

While the aforementioned biomarkers seem to have a role in the modulation of immune response and prognostic significance in solid tumors, including NSCLC, their role in predicting response to immune checkpoint inhibitors has to be clarified yet; hence, our aim was to assess the association between the expression of these biomarkers and benefit from nivolumab in advanced NSCLC.

AIM OF THE PRESENT STUDY

Our study explores the potential correlations between intra-tumor expression of a panel of immune-related biomarkers (PD-L2, PD-1, B7-H3, and B7-H4) and clinical outcomes of advanced NSCLC patients treated with nivolumab for advanced NSCLC (Nivolumab Cohort). In order to define whether any meaningful biomarker identified in the Nivolumab Cohort might have a role in predicting the efficacy of immunotherapy, rather than a plain prognostic role, we retrospectively assessed the correlations between these biomarkers and outcomes of a population of patients who had been treated with platinum-based chemotherapy and had not subsequently received any immune checkpoint inhibitor (Chemotherapy Cohort).

MATERIALS AND METHODS

NIVOLUMAB COHORT

The Nivolumab Cohort included 46 patients affected by advanced NSCLC treated within the Italian Nivolumab Expanded Access Program (NCT02475382) and enrolled in a mono-institutional translational research study approved by our Local Ethics Committee (registry number: P.R. 191REG2015) [53,54]. The patients were eligible if they met the following criteria: *i)* cytologically or histologically confirmed advanced/metastatic NSCLC, *ii)* progression after at least one line of platinum-based chemotherapy, *iii)* Eastern Cooperative Oncology Group Performance Status (ECOG-PS)= 0-2, *iv)* no previous treatment with immune checkpoint inhibitors, *iv)* any brain metastasis had to be treated and clinically stable for at least 14 days before starting nivolumab, v) no treatment with corticosteroids at a dose higher than 10 mg/day of prednisone or equivalent. Eligible patients received nivolumab at 3 mg/kg every 14 days, with assessment by computed tomography scan (CT-scan) every 8 weeks. Nivolumab was administered until onset of unacceptable toxicities, patient's refusal, death or up to 96 weeks from the start of treatment; treatment beyond tumor progression was allowed based on Investigators' judgment as long as clinical benefit was perceived. Objective responses and progression-free survival (PFS) were determined according to the Response Evaluation Criteria in Solid Tumors (RECIST) *v.*1.1; due to the peculiar mechanism of action of nivolumab, we assessed objective responses also with Immune-Related Response Criteria (irRC). Progression-free survival (PFS) and OS were calculated from the first administration of nivolumab to progression/death.

CHEMOTHERAPY COHORT

The Chemotherapy Cohort included 27 treatment-naive patients with histologically or cytologically confirmed advanced non-squamous NSCLC and ECOG PS= 0-1 treated between 2011 and 2015 and drawn from a wider population of patients (n= 90) enrolled in a mono-institutional translational research study (NCT02055144) on the basis of available stored tissue for biomarker analyses. The regimen of choice was cisplatin plus pemetrexed for up to 4 cycles, followed by maintenance with pemetrexed. Carboplatin was administered in place of cisplatin to patients with a creatinine clearance < 60 ml/min). Tumor response was assessed with RECIST *v.1.1* every 2 cycles. Chemotherapy was administered until unacceptable toxicity, patient's refusal, progression, or death. The aforementioned translational research study admitted patients with both squamous and non-squamous histology; however, to date, only the population affected by non-squamous histology completed accrual and was hence available for this analysis [55,56].

IMMUNOHISTOCHEMISTRY (IHC)

Sections of formalin-fixed, paraffin embedded (FFPE) tissue were cut at 2 μm and mounted on positively charged, adhesive glass slides (Superfrost Plus Gold, Thermo Scientific, Braunschweig, Germany). The IHC was carried out manually, miming the automated staining steps performed by Dako Autostainer Link 48, as indicated by the approved FDA protocol (PMA P150025; validated automated assay with rabbit monoclonal anti Human PD-L1 antibody clone 28-8 Pharm DX Dako). The sections were heated at 60°C for 15 minutes and washed with xylene (2 x 10 minutes) and ethanol (2 x 10 minutes) to remove paraffin. Antigen retrieval and primary antibody incubation were carried out at room temperature in

a hydrate chamber. Two sections of human placenta were included in each run as control; one section was incubated with the primary antibody containing the PD-L1 rabbit monoclonal antibody, while a second section was incubated with the negative control reagent of the kit, an IgG rabbit monoclonal antibody in a buffer solution. A two-step immunoperoxidase staining method was used for all the antibodies (Dako EnVision + Dual Link System – HRP – DAB+) as follows: B7-H4 (mouse monoclonal, clone MIH43, Abcam - dilution 1:60), B7-H3 (rabbit polyclonal, NovusBio - dilution 1:25), PD-L2 (mouse monoclonal, clone 8G8, LSBio - dilution 1:50), and PD-1 (rabbit polyclonal, Abcam - dilution 1:50). Each run contained a positive control (on-slide tonsil tissue for PD-1 and PD-L2, prostate cancer for B7-H3; breast carcinoma for B7-H4) and a negative control (no primary antibody). The 22C3 antibody was obtained from the commercially available PD-L1 PharmDX kit on the BenchmarkULTRA (Ventana Medical Systems/Roche, Tucson, AZ) platform, using the UltraView detection kit [57].

IHC SCORING

The percentage of stained positive tumor cells was evaluated for each sample under light microscope by two pathologists; positive staining was defined as complete or partial circumferential membrane staining at any intensity or diffuse cytoplasmic staining.

STATISTICAL ANALYSIS

In Nivolumab Cohort, response categories according to RECIST *v.1.1* and irRC were compared with the expression of each biomarker under study with Fisher's test or Chi Square test, as appropriate. Survival curves were compared between patient subgroups based on each biomarker expression by using Kaplan-Meier estimator. Cox’s Proportional Hazard Model was used for multivariate survival

analyses, starting from a model that included clinical and pathological characteristics, as well as the expression of the immune-related biomarkers. The final model was reached at by means of a stepwise regression with backward elimination of variables not significantly associated with PFS or OS, respectively, based on the Likelihood Ratio test. The same analyses were then repeated in the Chemotherapy Cohort, the rationale being that any difference between the 2 cohorts in the prognostic role of a specific biomarker would suggest an association between the expression of said biomarker and the efficacy of immunotherapy. Due to the exploratory aims of these analyses, only Odds Ratio for objective response and HR for PFS and OS for each of the 5 biomarkers in the 2 cohorts are reported, with their 95% CI, and no formal statistical comparison was planned. The analyses were carried out by using SPSS (v.23.0.0.0) and XLSTAT (v.19.03.44845).

RESULTS

NIVOLUMAB COHORT

The Nivolumab Cohort included 46 evaluable patients, whose clinical characteristics are summarized in **Table 1**. PD-L1 and PD-1 were not evaluable in one and two samples, respectively, due to excessive background. B7-H4, PD-1 and PD-L2 stainings were observed in cytoplasm, while B7-H3 was detected in both cell membrane and cytoplasm and PD-L1 was exclusively expressed in cell membrane. Representative images of IHC staining of PDL-1 and B7-H4 are reported in **Figure 1**, while the expression of each immune-related parameter is reported in **Table 2** and in **Supplementary Table 1**. All the biomarkers apart from PD-1 showed an expression < 1% in most samples; consistently with the cut-off values of previous studies involving immune checkpoint inhibitors in pre-treated NSCLC patients [58,59,60], we selected the value of 1% as an appropriate cuf-off for defining positive (≥ 1%) *vs.* negative (< 1%) samples for each potential biomarker. No significant correlations were observed among the expressions of the biomarkers.

When clinical characteristics were compared with each biomarker, PD-L2 expression was associated with ECOG PS= 0 (*p*-value= 0.038), while PD-1 expression was associated with age ≥70 years (*p*-value= 0.026) and B7-H3 expression was associated with squamous histology (*p*-value= 0.023).

No statistically significant association between the expression of the immune-related biomarkers and the proportion of ORR and DCR was observed, although, notably, none of the six patients expressing B7-H3 achieved objective response.

Progression-free survival was evaluable for 44 out of 46 patients according to RECIST. One patient discontinued treatment before the first assessment and did not undergo further CT scans after baseline; another patient had non-measurable

disease according to RECIST, but was considered evaluable by irRC, as the baseline lesions met the requirements for measurability with such criteria. Seven patients died before undergoing the first response evaluation. The overall outcome data are reported in **Supplementary Table 2**. With regards to univariate PFS analysis, B7-H3 expression was associated with significantly lower RECIST-PFS (median PFS 1.6 vs. 2.0 months; *p*-value= 0.009); while this result should be considered with caution due to the fact that only six out of 44 patients expressed B7-H3, the rapid progression of all these patients was noteworthy. More importantly, B7-H4 expression was associated with significantly reduced RECIST-PFS (median 1.7 vs. 2.0 months; *p*-value= 0.026); no other biomarker was associated with differences in terms of RECIST-PFS. The irRC-PFS analyses based on each immune-related biomarker were generally consistent with RECIST-PFS analyses, although the irRC-PFS difference based on B7-H3 expression fell short of statistical significance (*p*-value= 0.057). In the multivariate RECIST-PFS analysis, the only variables significantly associated with shorter PFS were B7-H3 expression (HR= 4.14; 95% CI: 1.44-11.9; *p*-value= 0.019) and B7-H4 expression (HR= 2.28; 95% CI= 1.16-4.48; *p*-value= 0.021). The multivariate irRC-PFS analysis was consistent with the RECIST-PFS analysis.

In the univariate OS analyses, no significant association with immune-related biomarkers was observed, although the association between B7-H4 expression and reduced OS was close to significance (4.37 vs. 9.83 months; *p*-value= 0.064). In multivariate analysis, OS was significantly reduced in patients with ECOG-PS= 1-2 vs. PS= 0, (HR= 2.73; 95% CI= 1.21-6.15; *p*-value= 0.01) and in patients with B7-H4 expression (HR= 2.38; 95% CI= 1.16-4.91; *p*-value= 0.022). A weak, non-significant association between PD-L1 expression and OS was observed (HR = 0.60; 95% CI= 0.15 -2.37 *p*-value= 0.460).

The Kaplan-Meier curves for RECIST-PFS and OS according to the expression of B7-H4 and the other biomarkers are reported in **Figures 2-3**, and in **Supplementary Figures 5-17**, respectively.

VALIDATION OF PD-L1 IHC IN THE NIVOLUMAB COHORT

We have noticed that our proportion of patients expressing PD-L1 determined by using our manual staining based on the 28-8 clone was low (15.21%), compared to what has been reported in other studies, ranging from 20% to over 50% [61]. Hence, in order to validate our proportion of PD-L1-expressing patients, we subsequently performed an automated staining; since the automated staining available within our Institution is based on the 22C3 pharmDx assay, which has been approved as a companion diagnostic assay for the use of pembrolizumab in NSCLC [62], we used this clone, hence being also able to compare the two clones in our population. Globally, 34 specimens had sufficient neoplastic areas for this additional analysis; 31 specimens (91.18%) had the same PD-L1 expression level with 28-8 and 22C3 assays. Among the three discordant samples, two resulted <1% with the 22C3 assay, while the 28-8 assay identified an expression between 1-9%; by contrast, one sample was categorized as ≥50% according to the 22C3 assay and between 10-49% according to the 28-8 assay. When the two assays were compared using the cut-off of 1% for positivity, a substantial concordance was observed (Cohen's Kappa= 0.767)

CHEMOTHERAPY COHORT

Tumor samples from 27 NSCLC patients within the Chemotherapy Cohort were collected. Since the available tissue was limited, we focused this analysis on B7-H4 and PD-L1 on the basis of the results concerning OS observed in the Nivolumab Cohort. The patients' characteristics are summarized in **Table 1**, while

the expressions of PD-L1 and B7-H4 are reported in **Table 2** and in **Supplementary Table 3**. We applied the same cut-off for positivity already employed in the Nivolumab Cohort (≥ 1% *vs.* < 1%). No correlation was observed between PD-L1 and B7-H4 expression. PD-L1 positivity was not associated with any clinical feature, while B7-H4 expression was associated with female gender (*p*-value= 0.008). The global outcome data for the Chemotherapy Cohort are reported in **Supplementary Table 4**. No significant correlation was observed between the expression of any biomarker and ORR or DCR, as reported in **Supplementary Figures 18-19**. No association between biomarker expression and survival was observed: in particular, at the univariate analysis, patients with B7-H4 expression ≥ 1% *vs.* < 1% had similar median RECIST-PFS (3.3 *vs.* 3.4 months; *p*-value= 0.274) and OS (8.7 *vs.* 8.2 months; *p*-value= 0.284); similarly, PD-L1 expression did not significantly affect neither RECIST-PFS (4.8 *vs.* 3.3 months; *p*-value= 0.444), nor OS (12.8 *vs.* 7.4 months; *p*-value= 0.406). At the multivariate analysis, RECIST-PFS was not associated with B7-H4 expression (HR= 0.64; 95% CI= 0.29-1.44; *p*-value= 0.275) or PD-L1 expression (HR= 0.74; 95% CI= 0.29-1.90; *p*-value= 0.446); likewise, OS was not associated with B7-H4 expression (HR= 0.85; 95% CI= 0.36-2.03; *p*-value= 0.287) or PD-L1 expression (HR= 0.50; 95% CI= 0.18-1.38; p-value= 0.408).

The Kaplan-Meier curves of PFS and OS based on B7-H4 expression are reported in **figure 4**, while those based on PD-L1 expression are reported in **supplementary figures 20-21**.

DISCUSSION

Our study focused on a panel of potential immune-related biomarkers with the aim of identifying possible correlations with the oucomes of a population of patients receiving nivolumab for advanced NSCLC. Interestingly, meaningful correlations were found between the expression of B7-H4 and survival of patients reeiving nivolumab. Notably, while the association between B7-H4 and OS did not reach statistical significance, (p= 0.064), 10 patients who had B7-H4 expression <1% were alive, hence censored at the time of our analysis, compared to only 2 patients in the B7-H4-positive group, supporting the observation of a difference between the two sub-populations. Furthermore, no difference in terms of PFS or OS was observed when B7-H4 expression was assessed in a cohort of patients receiving chemotherapy for advanced NSCLC.

While the clinical meaning of B7-H4 in NSCLC has been considered debatable for many years, a recent meta-analysis comprising 9 studies, for a total of 1444 patients with NSCLC at any stage, demonstrated a correlation between B7-H4 expression and clinicopathological features such as poor differentiation, advanced disease stage and poor survival, suggesting a prognostic role of this biomarker. However, this meta-analysis has some limitations, deriving from possible patient selection biases and from the fact that only the results of few studies were evaluable for survival analysis [63]; furthermore, the nature of a meta-analysis including all stages of NSCLC limits the available information on the effects of B7-H4 on specific treatments. Our experience, while developed within a mono-institutional study, suggests that the role of B7-H4 in advanced NSCLC might depend on the administered treatment, with particular reference to immunotherapy. We acknowledge that the two populations of our study differ as Nivolumab Cohort included patients with both histo-types (squamous and non-

squamous NSCLC) and who had received previous antineoplastic treatments for advanced disease, while Chemotherapy Cohort included only patients with adenocarcinoma receiving first-line chemotherapy, thus limiting formal statistical comparisons between the two cohorts. However, the clearly different behaviour of B7-H4 in the two cohorts is noticeable. PD-L1 and B7-H4 seem to have opposite effect in our population of patients receiving nivolumab and they are both known to promote immune tolerance, and subsequently the ability of tumor cells to escape the immune system; more specifically, while the immuno-regulatory role of PD-1/PD-L1 axis is widely known [64], B7-H4 appears to inhibit the proliferation of T-killer and T-helpers and to favor the proliferation of regulatory T cells (Tregs) with inhibitory function in different malignancies, although its receptor has not been identified yet [65,66]. While available information is currently limited, on the basis of our findings and previous publications we might speculate that, while PD-L1 expression has a favoring effect on the treatment with PD-1/PD-L1 immune checkpoint inhibitors, B7-H4 expression might promote immune tolerance through a completely different pathway, hence favoring immune tumor escape in spite of PD-1/PD-L1 blockade.

While our findings are mostly focused on B7-H4 and PD-L1, no meaningful result involving PD-L2 and PD-1 was observed. Notably, although B7-H3 expression brought interesting results in terms of decreased PFS within our Nivolumab Cohort, in line with other publications [67], the number of B7-H3-positive patients was globally low; therefore the conclusions we could draw about this biomarker in our experience were limited and require further assessments in this setting.

We are aware that our study has some limitations, mostly deriving from its nature of being a mono-institutional, retrospective study based on two different cohorts

of patients affected by advanced NSCLC. In first place, there are clinically meaningful differences in the cohorts, resulting in a limitation to the available aggregate analyses; howerer, the mono-institutional approach ensured that all the clinical assessments, including ORR and PFS data collection, were performed consistently among all the patients, and the same concept applies to IHC staining and analysis. In second place, the global number of patients is relatively limited, especially for Chemotherapy Cohort; this occurrence is related to the limited amount of available specimens which were suitable for IHC, since different biomarkers had to be explored in Nivolumab Cohort, while in the case of Chemotherapy Cohort we must take into account that, since these patients were enrolled in a translational research study, their samples had undergone multiple different analyses in the course of time, both for clinical reasons and for research purpose, thus reducing the number of specimens that could be properly analyzed for PD-L1 and B7-H4 expression. Notably, this issue also affects current clinical practice in NSCLC, as collecting the proper amount of tissue is still a challenging medical need. Furthermore, the analysis was mostly performed on archival samples, but this also reflects the current practice in NSCLC, as the collection of repeated biopsies is not mandatory for first-line chemotherapy or for the administration of nivolumab in subsequent lines.

With regards to IHC, although the employment of manual technique may be considered a limit for our study, we have adapted the kit provided by DAKO company to meet laboratory requirements by developing a protocol that simulates the steps taken on the recommended platform for the development of the immunohistochemistry reaction; additionally, every staining run was supported by proper positive and negative controls and a careful interpretation of results, resulting in the exclusion the samples which were not adequate for our analysis.

Furthermore, while we have observed a lower proportion od PD-L1-positive patients compared to other publications, the automated validation performed by using the 22C3 assay substantially confirmed our findings, and was substantially concordant with our initial assessment, with only three reported discordant cases, two of which switched from border-line negativity (<1%) to bordel-line positivity (≥1%) and one of which being positive with both assays (10-49% with 28-8 and ≥50% with 22C3). A possible explanation for our low proportion of PD-L1 ≥1% specimens compared to other publications may lie in the intra-tumoral heterogeneity of PD-L1 expression, especially when we consider the tissue collected through biopsies, rather than surgical specimens [68,69].

In conclusion, to our knowedge, this is the first clinical study suggesting a potential role of B7-H4 expression as predictor of benefit from PD-1 blockade with nivolumab in NSCLC; while limited due to the nature of the study and the number of evaluable patients, our findings strongly encourage future prospective studies designed to define and eventually confirm the predictive role of B7-H4 expression for patients receiving immune checkpoint blockade for advanced NSCLC.

FIGURES

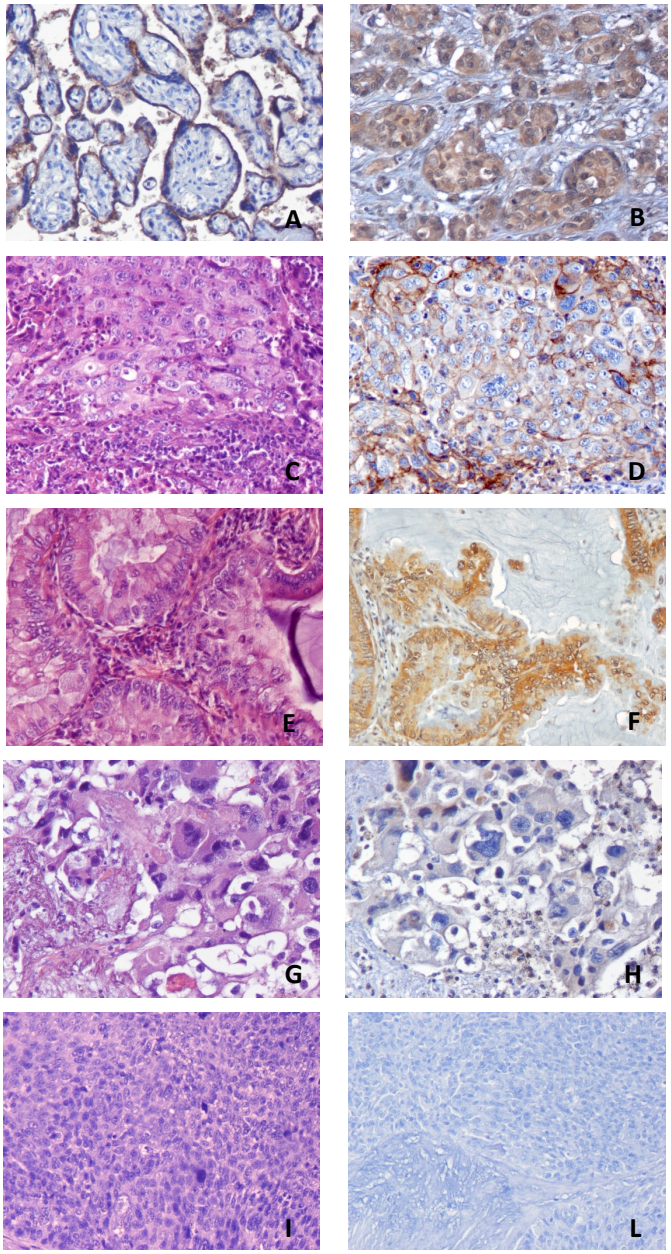


Figure 1. Representative images of IHC positive controls (A-B) and tumor samples from the Nivolumab Cohort (C-L). A: positive PD-L1 control (placenta); B: positive B7-H4 control (breast carcinoma); C-D: EE and IHC positive staining of PD-L1; E-F: EE and IHC positive staining of B7-H4; G-H: EE and IHC negative staining of PD-L1; I-L: EE and IHC negative staining of B7-H4. All the images are at magnification 20x.

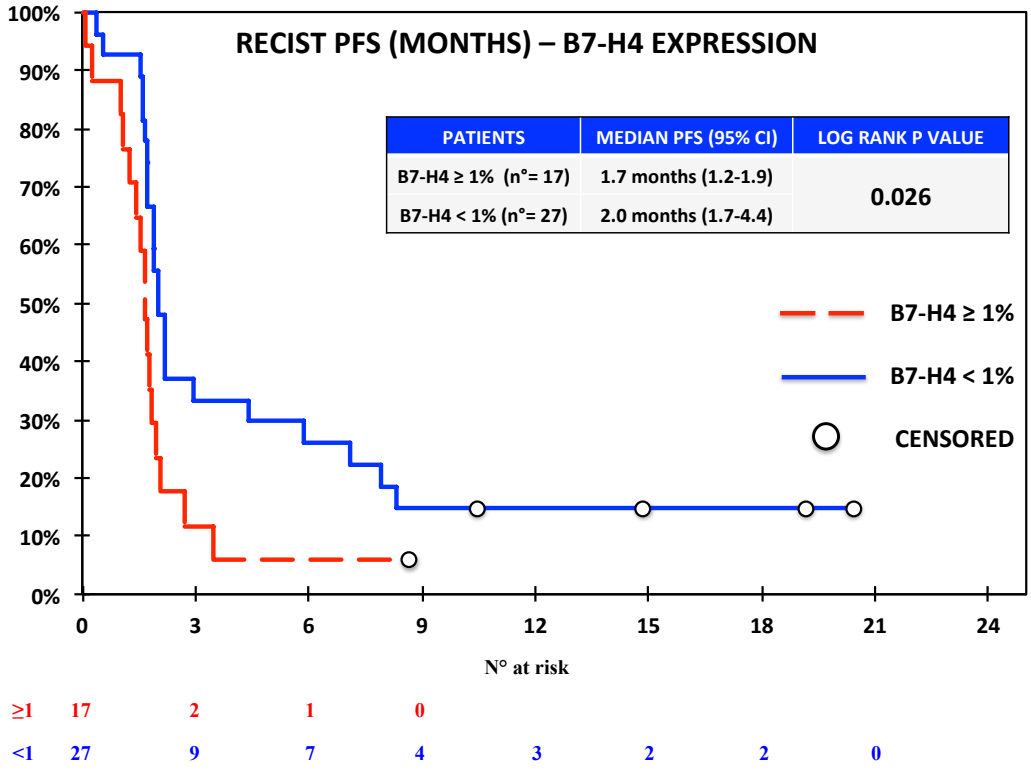


Figure 2. Kaplan-Meier curve for RECIST-PFS based on the expression of B7-H4 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.

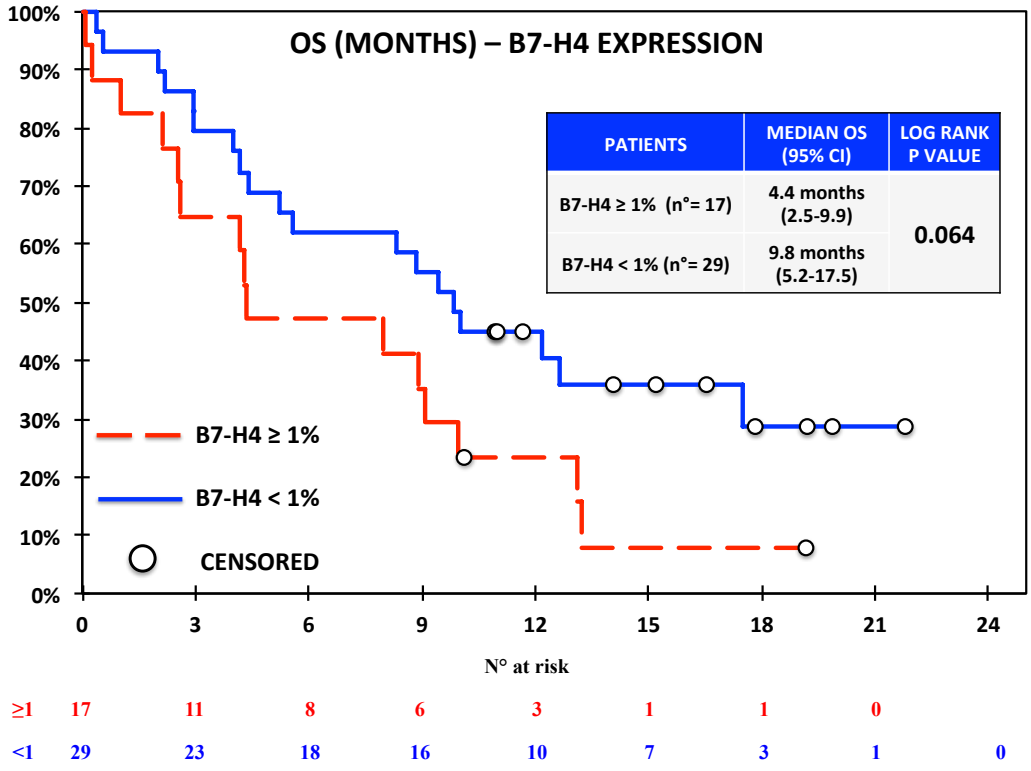


Figure 3. Kaplan-Meier curve for OS based on the expression of B7-H4 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.

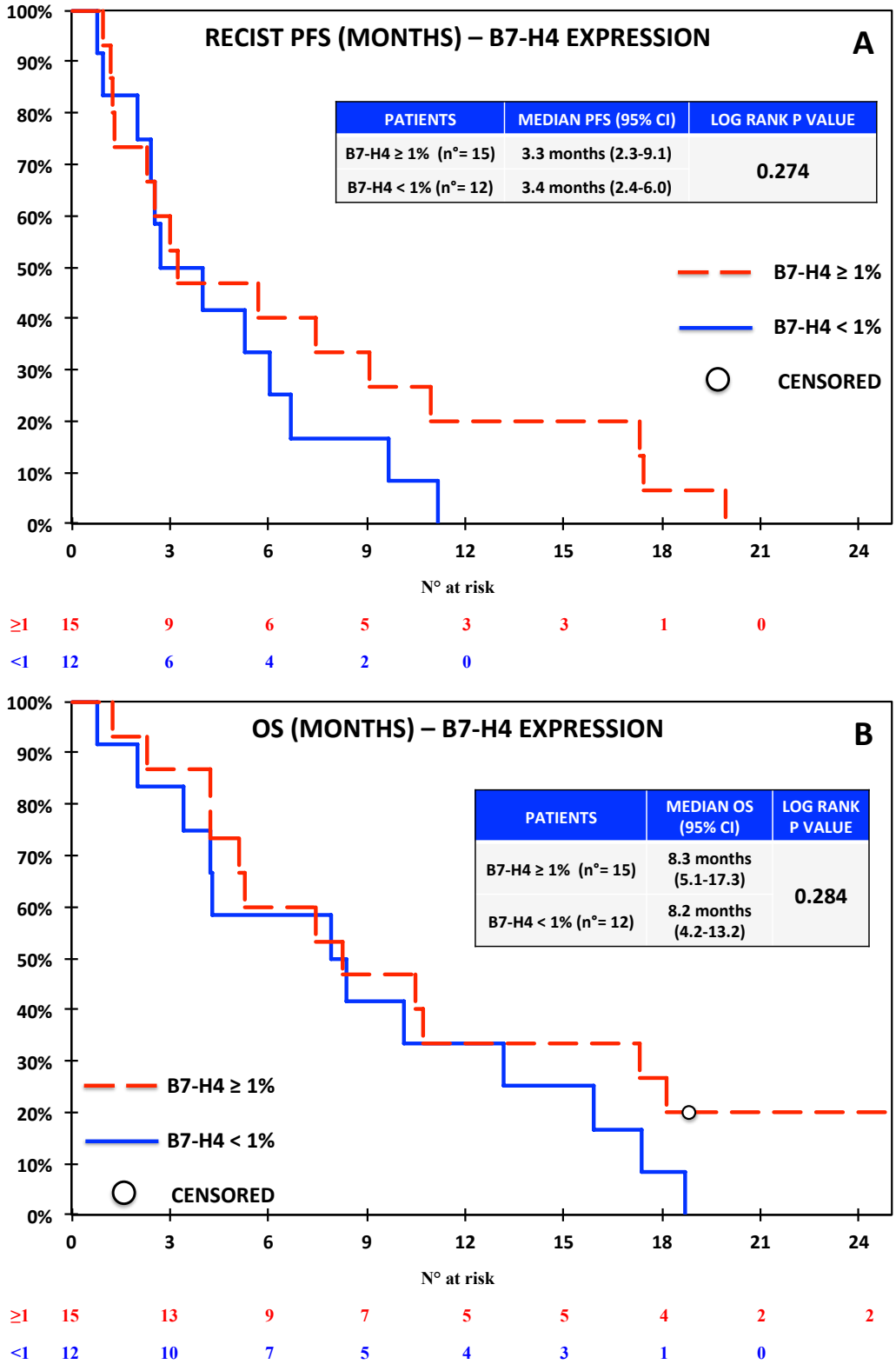


Figure 4. Kaplan-Meier curves for RECIST-PFS (A) and OS (B) based on the expression of B7-H4 defined as ≥ 1% vs. < 1% in the Chemotherapy Cohort.

TABLES

	CLINICAL CHARACTERISTICS NIVOLUMAB COHORT (N°= 46)			CLINICAL CHARACTERISTICS CHEMOTHERAPY COHORT (N°= 27)		
Gender	**Male** 34 (73.9%)		**Female** 12 (26.1%)	**Male** 20 (74.1%)		**Female** 7 (25.9%)
Age (years)	**Range** 44-82		**Median** 70	**Range** 46-81		**Median** 69
Smoking habit	**Current** 13 (28.3%)	**Former** 24 (52.2%)	**Never** 9 (19.5%)	**Current** 12 (44.4%)[1]	**Former** 12 (44.4%)[1]	**Never** 3 (11.1%)[1]
Histology[2]	**Non-squamous** 35 (76.1%)		**Squamous** 11 (23.9%)	**Non-squamous** 27 (100.0%)		**Squamous** 0 (0.0%)
Stage	**IIIB** 2 (4.4%)		**IV** 44 (95.6%)	**IIIB** 0 (0.0%)		**IV** 27 (100.0%)
ECOG PS[2]	**PS= 0** 17 (37.0%)	**PS= 1** 26 (56.5%)	**PS= 2** 3 (6.5%)	**PS= 0** 6 (22.2%)		**PS= 1** 21 (77.8%)
Previous lines for advanced disease[2]	**Range** 1-6		**Median** 2	**None**		
	MOLECULAR ALTERATIONS NIVOLUMAB COHORT (only non-squamous NSCLC; N°= 35)			**MOLECULAR ALTERATIONS CHEMOTHERAPY COHORT**[2] **(N°= 27)**		
EGFR	**Evidence of mutation** 3 (8.6%)[3]		**No evidence of mutation** 32 (91.4%)	**Evidence of mutation** 1 (3.7%)[4]		**No evidence of mutation** 26 (96.3%)
ALK	**Evidence of rearrangement** 0 (0.0%)		**No Evidence of rearrangement** 35 (100.0%)	**Evidence of rearrangement** 0 (0.0%)		**No Evidence of rearrangement** 27 (100.0%)

Table 1. Clinical and molecular characteristics of the evaluable patients in the Nivolumab Cohort and in the Chemotherapy Cohort.
[1] Total = 99.99% due to approximation.
[2] The Chemotherapy Cohort includes only chemotherapy-naïve patients affected by non-squamous NSCLC with ECOG PS= 0-1.
[3] Two patients in the Nivolumab Cohort had exon 19 deletion; one patient had exon 19 deletion in association with exon 20 insertion.
[4] One patient in the Chemotherapy Cohort had exon 21 deletion, which was identified after first-line treatment.

	NIVOLUMAB COHORT (N°= 46)						
BIOMARKER	**< 1%**	**1-9%**	**10-49%**	**≥ 50%**	**ND**	**POSITIVE**	**NEGATIVE**
PD-L1	38 (82.6%)	4 (8.7%)	2 (4.3%)	1 (2.2%)	1 (2.2%)	7 (15.6%)	38 (84.4%)
PD-L2	38 (82.6%)	2 (4.3%)	5 (10.9%)	1 (2.2%)	0 (0.0%)	8 (17.4%)	38 (82.6%)
PD-1	13 (28.3%)	8 (17.4%)	5 (10.9%)	18 (39.1%)	2 (4.3%)	31 (70.5%)	13 (29.5%)
B7-H3	40 (87.0%)	2 (4.3%)	3 (6.5%)	1 (2.2%)	0 (0.0%)	6 (13.0%)	40 (87.0%)
B7-H4	29 (63.0%)	5 (10.9%)	4 (8.7%)	8 (17.4%)	0 (0.0%)	17 (27.0%)	29 (63.0%)
	CHEMOTHERAPY COHORT (N°= 27)						
BIOMARKER	**< 1%**	**1-9%**	**10-49%**	**≥ 50%**	**ND**	**POSITIVE**	**NEGATIVE**
PD-L1	21 (77.8%)	5 (18.5%)	1 (3.7%)	0 (0.0%)	0 (0.0%)	6 (22.2%)	21 (77.8%)
B7-H4[1]	12 (44.4%)	3 (11.1%)	3 (11.1%)	9 (33.3%)	0 (0.0%)	15 (55.6%)	12 (44.4%)

Table 2. Expression of the potential immune-related biomarkers in the Nivolumab Cohort and in the Chemotherapy Cohort reported into each cut-off category; the evaluable samples from each cohort were then divided between positive (≥ 1%) and negative (< 1%) expression.
ND: Not determined.
[1] Total= 99.9% due to approximation.

SUPPLEMENTARY APPENDIX

NIVOLUMAB COHORT

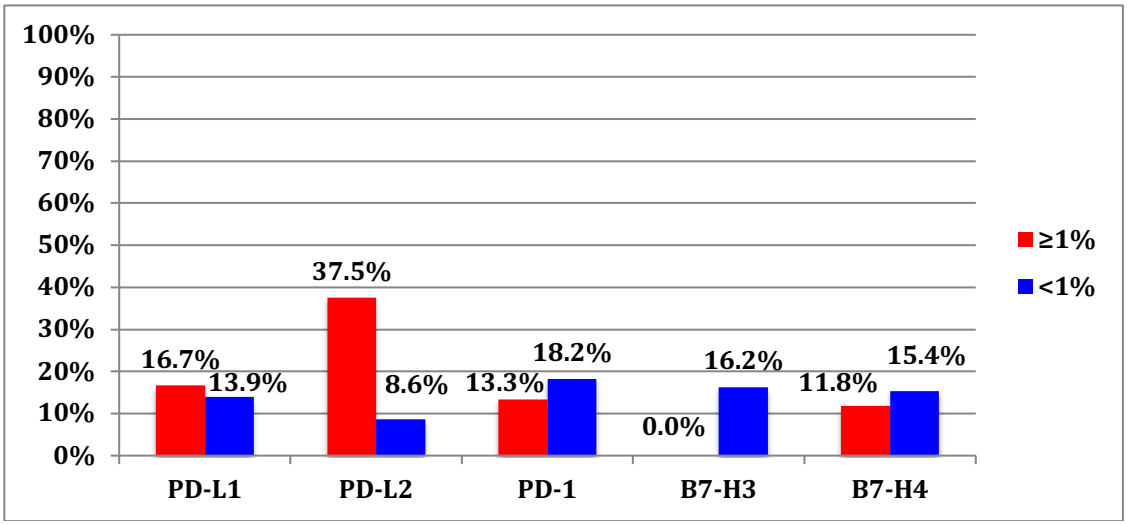
Supplementary Table 1: Distribution of the biomarker expressions among the samples collected from each patient within the Nivolumab Cohort. ND: not determined.

PATIENT	PD-L1	PD-L2	PD-1	B7-H3	B7-H4
1	<1%	10%-49%	<1%	<1%	<1%
2	<1%	<1%	1%-9%	<1%	1%-9%
3	<1%	<1%	1%-9%	<1%	≥50%
4	<1%	<1%	10%-49%	<1%	<1%
5	<1%	<1%	≥50%	<1%	<1%
6	<1%	<1%	≥50%	10%-49%	≥50%
7	<1%	<1%	≥50%	<1%	≥50%
8	<1%	<1%	<1%	<1%	<1%
9	<1%	<1%	<1%	10%-49%	<1%
10	<1%	<1%	<1%	<1%	<1%
11	1-9%	<1%	1%-9%	<1%	1%-9%
12	<1%	<1%	≥50%	1%-9%	<1%
13	<1%	<1%	<1%	<1%	<1%
14	<1%	<1%	1%-9%	<1%	<1%
15	<1%	<1%	≥50%	<1%	<1%
16	≥50%	<1%	≥50%	<1%	≥50%
17	<1%	<1%	10%-49%	<1%	≥50%
18	<1%	<1%	≥50%	<1%	10%-49%
19	<1%	<1%	≥50%	<1%	<1%
20	<1%	<1%	ND	≥50%	1%-9%
21	<1%	<1%	10%-49%	10%-49%	10%-49%
22	<1%	<1%	1%-9%	<1%	≥50%
23	<1%	1%-9%	10%-49%	<1%	<1%
24	<1%	<1%	<1%	<1%	<1%
25	<1%	10%-49%	<1%	<1%	<1%
26	1%-9%	<1%	≥50%	<1%	<1%
27	10%-49%	10%-49%	≥50%	<1%	10%-49%
28	<1%	<1%	<1%	<1%	<1%
29	<1%	<1%	≥50%	<1%	<1%
30	<1%	<1%	≥50%	<1%	<1%
31	1%-9%	<1%	≥50%	<1%	<1%
32	<1%	<1%	<1%	<1%	<1%
33	<1%	<1%	<1%	<1%	≥50%
34	<1%	<1%	1%-9%	<1%	<1%
35	<1%	<1%	1%-9%	<1%	<1%
36	1%-9%	<1%	>=50%	<1%	<1%
37	<1%	1%-9%	1%-9%	<1%	<1%
38	<1%	10%-49%	<1%	<1%	<1%
39	10%-49%	<1%	10%-49%	<1%	≥50%
40	<1%	<1%	ND	<1%	<1%
41	<1%	<1%	<1%	1%-9%	1%-9%
42	ND	<1%	≥50%	<1%	<1%
43	<1%	<1%	≥50%	<1%	1%-9%
44	<1%	<1%	<1%	<1%	<1%
45	<1%	≥50%	≥50%	<1%	<1%
46	<1%	10%-49%	≥50%	<1%	10%-49%

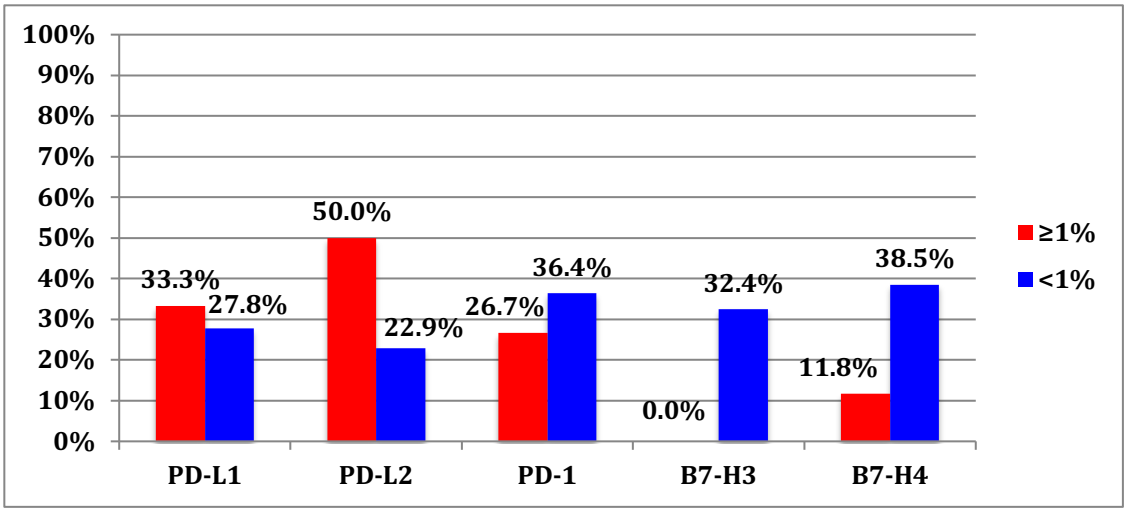
Supplementary table 2: global outcome data in the Nivolumab Cohort.
Among the patients from the Nivolumab Cohort, one was considered evaluable for irRC, but not for RECIST, based on measurable lesions; one additional patient was considered not evaluable as he/she discontinued treatment and did not undergo further CT scans after baseline; both patients were followed for overall survival.
Among the patients who were evaluable for PFS, one missed the first scheduled response assessment although subsequent scans were available for PFS; hence, this patient was excluded from ORR and DCR analysis.

Nivolumab Cohort	N= 46	
Median OS (95% IC)	8.9 months (4.4-12.2)	
Evaluable patients for PFS	**RECIST** N= 44	**irRC** N= 45
Median PFS (95% IC)	**RECIST** 1.9 months (1.7-2-2)	**irRC** 1.9 months (1.7-2.9)
ORR	**RECIST** 14.0%	**irRC** 13.6%
DCR	**RECIST** 27.9%	**irRC** 38.6%

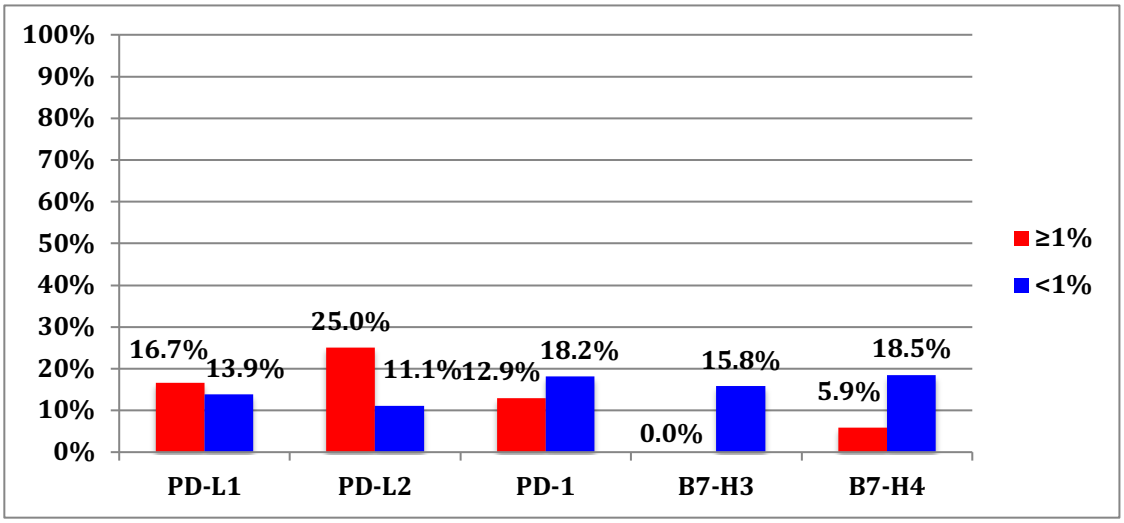
Supplementary figure 1: RECIST-ORR according to biomarkers expression at IHC in the Nivolumab Cohort. No statistically significant interaction was observed between each biomarker and ORR, although the correlation between PD-L2 expression and RECIST-ORR was close to significance (*p*-value= 0.067).



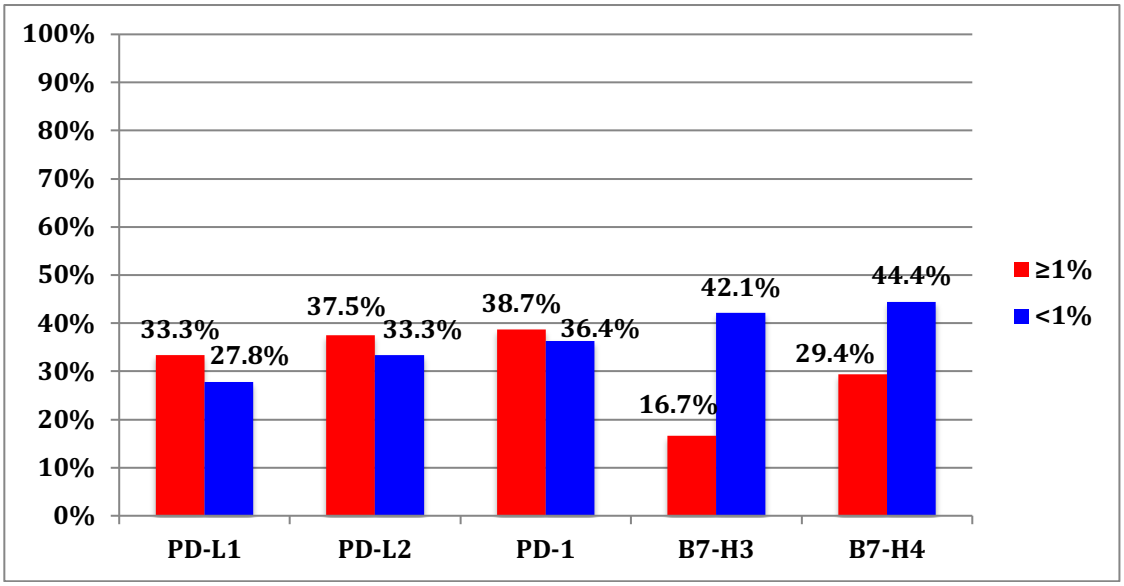
Supplementary figure 2: RECIST-DCR according to biomarkers expression at IHC in the Nivolumab Cohort. No statistically significant interaction was observed between each biomarker and DCR, although the correlation between B7-H4 expression and RECIST-DCR was close to significance (*p*-value= 0.085).



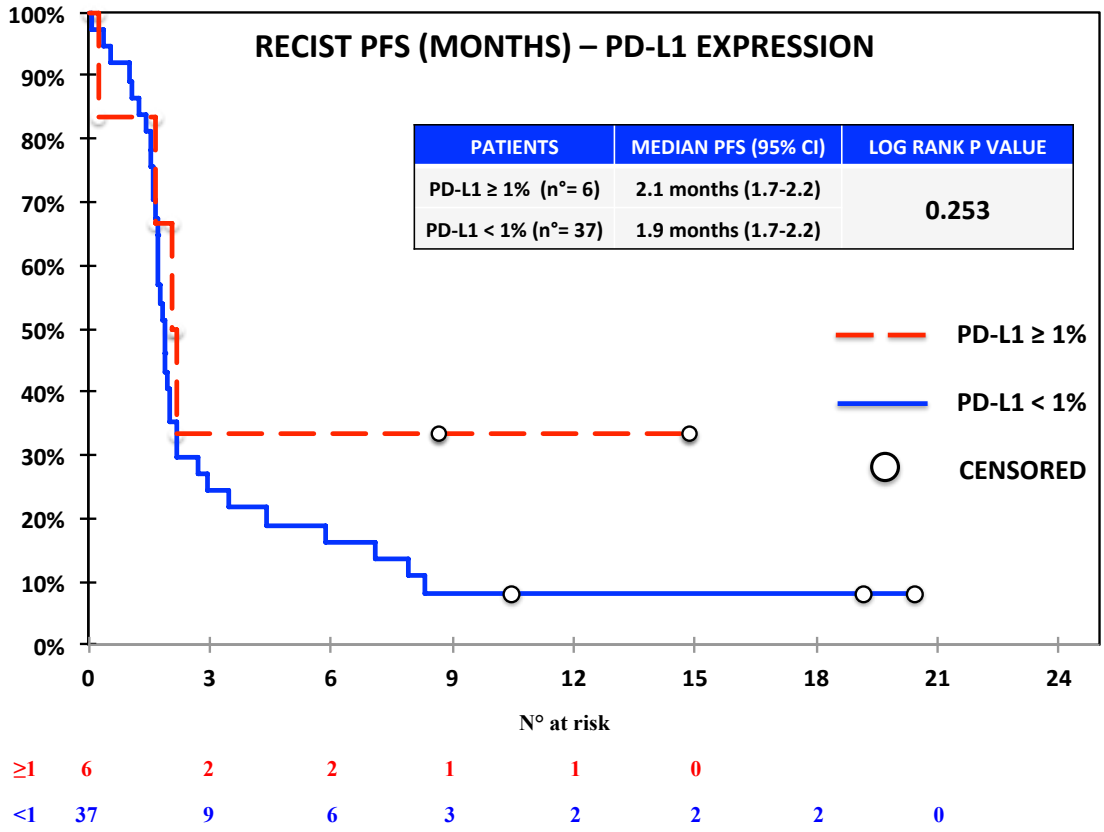
Supplementary figure 3: irRC-ORR according to biomarkers expression at IHC in the Nivolumab Cohort. No statistically significant interaction was observed between each biomarker and ORR.



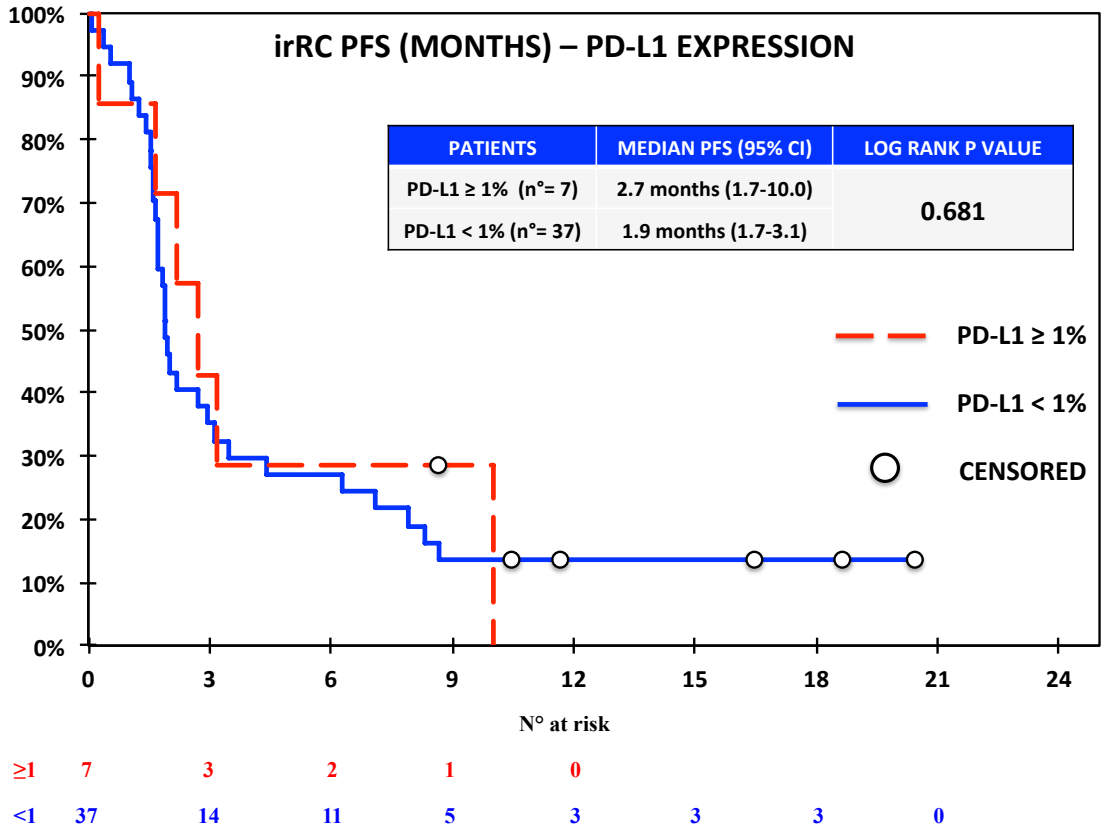
Supplementary figure 4: irRC-DCR according to biomarkers expression at IHC in the Nivolumab Cohort. No significant interaction was observed between each biomarker and DCR.



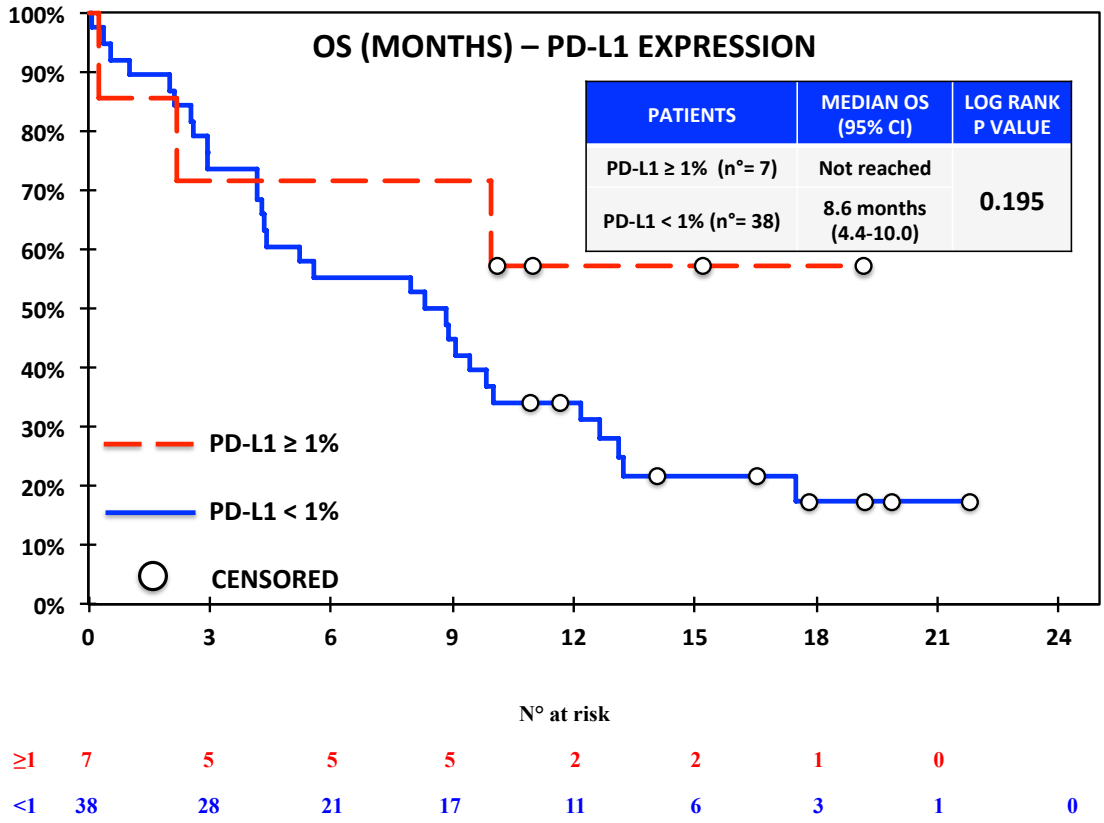
Supplementary figure 5: RECIST-PFS based on the expression of PD-L1 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



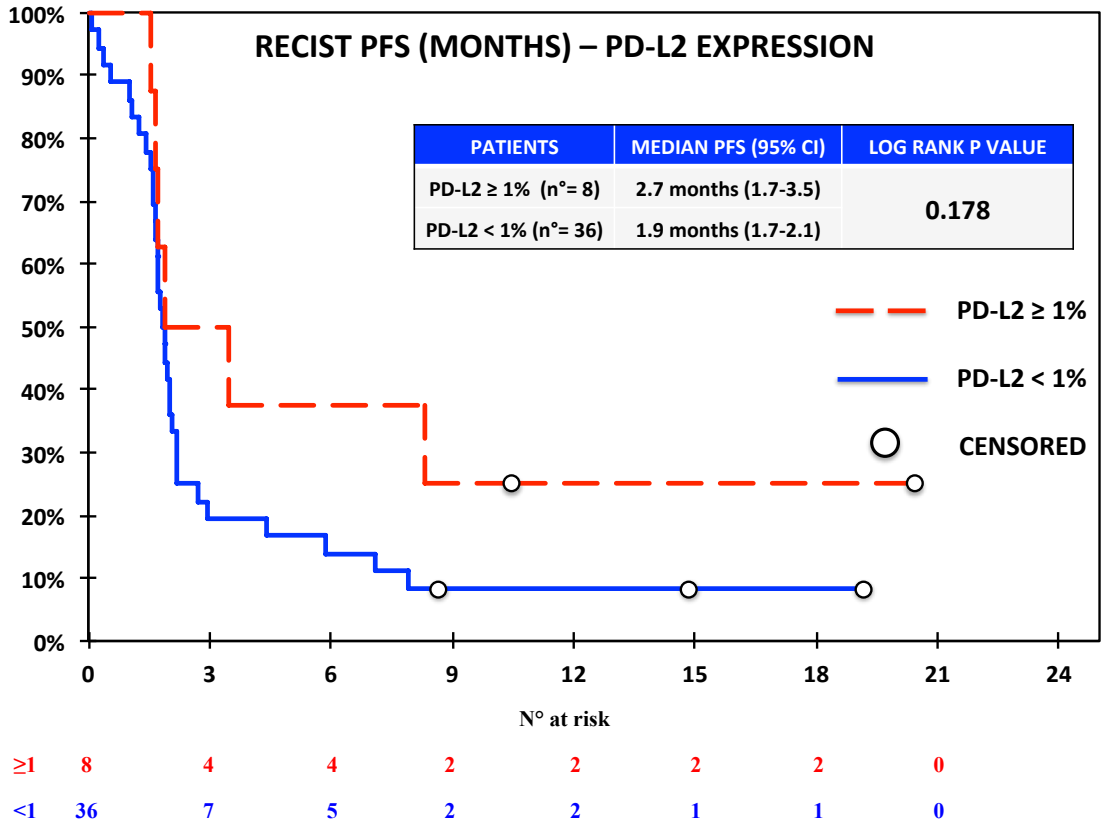
Supplementary figure 6: irRC-PFS based on the expression of PD-L1 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



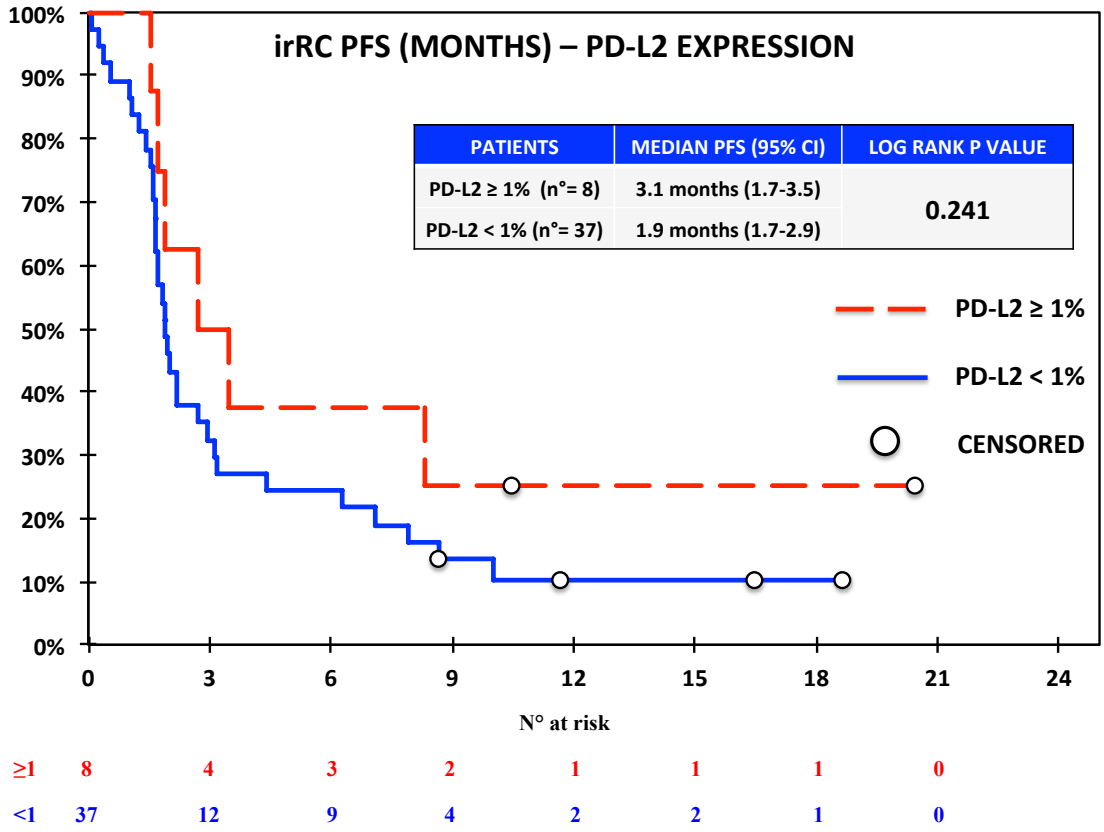
Supplementary figure 7: OS based on the expression of PD-L1 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



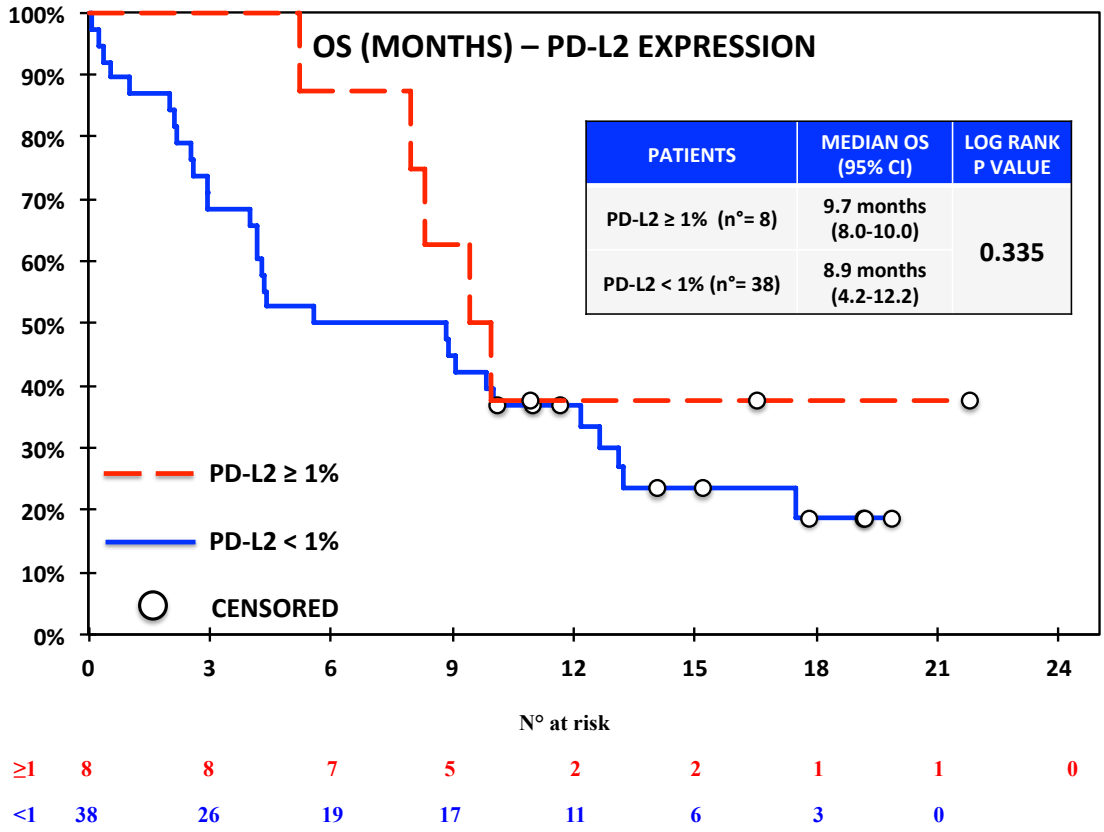
Supplementary figure 8: RECIST-PFS based on the expression of PD-L2 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



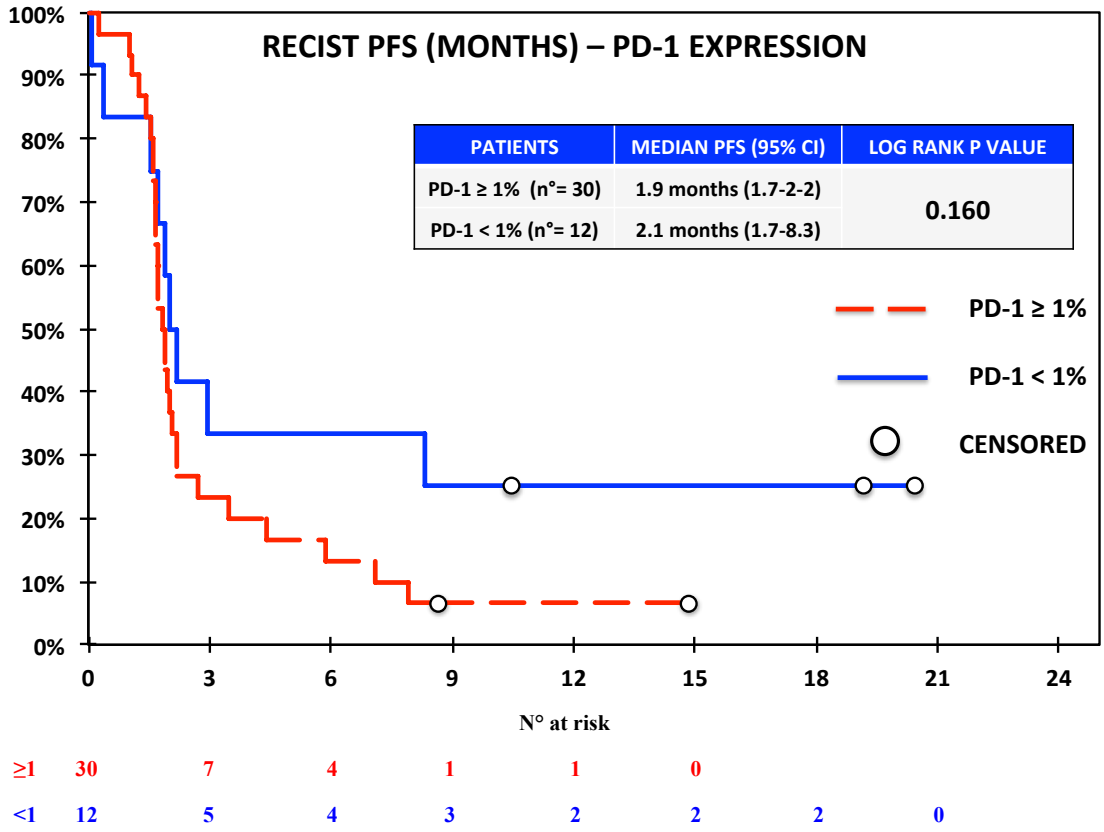
Supplementary figure 9: irRC-PFS based on the expression of PD-L2 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



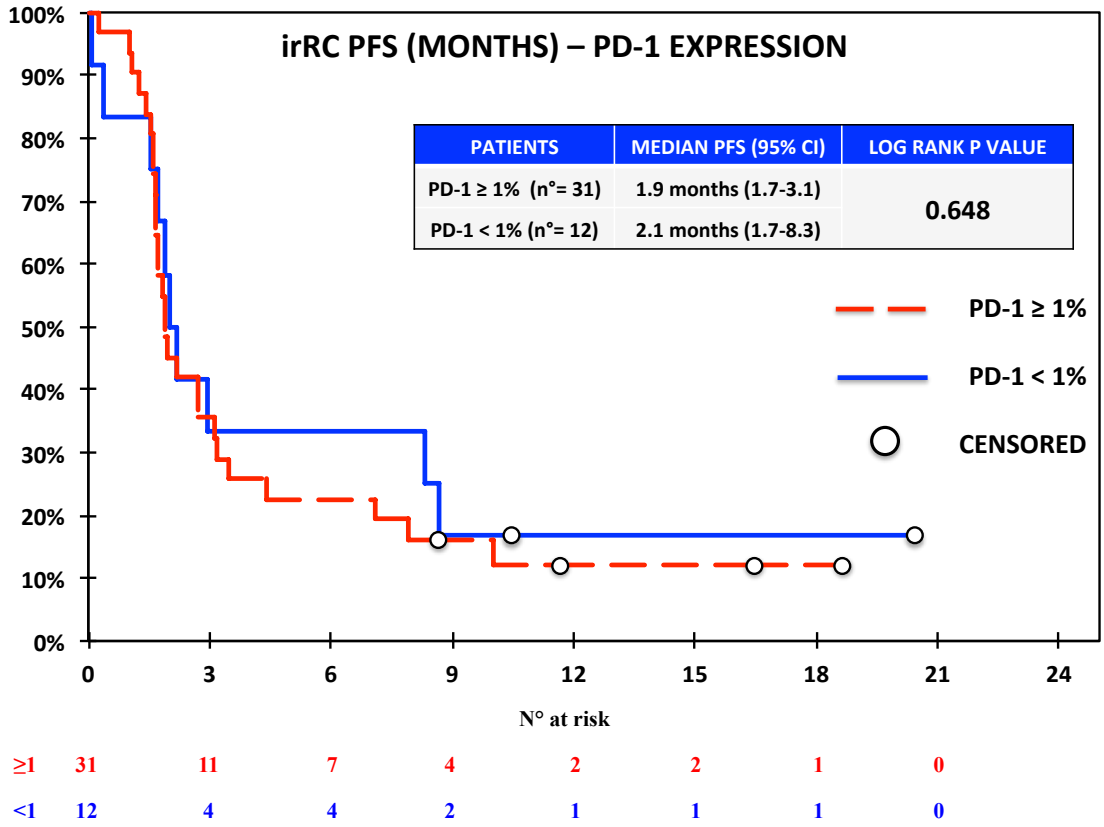
Supplementary figure 10: OS based on the expression of PD-L2 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



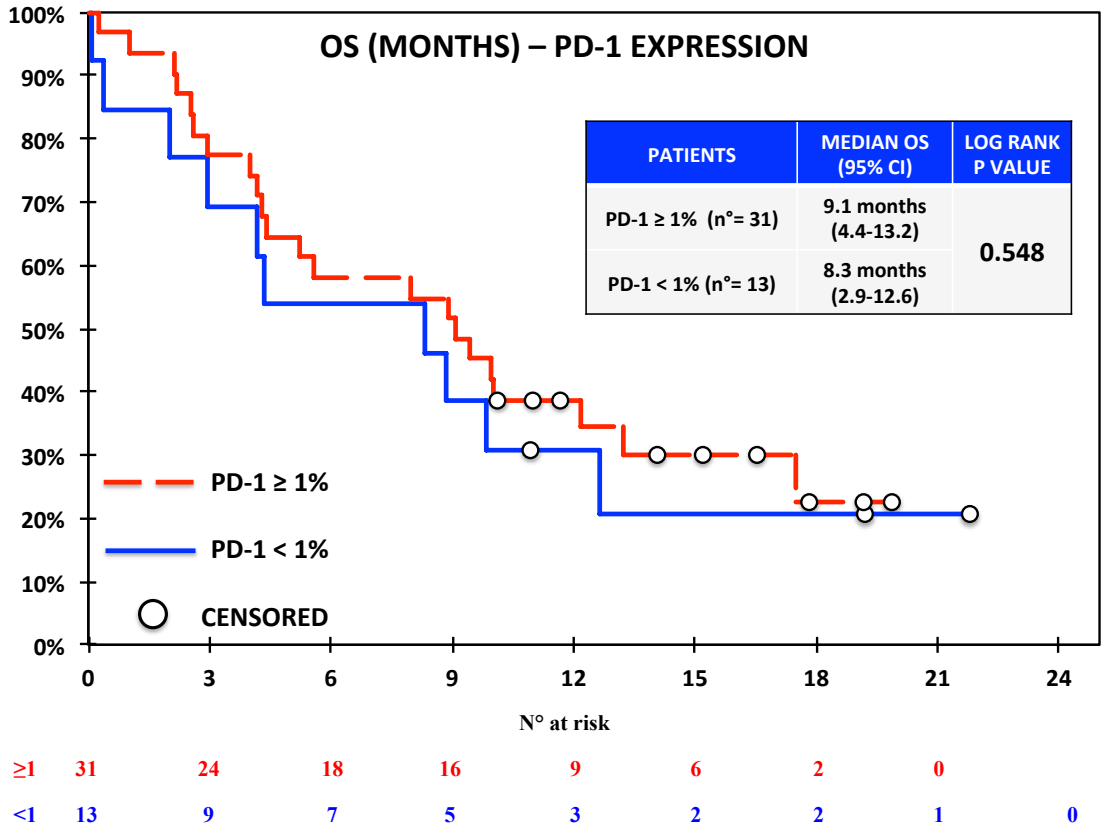
Supplementary figure 11: RECIST-PFS based on the expression of PD-1 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



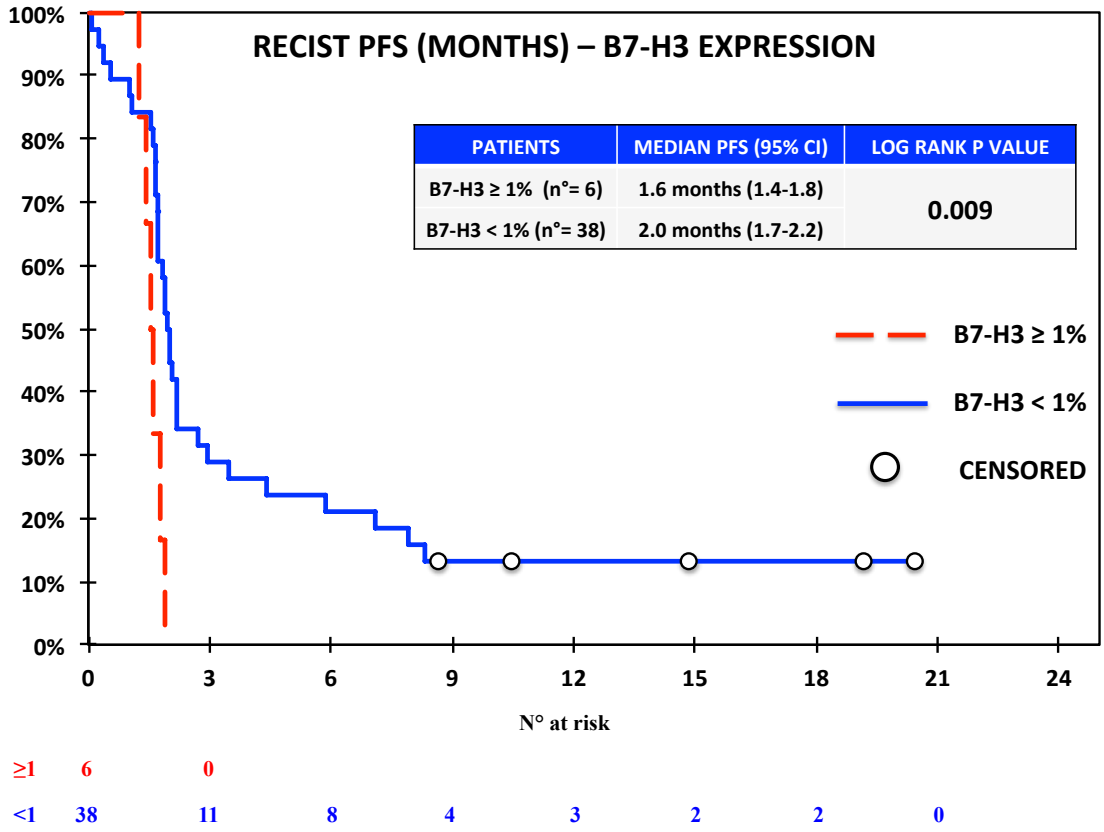
Supplementary figure 12: irRC-PFS based on the expression of PD-1 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



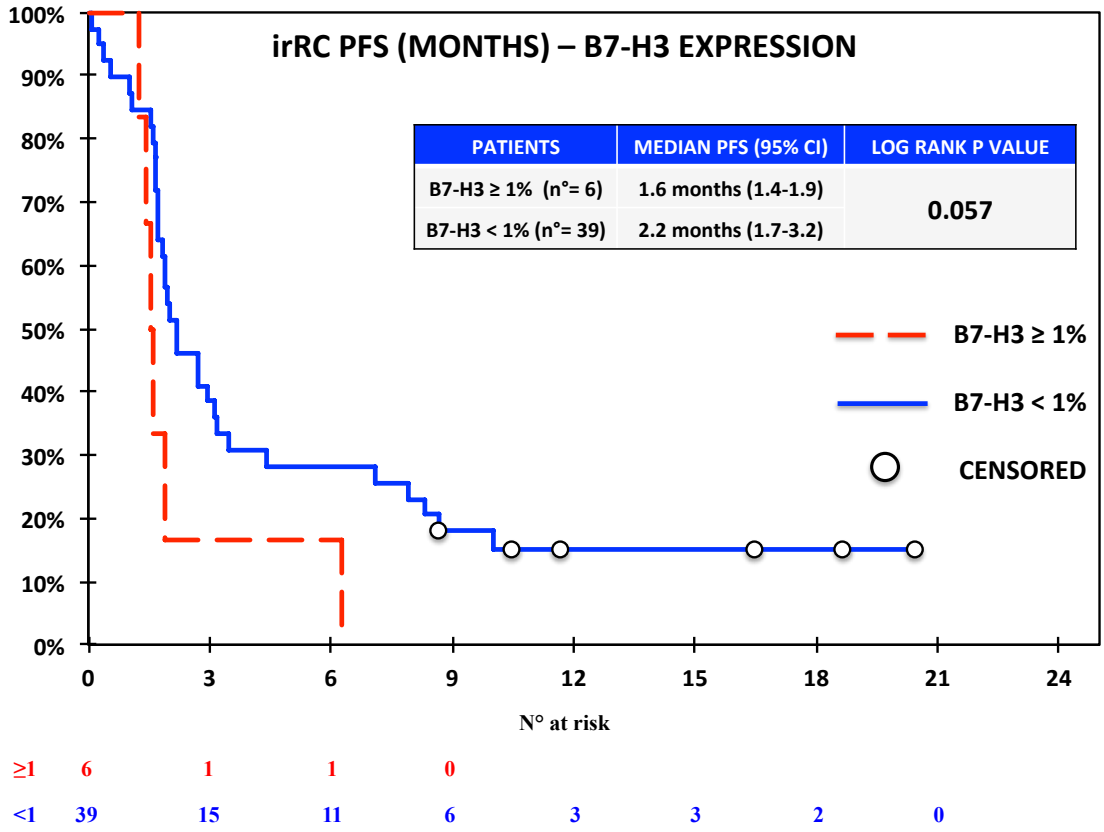
Supplementary figure 13: OS based on the expression of PD-1 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



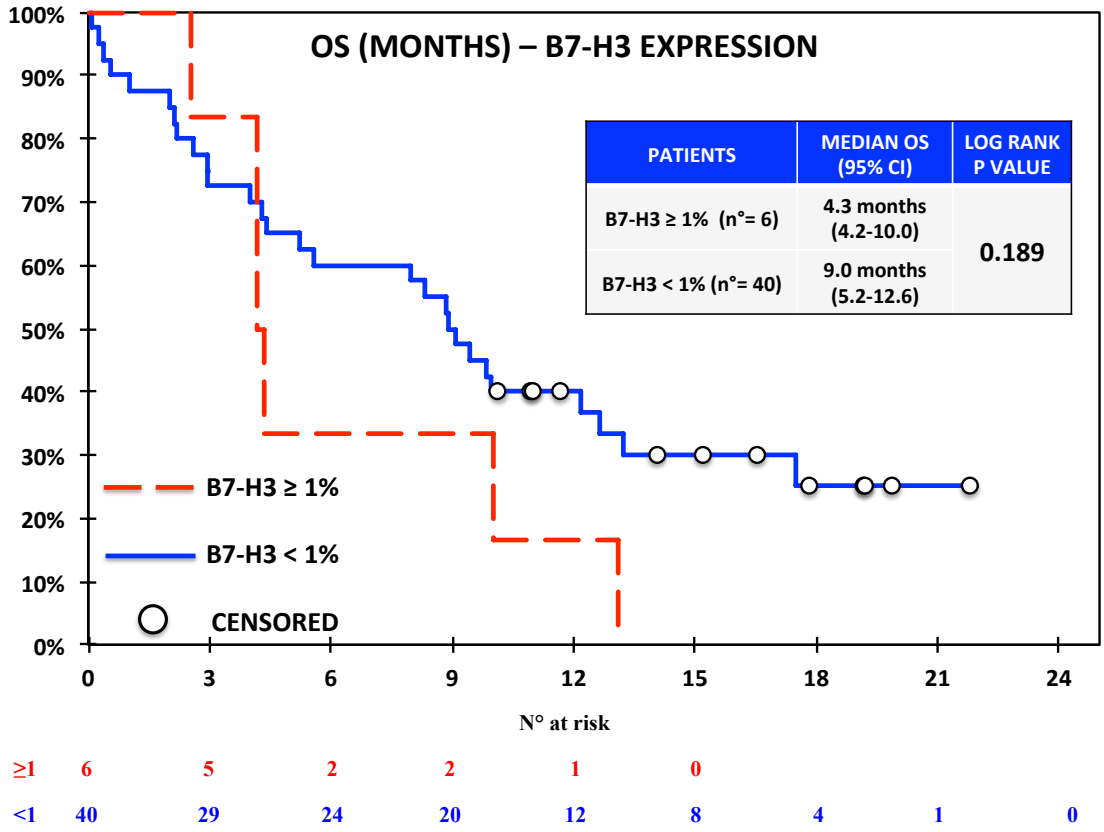
Supplementary figure 14: RECIST-PFS based on the expression of B7-H3 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



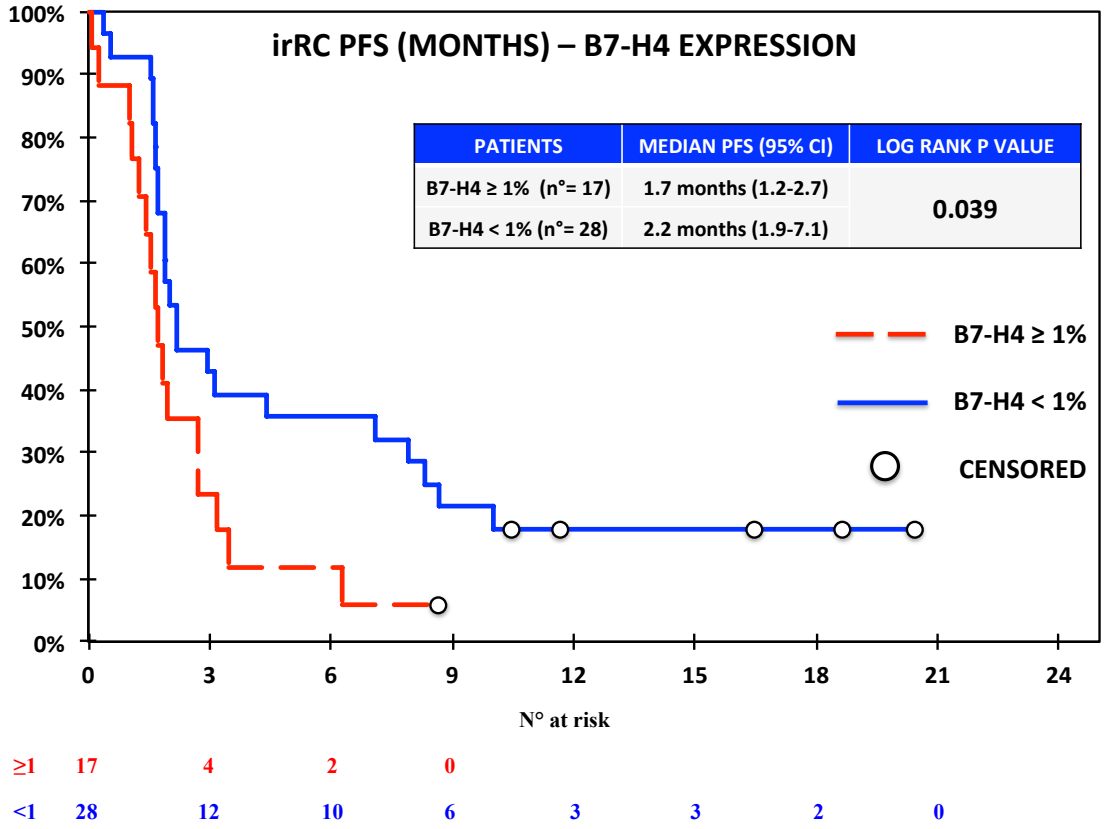
Supplementary figure 15: irRC-PFS based on the expression of B7-H3 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



Supplementary figure 16: OS based on the expression of B7-H3 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



Supplementary figure 17: irRC-PFS based on the expression of B7-H4 defined as ≥ 1% vs. < 1% in the Nivolumab Cohort.



CHEMOTHERAPY COHORT

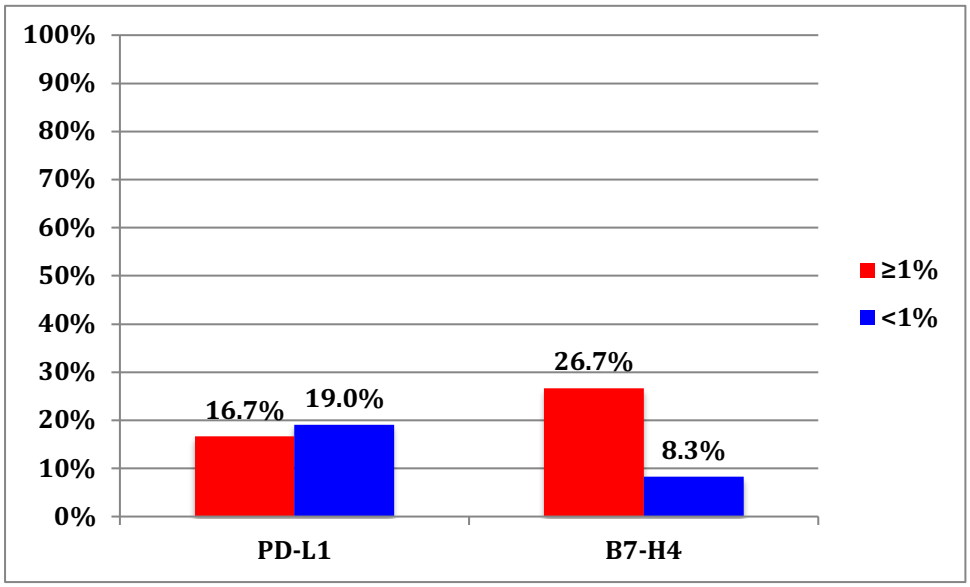
Supplementary Table 3: Distribution of the biomarker expressions among the samples collected from each patient within the Chemotherapy Cohort.

PATIENT	PD-L1	B7-H4
1	<1%	10%-49%
2	<1%	≥50%
3	<1%	≥50%
4	<1%	≥50%
5	1%-9%	1%-9%
6	<1%	<1%
7	<1%	<1%
8	<1%	<1%
9	<1%	1%-9%
10	<1%	≥50%
11	<1%	≥50%
12	<1%	<1%
13	10%-49%	<1%
14	1%-9%	≥50%
15	<1%	10%-49%
16	<1%	≥50%
17	<1%	<1%
18	<1%	1%-9%
19	<1%	10%-49%
20	1%-9%	<1%
21	<1%	<1%
22	<1%	≥50%
23	<1%	<1%
24	<1%	<1%
25	1%-9%	≥50%
26	1%-9%	<1%
27	<1%	<1%

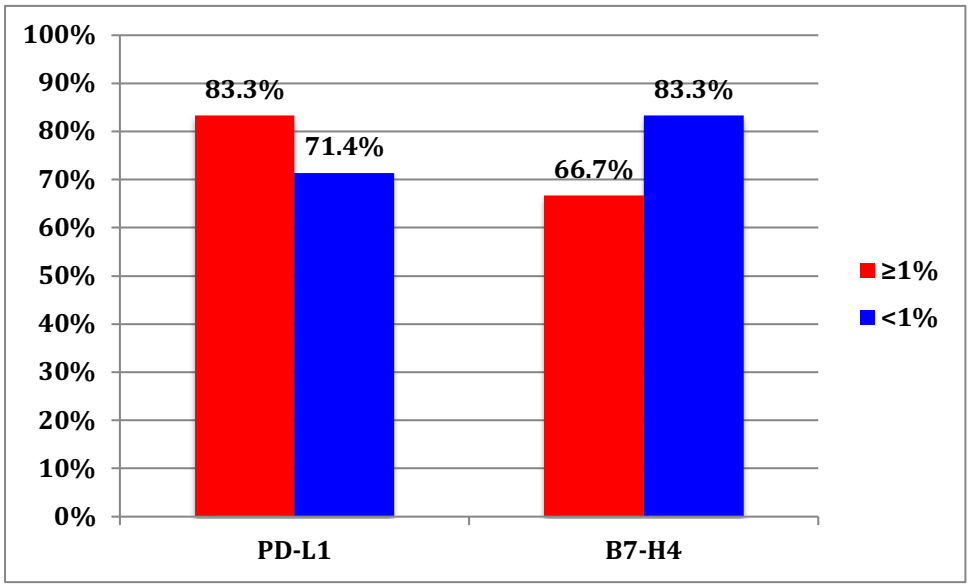
Supplementary table 4: global outcome data in the Chemotherapy Cohort.

Chemotherapy Cohort	N= 27
Median OS (95% CI)	8.3 months (4.3-13.2)
Evaluable patients for PFS	N= 27
Median PFS (95%C CI)	3.3 months (2.4-6.7)
ORR	18.5%
DCR	74.1%

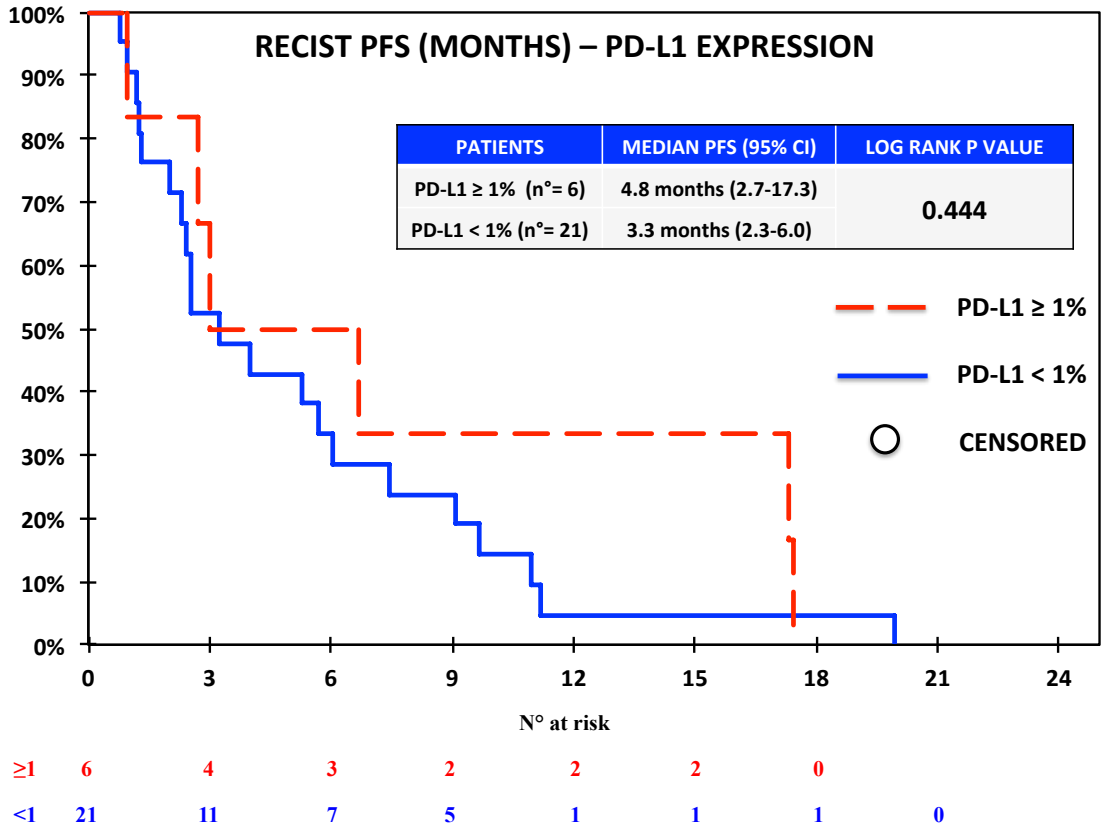
Supplementary figure 18: RECIST-ORR according to biomarkers expression at IHC in the Chemotherapy Cohort. No significant interaction was observed between each biomarker and ORR.



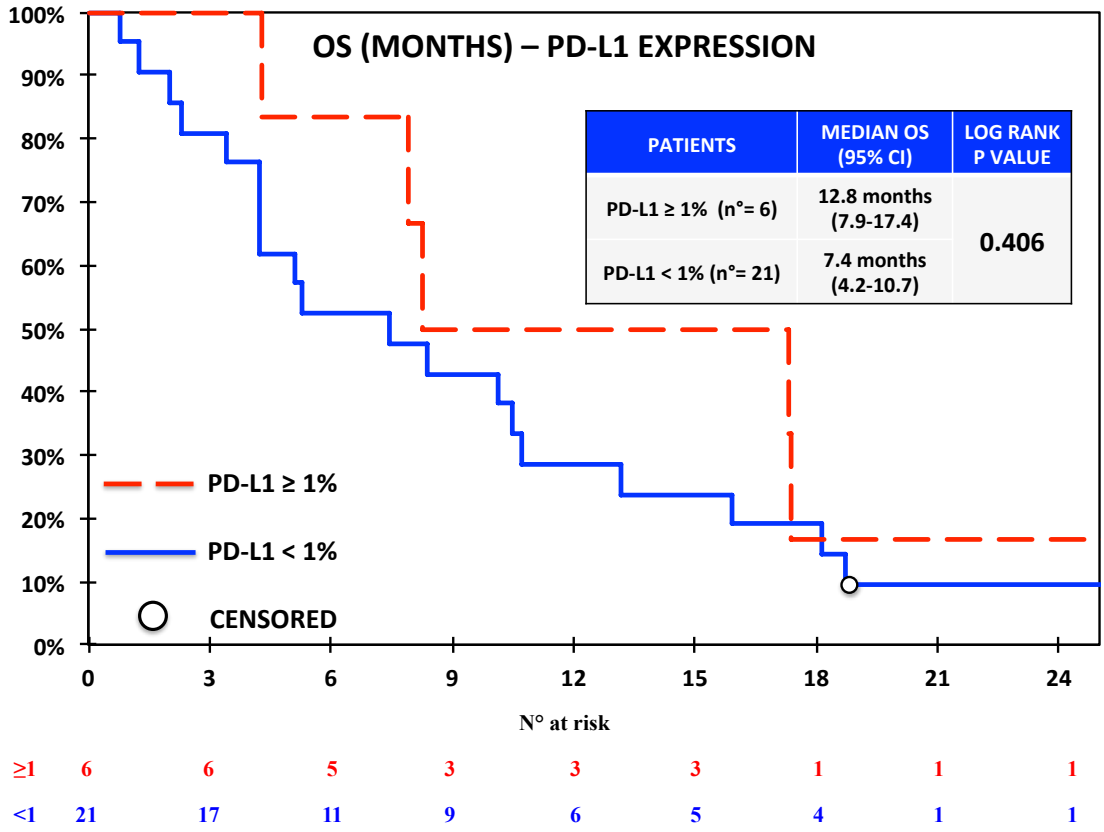
Supplementary figure 19: RECIST-DCR according to biomarkers expression at IHC in the Chemotherapy Cohort. No significant interaction was observed between each biomarker and DCR.



Supplementary figure 20: RECIST-PFS based on the expression of PD-L1 defined as ≥ 1% vs. < 1% in the Chemotherapy Cohort.



Supplementary figure 21: OS based on the expression of PD-L1 defined as ≥ 1% vs. < 1% in the Chemotherapy Cohort.



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