Impact of concomitant medication use and immune-related adverse events on response to immune checkpoint inhibitors

Shipra Gandhi^{*,1} , Manu Pandey¹, Nischala Ammannagari¹, Chong Wang², Mark J Bucsek³, Lamy Hamad^{4,5}, Elizabeth Repasky³ & Marc S Ernstoff^{1,4}

¹Department of Medicine, Roswell Park Comprehensive Cancer Center, Elm & Carlton Streets, Buffalo, NY 14263, USA

²Department of Biostatistics, Roswell Park Comprehensive Cancer Center, Elm & Carlton Streets, Buffalo, NY 14263, USA

³Department of Immunology & Cell Stress & Biophysical Therapies Program, Elm & Carlton Streets, Buffalo, NY 14263, USA

⁴Melanoma Program, Roswell Park Comprehensive Cancer Center, Elm & Carlton Streets, Buffalo, NY 14263, USA

⁵Clinical Pharmacy Service, Roswell Park Comprehensive Cancer Center, Elm & Carlton Streets, Buffalo, NY 14263, USA

*Author for correspondence: Tel.: +1 716 845 1486; Shipra.gandhi@roswellpark.org

Aim: Patients receiving checkpoint inhibitors (CPI) are frequently on other medications for co-morbidities. We explored the impact of concomitant medication use on outcomes. **Materials & methods:** 210 metastatic cancer patients on CPI were identified and association between concomitant medication use and immune-related adverse events with clinical outcomes was determined. **Results:** Aspirin, metformin, β -blockers and statins were not shown to have any statistically significant difference on clinical benefit. 26.3% patients with clinical benefit developed rash versus 11.8% without clinical benefit ($p < 0.05$) on multivariate analysis. **Conclusion:** Use of common prescription and nonprescription medications in patients with multiple co-morbidities appears safe and does not have an adverse effect on CPI efficacy. The presence of rash predicted for a better response.

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Inflammatory and regulatory pathways tightly control the immune system as it protects the host from pathogens and foreign cells. Immune checkpoints are part of the inhibitory pathways that dampen the immune systems' responses. Immune checkpoint inhibitors (CPI) have recently emerged as a promising treatment to reactivate the anticancer immune responses and unleash the immune system to destroy different types of cancer, resulting in durable responses. Monoclonal antibodies to cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein-1 (PD-1)/programmed death ligand-1 (PD-L1), two T-cell inhibitory checkpoint pathways with independent mechanisms of action have demonstrated improvement in overall survival (OS) and progression-free survival (PFS) in multiple tumor types [1].

Epidemiological studies have suggested the benefits of concomitant medications in cancer treatment. Aspirin, β -blockers, metformin and statins are commonly prescribed drugs in the world. Mounting evidence has shown the anticancer effect of these drugs. Preclinical studies of metformin demonstrate several anticancer molecular mechanisms including mTOR inhibition, cytotoxic effects and immunomodulation [2,3]. Aspirin was associated with a lower risk of colorectal, gastric and breast cancer by regulating various signaling pathways, including cyclooxygenase (COX)-1 and -2, AMP-activated protein kinase, mTOR and mitogen-activated protein kinase [4]. Antineoplastic effects of statins are demonstrated via inducing apoptosis, regulating cell cycle disturbance and suppressing the growth of tumors [5]. We have recently shown in preclinical models that β -2 adrenergic receptors (β -AR) play a role in regulating the antitumor immune response and that blocking β -AR with propranolol can enhance immune destruction of cancer and improve the efficacy of PD-1 checkpoint blockade [6].

Thus, we hypothesized that concomitant medications could have significant impact on outcome and sought to determine if metformin, β -blockers, aspirin or statins were associated with better outcomes in patients treated

with CPIs. Despite a wealth of information collected during prospectively designed clinical trials, no systematic evaluations of the impact of concomitant medications have been published to date.

During this investigation, we also sought to determine if there were other clinical (immune-related adverse events [irAE]) and biochemical parameters that might predict responses to immunotherapy. Biomarkers for outcome before/after immune-checkpoint blockade may influence individual treatment selection or sequence, and hence, could spare the side effects of a futile treatment to patients who would potentially not respond to the treatment, avoiding toxicity and cost of an unnecessary treatment [7].

Materials & methods

Eligibility criteria

This is a single institution retrospective study to examine the role of concomitant medications on outcome from therapy with CPI in patients with metastatic cancer. In addition, we sought to identify clinical and biochemical markers to identify clinical benefit to immunotherapy. Two hundred and sixteen consecutive patients treated at Roswell Park Comprehensive Cancer Center (RPCCC) with single-line/multiple lines of CPI from 2011 to 2016 were identified. The CPI consisted of the US FDA-approved CTLA-4-specific monoclonal antibody (mAb) ipilimumab (Yervoy®), two mAbs targeting PD-1, namely, nivolumab (Opdivo®) and pembrolizumab (Keytruda®).

Demographic details for patients were collected including age, sex, sites of metastatic disease and Eastern Cooperative Oncology Group performance status at the initiation of immunotherapy. Data on concomitant medication intake (aspirin, β -blocker, metformin, statins) were collected while the patient was on the first-line CPI (concomitant medication intake confirmed via chart review till the first response scan) and were correlated with response, PFS and OS.

All patient radiographs were reviewed and measured to determine Response Evaluation Criteria in Solid Tumors response rates (S Gandhi, M Pandey, MS Ernstoff). PFS is defined as the time interval from starting the first CPI to the date of progression or death. OS is defined as the time interval from starting the first CPI to the date of death. Patients were stratified into response groups based on the primary radiographic assessment by Response Evaluation Criteria in Solid Tumors version 1.1 criteria [8] that included complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD) or nonevaluable. The best overall response was defined as the best achieved response from the initiation of immunotherapy to the date of progressive disease or start of a new systemic treatment. Clinical benefit was defined as objective response (CR/PR) or stable disease with PFS ≥ 6 months. All the imaging studies within this time period were considered. Patients who had baseline scans performed more than 6 weeks before starting CPI or if the response scan was not done after completing treatment were considered nonevaluable. Responses were calculated ≥ 4 weeks after starting treatment with CPI unless patient had PD in less than 4 weeks that led to discontinuation of therapy. PFS and OS data were confirmed by radiologic review and calculated using baseline and follow-up imaging. PFS and OS were calculated only for the patient treated with single-line CPIs (as administration of second line and beyond CPIs could skew the results for survival).

Laboratory parameters, including white blood cell (WBC) count, absolute eosinophil count (AEC), absolute lymphocyte count (ALC), absolute basophil count (ABC), absolute neutrophil count (ANC), lactate dehydrogenase (LDH) were also collected. Laboratory parameters before starting treatment were taken as baseline. Follow-up labs were recorded at the beginning of each treatment cycle up to 9 weeks, unless a patient had progressive disease or started another treatment. However, if a patient had deviation from scheduled treatment, variables were collected at scheduled intervals (~ 2 – 3 weeks) and maximum values out of these four were considered. Autoimmune toxicities were reported using the Common Terminology Criteria for Adverse Events version 4.03 [9]. This study was approved by the institutional review board at RPCCC.

Statistical analysis

Summary statistical analyses are provided for the demographic features of the patients included in this study between tumor types. The differences of the outcomes between study groups are compared by Nonparametric Kruskal–Wallis Test/Generalized Linear Regression Model (for continuous variables) and Fisher's Exact Test/Logistic Regression Model (for categorical variable), and Log-rank Test/Cox Proportional Hazards Analysis for OS and PFS. OS/PFS are estimated by the Kaplan–Meier method. Since this a retrospective study and hence, frequency of intermediate and long-term follow-up was variable and not controlled, hence, making survival a poor outcome, especially since there could be changes in concomitant medications with long-term follow-up. The analyses for the association between clinical outcomes (clinical benefit) and each interested risk are conducted independently with univariate

Table 1. Use of concomitant medications among patients with clinical benefit and without clinical benefit (clinical benefit = CR + PR + SD with PFS $\geq$ 6 months).

	No clinical benefit	Clinical benefit	p-value
β -blocker	33	19 (36.5%)	0.08
No β -blocker	120	38 (24.1%)	
Metformin	19	4 (17.3%)	0.26
No metformin	134	53 (28%)	
Aspirin	41	17 (29.3%)	0.73
No aspirin	112	40 (26%)	
Statin	45	19 (29.7%)	0.68
No statin	108	39 (26%)	

CR: Complete response; PFS: Progression-free survival; PR: Partial response; SD: Stable disease.

analyses. To adjust for various parameters of the patients, a multivariate Cox regression model was adopted for clinical benefit. All related factors that are significant at level $\alpha = 0.10$ in the univariate analyses are included in the multivariate analyses. β -blocker status is imposed in the multivariate models for clinical interest. SAS version 9.4 (SAS Institute, NC, USA) are used for statistical analyses. All tests are two-sided and performed at a nominal significance level of 0.05.

Results

Patient characteristics

211 out of 216 (98%) patients had clinical response assessments after first-line CPI available. Our final analysis was performed on the 210 patients (one patient was excluded as the treatment was changed without any radiological scans). Various tumor types were included in the analysis – 101/210 (48.1%) melanoma, 68/210 (32.3%) lung, 22/210 (10.5%) kidney and renal pelvis, 10/210 (4.8%) bladder and 9/210 (4.3%) others (ocular melanoma, mesothelioma, colorectal cancer, base of tongue cancer). The median age of patients was 62 years (range: 24 to 88 years). Approximately, 65% were men, and 207/208 had an Eastern Cooperative Oncology Group performance score of 0 to 2 (data not available for two patients). **Supplementary Table 1** shows the distribution of demographic characteristics in this patient population.

Patients were treated with single CPI nivolumab, pembrolizumab or ipilimumab at FDA-approved doses of 3 mg/kg every 2 weeks, 2 mg/kg every 3 weeks or 3 mg/kg every 3 weeks, respectively. Combination ipilimumab and nivolumab was dosed as 3 and 1 mg/kg every 3 weeks. **Supplementary Table 1** also illustrates the first-line CPI used in our patient cohort. *Data on more than one line of treatment are not shown.*

Efficacy analysis

Responses are available for 210/216 patients after treatment with first-line CPI (which may include single CPI or combination of nivolumab/ipilimumab given as first line, **Supplementary Table 1**). The objective response rate was 37/210 – 17.6% (12 CR, 25 PR), 40/210 SD and 133/210 PD. **Supplementary Tables 2 and 3** show details about patient responses.

For OS and PFS, only patients treated with first-line CPI (178) were considered in the analysis (as treatment with multiple lines of CPI can skew the analysis). The median OS is 6.2 months in patients without clinical benefit (5.0–6.9 months) versus not reached in patients with clinical benefit ($p < 0.0001$). The median PFS is 3.7 months in patients without clinical benefit (3.1–4.9 months) versus not reached in patients with clinical benefit ($p < 0.0001$; data not shown).

Medication use

We analyzed the influence of concomitant medication use (β -blockers, metformin, aspirin, statins) with CPI on response.

A total of 52/210 (24.7%) patients were receiving β -blocker concomitantly with CPI. Clinical benefit was noticed in 19/52 (36.5%) patients on β -blockers versus 38/158 (24.1%) patients not on β -blockers ($p = 0.08$; **Table 1**). Majority of the patients ($n = 41$) were on β -1 selective blockers (metoprolol, atenolol, bisoprolol), with a large proportion on metoprolol ($n = 31$). On the other hand, the number of patients on nonselective β -blockers

(carvedilol, propranolol, nadolol, sotalol) was small, $n = 11$. We were not able to calculate the median dose of β -blockers as there are different β -blockers with varying bioequivalent dose.

Aspirin use was observed in 58/210 (27.6%) of our study group and was not associated with statistically significant change in clinical benefit (29.3 vs 26%; $p = 0.73$). Similarly, metformin (17.3 vs 28%; $p = 0.26$) and statin use (29.7 vs 26%; $p = 0.68$) was also not shown to have any statistically significant difference among the patients with clinical benefit and without clinical benefit (Table 1). No difference in the PFS or OS was observed with the use of aspirin, β -blockers, metformin and statins (data not shown).

Toxicity analysis

Treatment-related irAE, namely, hypothyroidism, diarrhea, colitis, hypophysitis, hepatitis, nephritis, liver dysfunction, vitiligo, uveitis, pneumonitis, myalgia/ myositis, arthralgia/ arthritis, rash and adrenal insufficiency were assessed based on the patient's chart review and physician documentation. They were graded per the Common Terminology Criteria for Adverse Events version 4.03. Grades per patient could be multiple if the patient received two or more CPI (Supplementary Table 4).

Occurrence of irAE with clinical benefit was analyzed (Table 2). 26.3% of patients with clinical benefit developed rash versus 11.8% without clinical benefit ($p = 0.01$) on univariate analysis and ($p < 0.05$) on multivariate analysis (Supplementary Table 5). The influence of irAE on survival (PFS and OS) was analyzed in a univariate analysis in 178 patients treated with a single CPI. We found that rash was associated with better OS ($p < 0.01$) and better PFS ($p < 0.05$; Figure 1). No significant influence of other irAE on survival was noted.

Laboratory parameters

Supplementary Table 6 shows the association between maximum median values and baseline values of AEC, ALC, ANC, LDH, WBC count, ABC, platelets and thyroid-stimulating hormone among patients with clinical benefit and without clinical benefit.

On univariate analysis, patients without clinical benefit were also noted to have higher baseline LDH >618 ($p = 0.0033$; 618 is our institutional upper limit of normal) and lower baseline platelet–lymphocyte ratio (<300) [10] ($p = 0.03$) than patients with clinical benefit. Higher median AEC and ALC were noted in patients with clinical benefit (380 and 1690/ μl in patients with clinical benefit vs 250 and 1370/ μl in patients without clinical benefit; $p = 0.0008$ and 0.0046).

Laboratory parameters were also analyzed for effect on PFS and OS. Cutoffs for AEC [11], ANC [12], WBC [13] and ALC [14] are based on previous publications. Eosinophilia and lymphocytosis were associated with an improved survival; while neutrophilia, leukocytosis and higher LDH were associated with worse survival. Table 3 details the values associated with statistically significant improvement in outcomes (Supplementary Figures 1 & 2).

Multivariate model

The presence of rash was associated with clinical benefit (OR: 3.11; $p = 0.045$). The multivariate analysis shows better OS and PFS with lower baseline LDH (<618 ; $p = 0.013$, 0.017). No association was noted between PFS and OS rate and other related risk factors (data not shown). β -blocker use was not associated with clinical benefit or an improvement in survival in the multivariate analysis (refer to Supplementary Table 5).

Discussion

We here report data on concomitant medication use along with some biochemical and clinical parameters and their potential to predict response and survival.

Influence of concomitant medication use with CPI

Several *in vitro* and animal studies have reported the effects of adrenergic stress on tumor growth and metastasis, with abrogation of effects with propranolol [15–17]. Observational studies have also linked the use of β -blocker to better survival in several malignancies [18–20]. Our group has shown in a mouse model that CD8⁺ T-cell frequency and function within the tumor microenvironment is regulated by β -AR and blocking β -AR can enhance the anticancer activity of anti-PD1. Additional support for the anticancer therapeutic activity of β -AR blockade comes from a recent prospective adjuvant study in melanoma patients that reported a 80% risk reduction of recurrence in patients who received propranolol [21]. Another study showed improved survival in patients with metastatic melanoma who received immunotherapy and nonselective β -blockers when compared with patients who did not

Table 2. Occurrence of immune-related adverse event in patients with clinical benefit.

irAE	No	Yes	p-value
Liver dysfunction			
No clinical benefit	111	42	0.3455
Clinical benefit	45	12	
Hypophysitis			
No clinical benefit	144	8	0.3478
Clinical benefit	52	5	
Hypothyroidism			
No clinical benefit	144	9	0.2452
Clinical benefit	51	6	
Diarrhea			
No clinical benefit	140	13	0.4061
Clinical benefit	50	7	
Colitis			
No clinical benefit	142	11	0.3250
Clinical benefit	55	2	
†Pneumonitis			
No clinical benefit	147	6	0.0254
Clinical benefit	50	7	
Myositis/myalgia			
No clinical benefit	149	4	0.7294
Clinical benefit	55	2	
Increased creatinine			
No clinical benefit	134	19	0.2052
Clinical benefit	46	11	
Arthralgia/arthritis			
No clinical benefit	148	5	0.2329
Clinical benefit	53	4	
Rash			
No clinical benefit	135	18	0.01
Clinical benefit	42	15	
Adrenal insufficiency			
No clinical benefit	150	3	0.9225
Clinical benefit	56	1	

†Pneumonitis is not reported as a significant result due to low incidence.
irAE: Immune-related adverse event.

Table 3. Laboratory parameters associated with significantly improved outcomes on univariate analysis.

PFS (baseline value)	OS (baseline value)	PFS (maximum median value)	OS (maximum median value)
AEC > 100/ μ l (p = 0.007)	LDH < 618 IU/l (p = 0.0003)	ANC < 7500/ μ l (p = 0.0003)	LDH < 618 IU/l (p = 0.004)
ANC < 7500/ μ l (p = 0.0004)	ANC < 7500/ μ l (p = 0.006)	WBC < 8750/ μ l (p = 0.006)	AEC > 100/ μ l (p = 0.0002)
LDH < 618 IU/l (p = 0.01)	AEC > 100/ μ l (p = 0.04)	LDH < 618 IU/l (0.04)	ANC < 7500/ μ l (p < 0.0001)
		AEC > 100/ μ l (p = 0.0041)	WBC < 8750/ μ l (p = 0.01)
			ALC > 1000/ μ l (p = 0.0002)

Cutoffs for absolute eosinophil count, absolute lymphocyte count, absolute neutrophil count, white blood cell count based on published literature; cutoff for lactate dehydrogenase based on institutional upper limit of normal.
AEC: Absolute eosinophil count; ALC: Absolute lymphocyte count; ANC: Absolute neutrophil count; LDH: Lactate dehydrogenase; OS: Overall survival; PFS: Progression-free survival; WBC: White blood cell count.

Figure 1. Rash and survival outcomes. (A) Kaplan–Meier survival curves showing median OS of 28.4 months (12.9, NR) with rash and median OS of 7.3 months (6.3–9.8) in the absence of rash with HR: 0.42 (CI: 0.22–0.81) and $p < 0.01$. **(B)** Kaplan–Meier survival curves showing median PFS of 10.3 months (3.1–28.4) with rash and median PFS of 5.3 months (4.6–6.3) in the absence of rash with HR: 0.60 (CI: 0.36–0.99) and $p < 0.05$. HR: Hazard ratio; NR: Not reached; OS: Overall survival; PFS: Progression-free survival.

receive any β -blocker and patients on β -1 selective blockers [22]. Though our data did not show the role of β -AR blockade as a therapeutic modality with improvement in outcomes in patients treated with CPI and β -AR-blocking agents, but there was a trend toward clinical benefit in patients on β -AR blockers on univariate analysis ($p = 0.08$; 36.5% with clinical benefit on β -AR blockers, while 24.1% with clinical benefit without β -AR blockers), which was lost on multivariate analysis. This may be attributable to the retrospective nature, the mix of histologies and variation of β -AR blockers. Our animal data that β -AR-mediated immune response is also supported by Watkins *et al.* report that there was a significant difference in median OS in ovarian cancer patients on nonselective β -blockers (94.9 months) versus selective β -1 AR selective agents (38 months) [23]. Our study was too small to tease out the role of selective β -AR-blocking agents. Although the numbers for high-grade toxicities among irAE were small, we are encouraged that no increase in high grade ($\geq$ grade 3) toxicities were seen in the patients concurrently treated with β -blockers.

The role of COX inhibition, statins and metformin as immune modulators and anticancer therapeutics have been reported by others, however, we were not able to demonstrate any impact on clinical outcome in our population [2,3,24–32]. No increases in high-grade ($\geq$ grade 3) toxicities were seen in the patients concurrently treated with aspirin, statins and metformin. There is a lack of incontrovertible evidence about efficacy of these agents and further studies to investigate their role as such remain to be seen. In our retrospective study we did observe a trend toward clinical benefit with β -AR blocker of 36.5 versus 24.1% without β -blocker ($p = 0.08$); however, this benefit was lost on multivariate analysis. Our study is limited by its retrospective design, small sample size along with heterogeneity in tumor subtype, treatment received and type of β -blocker. However, other retrospective studies have shown the benefit of β -blockers in combination with CPI [22] in addition to our laboratory study, which also supports the positive outcome of combining β -AR blocker with CPI [6]. This led us to initiate a prospective Phase I/II study combining propranolol with pembrolizumab in patients with metastatic melanoma (NCT03384836), which is actively recruiting.

IrAE & laboratory parameters associated with response & survival

In our study, we found that both the presence of irAE (rash) and certain lab parameters (eosinophil, lymphocyte, neutrophil, platelet–lymphocyte ratio and LDH) were associated with clinical benefit and an improvement in survival. It has been postulated that in the presence of CTLA-4 blockade, antigen presentation of melanoma-associated antigens on major histocompatibility antigens results in activation of melanoma-specific CD4⁺ T cells, which hone to the skin where melanocytes express the antigen, resulting in a rash [14]. Our data add to the chorus of others reporting similar observations supporting the mechanistic insights of these findings [11,14,20,33–36]. These predictive markers provide potential clues for additional targets, which may enhance the impact of CPI in cancer patients.

Conclusion

Concomitant medication use with CPI remains an area of continued interest with increasing adoption of immunotherapy in multitude of cancers with real-world clinical significance. While our findings do not support significant differences in clinical benefit with use of aspirin, statin, metformin and β -blockers, the data do support use of common prescription and nonprescription medications in the real-world population with multiple co-morbid conditions along with immunotherapy as they appear safe and no adverse effect on the efficacy of CPI was seen with the combination. This knowledge helps clinicians and patients to continue these medications without concern for significant interaction decreasing efficacy of CPIs.

While our observations are limited by the retrospective nature of the study, small sample size, the combination of multiple tumor types and different treatments received for efficacy analysis, and the shortcoming that duration of treatment may be prolonged due to irAE and is difficult to separate from patient with clinical benefit, it does provide support that laboratory changes and clinical adverse events represent ‘on-target’ effects of CPI therapy and should be considered as potential biomarkers and be analyzed as such and reported in the large prospective clinical trials.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/imt-2019-0064

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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.

Summary points

- There is a nonsignificant trend toward clinical benefit in patients on β -blockers along with checkpoint inhibitors (CPI) on univariate analysis, which is lost on multivariate analysis.
- No increase in high-grade ($\geq$ grade 3) toxicities were seen in the patients on CPI concurrently treated with β -blockers, aspirin, metformin and statins.
- Use of common prescription and nonprescription medications – aspirin, statins and metformin in the real-world in patients with co-morbidities appears to be safe and does not compromise the efficacy of CPI.
- Clinical trial Phase Ib/II combining propranolol and pembrolizumab in patients with advanced melanoma is actively recruiting (NCT03384836).
- 26.3% patients with clinical benefit developed rash versus 11.8% without clinical benefit ($p = 0.01$) on univariate analysis and ($p < 0.05$) on multivariate analysis.
- We found that rash was associated with better overall survival ($p < 0.01$) and better progression-free survival ($p < 0.05$) on univariate analysis but not significant on multivariate analysis.
- Immune-related adverse events like rash can be used a clinical parameter to predict response to CPI.
- On univariate analysis, patients without clinical benefit were noted to have higher baseline lactate dehydrogenase (LDH) >618 ($p = 0.0033$; 618 is our institutional upper limit of normal).
- The multivariate analysis shows better overall survival and progression-free survival with lower baseline LDH (< 618 ; $p = 0.013$, 0.017; 618 is our institutional upper limit of normal).
- On univariate analysis, patients without clinical benefit were noted to have lower baseline platelet–lymphocyte ratio (<300 ; $p = 0.03$) than patients with clinical benefit.
- Higher median absolute eosinophil count and absolute lymphocyte count were observed in patients with clinical benefit (380 and 1690/ μ l in patients with clinical benefit vs 250 and 1370/ μ l in patients without clinical benefit; $p = 0.0008$ and 0.0046) on univariate analysis.

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