ARTICLE

Application of a patient-centered reverse translational systems-based approach to understand mechanisms of an adverse drug reaction of immune checkpoint inhibitors

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Abstract

Immunotherapy became a key pillar of cancer therapeutics with the approvals of ipilimumab, nivolumab, and pembrolizumab, which inhibit either cytotoxic T-lymphocyte antigen-4 (CTLA-4) or programmed death-1 (PD-1) that are negative regulators of T-cell activation. However, boosting T-cell activation is often accompanied by autoimmunity, leading to adverse drug reactions (ADRs), including high grade 3–4 colitis and its severe complications whose prevalence may reach 14% for combination checkpoint inhibitors. In this research, we investigated how mechanistic differences between anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab and pembrolizumab) affect colitis, a general class toxicity. The data analytical platform Molecular Health Effect was utilized to map population ADR data from the US Food and Drug Administration (FDA) Adverse Event Reporting System to chemical and biological databases for hypothesis generation regarding the underlying molecular mechanisms causing colitis. Disproportionality analysis was used to assess the statistical relevance between adverse events of interest and molecular causation. We verified that the anti-CTLA-4 drug is associated with an approximately three-fold higher proportional reporting ratio associated with colitis than those of the anti-PD-1 drugs. The signal of the molecular mechanisms, including signaling pathways of inflammatory cytokines, was statistically insignificant to test the hypothesis that the severer rate of colitis associated with ipilimumab would be due to a greater magnitude of T-cell activation as a result of earlier response of the anti-CTLA-4 drug in the immune response. This patient-centered systems-based approach provides an exploratory process to better understand drug pair adverse events at pathway and target levels through reverse translation from postmarket surveillance safety reports.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

A key mechanism of action of the immunotherapies is to stimulate the immune system to help it recognize and attack cancer cells. The immune checkpoint inhibitors target CTLA-4 and PD-1, which act to control the tolerance of the immune system. However, boosting T-cell activation by applying immune checkpoint inhibitors often leads to adverse drug reactions (ADRs) including colitis.

WHAT QUESTION DID THIS STUDY ADDRESS?

By mapping pharmacovigilance and molecular descriptor data, several hypotheses were generated and tested regarding the underlying molecular mechanisms of immunotherapy-induced colitis.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

It was confirmed that downregulation of CTLA-4 would be more toxic with respect to colitis, compared to other pathways downregulating PD-1. There were limited data to confirm whether severer toxicity is observed due to the early response of the anti-CTLA-4 drug.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This patient-centered systems-based approach could be used to inform drug development pipelines through reverse translation from post-market surveillance safety reports to the molecular mechanisms and targets of ADR events.

INTRODUCTION

After a long battle vindicating its effectiveness, immunotherapies became one of the key pillars in cancer therapy with the approval, first, of ipilimumab to treat late-stage (metastatic) melanoma (March 25, 2011),[1] and later, nivolumab (December 22, 2014)[2] and pembrolizumab (September 4, 2014)[3] for patients with unresectable and metastatic melanoma who are no longer responding to other drugs. The US Food and Drug Administration (FDA) has further approved these drugs for other indications, including lung cancer and metastatic renal cell carcinoma. These are monoclonal antibodies that inhibit either cytotoxic T-lymphocyte antigen-4 (CTLA-4) or programmed death-1 (PD-1): ipilimumab inhibits CTLA-4 and the others inhibit PD-1.[4] Both CTLA-4 and PD-1 are negative regulators of T-cell activation. Thus, the immunotherapy drugs boost the immune system by increasing T-cell activation, and thus enhance anti-tumor responses (Figure 1). Yet, the battle with immunotherapies still confronts many challenges given the high variabilities in their exposure-response patterns, benefit-risk ratios, and safety profiles.[5] Sources of this high variability lie within the genetics of cancer as well as in the heterogeneous inter- and intrapatient variability in tumor expression and in the response to therapies.[6]

In a systems pharmacology view of a drug action, a drug interacts with multiple primary and secondary targets and pathways. These targets exist within a complex network that can mediate the response to the drug leading to both the therapeutic and adverse effects. Systems-based approaches can improve drug safety by enabling a more detailed and mechanistic understanding of adverse drug reactions (ADRs), which can provide feedback on how to mitigate future risks by rendering causal hypotheses and identifying biomarkers that can be used to predict ADRs before they occur, by delineating a strategy for targeting high-risk adverse events in clinical or postmarketing surveillance analysis, and by stratifying a population at the molecular level to identify risks for a particular ADR.

This paper presents an application following our previous proof-of-concept study[7] for a detailed investigation of adverse events by back translating ADR reports from the FDA Adverse Event Reporting System (FAERS)[8] to molecular pathway and target levels using the analytical platform Molecular Health Effect (MH Effect). Although it is known that boosting T-cell activation by downregulating CTLA-4 and PD-1 weakens the self-tolerance causing autoimmunity or inflammation, leading to colitis, which is one of the major ADRs induced by immunotherapies (Figure 1), the mechanism of immune-mediated colitis from immune checkpoint inhibitors is not fully understood.[9–11] In this research, we particularly focused on the mechanistic differences between CTLA-4 and PD-1 immune checkpoint inhibitors with respect to the incidence of colitis.
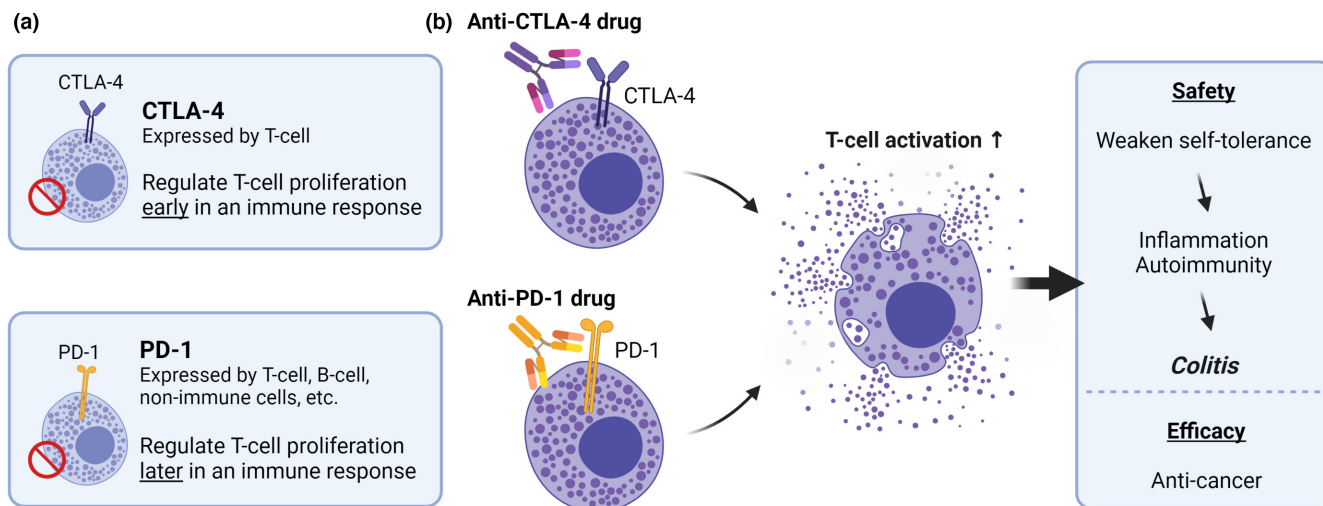
FIGURE 1 Overview of the mechanisms of action and toxicity. (a) Mechanistic differences between CTLA-4 and PD-1.[11,12] (b) The immune-mediated toxicity, colitis, and the mechanism of action of the immune checkpoint inhibitors. Created in BioRender.com

METHODS

FDA adverse event reporting system

FAERS is a postmarket surveillance database of ADRs submitted to the FDA.[8,12] It provides a rich source of ADR information submitted voluntarily by drug manufacturers, healthcare professionals, and consumers in the United States. Over 19,750,000 ADR reports were submitted from 1969 to the present, and the number of reports increase yearly.[8] The ADR reports are evaluated by clinical reviewers before being publicly released on a quarterly basis by the FDA.

Molecular health effect

The MH Effect is a data warehouse platform that contains and maps the population ADR data from FAERS with protein information, molecular targets and pathway data (Figure 2). It enables a comprehensive analysis of molecular targets and mechanisms associated with ADRs to untangle the complexity of the underlying molecular mechanisms of spontaneous ADRs.[13,14] Protein and pathway mappings are established based on information of entries from resources such as DrugBank,[15] PubChem,[16] UniProt,[17] NCI-Nature,[18] Reactome,[19] and BioCarta.[20]

In MH Effect version 1.7 used for this study, FAERS data were included up to Q4/2019. Reported ADRs associated with the immunotherapy drugs, namely, ipilimumab, nivolumab, and pembrolizumab, were collected from Q3/2015 to Q4/2019. Data were collected from ~ 6 months post the latest approval date among the three investigated

drugs (i.e., December 22, 2014, for nivolumab) in order to reduce statistical bias driven by the gap between the different approval and prescription times among the drugs of interest. The cohorts of adverse events that contained the respective FAERS cases reporting each drug were determined by searching over the synonyms of structured medication records that contain both brand and generic drug names. For example, searching for either "Nivolumab" or its brand name "Opdivo" gives the same search results.

There is a total of eight combinations of cohorts that were examined in this analysis. ADR cases associated with each of the three investigated drugs were collected (cohort A). In the ADR cases associated with ipilimumab, one subset of cases was reported with nivolumab in combination (cohort A). In order to focus on molecular targets and mechanisms that might be synergistic to increase immunotherapy drug-induced colitis, we further explored subsets of all four drug combinations after filtering out cases that do not include colitis among reported ADRs. The group of the subsets including colitis as one of the ADRs are referred to as cohort B in order to distinguish from cohort A in this paper (i.e., cohort A ⊃ cohort B).

Disproportionality analysis

The proportional reporting ratio (PRR) was used to assess the statistical relevance between the query entity and events of interest.[21] The PRR, commonly used in pharmacovigilance (PhVg), is one of the methods of disproportionality analysis that have the advantage of reducing uncertainty from the spontaneously reported data. MH Effect calculates the PRR according to its definition which
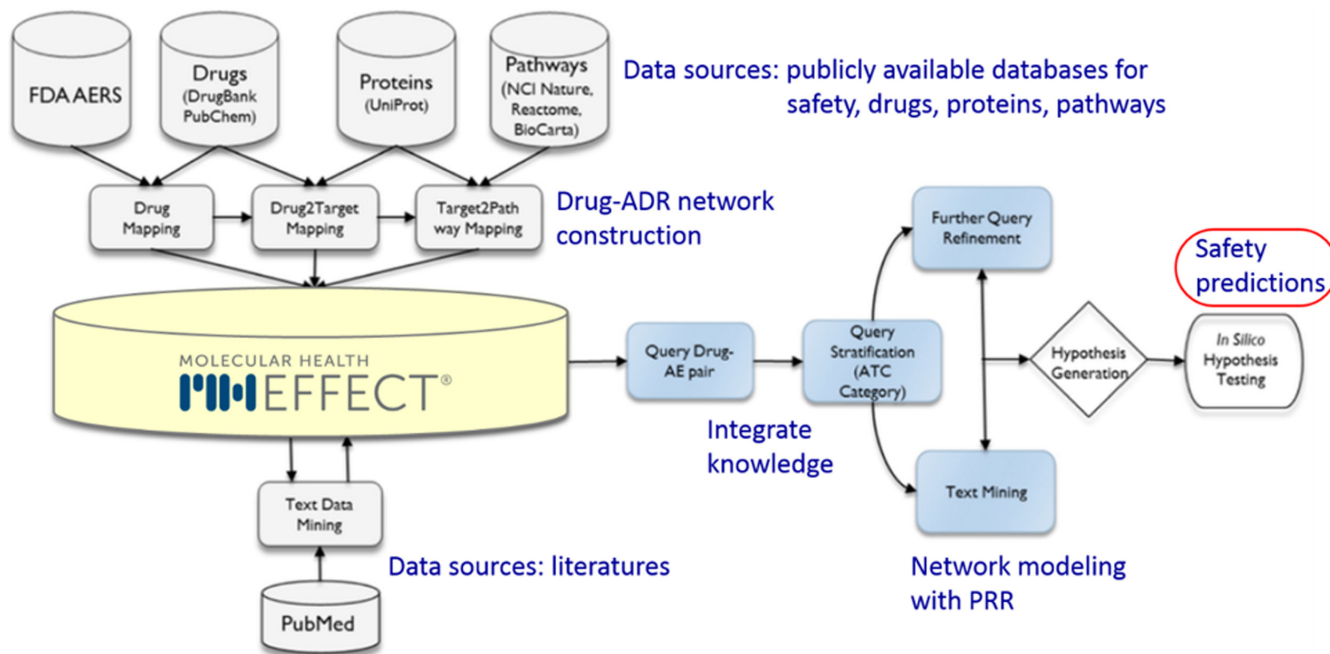
FIGURE 2 Overview of analysis workflow using Molecular Health Effect. This figure was adapted from Schotland et al.[42] ADR, adverse drug reaction; AE, adverse event; ATC, Anatomic Therapeutic Chemical; PRR, proportional reporting ratio

TABLE 1 Safety assessment overview presenting statistical associations between drug(s) and colitis

Drug(s)	N of all ADRs	N including colitis	PRR associated with colitis (95% CI)
Ipilimumab	14,448	1053	45.99 (43.31–48.82)
Nivolumab	41,783	1144	17.32 (16.33–18.37)
Pembrolizumab	19,022	404	12.96 (11.76–14.29)
Ipilimumab + nivolumab	9864	613	38.35 (35.48–41.46)

Abbreviations: ADR, adverse drug reaction; CI, confidence interval; N, number of case reports; PRR, Proportional Reporting Ratio and Data collection period, Q3/2015 to Q4/2019.
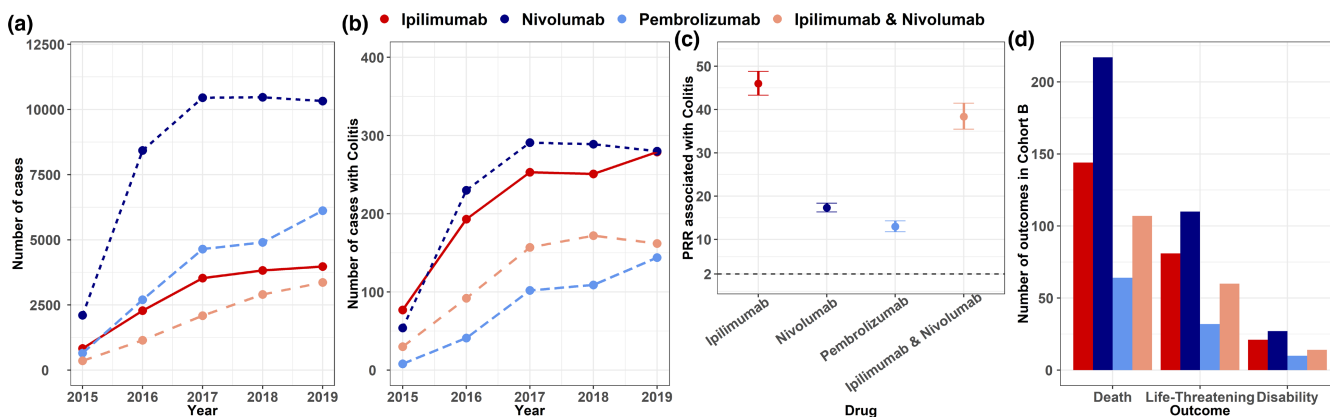

FIGURE 3 Graphical overview of cohort characteristics. (a) Number of cohort A case reports per year; (b) number of cohort B case reports per year; (c) comparison of the PRRs associated with colitis between anti-CTLA-4 (ipilimumab), anti-PD-1 (nivolumab and pembrolizumab) drugs, and the ipilimumab plus nivolumab regimen – the dashed line represents PRR = 2.0; and (d) distribution of the most severe outcomes in cohort B (namely, "Death," "Life-Threatening," and "Disability") – a case may have more than one outcome reported. Data collection period = Q3/2015 to Q4/2019. PRR, proportional reporting ratio

TABLE 2 Classification of inflammatory signaling

Signaling type	
Pro-inflammatory signaling	IFNα[23,24]
	IFNγ[24-26]
	IL1[23,24]
	IL6[23,24,27]
	IL8[24,28]
	TNFα[23,29]
Anti-inflammatory signaling	IFNβ[25,26,30]
	IL12[23]
Pro and anti-inflammatory signaling	IL2 (pro,[31] pro and anti[32])
	IL23 (pro,[33] anti[23])
	IL27 (pro and anti[34])
	PI3K (pro,[35,36] anti,[37,38] pro and anti[39])
	mTOR (pro and anti[39])

Abbreviations: IFN, interferon; IL, interleukin; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; TNF, tumor necrosis factor.

is $a/(a + b)$ divided by $c/(c + d)$ where a is the number of cases reported with the query entities and events of interest, b is the number of cases reported with the query entities and all events except the events of interest, c is the number of cases reported with the events of interest and all others in the categories of the query entities in the database except the query entities, and d is the number of cases reported with all events in the database but without the query entities and events of interest.[21,22] In addition, we determined if the signal is statistically significant using two traditional PhVg criteria: (1) three or more of the number of the ADR case reports and (2) two or greater of the lower bound of the 95% confidence interval (CI) of the PRR.[21]

RESULTS

Safety assessment overview

Table 1 presents the safety assessment overview, including the total counts of all ADR cases associated with the drug name(s) in each cohort during the entire data collection period (i.e., from July 1, 2015, to December 31, 2019). Although nivolumab was approved most recently, the total number of all ADR reports including nivolumab is the highest among the four cohorts, followed by pembrolizumab, ipilimumab, and the combination regimen of ipilimumab plus nivolumab. The number of ADR reports increases yearly, but the total ADR report case number with nivolumab starts showing a steady-state level starting in 2017 and even shows a slight decreasing annual trend (<2%) in 2019 (Figure 3a).

Among the total ADR reports associated with ipilimumab, 7.3% include colitis, whereas 2.7% and 2.1% include colitis among the reports found for nivolumab and pembrolizumab, respectively (Table 1). Although the number of cases with nivolumab is approximately three-fold higher than those with ipilimumab (Figure 3a), the counts of colitis cases are similar between these two cohorts (Figure 3b). All analyzed immunotherapy drugs were found to have a statistically significant PRR associated with colitis (Table 1) based on the two traditional PhVg criteria.[21] Figure 3c visualizes this PRR difference together with respective confidence intervals among the four cohorts. The PRR of the anti-CTLA-4 drug (i.e., ipilimumab) is approximately three times higher than those of anti-PD-1 drugs (i.e., nivolumab or pembrolizumab). The PRR of the combination regimen of anti-CTLA-4 and anti-PD-1 drugs (i.e., ipilimumab plus nivolumab) is between those of anti-CTLA-4 drug and anti-PD-1 drugs. In an attempt to measure the severity of those cases, we retrieved the number of ADR cases that reported "Death," "Life-threatening," or "Disability" as patient outcomes (Figure 3d). Of these three, death was the main outcome reported in cohort B (14–19%), as compared to the 8–10% and 2–3% of life-threatening and disability outcomes, respectively.

Protein and pathway mapping

Inflammatory signaling pathways

Molecular signaling pathways, which play a key role in inflammation including inflammatory cytokines, were found and classified into pro-, anti-, or pro- and anti-inflammatory signaling based on published studies extracted from PubMed (Table 2).[23–39] We found, using the protein and pathway mapping analysis in MH Effect, that colitis is directly related to intestinal inflammation from CTLA-4 or PD-L1 blocking drugs. In cohort B, which contains colitis in the list of reactions of all case reports, the PRRs of the pro-inflammatory signaling pathways were higher compared to the corresponding values in cohort A (Table 3), whereas the PRRs of the anti-inflammatory signaling pathways were lower compared to the corresponding values in cohort A (Table 3). However, the signals of the molecular mechanisms found in MH Effect meet the traditional PhVg criteria partially to be statistically significant.[21] The number of the ADR case reports is three or more (i.e., fulfill the first criterion), but the lower bound of the 95% CI of the PRR is not greater or equal to two (i.e., does not satisfy the second criterion) in all the explored cohorts (Table 3).

TABLE 3 Protein and pathway mapping on pro- and anti-inflammatory signaling pathways

Drug	Total N of ADR cases	PRR	95% CI PRR
Pro-inflammatory signaling			
IL1-mediated signaling events			
Cohort A			
Ipilimumab	751	0.7	0.65–0.75
Nivolumab	1963	0.63	0.6–0.66
Pembrolizumab	828	0.59	0.55–0.63
Ipilimumab + nivolumab	606	0.83	0.77–0.89
Cohort B			
Ipilimumab	64	0.82	0.65–1.04
Nivolumab	76	0.89	0.72–1.11
Pembrolizumab	27	0.9	0.62–1.3
Ipilimumab + nivolumab	51	1.12	0.86–1.46
IL6-mediated signaling events			
Cohort A			
Ipilimumab	942	0.55	0.51–0.58
Nivolumab	2785	0.56	0.54–0.58
Pembrolizumab	1417	0.62	0.59–0.66
Ipilimumab + nivolumab	760	0.65	0.6–0.69
Cohort B			
Ipilimumab	70	0.56	0.44–0.7
Nivolumab	98	0.72	0.59–0.87
Pembrolizumab	46	0.95	0.73–1.25
Ipilimumab + nivolumab	57	0.78	0.61–1.0
TNF receptor signaling pathway			
Cohort A			
Ipilimumab	1212	0.45	0.43–0.48
Nivolumab	3175	0.41	0.4–0.43
Pembrolizumab	1333	0.38	0.36–0.4
Ipilimumab + nivolumab	982	0.54	0.51–0.57
Cohort B			
Ipilimumab	110	0.57	0.47–0.68
Nivolumab	139	0.66	0.56–0.77
Pembrolizumab	43	0.58	0.43–0.77
Ipilimumab + nivolumab	85	0.75	0.62–0.92
Anti-inflammatory signaling			
IL12-mediated signaling events			
Cohort A			
Ipilimumab	623	0.6	0.56–0.65
Nivolumab	1964	0.65	0.63–0.68

TABLE 3 (Continued)

Drug	Total N of ADR cases	PRR	95% CI PRR
Pembrolizumab	1055	0.77	0.73–0.82
Ipilimumab + nivolumab	538	0.76	0.7–0.82
Cohort B			
Ipilimumab	38	0.5	0.37–0.69
Nivolumab	53	0.65	0.5–0.84
Pembrolizumab	20	0.69	0.45–1.06
Ipilimumab + nivolumab	35	0.8	0.58–1.1
The information processing pathway at the IFN beta enhancer			
Cohort A			
Ipilimumab	2221	1.25	1.2–1.3
Nivolumab	5255	1.02	0.99–1.05
Pembrolizumab	1898	0.81	0.78–0.84
Ipilimumab + nivolumab	1867	1.54	1.47–1.6
Cohort B			
Ipilimumab	178	1.37	1.2–1.57
Nivolumab	209	1.48	1.31–1.68
Pembrolizumab	56	1.12	0.88–1.43
Ipilimumab + nivolumab	140	1.85	1.6–2.14

Note: Data collection period, Q3/2015 to Q4/2019.

Abbreviations: ADR, adverse drug reaction; and IFN, interferon; CI, confidence interval; CXCR1, IL8 receptor alpha; CXCR2, IL8 receptor beta; IL, interleukin; N, number of case reports; PRR, Proportional Reporting Ratio; TNF, tumor necrosis factor.

Early stage of immune response

The molecular mechanisms related to the early stages of immune response show similar values of the PRRs among all explored cohorts (Table 4). The PRRs, however, did not fully satisfy the criteria to be statistically significant. The number of the ADR case reports is three or more (i.e., fulfill the first criterion), but the lower bound of the 95% CI of the PRR is not greater or equal to two (i.e., does not satisfy the second criterion) in all the explored cohorts (Table 4).

DISCUSSION

In this paper, the three negative regulators of T-cell activation, ipilimumab, nivolumab, and pembrolizumab, were compared with respect to the incidence of colitis as an ADR. Molecular mechanisms underlying immunotherapy-induced colitis were also investigated. For this study, the

TABLE 4 Protein and pathway mapping on early stage of immune response

Drug	Total N of ADR cases	PRR	95% CI PRR
TCR signaling in naïve CD8+T cells			
Cohort A			
Ipilimumab	689	0.57	0.53–0.62
Nivolumab	1866	0.54	0.51–0.56
Pembrolizumab	912	0.58	0.54–0.62
Ipilimumab + nivolumab	548	0.67	0.62–0.73
Cohort B			
Ipilimumab	52	0.6	0.46–0.78
Nivolumab	71	0.75	0.6–0.94
Pembrolizumab	23	0.69	0.46–1.02
Ipilimumab + nivolumab	39	0.77	0.57–1.04
TCR signaling in naïve CD4+T cells			
Cohort A			
Ipilimumab	726	0.58	0.54–0.62
Nivolumab	2073	0.57	0.55–0.59
Pembrolizumab	918	0.55	0.52–0.64
Ipilimumab + nivolumab	584	0.68	0.63–0.74
Cohort B			
Ipilimumab	52	0.57	0.44–0.74
Nivolumab	77	0.77	0.62–0.96
Pembrolizumab	23	0.65	0.44–0.97
Ipilimumab + nivolumab	39	0.73	0.54–0.99
Immunoregulatory interactions between a lymphoid and a non-lymphoid cell			
Cohort A			
Ipilimumab	664	0.37	0.35–0.4
Nivolumab	2142	0.41	0.4–0.43
Pembrolizumab	872	0.37	0.35–0.4
Ipilimumab + nivolumab	500	0.41	0.38–0.45
Cohort B			
Ipilimumab	39	0.3	0.22–0.41
Nivolumab	56	0.4	0.31–0.51
Pembrolizumab	27	0.54	0.38–0.78
Ipilimumab + nivolumab	21	0.28	0.18–0.42

Abbreviations: ADR, adverse drug reaction; CI, confidence interval; N, number of case reports; PRR, Proportional Reporting Ratio and Data collection period, Q3/2015 to Q4/2019.

data warehouse platform MH Effect was used, which links each ADR report from FAERS to associated biomolecules and molecular mechanisms.[13,14] Disproportionality analysis using the PRR metric was applied to quantitatively measure the statistical relevance between the ADR of interest (i.e., colitis), and the three drugs selected for the safety assessment overview (Table 1 and Figure 3). The PRR was

also applied to investigate the statistical relevance between colitis and molecular mechanisms in each cohort (Tables 3 and 4). Investigated cohorts referred to events between July 1, 2015, and December 31, 2019.

Our safety assessment overview (Table 1 and Figure 3) shows that the anti-CTLA-4 drug (i.e., ipilimumab) has a stronger association with colitis than anti-PD-1 drugs (i.e., nivolumab and pembrolizumab). This finding is in line with the review by Som et al., which compared percentage ranges of all grade immune-related common adverse events, including colitis, due to each of the different classes of the checkpoint inhibitors.[40]

The key mechanism of action of immunotherapies is to stimulate the immune system by blocking signaling via either CTLA-4 or PD-1 pathways to help recognize and promote an antitumor immune response.[39,40] The immune checkpoint inhibitors target CTLA-4 and PD-1, which act to control the tolerance of the immune system.[11] Therefore, boosting T-cell activation using immune checkpoint inhibitors could weaken immunological self-antigens (i.e., tolerance), leading to ADRs, including colitis.[38,41] Our protein and pathway analysis revealed that for most examined associations the PRRs did not satisfy the set PhVg criteria regarding statistical significance. Yet, results support the connection between colitis and inflammatory signaling pathways (Table 3). We further tested the hypothesis that CTLA-4 and PD-1 would regulate T-cell activation at different time periods; downregulation by CTLA-4 might occur at the early stage, whereas PD-1 would act late.[11,38] However, there was no clear difference observed in the molecular mechanisms of the early stage of immune response for anti-CTLA-4, as compared to anti-PD-1 drugs (Table 4). In Tables 3 and 4, there were minor differences consistently observed between the results for cohorts A and B. However, these could also be "circumstantial," attributed to the smaller number of cases in cohort B.

Lack of data regarding the exact chronological administration of therapies or the degree of severity may partly explain inconclusive findings, as well as reduced numbers of events reported in some cases. As previously noted in our proof-of-concept case study using the same approach,[7] there are several limitations of our study mainly driven by the structure and de facto content of FAERS data, which may among others include possible misstatement of indications, sparse of partly reported information, lack of details regarding condition severity (or grading), ADR reporting bias, co-administered medications and other difficulties in the risk assessment of drugs, and the establishment of a causal relationship with a given ADR.[41–45] In the current study, for example, important (but largely or completely) inaccessible information with respect to previous immuno-oncological exposure, specific treatment regimen timings, time to onset, or ADR-specific outcome

data would provide additional critical information of great relevance to the assessment of our findings. Therefore, based only on the available data our findings do not necessarily indicate or prove the tested hypothesis, according to which the anti-CTLA-4 drug could induce a more severe rate of colitis than anti-PD-1 drugs due to a greater magnitude of T-cell activation as a result of the early response of the anti-CTLA-4 drug in the immune response.

We expect that in the future, when more detailed adverse event data options will become available, we will be able to examine more immunotherapy specific intricacies, such as (previous) patient condition (e.g., cytokine release syndrome), monotherapy consideration, or co-administration with other immunosuppressive drugs (e.g., IL6 inhibitors, such as siltuximab and/or anti-IL6R monoclonal antibodies such as tocilizumab), other (pre-) treatment history metrics that may affect molecular components (like interference from steroids, T-cell activity, interleukin/cytokine levels, and inflammatory signaling), and severity at the ADR rather than at the patient level only (e.g., outcomes). Nonetheless, we find that our current dataset was largely representative of immunotherapy with the examined agents, based on reports coming from skilled experts (Figure S1 and Table S1).

Finally, at the time our analysis took place, we could benefit from FAERS data released only until Q4/2019. Hence, our work highlights the importance and value of the significant efforts put into integrating and updating adverse event data into platforms that allow accommodating feasible, systematic analytics over these large data sets.

In conclusion, we applied the established patient-centered systems-based approach[7] of reverse translation of adverse event reports to immunotherapy-induced colitis. Although data limitations (e.g., lack of information regarding details on some aspects, or reduced adverse event numbers) prevented us from assessing whether more severe toxicity may relate to the early response of the anti-CTLA-4 drug[46] or may have deemed some findings statistically insignificant, safety profiling at the drug level provided clear trends towards the observation that downregulation of T-cell activation via the CTLA-4 checkpoint pathway may increase toxicity and induce colitis, as compared to other checkpoint pathways downregulating PD-1. Importantly, our safety profiling methodology may be applied to other drugs or novel therapeutics under development with similar molecular mechanisms. With much effort being made to improve the accuracy of safety evaluation using data from spontaneous reporting databases,[47] we find that this patient-centered systems-based approach could be used to further inform drug development pipelines through reverse translation from postmarket surveillance safety reports to the molecular mechanisms and targets of ADR events.

AUTHOR CONTRIBUTIONS
S.K., G.L., M.V., D.B.J., T.G.S., L.J.L., and M.N.T. wrote the manuscript. S.K., L.J.L., and M.N.T. designed the research. S.K. and M.N.T. performed the research. S.K., G.L., M.V., D.B.J., T.G.S., L.J.L., and M.N.T. analyzed the data. D.B.J. and T.G.S. provided new reagents/analytical tools.

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SUPPORTING INFORMATION

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