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Dynamic changes in PD-L1 expression and CD8$^+$ T cell infiltration in non-small cell lung cancer following chemoradiation therapy

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ABSTRACT

Objectives: The treatment for stage III non-small cell lung cancer (NSCLC) is quite variable because stage III NSCLC is a heterogenous disease. Programmed death ligand 1 (PD-L1) and CD8$^+$ tumor infiltrating lymphocytes (TILs) are thought to be related to treatment outcome in many tumors. To improve treatment outcome in stage III NSCLC, it is necessary to obtain data on PD-L1 expression and CD8$^+$ TIL counts following CCRT and their relationship to treatment outcome.

Materials and methods: We retrospectively enrolled 43 patients with stage III NSCLC treated with neoadjuvant CCRT followed by surgery at Yonsei Cancer Center Severance Hospital in Korea between June 2008 and October 2010. PD-L1 level and CD8$^+$ TIL numbers in tumors following CCRT were analyzed by immunohistochemistry, and their association with patient survival was evaluated with Kaplan-Meier method.

Results: More than half patients (52%) showed up- or downregulation of PD-L1 expression, and most patients (81%) showed change in CD8$^+$ TIL counts by CCRT. Patients with PD-L1 expression following CCRT tended to have shorter recurrence free survival (RFS) (P = 0.182) or overall survival (OS) (P = 0.215) compared to the ones without PD-L1 expression. In the survival analysis with pre-CCRT specimens, neither RFS nor OS showed statistically significant differences. Patients with increased CD8$^+$ TIL counts following CCRT regardless of pathological response strongly showed longer OS (median: not reached vs. 14.2 months for others; P = 0.017).

Conclusions: CCRT dynamically alters PD-L1 expression and CD8$^+$ TIL numbers in stage III NSCLC. Our data provide a rationale for combining CCRT and immunotherapy for the treatment of potentially resectable NSCLC.

1. Introduction

Lung cancer is the leading cause of death from cancer worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all cases and has a 5-year survival rate of 15% [2]. Over 30% of NSCLC patients are diagnosed at a locally advanced stage such as stage III [3], for which the standard treatment is concurrent chemoradiation therapy (CCRT) and surgery with or without adjuvant therapy [4]. Despite many efforts to improve treatment outcome, the 5-year survival rate for stage III NSCLC remains between 14%–24% [5].

Abbreviations: AUC, area under the curve; CCRT, concurrent chemoradiation therapy; CD8, cluster of differentiation 8; CR, complete response; DAMP, damage-associated molecular pattern; IC, immune cell; MHC I, class I major histocompatibility complex; NSCLC, non-small cell lung cancer; OS, overall survival; pCR, pathologic complete response; PD-1, programmed death 1; PD-L1, programmed death ligand 1; PR, partial response; RFS, recurrence-free survival; TC, tumor cell; TIL, tumor-infiltrating lymphocyte

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Programmed death (PD)-1/PD ligand (PD-L1) inhibitors have shown anti-tumor activity in stage IV NSCLC and are approved as standard therapy [6–10]. The PD-L1 inhibitor durvalumab was shown to suppress tumor progression following completion of CCRT in patients with locally advanced and unresectable stage III NSCLC [11,12]. Based on the results of the phase III PACIFIC trial [11], the U.S. Food and Drug Administration approved durvalumab for the treatment of patients with locally advanced, unresectable NSCLC, where disease had not progressed following platinum-based CRT. Although the mechanisms underlying the interaction between immunotherapy and CRT are not fully understood [11], it has been suggested that combining these approaches can have beneficial synergistic effects [13–15].

The available information on changes in PD-L1 expression following chemotherapy is inconsistent [16–18], and data on the effects of CCRT on the tumor microenvironment in NSCLC patients are lacking [19–31]. The T cell phenotype of NSCLC—which includes high PD-L1 expression and $CD8^+$ tumor infiltrating lymphocytes (TILs)—favors immune escape and has been known for favorable predictive factor for PD-1 blockade [32]. To improve treatment outcome in stage III NSCLC, more detailed data on PD-L1 expression and TIL numbers after CCRT and their relationship to treatment outcome are needed. To this end, the current study investigated the dynamics of PD-L1 expression and $CD8^+$ T cell infiltration into lung tumors following CCRT in patients with stage III NSCLC, and how these are correlated with patient survival.

2. Materials and methods

2.1. Patients

We retrospectively analyzed patients who were diagnosed with stage III NSCLC and who underwent neoadjuvant CCRT followed by curative surgery at Yonsei Cancer Center, Severance Hospital, between June 2008 and October 2010. Patients were classified based on clinical stage according to the 7th edition TNM classification [33]. Detailed information on patient characteristics was obtained from an electronic medical database. Those who reported never having smoked were defined as never smokers, and the rest were considered smokers including ex- and current smokers. Pre-CCRT specimens were obtained by biopsy at the time of initial diagnosis, and post-CCRT specimens were obtained at the time of surgery. Recurrence-free survival (RFS) was defined as the period from the day of surgery until any recurrence of lung cancer, death from any cause, or the end of the follow-up. Overall survival (OS) was defined as the period from the day of surgery until death from any cause or the end of the follow-up. The observation period lasted until January 2017. This study was approved by the Institutional Review Board of Severance Hospital.

2.2. Treatment

A total of 43 patients with stage III NSCLC were included in the analysis. Weekly standard chemotherapy with five or six cycles of CCRT included docetaxel (40 mg/m^2) plus cisplatin (40 mg/m^2) or paclitaxel (45 mg/m^2) plus carboplatin (area under the curve [AUC] 2). The radiation dose was between 40 and 50 Gy. One patient underwent lobectomy before completing CCRT due to a lung abscess requiring surgery, and was included in all analyses except response to CCRT. The total radiation dose for the patient was 10 Gy. Computed tomography scans were performed every 3–4 months. Response to CCRT was evaluated based on the Response Evaluation Criteria in Solid Tumors Guideline v.1.1 [34] prior to surgery and after completion of CCRT. Patients who showed partial response (PR) or complete response (CR) to CCRT were defined as responders.

2.3. Immunohistochemistry analysis of PD-L1 expression in tumor cells (TCs), immune cells (ICs) and $CD8^+$ TILs

Formalin-fixed paraffin-embedded tissue sections were cut at a thickness of 4 μm and stained with hematoxylin and eosin. Immunohistochemistry was performed according to the 22C3 pharmDx protocol using the Dako Automated Link 46 platform (Agilent Technologies, Santa Clara, CA, USA) [35] or Ventana Bench Mark XT Autostainer (Ventana Medical Systems, Tucson, AZ, USA) and SP263 antibody (Ventana; 1:100 dilution) [36]. Thirty-three patients (77%) were tested with 22C3, and 10 patients were tested with SP263, and same assay was used to the paired samples. PD-L1 positivity was defined as membranous staining intensity ≥ 1%, when the number of tumor cells was at least one hundred. Unchanged expression of PD-L1 was defined as the same percentage of stained TCs in specimens collected before and after CCRT from the same patient. The change in PD-L1 expression after as compared to before CCRT was evaluated in each patient.

The presence of $CD8^+$ TILs in NSCLC specimens was assessed by immunohistochemistry using pre-diluted primary $CD8^+$ antibody from Dako (C8/144B). The percentage of $CD8^+$ lymphocytes compared to the total number of nucleated cells in stromal compartments was assessed using the following cutoff values: low density, ≤ 25%; intermediate density, > 25% to 50%; and high density, > 50%. An increase or decrease in the number of $CD8^+$ lymphocytes was defined as an increased or decreased percentage of labeled cells in post- as compared to pre-CCRT specimens from the same patient.

Appropriate positive and negative controls for immunohistochemistry were included. Formalin-fixed, Paraffin-embedded tissue of tonsil was used for control specimen. For PD-L1, positive control was strong membrane staining in proportions of the crypt epithelium and weak to moderate membranous staining of the germinal center macrophages. For CD8, cytoplasmic staining in the paracortical T-cells was considered as appropriate positive staining. Endothelium, fibroblasts, and the surface epithelium of tonsil were used as negative control for both PD-L1 and $CD8^+$ TILs. Two pathologists independently reviewed all hematoxylin and eosin and immunohistochemistry slides. After independent review, discordant results were resolved by consensus.

2.4. Statistical analysis

Association was analyzed with the χ^2 test or Fisher's exact test for categorical variables. The Wilcoxon signed-rank test was used to compare $CD8^+$ TILs and PD-L1 expression on TCs between matched pre- and post-CCRT specimens, and the sign test was used to determine the trend of changes in PD-L1 expression on TCs or $CD8^+$ TIL abundance induced by CCRT (e.g., decrease or increase). $CD8^+$ TILs or tumor cellularity according to changes in PD-L1 expression on ICs (no change, decrease, and increase) was analyzed by one-way ANOVA. The Kaplan-Meier method was used to estimate survival outcome and inter-group comparisons were performed with the log-rank test. The correlation between PD-1 expression and $CD8^+$ TIL infiltration was analyzed with Spearman's rank-order correlation test. The correlation between the residual tumor cellularity and $CD8^+$ TILs after CCRT was analyzed by linear regression. The comparison of the residual tumor cellularity after CCRT was performed by two-sample t-test. A P value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS v.20.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Patient characteristics and CCRT outcome

A total of 43 patients with stage III (IIIA: 37; IIIB: 6) NSCLC were included in this study. The enrolled patients received neoadjuvant

CCRT followed by surgery with curative intent. The median follow up time was 31 months (range: 3–105.5 months), and the median period from the end date of CCRT to the date of surgery was 45 days (interquartile range: 34–56 days). Patient characteristics are summarized in Table S1. The median age of patients at diagnosis was 62 years (range: 42–73 years); 30 (70%) were men and 13 (30%) were women. There were 17 nonsmokers (40%), and the remaining 26 patients (60%) were smokers. Over half of patients (n = 26, 60%) had squamous cell carcinoma. Among the 17 adenocarcinoma patients (n = 17, 40%), eight harbored *epidermal growth factor receptor* (*EGFR*) mutations including exon 19 deletion (n = 5) and L858R (n = 3). All patients received standard weekly platinum-based chemotherapy (n = 43); 27 showed PR (64%) to CCRT, 18 experienced down-staging (42%), and no radiological CR was observed. Association between PD-L1 expression/CD8$^+$ TIL infiltration and baseline T and N status was not significant (Table S2).

3.2. PD-L1 expression on TCs and ICs and CD8$^+$ TILs before and after CCRT

Representative images of PD-L1 expression on the membrane of TCs and CD8$^+$ TILs are shown in Fig. 1A and B. After CCRT, seven patients achieved pathologic pCR (16%, 7/43). Baseline PD-L1 and pCR was not associated (P = 0.6889). We intended to evaluate to what extent the pathological response is correlated to the amount and morphology of immune infiltrate found after CCRT as well. There was no correlation between residual tumor cellularity and CD8$^+$ TIL infiltration when analyzed by linear regression. Moreover, we divided patients into two groups based on the residual tumor cellularity after CCRT: pCR/≤ 5% (n = 18) cellularity vs > 5% cellularity (n = 19). However, no difference was found in CD8 + TIL infiltration between the two groups (pCR/≤ 5%: 23.39% vs. > 5%: 23.42%; P = 0.996).

We were unable to evaluate post-CCRT PD-L1 expression on TCs in three of the patients due to a lack of specimens (7%, 3/43). These 10 patients were excluded from the calculation of percent PD-L1 expression or PD-L1 positivity in post-CCRT specimens. A total of 16 patients (48%, 16/33) were positive for PD-L1 expression on TCs and the other 17 were negative on TCs (52%, 17/33) (Table 1). Thus, PD-L1 positivity was unchanged by CCRT (49% vs. 48%, P = 0.155; Table 1). There was no association between PD-L1 positivity on TCs after CCRT and any patient characteristic (Table 1).

Changes in PD-L1 expression after CCRT were assessed based on the percentage of PD-L1 expression on TCs in matched specimens from the same patient before and after CCRT. Of the 33 patients, 16 (48%, 16/33), 7 (21%, 7/33), and 10 (30%, 10/33) showed no change in PD-L1 expression or up- or downregulation, respectively, after CCRT (P =

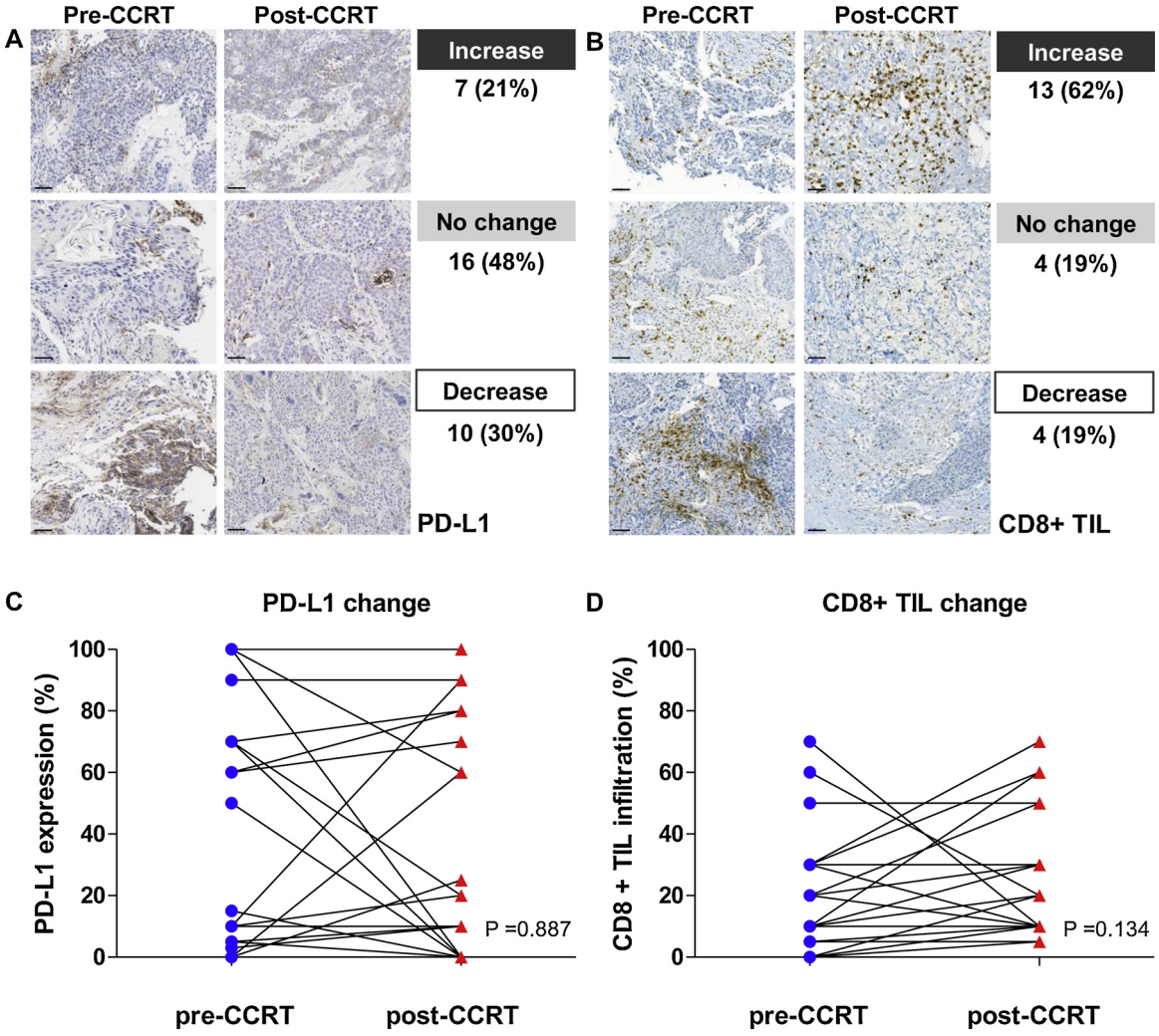

Fig. 1. Representative images of PD-L1 expression on TCs and CD8$^+$ TILs following CCRT in patients with NSCLC, as determined by immunohistochemistry. (A, B) The number of patients, dynamics of PD-L1 expression (increase, no change, or decrease) (A), and density of infiltrating CD8$^+$ TILs (B) before and after CCRT are shown. (C, D) Changes in PD-L1 expression (C) and density of CD8$^+$ TILs (D) were assessed based on the percentage of PD-L1 expression on TCs and CD8$^+$ TIL count in matched specimens from the same patient before and after CCRT. Each scale bar is 50 μm.

Table 1
PD-L1 positivity status on TCs pre and post-CCRT.

Characteristics	N (%)	Cases with positive PD-L1 on TCs		
		Pre-CCRT	Post-CCRT*	*p*-value
Total	43	21 (49)	16 (48)	0.155
Age				
< 62	21 (49)	11 (52)	10 (63)	0.138
≥62	22 (51)	10 (45)	6 (38)	0.522
Sex				
Male	30 (70)	16 (53)	11 (50)	0.266
Female	13 (30)	5 (38)	5 (45)	0.729
Smoking				
No	17 (40)	7 (41)	7 (50)	0.494
Yes	26 (60)	14 (54)	9 (47)	0.47
Histologic diagnosis				
Squamous	26 (60)	15 (58)	8 (44)	0.343
Non-squamous	17 (40)	6 (35)	8 (53)	0.342
Types of *EGFR* mutation				
Mutant	8 (19)	3 (38)	2 (29)	1
Wild-type	9 (21)	5 (56)	6 (86)	1
Concurrent chemotherapy regimen				
Docetaxel/cisplatin	19 (44)	9 (47)	6 (33)	0.274
Paclitaxel/carboplatin	24 (56)	12 (50)	10 (67)	0.914
Down-staging				
Yes	18 (42)	12 (67)	6 (46)	0.47
No	25 (58)	9 (36)	8 (40)	0.253
Response to CCRT				
Partial response	27 (64)	15 (56)	7 (37)	0.432
Stable	15 (36)	5 (33)	6 (46)	0.454

* For PD-L1 positivity, cases of pathologic CR and insufficient tissue were excluded (10 cases).

0.887, Wilcoxon signed-rank test; P = 0.629, sign test). Among the 16 patients with no change in PD-L1 expression, 13 (81%) did not express PD-L1 on TCs (0% PD-L1) either pre- or post-CCRT, whereas the other three (19%) had 10%, 90%, and 100% PD-L1 expression after as well as before CCRT (Fig. 1C).

We also reviewed the PD-L1 expression on ICs. Comparison between matched IHC slides was available in 25 cases. We divided patients into three groups according to the ΔPD-L1 on ICs after CCRT: increase, decrease, no change. Although statistical significance was not found, the PD-L1 decrease group showed lower tumor cellularity (P = 0.560). The same group showed highest $CD8^+$ TIL abundance (P = 0.041).

We analyzed the density of $CD8^+$ TILs in matched pre- and post-CCRT specimens, which were available for 21/43 patients. After CCRT, the number of $CD8^+$ TILs was increased in 13 (62%, 13/21), decreased in four (19%, 4/21), and unchanged in four (19%, 4/21) patients (P = 0.134, Wilcoxon signed-rank test; P = 0.049, sign test) (Fig. 1D). These results suggest that CCRT increased the density of $CD8^+$ TILs.

The relationship between PD-L1 expression and $CD8^+$ TIL infiltration was analyzed in pre- and post-CCRT specimens. In the former, PD-L1 expression and $CD8^+$ TIL infiltration were positively correlated (r = 0.459, P = 0.036; Fig. S1A). The correlation between the two parameters was also positive in post-CCRT specimens, but this was not statistically significant (r = 0.335, P = 0.137; Fig. S1B). The change in density of infiltrating $CD8^+$ TILs and PD-L1 expression following CCRT was negatively correlated, but without statistical significance (r = −0.115, P = 0.618, data not shown). Regardless of the change in PD-L1 expression (increase, decrease, or no change), most patients showed increased $CD8^+$ TIL density after CCRT (71% of patients with decreased PD-L1, 75% of those with increased PD-L1, and 50% of those with no change in PD-L1).

3.3. Association between PD-L1 expression and survival outcome

We evaluated treatment outcome according to PD-L1 expression in greater detail and found that the post-CCRT PD-L1-positive group showed a trend of shorter RFS compared to the PD-L1-negative group, although the difference was not statistically significant (median: 15.5 months for PD-L1-positive vs. not reached for PD-L1-negative, P = 0.182; Fig. 2A). The median OS was not reached after CCRT, and the post-CCRT PD-L1-positive group showed a trend of shorter OS with no statistical significance (not reached for both PD-L1-positive and -negative groups, P = 0.215; Fig. 2B).

In the survival analysis based on PD-L1 expression in pre-CCRT specimens, the median RFS was not reached, and there was no statistically significant difference in RFS between pre-CCRT PD-L1-positive and -negative groups (P = 0.423; Fig. 2C). The median OS was not reached, and there was no difference in OS between PD-L1-positive and -negative groups (P = 0.973; Fig. 2D).

We examined the association between survival outcome and changes in PD-L1 expression following CCRT. There was no obvious trend in RFS; the median RFS was not reached by patients showing no change or increased PD-L1 expression and was 16.1 months for patients with decreased expression (P = 0.817; Fig. 2E). None of the three groups reached the median OS (P = 0.220), while the group with increased PD-L1 expression after CCRT showed the steepest survival curve in the OS analysis (Fig. 2F).

3.4. Association between CD8+ TIL density and survival outcome

Patients were categorized based on the change in $CD8^+$ TIL density after CCRT, and the association between this parameter and patient survival was analyzed. The median value of RFS was 27.0 months. Patients who had increased $CD8^+$ TIL density after CCRT showed a tendency for longer RFS as compared to those with no change or decreased $CD8^+$ TIL density (median: 36.1 vs. 11.9 months, P = 0.088) (Fig. 3A). Although the median value of OS was not attained, increased $CD8^+$ TIL density after CCRT was significantly associated with longer OS (median: not reached for increase vs. 14.2 months for others; P = 0.017) (Fig. 3B). The increase of $CD8^+$ TILs regardless of pathological response strongly correlated with improved OS.

4. Discussion

This study reports on dynamic changes in PD-L1 expression and $CD8^+$ T cell infiltration into the tumor microenvironment after neoadjuvant CCRT and their prognostic significance in NSCLC patients. We found that 21% of patients showed increased and 30% showed decreased PD-L1 expression. Furthermore, RFS and OS were reduced to a greater extent in the post-CCRT PD-L1-positive as compared to the PD-L1 negative group. Notably, 62% of patients had increased $CD8^+$ TIL density after CCRT, which was associated with improved RFS and OS. Their results suggest that CCRT modulates the tumor microenvironment and thereby influences patient survival. The percentage of PD-L1 positive tumor cells significantly decreased after CCRT in stage II and III NSCLC patients [17]. The RFS of patients with decreased or unchanged PD-L1 was superior to that of patients with increased PD-L1 expression. $CD8^+$ TIL density increased after CCRT, but change of $CD8^+$ TILs was not associated with survival time unlike our study [17]. Neoadjuvant chemotherapy in stage III NSCLC also resulted in inconsistency of PD-L1 expression, and the negative-to-positive switch of PD-L1 status following chemotherapy was significantly associated with impaired disease free survival, but no significant changes in tumor-infiltrating immune cells were observed [16]. We tested if there was any difference in $CD8^+$ TIL abundance with different pathological response, but $CD8^+$ TIL abundance did not seem to vary depending on pathological response. In case of breast cancer, $CD8^+$ TILs were reported as an independent predictive factor for pCR to primary systemic therapy

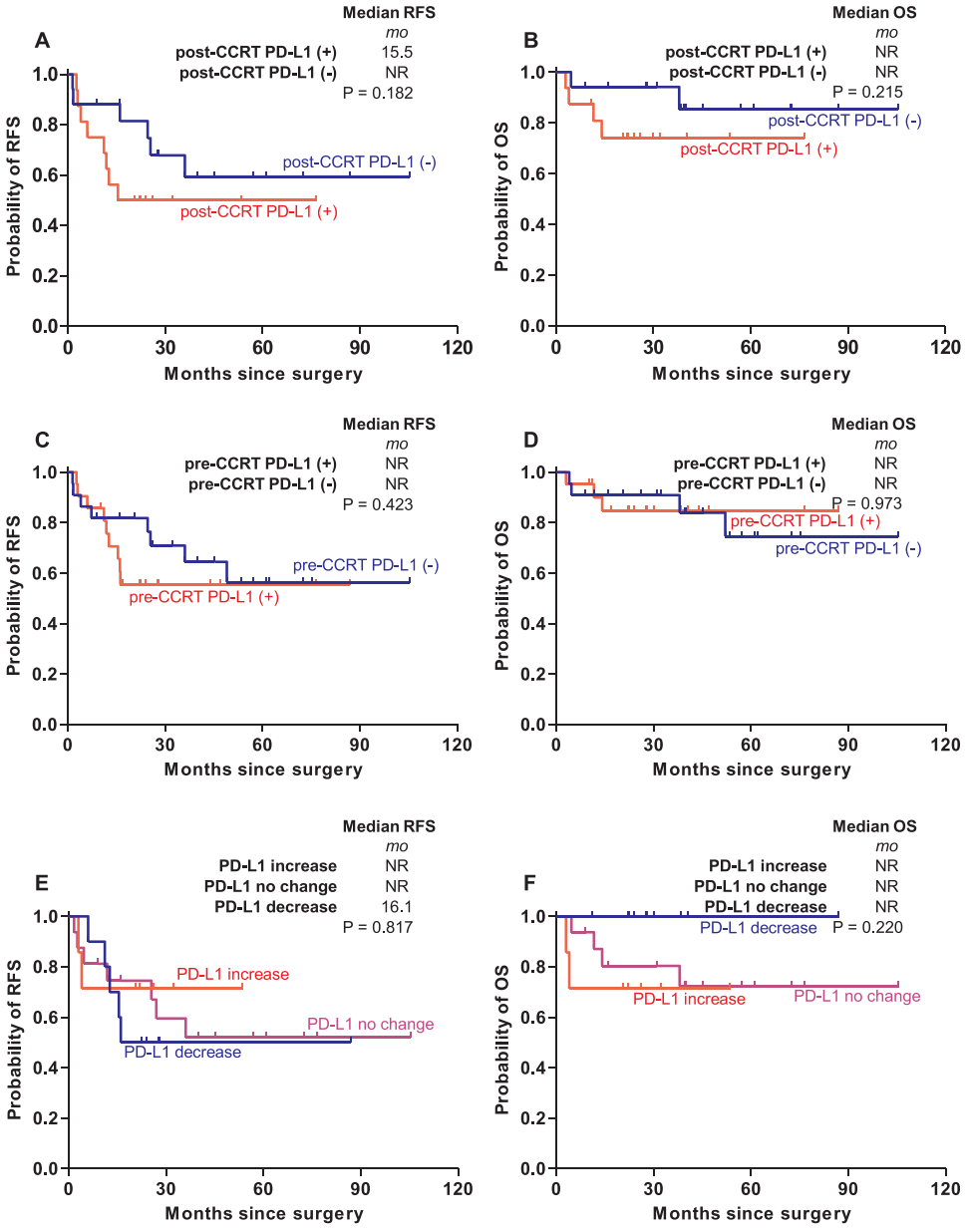

Fig. 2. Kaplan-Meier analysis of RFS and OS based on PD-L1 expression. (A, C, E) RFS and (B, D, F) OS were determined according to post-CCRT or pre-CCRT PD-L1 expression or changes in PD-L1 expression following CCRT.

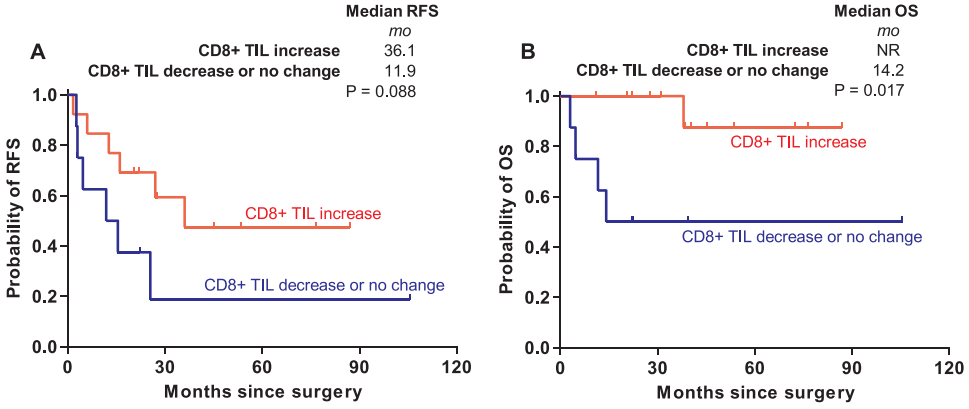

Fig. 3. Kaplan-Meier analysis of RFS and OS based on the change in $CD8^+$ TIL density following CCRT. (A) RFS and (B) OS.

[37]. We currently cannot explain why our data are different from those in breast cancer.

Chemotherapeutic agents exert their effects by eliciting *de novo* immune responses or reactivating pre-existing tumor-specific ones [38]. For example, cisplatin and gemcitabine broaden the range of tumor antigens that induce cytotoxic T lymphocytes *in vivo*. Our patients were treated with docetaxel/cisplatin or paclitaxel/carboplatin for CCRT, and most showed an increase in the density of $CD8^+$ TILs and longer OS, although the results were not statistically significant possibly due to the small sample size. Nonetheless, these results suggest that CCRT reduces the resistance of tumors in NSCLC to immune mechanisms that target cancer cells.

Radiotherapy not only has direct cytotoxic effects on TCs but also reprograms the tumor microenvironment to induce a potent antitumor immune response [39]. It can also enhance the efficacy of immunotherapy by stimulating the expression of class I major histocompatibility complex (MHC I), which is downregulated in many tumors as an immune evasion mechanism. Radiation also stimulates the release of damage-associated molecular patterns (DAMPs) which are essential for antigen-specific T cell responses in murine tumor models [40]. In patients who were otherwise unresponsive to immune checkpoint inhibitors, localized irradiation activated tumor-specific T cells to enhance the response to these therapies [41]. The beneficial synergistic effects of immune checkpoint inhibitors and anti-cancer therapies that cause immunogenic cell death (e.g., radiotherapy and/or chemotherapy) as first-line treatment for NSCLC were demonstrated by the Keynote 189 trial [42]. In the PACIFIC trial, consolidation therapy with durvalumab after CCRT improved survival in stage III, locally advanced, unresectable NSCLC [12,43]. There are currently over a dozen clinical trials that are evaluating anti-PD-1 and -PD-L1 antibodies in combination with radiation for cancer treatment [39]. In our study, PD-L1 expression was dynamic, and post-CCRT PD-L1 expression or increased PD-L1 expression following CCRT in the tumor microenvironment was correlated with worse prognosis. Our hypothesis is that balance between stimulation and inhibition of antitumor immunity was skewed towards inhibition and adaptive immune resistance was developed in that population during CCRT. The change in density of infiltrating $CD8^+$ TILs and PD-L1 expression following CCRT was negatively correlated in our study in line with this hypothesis. We speculate that CCRT converts non-T cell-inflamed into T cell-inflamed tumors and that combination with a PD-1/PD-L1 axis inhibitor further enhances the immune response to tumors. The adaptive resistance possibly brought about during CCRT also might be overcome by a PD-1/PD-L1 axis inhibitor. We will soon be initiating a clinical trial combining neoadjuvant CCRT with the PD-L1 inhibitor durvalumab in potentially resectable NSCLC (NCT03694236).

This study had certain limitations such as a relatively small sample size, the retrospective nature, differences in RT dose and chemotherapy agents used for systemic therapy, heterogeneity in sampling tumor, different PD-L1 assay, and selection bias since patients were recruited from a single institution in Korea. Nonetheless, our data on stage III NSCLC patients who received neoadjuvant CCRT and underwent surgery provide a rationale for combining CCRT and immunotherapy in the treatment of potentially resectable NSCLC.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.07.027.

References

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2016, CA Cancer J. Clin. 66 (1) (2016) 7–30.
[2] J.R. Molina, P. Yang, S.D. Cassivi, S.E. Schild, A.A. Adjei, Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship, Mayo Clin. Proc. 83 (5) (2008) 584–594.
[3] L. Crino, W. Weder, J. van Meerbeeck, E. Felip, E.G.W. Group, Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 21 (Suppl 5) (2010) v103–v115.
[4] P.E. Postmus, K.M. Kerr, M. Oudkerk, S. Senan, D.A. Waller, J. Vansteenkiste, C. Escriu, S. Peters, E.G. Committee, Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 28 (suppl_4) (2017) iv1–iv21.
[5] S.P. Saha, R.J. Kalathiya, D.L. Davenport, V.A. Ferraris, T.W. Mullett, J.B. Zwischenberger, Survival after pneumonectomy for stage III non-small cell lung cancer, Oman Med. J. 29 (1) (2014) 24–27.
[6] H. Borghaei, L. Paz-Ares, L. Horn, D.R. Spigel, M. Steins, N.E. Ready, L.Q. Chow, E.E. Vokes, E. Felip, E. Holgado, F. Barlesi, M. Kohlhaufl, O. Arrieta, M.A. Burgio, J. Fayette, H. Lena, E. Poddubskaya, D.E. Gerber, S.N. Gettinger, C.M. Rudin, N. Rizvi, L. Crino, G.R. Blumenschein Jr., S.J. Antonia, C. Dorange, C.T. Harbison, F. Graf Finckenstein, J.R. Brahmer, Nivolumab versus docetaxel in advanced non-squamous non-small-cell lung cancer, N. Engl. J. Med. 373 (17) (2015) 1627–1639.
[7] J. Brahmer, K.L. Reckamp, P. Baas, L. Crino, W.E. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E.E. Vokes, E. Holgado, D. Waterhouse, N. Ready, J. Gainor, O. Aren Frontera, L. Havel, M. Steins, M.C. Garassino, J.G. Aerts, M. Domine, L. Paz-Ares, M. Reck, C. Baudelet, C.T. Harbison, B. Lestini, D.R. Spigel, Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer, N. Engl. J. Med. 373 (2) (2015) 123–135.
[8] R.S. Herbst, P. Baas, D.W. Kim, E. Felip, J.L. Perez-Gracia, J.Y. Han, J. Molina, J.H. Kim, C.D. Arvis, M.J. Ahn, M. Majem, M.J. Fidler, G. de Castro Jr., M. Garrido, G.M. Lubiniecki, Y. Shentu, E. Im, M. Dolled-Filhart, E.B. Garon, Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial, Lancet (Lond. Engl.) 387 (10027) (2016) 1540–1550.
[9] L. Fehrenbacher, A. Spira, M. Ballinger, M. Kowanetz, J. Vansteenkiste, J. Mazieres, K. Park, D. Smith, A. Artal-Cortes, C. Lewanski, F. Braiteh, D. Waterkamp, P. He, W. Zou, D.S. Chen, J. Yi, A. Sandler, A. Rittmeyer, Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial, Lancet (Lond. Engl.) 387 (10030) (2016) 1837–1846.
[10] M. Reck, D. Rodriguez-Abreu, A.G. Robinson, R. Hui, T. Csoszi, A. Fulop, M. Gottfried, N. Peled, A. Tafreshi, S. Cuffe, M. O'Brien, S. Rao, K. Hotta, M.A. Leiby, G.M. Lubiniecki, Y. Shentu, R. Rangwala, J.R. Brahmer, Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer, N. Engl. J. Med. 375 (19) (2016) 1823–1833.
[11] S.J. Antonia, A. Villegas, D. Daniel, D. Vicente, S. Murakami, R. Hui, T. Yokoi, A. Chiappori, K.H. Lee, M. de Wit, B.C. Cho, M. Bourhaba, X. Quantin, T. Tokito, T. Mekhail, D. Planchard, Y.C. Kim, C.S. Karapetis, S. Hiret, G. Ostoros, K. Kubota, J.E. Gray, L. Paz-Ares, J. de Castro Carpeno, C. Wadsworth, G. Melillo, H. Jiang, Y. Huang, P.A. Dennis, M. Ozguroglu, Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer, N. Engl. J. Med. 377 (20) (2017) 1919–1929.
[12] S.J. Antonia, A. Villegas, D. Daniel, D. Vicente, S. Murakami, R. Hui, T. Kurata, A. Chiappori, K.H. Lee, M. de Wit, B.C. Cho, M. Bourhaba, X. Quantin, T. Tokito, T. Mekhail, D. Planchard, Y.C. Kim, C.S. Karapetis, S. Hiret, G. Ostoros, K. Kubota, J.E. Gray, L. Paz-Ares, J. de Castro Carpeno, C. Faivre-Finn, M. Reck, J. Vansteenkiste, D.R. Spigel, C. Wadsworth, G. Melillo, M. Taboada, P.A. Dennis, M. Ozguroglu, P. Investigators, Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC, N. Engl. J. Med. 379 (24) (2018) 2342–2350.
[13] J. Domagala-Kulawik, The role of the immune system in non-small cell lung carcinoma and potential for therapeutic intervention, Transl. Lung Cancer Res. 4 (2) (2015) 177–190.
[14] C. Tang, X. Wang, H. Soh, S. Seyedin, M.A. Cortez, S. Krishnan, E. Massarelli, D. Hong, A. Naing, A. Diab, D. Gomez, H. Ye, J. Heymach, R. Komaki, J.P. Allison, P. Sharma, J.W. Welsh, Combining radiation and immunotherapy: a new systemic therapy for solid tumors? Cancer Immunol. Res. 2 (9) (2014) 831–838.
[15] S.J. Dovedi, A.L. Adlard, G. Lipowska-Bhalla, C. McKenna, S. Jones, E.J. Cheadle, I.J. Stratford, E. Poon, M. Morrow, R. Stewart, H. Jones, R.W. Wilkinson, J. Honeychurch, T.M. Illidge, Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade, Cancer Res. 74 (19) (2014) 5458–5468.
[16] J. Sheng, W. Fang, J. Yu, N. Chen, J. Zhan, Y. Ma, Y. Yang, Y. Huang, H. Zhao, L. Zhang, Expression of programmed death ligand-1 on tumor cells varies pre and post chemotherapy in non-small cell lung cancer, Sci. Rep. 6 (2016) 20090.
[17] D. Fujimoto, K. Uehara, Y. Sato, I. Sakanoue, M. Ito, S. Teraoka, K. Nagata, A. Nakagawa, Y. Kosaka, K. Otsuka, Y. Imai, H. Hamakawa, Y. Takahashi, M. Kokubo, K. Tomii, Alteration of PD-L1 expression and its prognostic impact after concurrent chemoradiation therapy in non-small cell lung cancer patients, Sci. Rep. 7 (1) (2017) 11373.
[18] T. Tokito, K. Azuma, A. Kawahara, H. Ishii, K. Yamada, N. Matsuo, T. Kinoshita, N. Mizukami, H. Ono, M. Kage, T. Hoshino, Predictive relevance of PD-L1 expression combined with CD8+ TIL density in stage III non-small cell lung cancer patients receiving concurrent chemoradiotherapy, Eur. J. Cancer 55 (2016) 7–14.
[19] C.Y. Mu, J.A. Huang, Y. Chen, C. Chen, X.G. Zhang, High expression of PD-L1 in

lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation, Med. Oncol. 28 (3) (2011) 682–688.
[20] K. Azuma, K. Ota, A. Kawahara, S. Hattori, E. Iwama, T. Harada, K. Matsumoto, K. Takayama, S. Takamori, M. Kage, T. Hoshino, Y. Nakanishi, I. Okamoto, Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer, Ann. Oncol. 25 (10) (2014) 1935–1940.
[21] Y.B. Chen, C.Y. Mu, J.A. Huang, Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: a 5-year-follow-up study, Tumori 98 (6) (2012) 751–755.
[22] Y. Mao, W. Li, K. Chen, Y. Xie, Q. Liu, M. Yao, W. Duan, X. Zhou, R. Liang, M. Tao, B7-H1 and B7-H3 are independent predictors of poor prognosis in patients with non-small cell lung cancer, Oncotarget 6 (5) (2015) 3452–3461.
[23] Y. Tang, W. Fang, Y. Zhang, S. Hong, S. Kang, Y. Yan, N. Chen, J. Zhan, X. He, T. Qin, G. Li, W. Tang, P. Peng, L. Zhang, The association between PD-L1 and EGFR status and the prognostic value of PD-L1 in advanced non-small cell lung cancer patients treated with EGFR-TKIs, Oncotarget 6 (16) (2015) 14209–14219.
[24] C. Zhou, J. Tang, H. Sun, X. Zheng, Z. Li, T. Sun, J. Li, S. Wang, X. Zhou, H. Sun, Z. Cheng, H. Zhang, H. Ma, PD-L1 expression as poor prognostic factor in patients with non-squamous non-small cell lung cancer, Oncotarget 8 (35) (2017) 58457–58468.
[25] Y. Zhang, L. Wang, Y. Li, Y. Pan, R. Wang, H. Hu, H. Li, X. Luo, T. Ye, Y. Sun, H. Chen, Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma, OncoTargets Ther. 7 (2014) 567–573.
[26] J. Koh, H. Go, B. Keam, M.Y. Kim, S.J. Nam, T.M. Kim, S.H. Lee, H.S. Min, Y.T. Kim, D.W. Kim, Y.K. Jeon, D.H. Chung, Clinicopathologic analysis of programmed cell death-1 and programmed cell death-ligand 1 and 2 expressions in pulmonary adenocarcinoma: comparison with histology and driver oncogenic alteration status, Mod. Pathol. 28 (9) (2015) 1154–1166.
[27] V. Velcheti, K.A. Schalper, D.E. Carvajal, V.K. Anagnostou, K.N. Syrigos, M. Sznol, R.S. Herbst, S.N. Gettinger, L. Chen, D.L. Rimm, Programmed death ligand-1 expression in non-small cell lung cancer, Lab. Invest. 94 (1) (2014) 107–116.
[28] W.A. Cooper, T. Tran, R.E. Vilain, J. Madore, C.I. Selinger, M. Kohonen-Corish, P. Yip, B. Yu, S.A. O'Toole, B.C. McCaughan, J.H. Yearley, L.G. Horvath, S. Kao, M. Boyer, R.A. Scolyer, PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma, Lung Cancer 89 (2) (2015) 181–188.
[29] C.Y. Yang, M.W. Lin, Y.L. Chang, C.T. Wu, P.C. Yang, Programmed cell death-ligand 1 expression in surgically resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes, Eur. J. Cancer 50 (7) (2014) 1361–1369.
[30] A. D'Incecco, M. Andreozzi, V. Ludovini, E. Rossi, A. Capodanno, L. Landi, C. Tibaldi, G. Minuti, J. Salvini, E. Coppi, A. Chella, G. Fontanini, M.E. Filice, L. Tornillo, R.M. Incensati, S. Sani, L. Crino, L. Terracciano, F. Cappuzzo, PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients, Br. J. Cancer 112 (1) (2015) 95–102.
[31] J.M. Boland, E.D. Kwon, S.M. Harrington, J.A. Wampfler, H. Tang, P. Yang, M.C. Aubry, Tumor B7-H1 and B7-H3 expression in squamous cell carcinoma of the lung, Clin. Lung Cancer 14 (2) (2013) 157–163.
[32] J.A. Trujillo, R.F. Sweis, R. Bao, J.J. Luke, T cell-inflamed versus non-T cell-inflamed tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection, Cancer Immunol. Res. 6 (9) (2018) 990–1000.
[33] P. Goldstraw, J. Crowley, K. Chansky, D.J. Giroux, P.A. Groome, R. Rami-Porta, P.E. Postmus, V. Rusch, L. Sobin, C. International Association for the Study of Lung Cancer International Staging, I. Participating, The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours, J. Thorac. Oncol. 2 (8) (2007) 706–714.
[34] E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij, New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1), Eur. J. Cancer 45 (2) (2009) 228–247.
[35] C. Roach, N. Zhang, E. Corigliano, M. Jansson, G. Toland, G. Ponto, M. Dolled-Filhart, K. Emancipator, D. Stanforth, K. Kulangara, Development of a companion diagnostic PD-L1 immunohistochemistry assay for pembrolizumab therapy in non-small-cell lung cancer, Appl. Immunohistochem. Mol. Morphol.: AIMM 24 (6) (2016) 392–397.
[36] US Food and Drug Administration. VENTANA PD-L1 (SP263) Assay. https://www.accessdata.fda.gov/ccrh_docs/pdf16/p160046c.pdf.
[37] A.N. Seo, H.J. Lee, E.J. Kim, H.J. Kim, M.H. Jang, H.E. Lee, Y.J. Kim, J.H. Kim, S.Y. Park, Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer, Br. J. Cancer 109 (10) (2013) 2705–2713.
[38] L. Zitvogel, L. Galluzzi, M.J. Smyth, G. Kroemer, Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance, Immunity 39 (1) (2013) 74–88.
[39] R.R. Weichselbaum, H. Liang, L. Deng, Y.X. Fu, Radiotherapy and immunotherapy: a beneficial liaison? Nat. Rev. Clin. Oncol. 14 (6) (2017) 365–379.
[40] A.B. Sharabi, M. Lim, T.L. DeWeese, C.G. Drake, Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy, Lancet Oncol. 16 (13) (2015) e498–509.
[41] C. Vanpouille-Box, K.A. Pilones, E. Wennerberg, S.C. Formenti, S. Demaria, In situ vaccination by radiotherapy to improve responses to anti-CTLA-4 treatment, Vaccine 33 (51) (2015) 7415–7422.
[42] L. Gandhi, D. Rodriguez-Abreu, S. Gadgeel, E. Esteban, E. Felip, F. De Angelis, M. Domine, P. Clingan, M.J. Hochmair, S.F. Powell, S.Y. Cheng, H.G. Bischoff, N. Peled, F. Grossi, R.R. Jennens, M. Reck, R. Hui, E.B. Garon, M. Boyer, B. Rubio-Viqueira, S. Novello, T. Kurata, J.E. Gray, J. Vida, Z. Wei, J. Yang, H. Raftopoulos, M.C. Pietanza, M.C. Garassino, K.- Investigators, Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer, N. Engl. J. Med. 378 (22) (2018) 2078–2092.
[43] S.J. Antonia, A. Villegas, D. Daniel, D. Vicente, S. Murakami, R. Hui, T. Yokoi, A. Chiappori, K.H. Lee, M. de Wit, B.C. Cho, M. Bourhaba, X. Quantin, T. Tokito, T. Mekhail, D. Planchard, Y.C. Kim, C.S. Karapetis, S. Hiret, G. Ostoros, K. Kubota, J.E. Gray, L. Paz-Ares, J. de Castro Carpeno, C. Wadsworth, G. Melillo, H. Jiang, Y. Huang, P.A. Dennis, M. Ozguroglu, P. Investigators, Durvalumab after chemoradiotherapy in stage III non-small-cell lung cancer, N. Engl. J. Med. 377 (20) (2017) 1919–1929.