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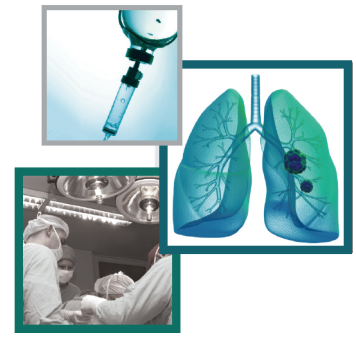
Lung Cancer Management



Clinical outcomes in non-small-cell lung cancer patients receiving concurrent metformin and immune checkpoint inhibitors

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Aim: To study the clinical benefits of concurrent metformin and immune checkpoint inhibitors (ICIs) in non-small-cell lung cancer patients. **Materials & methods:** This is a retrospective review of 50 non-small-cell lung cancer patients receiving ICIs with metformin (cohort A) or without metformin (cohort B). Patients were also stratified by ICIs as second-/third-line therapy. **Results:** Overall response rate and disease control rate were higher in cohort A (41.1 vs 30.7%, p = 0.4 and 70.5 vs 61.6%, p = 0.5, respectively). Median overall survival and progression-free survival were also higher in cohort A (11.5 vs 7.6 months, p = 0.5 and 4.0 vs 3.0 months, p = 0.6, respectively). On subset analysis (second-/third-line ICIs), overall response rate, disease control rate, median overall survival, progression-free survival were also higher in cohort A. **Conclusion:** Despite the small-sample size, we observed improved clinical outcomes in patients who received ICIs in combination with metformin.

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Keywords: anti-PD-1/anti-PD-L1 • atezolizumab • metformin • nivolumab • non-small-cell lung cancer • pembrolizumab • second-line ICI • third-line ICI

Lung cancer is one of the most common cancers and a leading cause of cancer-related deaths. In the USA, 234,030 new cases of lung cancer (13.5% of all new cancer cases) are estimated to be diagnosed in 2018. Estimated lung cancer-related deaths are 154,050 in 2018. Non-small-cell lung cancer (NSCLC) is estimated to be 80% of all lung cancer cases per National Cancer Institute data [1].

Platinum-based chemotherapies, such as carboplatin and cisplatin combined with a second agent, have been the most commonly used therapies in lung cancer for many years [2–7]. Recently, immune checkpoint inhibitors (ICIs) and monoclonal antibodies against immune checkpoints, such as anti-CTLA-4, anti-PD-1 and anti-PD-L1, have been studied in various type of cancers and are routinely being used in clinical practice [8,9]. Nivolumab was the first monoclonal antibody targeting PD-1 receptors that was tested in solid tumors such as melanoma, NSCLC or renal cell carcinoma [10,11]. In NSCLC, antibodies targeting PD-1/PD-L1 have shown promising results [12]; Pembrolizumab and nivolumab are anti-PD-1 ICIs where as atezolizumab is anti-PD-L1 ICI [12]. These ICIs have been used in combination with different chemotherapies in NSCLC in prospective Phase II and III clinical trials as well as retrospective studies; all these studies have shown promising results [13–15]. The combination of carboplatin, pemetrexed and pembrolizumab is approved by the US FDA for first-line treatment of nonsquamous cell lung carcinoma.

Although these ICIs have shown improved outcomes, a long-term follow-up of the patients on clinical trials has shown the delayed progression in patients who have had achieved response once. This is thought to be as a result of underlying innate (primary) and acquired (secondary) resistance [16]. Evaluation of patients in clinical trials has suggested three types of patient subsets: those who have never shown any response to therapy (primary resistance), those who have responded initially and continue to respond in long-term follow-up (responders), and

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those who responded initially and then progressed eventually (acquired resistance) [17–21]. Several mechanisms have been considered as the cause of ICI therapy failure. This may be due to insufficient generation of antitumor T cells, inadequate function of tumor-specific T cells or impaired formation of T-cell memory [20–22].

The continued emergence of resistance and relapse after long-term follow-up after ICIs treatment has encouraged scientists to look further therapeutic modalities in lung cancer patients. Recently, there has been a trend toward the use of anticancer properties in non-anticancer drugs. The idea is to evaluate the direct cytotoxic effects of these drugs as well as to improve the antitumor effect of the chemotherapeutic and immunotherapeutic drugs when used in combination [23]. An observational study by Tian *et al*. has shown that metformin, a commonly used drug for diabetes, decreases the incidence of lung cancer [24]. Several retrospective studies have shown improved cancer-related outcomes in patients with NSCLC who have received metformin concurrently with platinum-based chemotherapy, radiation therapy or EGFR-TKI inhibitors [25–27]. Recently, a randomized Phase II trial by Marrone *et al*. examined a combination of carboplatin/paclitaxel/bevacizumab with concurrent metformin in NSCLC and demonstrated a significant improvement in progression-free survival (PFS) [28].

It has also been reported that metformin may enhance the PD-1 blockage by anti-PD-1 antibodies, as has been suggested by mouse models. Such models have shown that metformin reduces the tumor hypoxia that results in the enhancement of PD-1 blockage [29]. Metformin and phenformin (another biguanide) also inhibit the myeloid-derived suppressor cells (MDSCs) and, as a result, enhance the antitumor activity of PD-1 blockers [30,31]. MDSCs are the type of immune cells that promote tumor-induced immune suppression. This immune suppression by MDSCs assists in the evasion of the tumor cells and the emergence of resistance [32]. Although metformin has been used concurrently with many cytotoxic agents in NSCLC, on further literature review, we were unable to find any study evaluating the efficacy of metformin when used in combination with ICIs.

Based on these observations, we conducted a retrospective review to observe clinical outcomes in patients who have received metformin concurrently with ICIs in NSCLC treatments. To our knowledge, this is the first of such study in the literature on NSCLC patients receiving ICIs.

Materials & methods

This study was conducted at Dartmouth Hitchcock Medical Center and the Norris Cotton Cancer Center in Lebanon, NH, USA. This is a retrospective chart review study for which nonprobability convenience sampling was done. The Dartmouth Hitchcock Medical Center coding department was contacted to identify patients of 18 years of age or higher who have been diagnosed with stage IV NSCLC and have received FDA-approved nivolumab, pembrolizumab or atezolizumab with metformin (cohort A) or without metformin (cohort B) between 1 January 2015 until 30 May 2018. Patients who have received metformin and ICI for at least 1 week were included in cohort A. Only cohort A had patients with history of diabetes mellitus. Patients with a history of prior ICI use, chemotherapy or history of autoimmune disease were not excluded from the study. Due to the retrospective nature of this study, there was no direct patient contact. The Dartmouth College Institutional Review Board granted an exemption from the informed consent. Patients less than 18 years of age or who had received concomitant metformin for less than 1 week were excluded.

Electronic chart review was performed to extract data that included basic demographics (age, sex, race), time of diagnosis, clinical stage at diagnosis (AJCC 8th edition), PD-L1 status, mutation status, diabetic status, prior adjuvant therapy, concurrent chemoradiation treatment, cancer-related surgery, metformin use, number of cycles of ICIs, best radiographic response, in other words, complete remission (CR), partial response (PR), stable disease and progressive disease (PD). Radiographic response was determined using the RECIST v. 1.1 criteria. Additional chemotherapy or ICI, progression, current status (dead/alive) were recorded as well. Overall response rate (ORR) was calculated and defined as the percentage of patients who achieved either PR or CR. Disease control rate (DCR) was defined as the total percentage of patients achieving CR, PR and stable disease. Overall survival (OS) from the initiation of the therapy till the date of last follow-up (15 June 2018), or the date of death, and PFS from the date of therapy initiation till the last follow-up (15 June 2018), progression or date of death, were both calculated. Basic laboratory data, Eastern Cooperative Oncology Group Performance Status score, reported immune-related adverse events (IrAEs), for example, fatigue, rash/mucositis, transaminitis, colitis/diarrhea, etc., were collected. We also recorded if patients received any steroid therapy for the reported side effects. Besides that, we recorded the anatomic sites involved with metastases before and during or after the therapy.

The independent variables of the study are age, sex, presence of driver mutation, anatomical sites involved with metastases and category of therapy (nivolumab or pembrolizumab, atezolizumab with or without metformin).

The dependent variables are the best response, ORR, OS, PFS, reported side effects of the therapy and Eastern Cooperative Oncology Group scores. ORR is the primary end point. OS, PFS, DCR are the secondary end points.

Statistical analysis

Due to the small-sample size of cohort A, nonprobability convenience sampling was done for cohort B via a random number generator online tool [33]. Summary measures of continuous data such as age at diagnosis of stage IV, OS and PFS (in months), mean (or geometric mean, as appropriate), median, standard deviation and interquartile range were calculated. Histograms and Q–Q plots of continuous end points were used to evaluate distributional assumptions. To evaluate the survival analyses (OS and PFS, with 95% CI), the Kaplan–Meier method and the log-rank test were applied. However, to account for potential variables affecting the survival data (such as age at diagnosis, sex, KRAS mutations, number of mutations present, prior malignancy, prior therapy, metformin duration, metformin dose), Cox regression analyses were done. Multiple-linear regression analyses were performed to investigate the effect of duration of metformin therapy on OS and PFS. Chi-square and Fisher exact tests were applied to compare the categorical variables and calculate the p-value. T-tests were applied to analyze the continuous variables and to calculate the p-values.

Results

General characteristics

Fifty patients were included in the final analysis, of which 21 (42%) patients were in cohort A and 29 (58%) patients were in cohort B. One patient was lost during follow-up in cohort A and five patients were lost in cohort B. Overall median age at the time of diagnosis was 68.5 years. Overall gender distribution was similar (50% male and 50% female). None of the patients had EGFR mutation in either of the cohorts.

Mean age was higher in cohort A (A: 70.1 vs B: 66.7 years, p = 0.4). Gender distribution was similar between both cohorts (p = 0.8). All patients had stage IV disease at the time of initiation of ICIs in both cohorts. A slightly higher proportion of patients passed away in cohort B (A: 61.9 vs B: 68.9%, p = 0.4). KRAS was the most common mutation and was more prevalent in cohort B (A: 25 vs B: 41.6%, p = 0.2). Overall, PD-L1 status was known in 58% of the patients. Of the patients with known PD-L1 status, a higher proportion of patients in cohort B had greater than 50% PD-L1 expression (A: 33.3 vs B: 41.1%, p = 0.6); however, the overall difference in PD-L1 expression was not significant between both cohorts (p = 0.8).

A similar proportion of patients received ICI only as second- or third-line therapy in both cohorts (A: 57.1 vs B: 58.6%, p = 0.9). Among these patients, one patient in cohort A and three patients in cohort B had received prior chemotherapy as a part of adjuvant therapy. Eighteen patients (85.7%) in cohort A and 24 patients (82.8%) in cohort B received anti-PD-1 (pembrolizumab, nivolumab), whereas three patients (14.3%) in cohort A and five patients (17.2%) in cohort B had anti-PD-L1 (atezolizumab). Three patients (14.3%) received carboplatin/pemetrexed with pembrolizumab as the first-line treatment in cohort A versus six patients (20.7%) in cohort B, p = 0.5.

All patients in cohort A either had diabetes or had steroid-induced hyperglycemia requiring metformin. None of the patients in cohort B had diabetes mellitus. Overall, the median duration of metformin therapy was 18.8 months. The median duration that patients received metformin and ICI concurrently was 2.3 months. Median metformin dose was 500 mg twice daily. The range of doses was 500 mg daily to 1000 mg twice daily (Table 1).

The mean number of metastatic sites involved with malignancy before starting ICI was similar between cohorts. However, the mean number of new metastatic sites appearing while on ICI therapy was higher in cohort B (2.3 vs 1.7, p = 0.2). New brain metastases appearing while on therapy were also higher in cohort B but new skeletal metastases appearing while on therapy were higher in cohort A (Table 2).

Both cohorts tolerated the therapies well. Fatigue was the most common side effect experienced, though was significantly higher in cohort B (B: 96.5 vs A: 76.2%, p = 0.02). The number of patients experiencing IrAE (excluding patients with fatigue) was slightly higher in cohort B (B: 44.8 vs A: 38.1%, p = 0.6). The proportion of patients who received prednisone for IAEs were higher in cohort B as well (B: 41.3 vs A: 28.5%, p = 0.3). One patient did not receive prednisone for IrAE in cohort A, death was reported due to therapy-related adverse events (Table 2).

Table 1. General patient characteristics

Variables	Cohort A (metformin) N = 21 (42%)	Cohort B (no metformin) N = 29 (58%)	
Mean age at diagnosis (years)	70.1 ± 10.3	66.7 ± 10.3	p = 0.2
Sex – Male – Female	 12 (57.1%) 09 (42.9%)	 16 (55.2%) 13 (44.8%)	p = 0.8
ECOG performance status – 0 – 1 – 2 – 3	 4 (19%) 10 (47.6%) 6 (28.6%) 1 (4.8%)	 4 (13.8%) 13 (44.8%) 6 (20.7%) 6 (20.7%)	p = 0.4
Smoking history	19 (90.5%)	29 (100%)	p = 0.09
KRAS mutations	4/16† (25%)	10/24† (41.6%)	p = 0.2
PD-L1 status known	12 (57.1%)	17 (58.6%)	
PD-L1 status (only patients with known status) – <1 % – 1–50% – >50%	 6 (50%) 2 (16.7%) 4 (33.3%)	 7 (41.1%) 3 (17.6%) 7 (41.1%)	p = 0.8
History of diabetes/hyperglycemia	21 (100%)	0 (0%)	p < 0.001‡
Median metformin dose	500 mg BID (range: 500 mg daily–1000 mg BID)		
Median duration of metformin therapy (months)	18.8 (1–110.3)		
Median duration of concurrent metformin and ICI (months)	2.3 (0.4–18.3)		
History of concurrent chemoradiation therapy	7 (33.3%)	7 (24.1%)	p = 0.4
ICIs as second- or third-line therapy	12 (57.1%)	17 (58.6%)	p = 0.9
Surgery	2 (9.5%)	3 (10.3%)	p = 0.5
History of malignancy	3 (14.3%)	4 (13.8%)	p = 0.9
ICIs – Anti-PD-1 (pembrolizumab, nivolumab) – Anti-PD-L1 (atezolizumab)	 18 (85.7%) 3 (14.3%)	 24 (82.8%) 5 (17.2%)	p = 0.7
Mean number of cycles	6.6 ± 5.9	5.8 ± 5	p = 0.6
ICI+ chemotherapy	3 (14.2%)	6 (20.7%)	p = 0.5
Progression	13 (61.9%)	19 (65.5%)	p = 0.7
Mean number of metastatic sites involved before starting therapy	3 ± 0.8	2.9 ± 1	p = 0.7

† Of the patients in which KRAS mutation was checked.
‡ Signifies statistical significance.
BID: Twice daily; ECOG: Eastern Cooperative Oncology Group; ICI: Immune checkpoint inhibitor; KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog.

Efficacy analyses

Best radiographic response

The best radiographic response was determined by using RECIST criteria v1.1. Four patients in cohort A and three in cohort B did not have the radiographically evaluable disease. ORR was higher in cohort A, 41.1%, than cohort B, 30%, but the difference was not statistically significant (p = 0.4). Moreover, there was no statistically significant difference in DCR between both cohorts, although the DCR was higher in A (A: 70.6 vs B: 61.6% p = 0.5). The median time to achieve the best response was longer in cohort B (3.2 vs 2.7 months; Table 3).

We further investigated the objective response in patients who have received ICI as second- or third-line therapy. ORR was significantly higher in this subset of cohort A compared with that of cohort B (A: 44.4 vs B: 6.7%, p = 0.02). DCR was also higher in subset of cohort A (A: 77.7 vs B: 46.7%, p = 0.1; Table 4).

When the one patient in cohort A and the three patients in cohort B who received prior chemotherapy as adjuvant therapy were excluded from the subset analyses, the ORR was still significantly higher in cohort A (A: 37.5 vs B: 0%, p = 0.02). DCR was also higher in cohort A (A: 75 vs B: 41.6%, p = 0.1).

Table 2. Treatment-related adverse outcomes

Variables	Cohort A (metformin) N = 21 (42%)	Cohort B (no metformin) N = 29 (58%)	
Mean number of new metastatic site appeared while on therapy	1.7 ± 1.6	2.3 ± 2	p = 0.2
New skeletal metastasis on therapy	7 (33.3%)	7 (24.1%)	p = 0.4
New brain metastasis on therapy	1 (4.8%)	5 (17.2%)	p = 0.1
Fatigue	16 (76.2%)	28 (96.6%)	**p = 0.02**
Rash	1 (4.8%) (G 1)	1(3.4%) (G 1)	p = 0.8
Pneumonitis	1 (4.8%) (G 3)	5 (17.2%) (G 3–4)	p = 0.1
Acute kidney injury	0 (0%)	1 (3.4%) (G 3)	p = 0.2
Transaminitis	0 (0%)	2 (6.8%) (G 3–4)	p = 0.2
Colitis	3 (14.3%) (G 2–4)	3 (10.3%) (G 2–4)	p = 0.6
Hospitalization due to treatment	3 (14.3%)	7 (24.1%)	p = 0.3
IrAE (excluding fatigue)	7 (38.1%)	13 (44.8%)	p = 0.6
Prednisone required for IrAEs	6 (28.5%)	12 (41.3%)	p = 0.3

IrAE: Immune-related adverse event, G (Grade of IrAE)

Table 3. Overall best response per RECIST V 1.1.

	Cohort A (metformin) N = 17†	Cohort B (no metformin) N = 26†	
Best response – CR – PR – SD – PD	 0 (0%) 7 (41.1%) 5 (29.4%) 5 (29.4%)	 0 (0%) 8 (30.8%) 8 (30.8%) 10 (38.5%)	 p = 0.7
ORR	41.1%	30.7%	p = 0.4
DCR	70.5%	61.6%	p = 0.5
Median time to achieve best response (months)	2.7	3.2	

†Radiographically evaluable patients. Three patients in cohort A and four patients in cohort B were not evaluable.
CR: Complete response; DCR: Disease control rate; ORR: Objective response rate; PD: Progressive disease; PR: Partial response; SD: Stable disease.

Table 4. Overall best response per RECIST V 1.1 in patients who have received immune checkpoint inhibitor as second- or third-line therapy.

	Cohort A (metformin) N = 9†	Cohort B (no metformin) N = 15†	
Best response – CR – PR – SD – PD	 0 (0%) 4 (44.4%) 3 (33.3%) 2 (22.2%)	 0 (0%) 1 (6.7%) 6 (40%) 8 (53.3%)	 p = 0.1
ORR	44.4%	6.7%	**p = 0.02**
DCR	77.7%	46.7%	p = 0.1
Median time to achieve best response (months)	3	4.6	

†Radiographically evaluable patients. Two patients in cohort A and three patients in cohort B were not evaluable.
CR: Complete response; DCR: Disease control rate; ORR: Objective response rate; PD: Progressive disease; PR: Partial response; SD: Stable disease.

Overall survival

Median OS was 11.5 months in cohort A and 7.6 months in cohort B, the difference was not statistically significant between both cohorts (p = 0.5; HR: 0.8; 95% CI: 0.39–1.63; Figure 1A). 42.8% of patients were alive by the end of the first year in cohort A versus 32.7% in cohort B; this difference in percentage survival was reduced by the end of second year (28.5% in cohort A vs 26.2% in cohort B; Table 5).

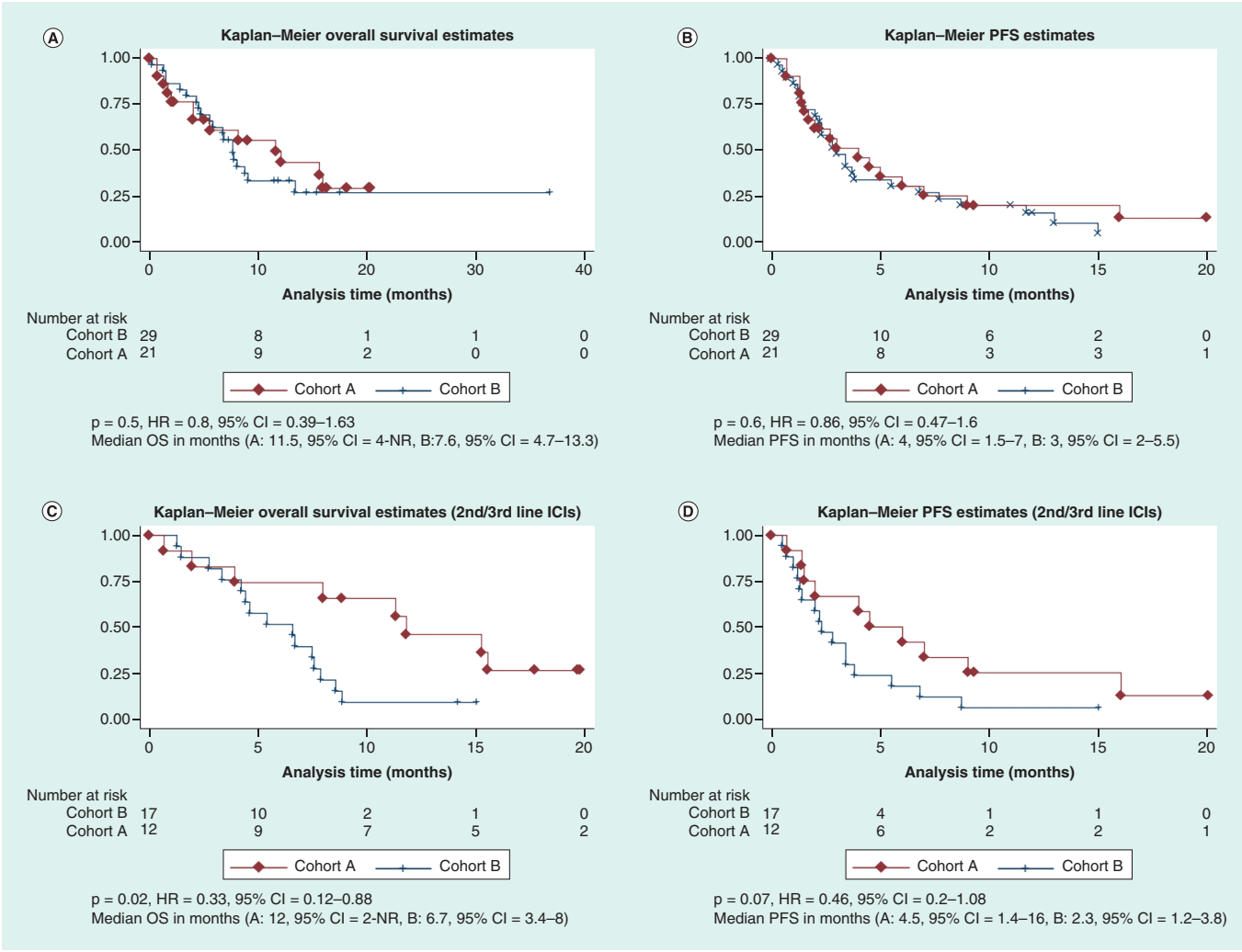

Figure 1. Kaplan–Meier Curves. OS and PFS **(A & B)** and OS and PFS in patients receiving ICI as second- or third-line therapy **(C & D).**
ICI: Immune checkpoint inhibitor; OS: Overall survival; PFS: Progression-free survival.

Table 5. Overall and progression-free survival (all patients).

Cohort A (metformin)	Cohort B (no metformin)
Overall survival	
42.8% patients alive at 1 year	32.7% patients alive at 1 year
28.5% patients alive at 2 years	26.2% patients alive at 2 years
Progression-free survival	
15.5% patients free from progression at 1 year -10.3% patients free from progression at 2 years	16.5% patients free from progression at 1 year 5.5% patients free from progression at 2 years

On univariate and multivariate analyses, the duration of concurrent metformin and ICI therapy had significant impact on OS [(p = 0.01; Hazard ratio (HR): 0.75; 95% CI: 0.59–0.9) and (p = 0.009; HR: 0.63; 95% CI: 0.45–0.89), respectively]. Overall duration of metformin therapy and metformin dose had no impact on OS on both type of analyses (p = 0.19 and 0.7, respectively).

In patients who had received ICIs as second- or third-line therapy, median OS was 12 months in cohort A, and 6.7 months in cohort B, a significant difference (p = 0.02, HR 0.33; 95% CI: 0.12–0.88; Figure 1C).

Progression-free survival

The median PFS was 4 months in cohort A, and 3 months in cohort B (Figure 1B). The PFS was not statistically different between both cohorts (p = 0.6; HR: 0.86; 95% CI: 0.47–1.6). A similar proportion of patients were free from progression by the end of the first year in both cohorts; however, higher proportion of patients were free from progression by the end of the 2nd year in cohort A (A: 10.3 vs B: 5.5%; Table 5).

On univariate and multivariate analyses, the duration of concurrent metformin and ICI therapy had a statistically significant impact on PFS as well [(p = 0.03; HR: 0.86; 95% CI: 0.76–0.98) and (p = 0.002; HR: 0.49; 95% CI: 0.31–0.77), respectively]. However, metformin dose and overall duration of therapy had no impact on PFS (p = 0.13 and 0.24, respectively).

Median PFS was higher in subset of cohort A for patients who had received ICI as second- or third-line therapy, but the difference was not statistically significant (A: 4.5, B: 2.3 months, p = 0.07; HR: 0.46; 95% CI: 0.2–1.08; Figure 1D).

Discussion

Use of metformin in various studies has demonstrated its antitumor properties [34,35]. Metformin regulates the adenosine monophosphate-activated protein kinase (AMPK)/liver kinase B1 (LKB1) and inhibits the gluconeogenesis from the liver [36–38]. The AMPK/LKB1 pathway regulates the cell cycle via control of protein synthesis and cell proliferation by manipulating the energy required by the cells [30,39]. This manipulation of the cell cycle and proliferation in return inhibits the cancerous cells and induces apoptosis [39]. Metformin also inhibits the unfolded protein synthesis, activates the immune response targeting cancer cells, inhibits the expression of CD 39/73 on MDSCs and prevents the development of immune tolerance of cancer cells [30,39,40]. Metformin also potentiates the blockage of PD-1 by anti-PD-1 antibodies as well [29]. Eikawa *et al.* studied the direct effect of metformin on cytotoxic T-lymphocytes. The authors reported that metformin targets the $CD8^+$ tumor-infiltrating leukocytes (TILs). Metformin prevents the exhaustion of the $CD8^+$ TILs that will lead to decreased production of various cytokines such as TNF-α, TNF-γ, IL-2, etc. A diminished supply of these cytokines in TME will result in immune tolerance and progression of the cancer. Metformin further prevents the apoptosis of $CD8^+$ TILs in TME irrespective of the PD-1 or Tim-3 expression and promotes the rejection of tumor cells [41]. Metformin has also been shown to have direct cytotoxic effect and can directly kill NSCLC cells as described by Kalender *et al.* and Song *et al.* in their respective studies [42–44]. Although, in literature, metformin use has been reported in combination with various cytotoxic chemotherapeutic agents in different solid tumors, including lung cancer [24–28,44], to our knowledge, its use in combination with ICIs has not been reported.

Overall, we report improved ORR in the patients who received metformin (cohort A) compared with those who did not receive metformin (cohort B) (A: 41.1 vs B: 30%, p = 0.4). DCR was also considerably higher in cohort A (A: 70.6 vs B: 61.1%, p = 0.5). These differences are not statistically significant, likely due to small-sample size; however, we can see a trend toward increased responsiveness and disease stability in patients who have received ICIs and metformin. Tan *et al.* also reported better ORR and DCR in patients who have received chemotherapy + metformin, although the difference was not statistically significant in this study either [25]. In their study, ORR was 35.9% in patients receiving metformin (N = 39), which is less than 41.1% in cohort A of our study. However, reported DCR was higher in the study by Tan *et al.* (82%) versus 70.6% in cohort A of our study [25]. Chen *et al.* evaluated the synergistic effect of metformin in combination with EGFR-TKI in NSCLC Chinese patients. They reported significantly improved ORR and DCR in patients receiving metformin and EGFR-TKI combination (70.5 vs 45.7%, p = 0.017 and 97.7 vs 80.4%, p = 0.009, respectively, N = 46) [27]. Although Chen *et al.* and Tan *et al.* reported better survival results in their studies, it is noteworthy here that the patients included in both studies had AJCC clinical stage II–IV, whereas all of the patients included in our study have AJCC clinical stage of IV. It is also important to note that in these studies, only chemotherapy-naive patients were included, whereas in both of our cohorts more than 50% patients have received ICIs as second- or third-line therapy (A: 57.1 vs B: 58.6%). Patients who have received prior chemotherapy did better in our cohort A. In NSCLC, the response rate to second-line therapies has been within a range of 1–15% in various studies [45–48]. In our study, of the subset of patients who have received ICIs as second- or third-line therapy, the response rate in cohort B is in line with this observation (ORR = 6.7%) though the cohort A patients did much better with ORR of 44.4% (p = 0.02). It is unclear what may have led to this improved ORR but increased PD-1 blockade by metformin [29] coupled with increased PD-1 expression by prior chemotherapy use may have a role here [49,50]. It will be interesting to investigate this observation in further basic and clinical studies.

While there was a trend toward improved secondary end points, PFS (A: 4 vs B: 3 months) and especially OS (A: 11.5 vs B: 7.6 months), in cohort A, the differences were not statistically significant between both cohorts (p = 0.5; HR: 0.8; 95% CI: 0.39–1.63 and p = 0.6; HR: 0.86; 95% CI: 0.47–1.6, respectively). These median OS and PFS are also lower than what have been reported in the studies mentioned above. Tan *et al.* reported a median OS of 20 months in patients receiving chemotherapy + metformin. The difference in OS was also significant in this group compared with the patients who have not received metformin [25]. In this study, the median PFS was 8.4 months in patients who have received chemotherapy + metformin, and the difference in PFS was also statistically significant [25]. Chen *et al.* reported improved median PFS and median OS in patients receiving metformin and EGFR-TKI combination (19.0 vs 8.0 months, p = 0.005 and 32.0 vs 23.0 months, p = 0.002, respectively) [27]. In our study, 1-year OS was higher in cohort A (A: 42.8 vs B: 32.7%). In Tan *et al.*'s study, at year 1, OS was 53.8%, and PFS was 30.8% in patients receiving chemotherapy + metformin [25]. Whereas PFS at first year was only 15.5% in cohort A of our study. A randomized Phase II study used metformin + paclitaxel/carboplatin/bevacizumab in chemotherapy-naive NSCLC patients. In this study, PFS at a first year was 47% (N = 18) in patients receiving the metformin combination. The median OS was 15.9 months (NCT01997775) [28]. Sayed *et al.* conducted a Phase II clinical trial on nondiabetic chemo-naive stage IV patients with NSCLC [51]. Arm A received gemcitabine/cisplatin + metformin while arm B received gemcitabine/cisplatin. Fifteen patients were included in each study. ORR and median OS in arms A and B were 46.7 versus 13.3%, respectively, p = 0.109 and 12 versus 6.5 months, respectively, p = 0.119. Median PFS in arms A and B was 5.5 versus 5 months, p = 0.062 [51]. In all these studies, only chemotherapy-naive patients were included; however, in both cohorts of our study more than 50% patients were already progressed on at least one chemotherapy and their treatment was switched to ICI. A poor response to first-line chemotherapy is the poor predictor of survival and response to second-line therapy [52]. However, on performing survival analyses only on the subset of the patients who have received ICI as second- or third-line therapy, those in cohort A had better outcomes as shown with improved median OS (A: 12 vs B: 6.7 months, p = 0.02) and improved PFS (A: 4.5, B: 2.3 months, p = 0.07).

The median duration of overall metformin therapy was 18 months, and the median duration of concomitant metformin and ICI was 2.3 months. The median duration of metformin therapy was 67 months in Chen *et al.*'s study [27] and was 36 months in Tan *et al.*'s [25]. Duration of concurrent metformin was 18 weeks in the study by Sayed *et al.* [51]. The overall duration of metformin therapy and metformin dosing had no impact on the OS and PFS in our study; however, the duration of concurrent ICI + metformin duration had a significant impact on OS and PFS. This is an interesting observation, as preclinical studies on mouse models have suggested a modulating effect of metformin and potentiation of anti-PD-1 blockage by these ICIs [29].

In their review article, Levy *et al.* conclude that, of the patients with a diagnosis of diabetes who have been on metformin treatment, there is a decrease incidence of cancer including that of lung cancer. They further report that the antitumor effect of metformin seems to be dose-dependent. They report that the higher the metformin dose, the more potent is its antitumor effect. This observation is based on the results of preclinical studies [44]. However, in our study, metformin dose had no impact on the clinical outcomes. Metformin is one of the safest hypoglycemic drugs used in Type 2 diabetic patients. It does not cause hypoglycemia and it has a favorable metabolic effect profile [53,54]. Metformin has been administered in various nondiabetic conditions. Different Phase I and Phase II studies have demonstrated the safety of metformin use in nondiabetic patients [55–59]. However, the potential side effects of metformin therapy include asthenia, diarrhea, flatulence, muscle pain, abdominal pain, lactic acidosis and low B12 levels [53,54]. Although metformin appears to be more efficacious at higher doses, there is no consensus regarding optimal dosing in nondiabetic patients. The majority of the studies have used 500 mg twice daily dosing in nondiabetic patients [57,59].

In our study, the differences in the primary end point (ORR) and secondary end points (OS, PFS and DCR) were not statistically significant but there was a trend toward better outcomes in patients receiving metformin while having almost similar baseline characteristics in both cohorts (such as median age, gender, clinical staging, type of ICIs received, number of patients receiving concurrent chemotherapy + ICI, prior use of chemotherapies and the mean number of metastatic sites involved before therapy). Distribution of skeletal and visceral metastasis was also similar between both cohorts. It has been reported that the emergence of IrAEs is associated with better clinical outcomes in patients receiving ICIs [60,61]. However, the proportion of patients experiencing IrAEs and the associated prednisone therapy for was higher in cohort B. Moreover, the mean number of new metastatic sites and new brain metastases appearing while on therapy was also lower in cohort A.

The major limitations of our study include the smaller sample size, the retrospective nature and the use of convenience sampling. Moreover, four patients in cohort A and three patients in cohort B did not have the radiographically evaluable disease. Although univariate and multivariate analyses showed a positive impact of duration of concurrent metformin + ICI on OS and PFS, it is likely because the patients who are to receive concomitant metformin and ICIs for less duration of time are expected to have unfavorable outcomes.

Conclusion

Improved clinical outcomes (ORR, DCR, OS) are observed in patients receiving ICI + metformin, although the differences are not statistically significant in overall patient population of our study, likely due to small-sample size. We need further prospective studies to evaluate the long-term clinical benefits of metformin when used in conjunction with ICIs. The linear relationship between the duration of metformin therapy and clinical outcomes also needs to be investigated in preclinical and clinical studies. Currently, a Phase II clinical trial is being conducted at Northwestern University evaluating nivolumab and metformin in patients with unresectable stage III–IV NSCLC (NCT03048500) [62]. Another Phase Ib clinical trial is being conducted at Okayama University on refractory/recurrent tumors using nivolumab and metformin (UMIN000028405) [63]. Despite our limited sample size, we observed a significant benefit of the combination of metformin with ICI in patients who have received ICIs as second- or third-line therapies in term of response rate, DCR and survival analyses. These are hypothesis generating results and larger retrospective and prospective studies are needed to understand the potential benefit of metformin in combination with ICIs in patients progressed on one line of therapy. Dose escalation Phase I studies will be beneficial in determining the optimal metformin dose with antitumor properties.

Summary points

- Although immune checkpoint inhibitors (ICIs) have shown improved outcomes, a long-term follow-up of the patients on clinical trials has shown the delayed progression in patients who have had achieved response once. This is thought to be as a result of underlying innate and acquired resistance. The response rate to second- or third-line therapy decreases to 0–20% after progression.
- Efforts are underway to identify potential noncancerous drugs with antitumor properties that can be used along with these chemotherapeutic and immunotherapeutic agents to improve treatment-related outcomes.
- Metformin, a hypoglycemic agent, has been shown to have antitumor properties. Use of metformin has been shown to decrease the incidence of lung cancer.
- Metformin exerts its antitumor effect by inhibiting myeloid-derived suppressor cells, regulating a cell cycle via a control of protein synthesis and cell proliferation, potentiating the blockage of PD-1 and preventing the $CD8^+$ tumor-infiltrating leukocyte exhaustion.
- In our study, we observed an increase in response rate, median overall survival and median progression-free survival in patients who received ICI (alone or in combination with carboplatin/pemetrexed) along with metformin compared with the patients who have received ICI (alone or in combination with carboplatin/pemetrexed) only therapy.
- These outcomes were more favorable and significant in the subset of patients who have received ICI as second- or third-line therapy along with metformin.

Author contributions

K Shirai and K Dragnev are the primary oncologists for more than 90% of the patients in this study. K Shirai, MZ Afzal designed the study, collected data, performed data analysis and wrote manuscript. T Sarwar performed data analysis and wrote part of the manuscript. K Dragnev wrote the manuscript.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human

subjects, informed consent has been obtained from the participants involved. This study was approved by institutional review board (IRB) of Dartmouth-Hitchcock Medical center/Dartmouth College. Due to retrospective nature of the study, request for informed consent waiver was approved by IRB.

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