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Treatments for the Mitigation of Immunotherapy-related Neurotoxicities in Patients with Non-small Cell Lung Cancer

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Abstract

Lung cancer is the most common cancer type worldwide, with one of the highest mortality rates across all cancer types. Cancer immunotherapeutics such as immune checkpoint inhibitors (ICIs) have improved cancer treatment by promoting the body's natural immune response to tumor development while avoiding toxic effects associated with traditional cytotoxic chemotherapy. However, immunotherapies have been associated with unique toxic effects similar to autoimmune disorders known as immune-related adverse events (irAEs). 4.2% of immunotherapy-treated cancer patients reportedly developed neurological irAEs. In this review, different immunotherapies were studied, including ICIs and mechanism-specific novel therapies, in order to determine a combination therapy to most effectively mitigate the development of neurotoxicities, e.g. encephalitis, in patients with Non-small Cell Lung Cancer (NSCLC). Reviews were examined discussing the outcomes of monotherapy and combination therapy with ICIs in cancer types such as melanoma and NSCLC, and studies where immune antitumor response was enhanced using antibodies against specific elements of the immune system, such as Regulatory T cells and Interleukin-6 (IL-6), were investigated. Existing immunosuppressive treatments used to resolve neurotoxicities were also examined. Many ICIs were associated with neurotoxicities and varied treatment responses, but certain ICI combinations led to durable antitumor responses with relatively milder toxicity. Novel blockade of immune mechanisms can increase antitumor activity without directly targeting immune effector cells, and second-line immunosuppressives can resolve irAEs when they arise. Though research suggests certain treatment regimens are more effective with relatively lower associated toxicity, treatments such as CCL1/CCR8 antibody blockade have only shown results in mouse and in vitro models. Novel antibodies will need to undergo clinical trials before the use of these antibodies is considered in

cancer patients, and few studies have investigated the use of immunosuppressives used to treat adverse events, such as rituximab, in combination with first-line immunotherapeutics.

Keywords: non-small cell lung cancer, immunotherapy, immune-related adverse event, neurotoxicity, immune checkpoint inhibition, monoclonal antibodies, encephalitis, PD-1, CTLA-4

Treatments for the Mitigation of Immunotherapy-related Neurotoxicities in Patients with Non-small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) is responsible for the most cancer fatalities both in the United States and worldwide. 2017 data from the CDC and NCI found that lung and bronchus cancers were the third most common cancer in the U.S. at a rate of 55.2 cases per 100,000 people, and the most common cause of cancer death, at a rate of 36.7 cases per 100,000 (U.S. Cancer Statistics Working Group, 2020). Existing treatment methods include surgical tumor resection, radiotherapy, cytotoxic chemotherapy, and immunotherapy—a continually expanding modern development in cancer treatment. However, while the use of cancer immunotherapy continues to increase, the 5-year survival rate for NSCLC remains at approximately 17.4% (p. S33). NSCLC also accounts for between 80 and 85% of all lung cancer cases, making NSCLC the large majority of lung cancer types (Xia et al., 2019, p. S33).

Though immunotherapies, particularly immune checkpoint inhibitors (ICIs), represent one of the leading developments in modern cancer treatment, the poor response rates to ICI treatment and tendency for immune-related toxicities presents a significant challenge to overcome before immunotherapy can replace contemporary treatment options. ICIs are monoclonal antibodies that blockade receptors or ligands involved in immune tolerance to tumor cells, for example programmed cell death protein 1/programmed cell death-ligand 1 (PD-1/PD-L1) and T-lymphocyte associated protein 4 (CTLA-4). The trials KEYNOTE-189, IMpower150, IMpower130, and KEYNOTE-40 demonstrated improved overall survival in NSCLC patients being treated with PD-1/PD-L1 inhibitors in addition to chemotherapy, and the trials KEYNOTE-024, KEYNOTE-042, and CheckMate227 saw improved survival in NSCLC patients treated with PD-1/PD-L1 checkpoint inhibitor monotherapy or CTLA-4 inhibitors in

combination with chemotherapy (Lim et al., 2020, p. 2). Despite these clinical results, response rates remain as low as 14-20%, negating the relatively stronger antitumor capabilities of ICIs compared to traditional cytotoxic chemotherapy (Xia et al., 2019, p. S33). Because ICIs are not specific to any tissue or the tumor microenvironment (TME), immune checkpoint inhibition can also lead to immune-related adverse events (irAEs).

ICIs are known to cause inflammation in the gastrointestinal tract, endocrine glands, skin, lung, and liver, though complications can occur across most major body systems, including the nervous system (Chang et al., 2020, p. 2243). Adverse conditions resulting from immunotherapy are known as irAEs and are graded such that a 1-2 is mild/moderate, 3 is medically significant, and 4-5 is severe/life-threatening. While the exact mechanisms that lead to the development of neurological irAEs are unknown, suggested pathways include loss of regulation by Regulatory T cells (Tregs), cross-reactivity of immune cells to foreign antigens resembling autoantigens, the proliferation of secondary antigens unrelated to the original antigen, genetic factors, and microenvironmental factors (Vilariño et al., 2020, pp.1-3). Some studies have investigated the antitumor potential of targeting unique antigens associated with aspects of the immune system without affecting T cell cytotoxicity and potentially preventing autoimmunity, e.g. the antibody blockade of the CCR8/CCL1 pathway found in Tregs. However, studies have been limited to in vitro and mouse model studies (Hoelzinger et al., 2010; Villarreal et al., 2018).

ICIs are known to be associated with multiple neurological irAEs, including Guillain–Barre syndrome, Myasthenia Gravis, aseptic meningitis, encephalitis, and myelitis (Chang et al., 2020, p. 2243). Neurological irAEs reportedly occur in 4.2% of patients treated with immunotherapy for cancer with manifestations in both the central and peripheral nervous system. Neurological irAEs differ from paraneoplastic syndromes due to the nature of

presentation, in which paraneoplastic syndromes often arise prior to the detection of cancer and are not acute. Some common neurological irAEs have been considered “principal” neurological irAEs, one of which is peripheral neuropathy, a loss of sensory and/or motor neurological function that can be symmetrical or asymmetrical. The symptoms associated with peripheral neuropathy include numbness, paresthesia, areflexia, sensory ataxia, and cranial nerve palsies. Peripheral polyneuropathy reportedly occurs as a grade 1-2 irAE at 5% and 6% as a grade 3-4 irAE (Mirabile et al., 2019, pp. 5-8).

Another neurological irAE, Myasthenia Gravis (MG), has a 30.4% mortality and is suggested to develop from activating cytotoxic T cells. MG is a tendency of weakness or varying muscle weakness that is usually proximal and is sometimes associated with muscles of the eyes, mouth, neck, and lungs. (Mirabile et al., 2019, p. 9). One of the most common neurological irAEs is encephalitis, defined by the symptoms—when not caused by infection—of confusion, altered behavior, headaches, seizures, short-term memory loss, depressed levels of consciousness, focal weakness, and speech abnormalities. Encephalopathies occur in 19% of patients treated with immunotherapies, and encephalitis occurs in 1-3% (Mirabile et al., 2019, pp. 10-11).

While current first-line therapies do not preemptively combat the development of neurological adverse events, immunotherapeutics such as tocilizumab and rituximab have been used as second-line treatments to resolve irAEs and non-cancer related encephalitis and there is little data documenting their use in a first-line role or as part of initial treatment (Lee et al., 2016; Stroud et al., 2019). Because common immunotherapeutics—such as the ICIs nivolumab, ipilimumab, and pembrolizumab—for NSCLC have suboptimal response rates and are connected to multiple neurotoxicities, treatment of NSCLC involving a combination of select ICIs,

mechanism-specific, novel, targeted therapies, or treatments for the management of neurological irAEs may better treat NSCLC while mitigating the development of neurological irAEs due to the greater specificity of novel, targeted therapies and the ability of immunosuppressives to reduce the severity of neurological irAEs.

Toxicity of Immune Checkpoint Inhibitors

Because ICIs are known to cause toxicities across multiple organ systems (the nervous system, skin, digestive, and respiratory systems), selecting specific ICIs and treatment dosages that are associated with lesser toxicity may help mitigate the development of neurological irAEs.

Antonia et al. (2016) conducted a study of 102 participants treated with a combination of the ICIs durvalumab and tremelimumab with the goal to investigate the effectiveness of combining PD-L1 and CTLA-4 inhibitors as opposed to using them individually. Antonia et al. noted that the median duration of treatment was 11.6 weeks and listed the primary reasons and percentages for treatment cut-off as 26% due to adverse events, 21% due to disease progression, 15% due to death, and approximately 9% due to patient or study related reasons (p. 304). Despite Antonia et al. 's later claim that combination inhibition of multiple immune checkpoints is a more effective approach to cancer immunotherapy, the study still reported just over 25% of patients stopped treatment due to adverse events, and nearly a quarter of patients' cancers progressed.

According to Antonia et al., the cohort receiving durvalumab 20 mg/kg every 4 weeks plus tremelimumab 3 mg/kg reached the maximum tolerated dose. Of the total 102 patients, 82 had at least one adverse event, and the most common were diarrhea, fatigue, and pruritus (p. 304). The most common grade 3 or higher adverse events were diarrhea, colitis, and increased lipase (p. 304). Antonia et al. noted that 22 patients died within the study, with three deaths

attributable to treatment (p. 305). Antonia et al. claimed that the treatment-related deaths were due to complications from MG, pericardial effusion, and neuromuscular disorder. Though more than 75% of study patients developed adverse events, the most common were relatively mild; however, deaths which occurred during treatment were partly due to neurological irAEs (MG and neuromuscular disorder), indicating that while neurological irAEs were not markedly common, these irAEs proved to be fatal. Moreover, 80.3% experienced adverse events during treatment, while 26% of patients terminated treatment due to adverse events.

Stroud et al. (2019) conducted a study wherein a sample of 87 nivolumab-treated patients were treated with tocilizumab. According to Stroud et al., the previously reported incidence of irAEs of any grade with the PD-1 inhibitor nivolumab is 41-68%, and the reported incidence of irAEs of grades 3-4 is 4.5-7.7% (p. 552). Stroud et al. noted that in a study by Shoushtari et al. (2017) investigating toxicities resulting from nivolumab and ipilimumab treatment in patients with melanoma, the incidence of steroid-refractory irAEs was 25% (p. 552). Stroud et al.'s claims support the pattern observed in Antonia et al. (2016)'s study, in which close to 75% of patients developed irAEs, and a smaller but noteworthy fraction (2.9%) developed severe enough irAEs to be fatal.

Gandhi et al. (2018) conducted a double-blind clinical trial in which 616 patients with metastatic, nonsquamous NSCLC without EGFR or ALK mutations were treated with a combination of the cytotoxic chemotherapies pemetrexed and either cisplatin or carboplatin, and patients received either a placebo or the PD-1 inhibitor pembrolizumab. Pembrolizumab is used as a first-line therapy for patients with a 50% or greater tumor proportion of PDL-1-positive cells, but Gandhi et al. claimed that in patients with NSCLC, those who meet this standard are part of the minority (p. 2079). Gandhi et al. noted that adverse events of any kind occurred in

99.8% of patients in the pembrolizumab group and 99% of patients in the only-chemotherapy group (p. 2088). Grade 3 or higher adverse events occurred in 67.2% and 65.8% of patients respectively, and adverse events led to death in 6.7% of pembrolizumab group patients and 5.9% of only-chemotherapy patients (p. 2088). Gandhi et al. noted that adverse events of Grade 3 or higher included anemia, neutropenia, febrile neutropenia, and acute kidney injury resulting in discontinuation of treatment (p. 2088). While Gandhi et al. did not note any neurological irAEs in their study, the proportion of patients with relatively high grade irAEs was comparable to the findings of Antonia et al. (2016) and Stroud et al. (2019). Although—as discussed in Combination Therapy—the use of pembrolizumab in combination with chemotherapy improved patient survival and treatment response compared to chemotherapy alone, pembrolizumab treatment did not considerably reduce development of treatment-related toxicities compared to chemotherapy alone. Pembrolizumab has also been associated with neurotoxicity in at least one case report before (Niki et al. 2018). Because pembrolizumab targets the common immune checkpoint PD-1, similar results might be expected when comparing other PD-1/PD-L1-targeted ICIs with chemotherapy, however toxicities could be better reduced by avoiding the use of cytotoxic chemotherapeutics altogether.

Larkin et al. (2017) searched The Bristol-Myers Squibb Global Pharmacovigilance and Epidemiology database for neurological irAEs reported over an 8-year period in patients with advanced melanoma treated with nivolumab or nivolumab and ipilimumab and analyzed a sample of 3,763 patients (pp. 711-712). 35 out of the total 3,763 patients (0.93%) had neurological irAEs possibly related to treatment (p. 712). Larkin et al. claimed that 28 of the 35 patients had only one neurological irAE, six patients had two, and one patient had three, with five of the seven patients who had more than one neurological irAE having received combination

therapy and two having received nivolumab alone (p. 712). Larkin et al.'s findings suggest a particularly low incidence of neurological irAEs but present a possible correlation between combination therapy and the development of multiple neurological irAEs.

Larkin et al. stated that neuropathy was seen in 22 patients, noninfective meningitis in five, encephalitis in six, neuromuscular disorders in three, and "nonspecific events," including headache, seizure, confusion, and syncope, in seven (p. 712). 32 of the 43 observed neurological irAEs were grade 3 or 4, with one fatal case of grade 5 encephalitis (p. 713). According to Larkin et al., 26 out of 35 patients with neurological irAEs (74%) resolved their irAEs and recovered, while seven developed sequelae after resolution (p. 713). The median time for the onset of any grade neurological irAE was 45 days, and for grade 3-5 irAEs the median time of onset was 48 days, with a median time until resolution of 32 and 49 days for all neurological irAEs versus grade 3-5 irAEs, respectively (p. 713). The neurological irAEs observed by Larkin et al. align with those known to be associated with ICIs, and nearly 75% of the observed neurological irAEs were of at least grade 3, indicating that while the incidence of neurological irAEs is low, these irAEs are relatively severe when they develop.

Although the incidence of neurological irAEs, as noted by Antonia et al. (2016) and Larkin et al. (2017), in patient samples treated with ICIs was very low (2.0 & 0.93%, respectively), both studies connected neurological irAEs to fatalities, or otherwise observed those neurological irAEs among the more severe grade irAEs. General irAEs were observed across all studies, with a reported incidence as high as 80.3% by Antonia et al. (2016). Though ICI treatment benefits the survival and durable antitumor treatment response of cancer patients, it is clear that the use of ICIs contributes to the development of toxic effects. While the incidence of neurological irAEs secondary to immunotherapy is considerably low, the connection between

neurological irAEs and fatalities necessitates deeper consideration of the use of ICIs so that specific drugs and combinations least conducive of neurological irAEs may be identified.

Treatment Using Novel Antibody Targets

Because novel antibody targeting of specific pathways connected with immune system mechanisms, such as Tregs in the TME, has shown durable antitumor responses, novel antibody targeting could increase overall patient survival and slow or stop tumor growth in NSCLC patients while avoiding neurological irAEs.

Hoelzinger et al. (2010) tested the antitumor effect of treatment combinations consisting of CpG-ODN, CCL1 antibodies (αCCL1), and ITGαE antibodies (αITGαE) using a mouse model in which cells from the TUBO murine breast cancer cell line were used to form tumors (p. 6834). CpG-ODN functions by binding antigen-presenting cells and triggering release of a proinflammatory cytokine Interleukin-6 (IL-6), and according to Hoelzinger et al., chemokine ligand 1 (CCL1) was found to be particularly expressed on Tregs in the presence of IL-6.

Hoelzinger et al. noted that differences in survival rate between the treatment variations were significant ($\chi 2=23.21$, df 4, $p = 0.0001$), with 6 out of 9 mice treated with CpG-ODN and αCCL1 rejecting the tumor completely (p. 6836). CpG-ODN/αCCL1 treatment significantly slowed tumor growth compared to all other treatment variations, and 100% of mice treated with the regimen and exposed again to tumors of the same cell line rejected the tumor completely, manifesting no tumor regeneration in 12 months (p. 6836). CpG-ODN/αCCL1 treatment induced tumor rejection in 67% of mice treated, and though the overall sample of mice treated with the regimen was small, all mice treated showed significantly slower tumor growth, suggesting reliable antitumor response to CpG-ODN/αCCL1 treatment. Full tumor rejection following exposure to tumors after 12 months demonstrates the durability of antitumor response induced by

CpG-ODN/αCCL1 treatment and suggests that the combination could not only be effective in combating tumors, but also in maintaining a long-term immune memory of cancer cells.

Bjoern et al. (2016) found data in their study of 80 patients who received treatment for melanoma with the CTLA-4 inhibitor ipilimumab inconsistent with—while not statistically significantly—the results of Hoelzinger et al. (2010)'s mouse model. Bjoern et al. found that baseline levels of IL-6 did not predict patient overall outcome ($p = 0.5$) but higher levels of IL-6 by the 4th dose of treatment showed a non-significant association with poor survival ($p = 0.07$) (p. 5). Though the association was not statistically significant, further study of IL-6 levels could guide cancer treatment to avoid treatments affecting major changes in IL-6, as the increase of IL-6 induced by CpG-ODN used in Hoelzinger et al.'s (2010) study could potentially worsen patient conditions in clinical trials if the negative correlation to patient outcome is found to be significant.

Villarreal et al. (2018) investigated the antitumor effects of antibody blockade of the chemokine receptor CCR8, which binds CCL1, and its interactions with *Listeria*-based cancer vaccination in mice models and in vitro analyses of cells from the CT26 colorectal cancer cell line. According to Villarreal et al., CCR8 is primarily expressed on tumor-infiltrating Tregs but not on splenic Tregs, and CCR8 is not expressed on CT26 tumor cells (p. 5341). Treatment with αCCR8 led to a significant antitumor response and an improved long-term survival ($p < 0.0001$), and tumor growth curves showed that αCCR8 treatment slowed tumor growth compared to the control groups (p. 5341). Villarreal et al. tested the same conditions in an MC38 colon tumor model to verify their results, claiming that treatment with αCCR8 resulted in significantly slower MC38 tumor growth and increased survival ($p < 0.0001$) (p. 5341). Because CCR8 is a receptor for CCL1, Villarreal et al. and Hoelzinger et al.'s (2010) findings suggest that blockade of the

CCL1/CCR8 pathway can highly enhance antitumor treatment response, and the exclusivity of CCR8's expression on tumor-infiltrating Tregs suggests the potential of CCL1/CCR8 blockade as a highly specific treatment capable of avoiding off-target immune changes responsible for neurological irAEs. CCR8 blockade also appears to be adaptable to multiple cancer types, and further testing could establish the versatility of the blockade across cancer types.

Villarreal et al. noted that αCCR8 treatment significantly increased the frequency of AH1 tetramer-specific $CD8^+$, $IFN\gamma^+$ Effector, and multipurpose $CD8^+$ Effector T cells in the TME compared to the control group ($p < 0.05$) (p. 5342). Villarreal et al. also observed changes in the phenotypes of immune cells infiltrating the TME, noting that αCCR8 treatment increased the total measured CD45+ tumor-infiltrating lymphocyte count compared to the control group ($p < 0.05$) (p. 5342). In the population of CD45+ cells, αCCR8 treatment increased the percentage of tumor-infiltrating CD8+ and CD4+ T cells ($p < 0.05$), and both tumor-infiltrating CD8+ and CD4+ T cells expressed PD-1 and CTLA-4 at a significantly lower rate ($p < 0.05$) (p. 5342). Villarreal et al. claimed that αCCR8 treatment could improve the antitumor potency of T cells in the TME by preventing the depletion of T cells (pp. 5342-5343). αCCR8 treatment significantly mobilizes effector T cell populations in the TME, augmenting immune antitumor response, and significantly reduces tumor-infiltrating cells expressing the immune regulatory molecules PD-1 and CTLA-4, suggesting that αCCR8 treatment can promote the proliferation of T cells less prone to suppression by immune checkpoint regulation.

Villarreal et al. claimed that αCCR8 treatment significantly reduced the frequency of $CD4^+$ and "tumor-resident" $CCR8^+Foxp3^+CD4^+$ Tregs ($p < 0.01$) (p. 5343). The ratio of $CD8^+$ T cells to Treg cells within the TME significantly increased after αCCR8 treatment ($p < 0.01$), and αCCR8 treatment had significant effects on $CCR8^+$ populations but not on all T cell populations.

($p < 0.05$) (p. 5343). Villarreal et al. analyzed the effect of CCR8 blockade on Treg conversion, finding that αCCR8 treatment inhibited Treg induction, while the isotype antibody used for the control group manifested no changes ($p < 0.001$) (p. 5343). Tumor-infiltrating lymphocytes taken from CT26 tumors and cultured in the presence of αCCR8 showed significantly decreased percentages of Tregs compared with the control group after 72 hours (45% vs. 10%, respectively) ($p < 0.0001$) (p. 5343). Villarreal et al. noted that the frequency of CD8+ T cells was slightly increased in the cell culture of tumor-infiltrating cells treated with CCR8 antibodies ($p < 0.05$), which Villarreal et al. suggested could be due to the reduced Tregs in the environment ($p < 0.0001$) (p. 5343). αCCR8 intensifies infiltration of Effector T cells in the TME by reducing Tregs and inhibiting induction of new Tregs, and the effects of αCCR8 are specific to the $CCR8^+$ Tregs observed in the TME.

Villarreal et al. looked into the effect of αCCR8 on the suppressive function of Tregs, finding that Tregs reduced the proliferation of $CD8^+$ T cells, but that addition of αCCR8 to cell cultures containing Tregs and $CD8^+$ T cells significantly decreased the Treg cells' suppressive effect on the proliferation of $CD8^+$ T cells ($p < 0.001$) (p. 5344). Introducing αCCR8 antibodies to a culture of cells containing only $CD4^+$ or $CD8^+$ T cells induced no variation from the baseline rates of proliferation (p. 5344). αCCR8 not only decreases the population of tumor-infiltrating Tregs, but it also reduces Tregs' inhibitory effect on the proliferation of Effector T cells involved in antitumor response. αCCR8 also appears to be specific to Tregs, preventing the direct modulation of Effector T cell behavior that may result in autoimmune cytotoxicity such as in ICI treatment.

Chang et al. (2020) conducted a prospective study on 290 patients of the Seoul National University Hospital treated with PD-L1 inhibitor atezolizumab to determine a correlation

between treatment-induced encephalitis and the HLA-B*27:05 genotype. Seven patients out of the total 290 developed encephalitis after atezolizumab treatment, and five were enrolled into the study. All five enrolled patients had a score of 7 on the Naranjo adverse drug reaction probability scale (p. 2244). Chang et al. noted that the HLA-B27 genotype was present in 60% of the more severely affected patients, while the genotype was present in only 3.1% of individuals in the Korean population as a whole (p. 2246). After statistical correction, there was a significant connection between the HLA-B27 genotype and neurological irAEs of the central nervous system (p. 2246). The HLA-B27 protein presents as another potential novel target for antibody blockade due to its significant connection to neurological irAEs, and further study would benefit cancer patients if the role of HLA-B27 in neurological irAEs was understood, because targeting HLA-B27 could interfere with the mechanism of neurological irAE development.

Inhibition of the CCL1/CCR8 pathway *in vitro* and in mouse models using tumor cells of breast, colorectal, and colon cancers not only mobilizes a strong antitumor response by Effector T cells but also contributes to the reduction of and decreased suppression by tumor-infiltrating Tregs. The unique presentation of CCR8 and CCL1 by immune cells primarily within the TME also suggests that inhibition of CCL1/CCR8 may prevent the development of off-target adverse effects, such as neurological irAEs, by modulating immune behavior of cells specific to the TME. The lower rate of immune checkpoint molecules PD-1 and CTLA-4 observed on effector cells after αCCR8 treatment also presents CCL1/CCR8 inhibition as a potential replacement for ICIs, as the disinhibition of immune cells caused by ICIs could be achieved from the reduced presence of inhibitory surface antigens by αCCR8 treatment. The connection discovered between HLA-B27 and neurological irAEs also encourages further research into the potential for

HLA-B27 antibody blockade as well as determining other potential novel targets similar to CCL1 and CCR8.

Combination Therapy

Because the use of ICIs in immunotherapy is widely associated with irAEs, treatment combinations that are connected to lesser toxicity and strong antitumor effects may maximize the effectiveness of treatment of NSCLC while managing the development of irAEs. Immunological mechanisms besides immune checkpoints, for example IL-6 and Tregs, also have a role in immune tumor response, and combinations of ICIs and novel, targeted therapies could enhance the effect of treatment while potentially preventing or avoiding neurological irAEs.

According to Antonia et al. (2016), studies investigating nivolumab and ipilimumab combination therapy in melanoma and NSCLC patients reported durable responses to treatment in PD-L1-positive and PD-L1-negative patients (p. 299). Antonia et al. noted that out of 63 patients able to be assessed for tumor response, 11 showed an objective response, with 18 showing disease control at 24 weeks (p. 305). According to Antonia et al., no objective responses occurred in the lowest dose cohort of durvalumab 3 mg/kg every 4 weeks plus tremelimumab 1 mg/kg (p. 305). Relative to groups receiving tremelimumab 1mg/kg doses, those receiving tremelimumab 3mg/kg doses had more frequent adverse events, more grade 3 and 4 events, and serious events, without an increase in the degree of treatment response (p. 306). Though objective response was achieved in some patients with durvalumab/tremelimumab treatment, only 28.6% of patients achieved disease control after 24 weeks, and among the different dose cohorts, the lowest durvalumab dosage had no effect, and the highest tremelimumab dose resulted in the most irAEs. Toxicity associated with tremelimumab could also indicate a greater tendency to toxic effects for CTLA-4 inhibition than for PD-1 inhibition.

"Complete suppression" of free soluble PD-L1 was noted in most patients (p. 306), and according to Antonia et al., combination of durvalumab and tremelimumab promoted greater activation and proliferation of T cells than was seen in a study by Rizvi et al. (2015) conducted using durvalumab alone (p. 306). Complete suppression of free soluble PD-L1 likely indicated the effective binding of PD-L1 by durvalumab, and combination therapy with durvalumab and tremelimumab induced a stronger immune response than durvalumab alone. However, the low proportion of treatment-responsive patients and sensitivity of dosage highlights the ineffectiveness of ICIs alone, even in combination therapy, and indicates the need for supplementary drugs within the treatment regimen.

Gandhi et al. (2018) noted that pembrolizumab treatment improved overall patient survival across patients of all PD-L1 expression levels versus patients treated with combination chemotherapy alone. The 12-month survival rate with and without pembrolizumab treatment in patients with a tumor positivity score <1% was 61.7% vs. 52.2% (HR: 0.59, 95% CI, 0.38 to 0.92), 71.5% vs. 50.9% for patients with a score between 1 and 49% (HR: 0.55, 95% CI, 0.34 to 0.90), and 73.0% vs. 48.1% for patients with a score >50% (HR: 0.42; 95% CI, 0.26 to 0.68) (p. 2084). For pembrolizumab treatment with chemotherapy, progression free survival was greater than with chemotherapy alone, and the hazard ratios indicated strongly decreased risk across all tumor positivity scores.

While pembrolizumab combination treatment with chemotherapy presents as a promising modification to chemotherapy treatment alone, toxicities could be attributed to the use of chemotherapy itself. In a study by Spain et al. (2017) observing 352 patients treated with the common PD-1/PD-L1-targeted ICIs nivolumab, ipilimumab, and pembrolizumab, rates of neurotoxicity were comparable among the three (7%, 1%, and 2%, respectively), and the highest

rate of 14% in combination therapy with nivolumab/ipilimumab (p. 378). Thus, pembrolizumab monotherapy or in combination with other ICIs may still be favorable to combination treatment with cytotoxic chemotherapeutics.

Gandhi et al. noted that the disease control rate, defined as, "the proportion of patients with a confirmed complete or partial response or stable disease," was 84.6% in the pembrolizumab group and 70.4% in the only-chemotherapy group (p. 2084). Antitumor response rate in the pembrolizumab group was 47.6% (95% CI, 42.5 to 52.5) compared to the chemotherapy combination group's rate of 18.9% (95% CI, 13.8 to 25.0) ($p < 0.001$) (p. 2084). To combat the issue of poor treatment response among most ICIs, combination treatment may be a potential pathway to elicit a greater treatment response using ICIs.

Villarreal et al. (2018) noted that cancer vaccines using the bacterial vector *Listeria* reduce the influence of suppressive cells in the TME and increase activity of antitumor effector cells. Villarreal et al. tested the combination of *Listeria* vaccination, targeting the AH1 tumor-associated antigen, with αCCR8 treatment. Combination treatment was significantly associated with increased suppression of tumor growth, and the long-term survival of the combination group was approximately 20% greater than that of the control group ($p < 0.0001$) (p. 5344). Villarreal et al. claimed that when analyzing tumor-infiltrating leukocytes, the study observed "synergistically" enhanced responses against the AH1 antigen ($p < 0.001$) (p. 5344). Villarreal et al. noted that combination therapy significantly increased the production of IFNγ and TNFα by Effector CD8+ and CD4+ tumor-infiltrating lymphocytes, and the quantity of Tregs in the TME decreased ($p < 0.001$) (p. 5344). Combination treatment with the novel CCR8 antibody and *Listeria* vaccination is able to mobilize a strong immune response via CCR8 inhibition while reducing Tregs in the TME and inducing release of immune-activating

cytokines. Because αCCR8 treatment significantly increases the frequency of AH1-specific $CD8^+$ T cells, αCCR8/*Listeria* combination treatment also targeted the tumor-associated AH1 antigen coactively, revealing the potential effectiveness of a treatment combination targeted to the same antigen or pathway.

Multiple combinations of ICIs and traditional chemotherapies have proven to elicit greater response or induce a greater antitumor response in patients when compared to ICI monotherapies; however, due to the specificity of effect and ability to mobilize a very strong antitumor response, αCCR8/*Listeria* treatment could potentially induce the most reliable antitumor response while avoiding off-target effects. Current study of αCCR8/*Listeria* treatment has been limited to mouse and cell models, but further research into concurrent treatment with novel inhibitors and more common treatments such as ICIs may be beneficial to determine whether synergistic and specific treatment response could occur, enhancing response and/or reducing the incidence of neurological irAEs.

Treatments for Adverse Events

Because secondary treatments that suppress immune activity by targeting B cell adaptive immunity and the cytokine system have been used to treat adverse events secondary to cancer immunotherapy as well as autoimmune neurological conditions like encephalitis, the use of immunosuppressives as first-line immunotherapies could preemptively inhibit the development of neurotoxicities.

Lee et al. (2016) conducted a study on a sample of 80 patients diagnosed with Autoimmune Limbic Encephalitis (ALE), 12 of which exhibited tumors, in which they studied the effect of the CD20 antibody rituximab on neurological status and compared the results to a control of 81 patients not treated with rituximab. A median mRS score of 2 was observed at the

final follow-up (IQR 1–3). According to Lee et al., 61 patients' mRS scores improved and 55 had favorable (0-2) mRS scores, but 10 patients relapsed (p. 1687). The rituximab group had greater mRS improvement ($p = 0.011$) but not more favorable mRS at last follow-up ($p = 0.442$) (p.1687).

According to Lee et al., those who responded to first-line therapy (corticosteroids, intravenous immunoglobulin (IVIG), and plasmapheresis) had more favorable outcomes overall, but in those who did not respond, rituximab treatment led to more favorable outcomes ($p = 0.001$) (p. 1687). Rituximab treatment can improve neurological statuses of patients with ALE, with more than 75% of patients' mRS scores improving, and more than 50% having scores considered favorable after treatment, however rituximab did not significantly lead to more favorable mRS scores at last follow-up. Rituximab was also able to improve mRS scores in patients who did not respond to first-line treatment, demonstrating rituximab's usefulness as a stronger treatment in managing ALE.

Stroud et al. (2019) observed improvements in the conditions of 27 out of 34 patients (79.4%), and some patients required more than one dose of tocilizumab with 38.2% requiring two, 8.8% requiring three and one patient requiring four doses (p. 553). All tocilizumab doses were 4 mg/kg, except for one 8 mg/kg dose (p. 553). Stroud et al. did not find a significant difference in overall patient survival in NSCLC patients treated with nivolumab vs. those treated with both nivolumab and tocilizumab (8.7 vs. 6.1 months; $p = 0.37$) (p. 554). A survival difference was found between patients who were treated with nivolumab without tocilizumab and patients treated with tocilizumab who did not respond to tocilizumab treatment (HR: 3.44; 95% CI—1.38–8.59; $p = 0.0079$) (p. 554). Tocilizumab treatment induced improvements in more

than 75% of patients, making it a strong treatment for the management of irAEs, however treatment does not significantly increase overall patient survival.

Lee et al. (2019) found similar results to the studies of Lee et al. (2016) and Stroud et al. (2019). Lee et al. (2019) treated a sample of 79 patients, diagnosed with autoimmune encephalitis and confirmed by associated antibodies, with combination treatments of IVIG, rituximab, and tocilizumab. The variable most strongly associated with patient outcome was clinical responsiveness after three months (p. 4), and multivariate analysis showed an association between IVIG, rituximab, and tocilizumab combination and a higher frequency of patients who exhibited clinical responsiveness at three months (p. 4). The frequency of serious adverse effects was similar across all treatment regimens (p. 4). Rituximab and tocilizumab treatment in combination can improve clinical responsiveness of patients with autoimmune encephalitis after three months, suggesting the potential advantage of the combination's use against encephalitis and other neurological irAEs.

Chang et al. (2020) found similar results when treating patients who developed autoimmune encephalitis after treatment with ICIs. According to Chang et al., consciousness was recovered in between two and six days after patients were treated with methylprednisolone (1g daily for five days), IVIG (2g/kg total over 5 days), and in the case of two patients, rituximab and tocilizumab, but the mRS scores of patients did not change after recovery (pp. 2244-2245). In one instance, however, a patient ultimately died despite use of rituximab and tocilizumab. The patient had meningitis, limbic and brainstem encephalitis, cranial nerve palsies, and Guillain–Barre syndrome, and the patient was seen in the ICU for seizures. The patient's MRI scan showed T2 signal changes in the brainstem (p. 2246). Chang et al. claimed that steroids and IVIG were not adequate to treat the patient's condition, and rituximab and tocilizumab were used

to supplement the treatment, but the patient's condition did not improve. The patient died after seven months due to cancer progression (p. 2246). While death occurred in only one case, use of rituximab and tocilizumab should be considered as a first-line treatment for neurological irAEs.

Rituximab and tocilizumab have become established treatments for the management of irAEs, however their effectiveness in treating and resolving neurological irAEs is limited. Rituximab and tocilizumab's use remains primarily as second-line therapies, however further study exploring the potential benefit of employing rituximab and tocilizumab in a first-line role after development of irAEs or even in tandem with initial treatment could help determine a method of preventing the development of neurological irAEs or mitigating their severity as soon as possible.

Proposal for New Treatment Regimens and Future Clinical Study

Current combination and monotherapy regimens of immunotherapies are inconsistent for appreciable treatment responses, and the most common immunotherapeutics currently in use (nivolumab, ipilimumab, pembrolizumab, and other PD-1/PD-L1 and CTLA-4 inhibitors) are known to be associated with neurological irAEs, underscoring a need for further clinical trials and research to determine the most optimal immunotherapy treatment regimen for the mitigation of neurological irAEs.

ICIs are the primary immunotherapeutic used in the treatment of cancer, and the use of ICIs as monotherapies and in combinations often succeeds in promoting a stronger antitumor response compared to contemporary treatments. Exploration into the antibody blockade of novel targets such as the CCL1/CCR8 pathway found that blockade successfully mobilized a strong antitumor response by immune effector cells, reduced immune suppression by Tregs, and treatment was specific to immune cells within the TME. Despite the considerable potential for

CCL1/CCR8 inhibitors as an anticancer immunotherapeutic, current data is limited to in vitro and mouse model studies. Immunosuppressive treatments, namely rituximab and tocilizumab, have become established within the management plan of neurological irAEs as a second-line treatment, however their use as first-line treatments or even in tandem with initial treatment for cancer has not been adequately studied (Chang et al., 2020).

For the treatment of NSCLC with ICIs, no single treatment regimen appears to induce a markedly stronger antitumor response while also maintaining little or no toxicity. Combination treatment with durvalumab and tremelimumab revealed that treatment responses are sensitive to dosages and the incidence of irAEs can be noticeably affected by dose changes (Antonia et al., 2016, pp. 304-305). NSCLC patients may benefit from further studies investigating the most optimal dosage and ICI combinations associated with durable antitumor response and low incidence of neurotoxicity, for example through dose escalation studies in which treatment drugs are given to various dose cohorts from 3mg/kg to 20mg/kg and at frequencies of two to four weeks to establish the maximum tolerated dose, after which dose expansion would provide additional information about treatment characteristics. Despite the benefits of ICI treatment, the lack of specificity in the mechanism of function of ICIs suggests that treatment using ICIs may not be able to avoid the development of general or neurological irAEs. The genotypical and phenotypical diversity of NSCLC and cancer in general means that one combination of ICIs is unlikely to be consistently effective across all patients.

Further study determining reliable biomarkers may aid oncologists by guiding treatment using ICIs, because established biomarkers, such as PD-L1, are not completely reliable in predicting treatment response and behavior. For patients treated with pembrolizumab and chemotherapy, significantly different antitumor response rates between

pembrolizumab/chemotherapy and chemotherapy alone were observed across all PD-L1 tumor positivity scores (Gandhi et al., 2018, p. 2084). Based on data connecting HLA-B27 positivity to neurological irAEs, atezolizumab treatment should only occur after screening for the HLA-B27 genotype (Chang et al., 2020, pp. 2243-2250). The experimental study of similar biomarkers may help oncologists by informing treatment routes, and biomarkers may also provide a means of understanding pathogenesis of neurological irAEs, allowing certain treatment combinations or individual drugs to be phased out in favor of less harmful alternatives.

The antibody blockade of the novel, immune-related CCL1/CCR8 pathway, has proven to induce a potent and specific antitumor response *in vitro* and in mouse models of breast, colorectal, and colon cancer, mobilizing immune effector cells to the TME while reducing immune suppression by Tregs within the TME (Hoelzinger et al., 2010; Villarreal et al., 2018). Cancer treatment as a whole could benefit from further studies into the interactions of αCCL1/CCR8 treatment within the body and the treatment's safety for *in vivo* cancer treatment. New studies could investigate outcomes in mice or primate models before moving to clinical trials. Cancer patients could benefit greatly if αCCL1/CCR8 treatment is determined to be safe for testing in clinical trials, as durable tumor responses could be reached in a larger proportion of patients than with ICI therapy. The specificity of αCCL1/CCR8 treatment to tumor-infiltrating Tregs suggests that αCCL1/CCR8 treatment could induce durable antitumor effects without risking off-target autoimmunity resulting in irAEs, and αCCR8 treatment reduces the expression of inhibitory molecules on tumor-infiltrating Effector T cells, so αCCL1/CCR8 may treat cancer patients without requiring supplementary immune checkpoint inhibition and while avoiding the development of irAEs.

Combination therapy of αCCR8/*Listeria*-based vaccination has also proven to enhance antitumor response, and observed responses to targeted tumor-antigens were cooperatively strengthened (Villarreal et al., 2018, p. 5344). Further exploration into the interactions of cancer vaccinations with immunotherapies, both ICIs and novel treatments, would be beneficial to determine the relative toxicity associated with cancer vaccination and the degree of antitumor response. Due to the complex role of IL-6 in immune function, investigating potential novel combinations with existing treatments may also be valuable, for example the interactions of CpG-ODN/αCCL1 treatment and the IL-6 inhibitor tocilizumab. Targeting treatments towards parts of the immune system not directly involved in tumor cell cytotoxicity, such as Tregs and the cytokine system, may enhance antitumor response of effector cells without direct interactions, such as with ICI treatment.

Despite the established role of immunosuppressive drugs such as rituximab and tocilizumab in treating irAEs developed secondary to ICI therapy, the use of rituximab and tocilizumab in a first-line role remains limited, and both drugs have yet to be tested in a combination initial treatment with ICIs. Rituximab and tocilizumab are effective in improving conditions of patients with neurological irAEs though have limited benefit in creating more favorable outcomes or increasing survival (Lee et al., 2016; Lee et al., 2019; Stroud et al., 2019). Because of the limited benefits conferred by rituximab and tocilizumab treatment, incorporating one or both drugs into an initial treatment regimen could potentially prevent the development of major symptoms or neurological irAEs altogether.

Although the rate of neurological irAEs in NSCLC patients treated with immunotherapy is considerably low, the incidence of fatal neurological irAEs may be significant, as lung cancer and NSCLC account for the majority of cancer cases. Currently, ICIs remain the primary option

for cancer immunotherapy, however cancer treatment could be greatly improved by further clinical study exploring more effective treatment options with lesser associated toxicity. If clinical study can determine an ideal treatment regimen using ICIs, traditional cytotoxic chemotherapy could be phased out completely in favor of immunotherapy. αCCL1/CCR8 treatment or similar novel treatments also present as a potential replacement for ICIs as a whole if novel antibody blockade can induce treatment response while entirely avoiding toxic effects in clinical trials, but while new treatments are tested, further trials in expanding the use of rituximab and tocilizumab to first-line and initial treatment roles in combination with ICIs could help improve existing treatment regimens.

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