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A literature review on tumor microenvironmental immunosuppressive mechanisms on CD8+ T lymphocytes that contribute to tumor immune evasion

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Abstract

The concept of tumor immune evasion is a prevalent obstacle in cancer research and the development of immunotherapies. Increased insight into mechanisms that are responsible for the escape of cancer cells from the immune system will help to improve therapeutic approaches and provide understanding into slowing disease progression by utilizing the body's immune system. With immunotherapy development on the forefront of cancer research due to seemingly attenuated toxicity and resistance when compared to traditional chemoradiotherapy, analyzing these obstacles is essential for emerging roles and future directions in the discipline of immuno-oncology. Understanding the immunosuppressive molecules that contribute to the process of tumor-induced immune evasion, as well as the immunomodulatory molecules that may help bypass these barriers in cancer treatment, will help to provide insight into new therapeutic development and increase efficacy of existing treatments. A subtype of T-lymphocyte, CD8+ cytotoxic T cells, are key players in viral infections and in the tumor microenvironment, so when their effector functions are limited or inhibited, the body becomes less capable of fighting the cancer and more susceptible to disease progression and metastasis. Insight into how these mechanisms impact the activity within the tumor microenvironment is pertinent in the ongoing battle to develop novel, efficacious cancer treatments. In this review, a few key pathways that contribute to immune evasion and suppression of these CD8+ T cells will be discussed, as well as immunotherapies that are focused on combating the obstacles that these mechanisms introduce.

Introduction

Typically, the key players in the immune system are able to recognize foreign and potentially harmful antigens, initiate inflammatory responses, induce phagocytosis of possibly infected cells,

and develop adaptive responses in order to acquire memory against invaders. Adaptive responses involve antigen-specific immune cells and the subset that will be focused on in this review are known as CD8+ cytotoxic T lymphocytes. This subset is primarily responsible for detection and destruction of virally infected cells and cancer cells by recognizing foreign antigens presented on MHC I proteins, which are present on all nucleated cells. CD8+ T cells then secrete antimicrobial cytokines as well as cytotoxic granules, which are released into the targeted cell and shut down production of viral proteins while also inducing apoptosis, or programmed cell death. Due to the fact that cancer cells typically have foreign antigens presented on their surfaces, these mechanisms of action should allow CD8+ T cells to disrupt progression of disease. However, cancer cells utilize a wide variety of mechanisms at the microenvironmental level that result in tumor immune evasion. Tumor immune evasion refers to the phenomenon in which cancer can continue to progress and metastasize by avoiding the immune system's mechanisms of recognition and attack. Tumor-induced immunosuppression has various mechanisms, most of which revolve around the recruitment and activation of immunosuppressive cells in the tumor microenvironment which secrete factors that contribute to immune system evasion. Another mechanism of tumor-induced immunosuppression involves the expression of immunosuppressive molecules and receptors on the surface of tumor cells. These are known as immune checkpoints and their overexpression, commonly characterized by cancer cells, can lead to the inhibition of immune responses and therefore contribute to the progression of disease and resistance to treatment. Checkpoint blockade therapy has revolutionized the field of immuno-oncology as one of the most prominent and up-and-coming immunotherapies. These drugs are designed to interfere with inhibitory pathways that restrict immune responses against cancer cells, thus combating this tumor immune evasion and provoking a response. This review

will focus on a few key mechanisms that contribute to the suppression of CD8+ T cell effector functions in the tumor microenvironment, serving as a barrier to immune responses and cancer therapies.

Myeloid-derived suppressor cells

The myeloid lineage of immune cells plays a critical role in responses against infection, promoting inflammation, and linking the adaptive and innate immune responses. Fully differentiated cells of this lineage include dendritic cells, monocytes, mast cells, and granulocytes. A subset of these called myeloid derived suppressor cells are myeloid progenitors that have never fully differentiated into one of the previously mentioned cell types (Gabrilovich et. al., 2017). They are typically found in bone marrow, peripheral blood, spleen, liver, lungs, and certain tumor types (Gabrilovich et. al., 2017). Myeloid derived suppressor cells are thought to play a role in processes such as chronic inflammation, infection, autoimmune diseases, trauma, graft versus host disease, and are now a prevalent area of interest in tumor immunology (Gabrilovich et. al., 2017). MDSCs have been shown to contribute to immunosuppression significantly, with their main target being T cells. Tumor and host cells in the tumor microenvironment produce several pro-inflammatory molecules that activate and recruit MDSCs to the tumor microenvironment, driving their suppressive activity. MDSCs have been isolated from human cancer patients who exhibit high levels of these cells, and these high levels have shown correlation with clinical cancer staging and metastatic burden of disease (Law et al., 2020. Specific cancer types that frequently are associated with high levels of MDSCs include non-small cell lung cancer, multiple myeloma, non-Hodgkin's lymphoma, renal cell carcinoma, esophageal cancer, sarcomas, breast cancers, pancreatic adenocarcinoma, and colorectal cancer (Damuzzo et. al., 2014). The transcription factors in the STAT family, such as STAT1, STAT3,

STAT6, and NFκB, are significant regulators of MDSC recruitment and activation. Cancer cells and tumor-associated stromal cells play roles in activating these pathways (Law et al., 2020). Previous findings have shown a significant correlation between chronic inflammation and malignant growth, as well as the concept of inflammation-induced MDSC recruitment and activity. Interleukin-1β (IL-1β) is another key player in MDSC activity, as well as in the suppression of CD8+ T cell function. It is a pro-inflammatory cytokine that is secreted by many tumor types and other cells in the tumor microenvironment. Tumor-derived IL-1β secreted into the tumor microenvironment has been shown to induce the accumulation of MDSC possessing an enhanced capacity to suppress T cells (Elkabets et. al., 2010). The IL-1 pathway in CD8+ T cells is far from being fully understood, but it has been shown that IL-1R$^{-/-}$ CD8+ effector cells contain transcripts for perforin and granzyme, but only express perforin (Joeckel et al., 2012). Lack of granzyme results in inhibition of in vitro apoptosis by CD8+ T cells unless exogenous granzyme is present. Overall results of this study showed significant reduction in expansion of antigen-specific CD8+ T cells, leading to the conclusion that the IL-1/IL-1R pathway is crucial for full maturation of functionally competent CD8+ T cells (Joeckel et al., 2012). When the IL-1β pathway was perturbed in mice with 4T1 mammary carcinoma tumors by removing the IL-1β receptor, IL-1R, the extent of inflammation was manipulated. A delay in MDSC accumulation was observed, along with slowed tumor progression (Bunt et al., 2007). Essentially, when tumor cells and other cells of the tumor microenvironment secrete IL-1, it contributes to the recruitment and activation of these myeloid derived suppressor cells, which further the immunosuppressive activity that is seen as an obstacle for many cancer therapies. It was also hypothesized that IL-6, another pro-inflammatory mediator, is a downstream regulator of IL-1β's inflammatory and tumor-promoting functions, and that transfecting these same mice

with the human *IL-6* gene should compensate for the loss of IL-1R (Bunt et al., 2007). No delay in progression of 4T1/IL-6 tumors was observed in IL-1R$^{-/-}$ mice with the transfected *IL-6* gene, and they seemed to progress at a similar rate as 4T1/IL-6 tumors in the traditional mouse model (Bunt et al., 2007). Because of the link between IL-1 and IL-6 in primary tumor site activity, the metastatic activity of these tumors was also analyzed. Metastatic disease progression to the lungs also progressed in a similar fashion, proposing the idea that IL-6 also serves as a downstream mediator of IL-1 in metastatic mechanisms and that IL-6 does in fact compensate for the loss of IL-1R in disease-progressive mechanisms. To summarize these findings, IL-1 induced MDSC recruitment can lead to immunosuppressive activity in the tumor microenvironment as well as depletion of the population of functional CD8+ T cells. On another hand, LCK is a protein associated with the cytoplasmic tails of the co-receptors of CD4+ and CD8+ T cells and has been shown to aid in signaling and activation of these T cells (Feng, et al., 2018). MDSCs have been shown to be involved in the nitration of LCK, impairing activation and signaling pathways and contributing to CD8+ T cell-specific immunosuppression. LCK nitration has also shown reduced levels of IL-2 in the tumor microenvironment (Feng, et al., 2018). IL-2 is a common target for immunotherapies as it is strongly correlated with T cell differentiation and proliferation, acquisition of effector functions, and cytotoxic activity. Recombinant IL-2 therapy shows partial and complete regression of disease alone, and has been utilized as a combination therapy alongside other cancer treatments, showing positive clinical outcomes (Choudry, et al., 2018). Therefore, the inhibitory effects of MDSCs on IL-2 would hinder the regression of disease and limit the therapeutic potential of the cytokine. Another signaling cascade that is modified by the presence of MDSCs involves TCR-CD3 signaling. CD3 is a T cell co-receptor that is involved in activating both the CD4+ and CD8+ subsets of T cells. It is believed that the integrity of the

TCR-CD3 complex is altered by MDSCs, as well as the physical interaction between TCR and CD8+ (Nagaraj, et al., 2010). The obstruction of these signaling pathways prevents the immune system from generating tumor antigen-specific CD8+ T cells and serves as a significant mechanism of tolerance in systemic CD8+ T cells as well as the CD8+ T cell population of the tumor microenvironment (Nagaraj, et al., 2010). A promising area of immunotherapeutic research revolves around the induction of MDSC differentiation. All-trans-retinoic-acid (ATRA) has been utilized to inhibit retinoic signaling to promote differentiation and shift MDSCs into mature myeloid cells, such as macrophages and dendritic cells. ATRA has resulted in the attenuation of T cell suppression and mice and patient models of multiple types of cancers have exhibited reduced levels of circulating MDSCs, enhanced response to cancer vaccine treatments, improved dendritic cell function, and enhanced antigen-specific T cell response (Law, et al., 2020). Another potential treatment discussed in this study was the utilization of MDSC depletion combined with other immunotherapies. Checkpoint inhibitors anti-PD1 and anti-CTLA4 combined with epigenetic modulatory drugs, which have been used to functionally inhibit MDSCs, resulted in complete tumor regression and metastatic progression of TNBC cancer cell line 4T1 with over an 80% survival rate 100 days post-tumor implantation (Law, et al., 2020). When the two epigenetic modulatory drugs used in that study, entinostat and 5-azacytidine, were used together but not in combination with the checkpoint inhibitors, the primary and metastatic progression remained, leading to the hypothesis of the synergistic effects of combining checkpoint inhibitors and MDSC-targeted therapy. There are some complexities and challenges in targeting MDSC populations in cancer therapy. One of the most prevalent obstacles is the heterogeneity of MDSC composition. MDSCs have shown that they lack the markers associated with mature myeloid cells, so identifying the presence and overall impact of MDSCs in a cancer

patient has been difficult to execute (Damuzzo, et al., 2014). Research focused around identifying further markers and morphological characteristics of MDSCs in human and murine models is underway, which will provide more insight into utilizing the tendencies and pathways that we are aware of in these immunosuppressive cells for immunotherapeutic development.

The PD-1/PD-L1 pathway

The PD-1/PD-L1 pathway plays a crucial role in the promotion of self-tolerance and the inhibition of immune responses in the tumor microenvironment by attenuating and manipulating T cell activity, inhibiting apoptosis of regulatory T cells, and inducing apoptosis of antigen-specific T cells (Han et. al., 2020). PD-1 is a receptor on the surface of activated T cells, NK cells, B cells, macrophages, dendritic cells, and monocytes, while PD-L1, programmed death ligand 1, is its ligand that is present on antigen presenting cells, such as dendritic cells and macrophages, some activated T cells and B cells, and some epithelial cells under inflammatory conditions. Typically, PD-L1 is utilized as a co-inhibitory factor in this pathway to inhibit immune responses and self-tolerance to ensure that the immune system is only activated at appropriate times to minimize chronic inflammation and autoimmune responses (Qin et. al., 2019). The molecule serves a dual role as being beneficial and detrimental. It is beneficial in the way that its promotion of self-tolerance and inhibition of responses helps to downplay recognition of self, therefore limiting autoimmune responses. However, this pathway is largely responsible for cancer immune escape and resistance mechanisms to adaptive immune responses and cancer treatments. Certain proinflammatory cytokines present in the tumor microenvironment such as IFN-γ, IL-6, and TNF-α are responsible for the upregulation of expression of PD-L1 on tumor cells (Chan et al., 2019). This phenomenon results in attenuation of immune responses and progression of disease. Amplification and translocations present in the

genomic region of PD-L1 have been shown to lead to upregulation of PD-L1. These genomic rearrangements have been shown to play a role in tumor immune evasion in cancers such as primary mediastinal large B cell lymphoma (PMBCL), NSCLC, squamous cell carcinoma, and gastric adenocarcinoma (Green, et. al., 2010). While certain mechanisms have been studied that specifically impair the function of CD8+ T cells, there has also been a correlation seen between the inhibition of cytotoxic T cell activity and the amplification of immune-inhibitory cell types. In a study looking at PD-L1 expressing CD8+ T cells in melanoma patients, it was shown that an increase in PD-L1+ CD8+ T cells correlates with markers indicative of a negative immune climate. These markers include a decrease in plasmacytoid dendritic cells, an increase in myeloid derived suppressor cells, and a strong correlation with the presence of regulatory T cells (Brochez, et. al., 2018). This study also demonstrated an increase in PD-L1+ CD8+ T cells in patients who relapsed and/or died from melanoma, leading to the conclusion that increased PD-L1 expression on these lymphocytes is correlated with poor prognoses. An interesting mechanism of metastatic progression in many cancer cell lines is epithelial-mesenchymal transition (EMT). EMT enables carcinoma cells to suppress their epithelial features and begin expressing mesenchymal ones. This phenomenon gives tumor cells the ability to acquire mobility and migrate from the primary site of the tumor, resulting in disease progression and metastasis (Ramos et. al., 2017). The presence of EMT in cancer has been strongly associated with the exhaustion of CD8+ T cells in the tumor microenvironment (Romeo et. al., 2019), but also has been shown to be positively associated with PD-L1 levels (Chen et. al., 2017). In a study analyzing the role of PD-L1 in molecular pathways, specifically EMT, in a human esophageal cell line, it was found that tumors in the EMT positive subgroup also exhibited higher levels of PD-L1 expression than tumor samples in the EMT negative subgroup (Chen et. al., 2017). In this

same study, it was found that manipulating the cell lines to overexpress PD-L1 resulted in a significant promotion of cell viability, migration, and EMT phenotype compared to cell lines with ablation of PD-L1. These findings exhibit the correlation between PD-L1 overexpression and epithelial-mesenchymal transition, which is a significant contributing factor to disease progression and metastasis, while CD8+ T cell populations are exhausted and dysfunctional. It has been suggested that therapeutic approaches designed to reverse EMT induced immunosuppression may provide synergistic therapeutic effects combined with PD-L1 blockade (Jiang et. al., 2020). Alongside the PD-1/PD-L1-dependent indirect mechanisms in which T cell populations are attenuated and functions are suppressed in the tumor microenvironment, there is also data surrounding the direct effect of PD-L1 expression on CD8+ T cells. PD-L1 is upregulated on T cells in the tumor microenvironment following antigen presentation and as a response to inflammatory conditions (Diskin, et. al., 2020). Findings in this study indicate the suppression of neighboring T cells in the tumor microenvironment in the presence of PD-L1+ T cells, suggesting that the upregulation of PD-L1 on T cells maintains intra-tumoral immune tolerance via both innate and adaptive mechanisms. It was found that *Pdl1*$^{-/-}$ T cells were permissive of cytotoxic T cell activation, while *Pdl1*$^{+/+}$T cells had an inhibitory effect on cytotoxic T cell activation. To summarize, the expression of PD-L1 on T cells in the tumor microenvironment directly correlates with suppressive activity of CD8+ T cells in the tumor microenvironment, and the depletion of functional CD8+ T cells is observed secondary to disease progression stimulated by PD-L1 expression on other cell types. While there are significant obstacles presented by PD-L1 overexpression in the tumor microenvironment, the PD-1/PD-L1 pathway is a promising and progressive area of immunotherapeutic research. An approach that is frequently utilized is known as checkpoint blockade therapy, where anti-PD-1

antibodies are administered which bind to PD-1. This prevents PD-1/PD-L1 interaction, therefore inhibiting the immunosuppressive activity that is seen when the two bind (Martin-Ruiz, et. al., 2020). This approach has been widely tested and has shown significant, and very promising, effects on disease progression, recurrence, and patient survival in those with PD-L1 positive tumors. To date, five PD-1/PD-L1 targeted immunotherapies have been approved by the Food and Drug Administration, two anti-PD-1 antibodies, nivolumab and pembrolizumab, and three anti-PD-L1 antibodies, avelumab, atezolizumab, and durvalumab (Qin et. al., 2019). Nivolumab has been approved for the treatment of non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), bladder cancer (BC), colorectal cancer with microsatellite instability or mismatch repair deficiency (MSI-H/dMMR), hepatocellular carcinoma (HCC), classic Hodgkin's lymphoma (cHL), melanoma, and head and neck squamous cell carcinoma (HNSCC). Pembrolizumab has been approved for melanoma, stomach and gastroesophageal cancers, cervical cancer, BC, HNSCC, NSCLC, cHL, and all advanced solid tumors with MSI-H/dMMR (Qin et. al., 2019). Avelumab has been approved for Merkel cell carcinoma and BC. Atezolizumab has been approved for NSCLC and BC. Durvalumab has been approved for BC and stage III NSCLC (Qin et. al., 2019). Despite the fact that several cancer types are approved to be treated by these drugs, many of them overlap and there is limited potential for treatment using this approach for other cancer types not listed. The approval of these drugs by the FDA and promising results in clinical trials is a step in the right direction when it comes to PD-1/PD-L1-related immunotherapies, but significant obstacles are still present. The lack of long-term data, as well as minimal data about the utilization of these drugs in combination with other cancer therapies, makes it difficult to analyze what the most effective combination of therapies is for certain cancer types. Another obstacle is the development of immune-related

adverse events (irAEs). The addition of these immunotherapies to chemotherapy increases the presence of irAEs significantly. For example, a study showed that 53.6% of patients receiving atezolizumab in combination with chemotherapy developed irAEs, whereas only 29.1% of patients receiving chemotherapy alone presented with these adverse events (Qu, et. al., 2021). The final obstacle that will be touched on, that has minimal studies revolving around it, is the resistance to PD-1/PD-L1 therapies. The resistance mechanism is particularly tricky and there has been very limited work done on uncovering the tumor-related antigens associated with development of this resistance (Qu, et. al., 2021). With more research focused on the PD-1/PD-L1 therapy resistance mechanism and its associated antigens, the prevalence and prevention of irAEs, and insight to new combination therapies, the PD-1/PD-L1 pathway will likely be a frontline target for immunotherapeutic development and usage, and potentially in more cancer types.

Cytotoxic T-lymphocyte associated antigen (CTLA-4)

Cytotoxic T-lymphocyte associated antigen (CTLA-4) is a CD28 homolog molecule that binds to a costimulatory molecule, B7, on antigen presenting cells to heighten the activation threshold of T cells. When CD28 binds B7, this provides a secondary signal to activate the T cell after T-cell receptor:MHC I/antigen binding, while CTLA-4:B7 binding halts T cell activation (Rowshanravan et. al., 2018). Inhibition of T cell activation and proliferation leads to a decrease in the number of T cells capable of their effector functions, which further allows tumor immune evasion and disease progression. Aside from this function in the tumor microenvironment, it has also been hypothesized that CTLA-4 produces tonic inhibitory signals in the early stages of tumorigenesis, increasing T cell activation thresholds and weakening immune responses against the cancer cells (Intlekofer et. al., 2013). Along with PD-1, CTLA-4 is one of the most

commonly studied immune checkpoints in the tumor microenvironment, as well as one of the most influential on cancer progression and treatment response. Aside from the cancer perspective, CTLA-4 is also expressed on regulatory T cells and memory T cells and functions to prevent autoimmunity by inhibiting activation of self-reactive T cells (Wolchok et. al., 2008). Relatively early studies of this molecule showed that germline deletion of *Ctla4* has been associated with massive lymphoproliferation and fatal multiorgan tissue destruction (Tevol, et. al., 1995), while mutations of *Ctla4* were associated with immune dysregulation and ultimately autoimmune disorders (Kuehn, et. al., 2014). CTLA-4 expression is frequently upregulated on cancer cells as well as tumor infiltrating lymphocytes (TILs) in the tumor microenvironment. Expression levels are commonly used as an indicator of prognostic capabilities in malignant disease. In terms of expression, a study revolving around CTLA-4 expression on the dendritic cells of hepatocellular carcinoma (HCC) patients displayed that CD14+ DCs in these patients highly expressed CTLA-4. It was found to be crucial for production of molecules that significantly inhibit T cell function, as well as suppression of antitumor immune responses, therefore contributing to progression of disease in these patients (Han, et. al., 2013). There are also several findings showing the difference in expression levels of CTLA-4 between cancer patients and healthy volunteers. For example, non-small cell lung cancer (NSCLC) patients demonstrated a much higher proportion of regulatory T cells and significantly increased CTLA-4 expression when compared to healthy volunteers. This study also demonstrated a correlation between CTLA-4 expression levels and NSCLC disease staging and metastatic progression (Erfani, et. al., 2012). Due to the fact that Tregs and CTLA-4 are the main inhibitory cells and molecules of the immune system (Erfani, et. al., 2012), inhibition of CTLA-4 appears to be a promising area in immunotherapeutic development. There have been numerous preclinical

studies in murine models that have provided confirmation of CTLA-4-blockade induced enhancement of antitumor immunity as well as the potential of synergistic effects when used with other cancer therapies (Peggs, et. al., 2006). In one murine model study, it was hypothesized that temporary removal of CTLA-4–mediated inhibition may lead to the enhancement of anti-tumor immunity (Egen, et. al., 2002). Initial experiments in this study used two murine tumor cell lines, a colon carcinoma and a fibrosarcoma, and administration of antibodies that would block the interaction of CTLA-4 and B7 resulted in tumor rejection post-implantation. This phenomenon also came along with the findings of long lasting immunity in the absence of further treatment. These findings were less prominent in less immunogenic tumors, which showed a much weaker response to anti-CTLA-4 therapy (Wolchok, et. al., 2008). This study served as a proof-of-purpose study, and since the publication there has been a wealth of data and knowledge about this molecule in the context of immunotherapy. Two human monoclonal anti-CTLA-4 IgG antibodies have been produced using transgenic mouse technologies, ipilimumab and tremelimumab (Arce Vargas et. al., 2018). They have both been utilized in several cases of advanced stage cancers. Reports from human trials in melanoma, non-small cell lung cancer, mesothelioma, prostate, ovarian, breast and urothelial cancer treatment have displayed a level of efficacy (Arce Vargas et. al., 2018). Alongside the promising data, a variety of immune related adverse events (irAEs) have been observed in 60-65% of patients. These frequently impacted the skin, gastrointestinal tract, liver, and endocrine organs. Another observation has been the presence of autoimmunity in several other organs including the liver, eyes, nervous system, lungs, heart, kidneys, and joints (Jia et. al., 2020). These antibodies have also shown correlation with an increase in the diversity of T-cell receptor repertoire (Rowshanravan et al., 2018), which is consistent with the idea that inhibition of CTLA-4 would

lead to an increase in T cell activation and proliferation, which would come with an increase in diversity of T cells that could have the potential to combat cancer cells. Aside from the direct effects observed, immunological effects such as immune cell infiltration into tumors and tumor necrosis have also been witnessed. When compared with anti-PD-1 checkpoint blockade therapy, CTLA-4 comes with increased toxicity, limitations to fewer cancer types, and a lower recurrence-free survival rate (Liu, et. al., 2020). Another significant obstacle arises when the necessity for CTLA-4 in prevention of autoimmune disorders is considered. Future directions for CTLA-4 immunotherapy have been strongly contemplated. Some of these include acquiring further knowledge on other cancer types that may respond well to this therapy, maneuvering around the autoimmunity that comes with blockade of CTLA-4, prevention or attenuation of adverse events, and further studies on the mechanisms of overexpression of CTLA-4 found in the tumor microenvironment. Acquisition of knowledge in these areas will improve efficacy of drugs in the development phase, provide further understanding on the therapy and its mechanisms, and make CTLA-4 blockade a more promising target in the field of immunotherapeutic development.

Conclusion and future directions

Cancer continues to be among the leading causes of death worldwide with new cancers still being discovered, sometimes more aggressive and less responsive to treatment. Numbers are still predicted to rise in the coming years, however this increase in occurrence does not necessarily correlate with an increase in new therapeutic developments. New approaches and technologies in oncology are essential to combat the rising numbers, and with immuno-oncology on the forefront of cancer research, emerging immunotherapies are hoped, and expected, to yield promising clinical benefits as new knowledge is uncovered. To develop new therapies in this field, having a full understanding of the immune mechanisms that are in favor of or against disease progression

is crucial. Checkpoint blockade therapies have demonstrated encouraging data, both pre-clinically and clinically, and advancing this approach by gaining more insight into the immune-cell makeup and behavior in the tumor microenvironment is a promising future direction for the field of immuno-oncology. Gaining more understanding about disease responses and patient outcomes to immunotherapies in order to minimize toxicity and adverse events from these new therapies is essential to ensure safety of these treatments alongside efficacy. It will also be crucial to analyze general immune function in these patients during and after these treatments as long-term effects of immunotherapy on immune function are not well understood. An important aspect of this will be to study the duration of immunity by monitoring for recurrence of disease after successful treatment with immunotherapy. Similarly, monitoring for development of resistance to these therapies is a critical role of immunologists and oncologists as the utilization of these treatments becomes conventionalized. Immunotherapy provides a strong sense of optimism for those suffering from cancers that have shown significant responses to already-developed immunotherapies both clinically and pre-clinically, however there is still an abundance of work that can be done in terms of targeting other cancer types. As the field progresses, it will be vital to begin to target the previously mentioned checkpoints, as well as others, for a wider variety of cancers in order to maximize the number of patients able to benefit from immunotherapy. These future directions put us in a promising position to yield safe and efficacious approaches to the development of novel immunotherapeutics.

Citing Literature

1. Gabrilovich D. I. (2017). Myeloid-Derived Suppressor Cells. *Cancer immunology research*, *5*(1), 3–8. https://doi.org/10.1158/2326-6066.CIR-16-0297
2. Bunt, S. K., Yang, L., Sinha, P., Clements, V. K., Leips, J., & Ostrand-Rosenberg, S. (2007). Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. *Cancer research*, *67*(20), 10019–10026. https://doi.org/10.1158/0008-5472.CAN-07-2354
3. Joeckel, L. T., Wallich, R., Metkar, S. S., Froelich, C. J., Simon, M. M., & Borner, C. (2012). Interleukin-1R Signaling Is Essential for Induction of Proapoptotic CD8 T Cells, Viral Clearance, and Pathology during Lymphocytic Choriomeningitis Virus Infection in Mice. *Journal of Virology*, *86*(16), 8713–8719. https://doi.org/10.1128/jvi.00682-12
4. Feng, S., Cheng, X., Zhang, L., Lu, X., Chaudhary, S., Teng, R., Frederickson, C., Champion, M. M., Zhao, R., Cheng, L., Gong, Y., Deng, H., & Lu, X. (2018). Myeloid-derived suppressor cells inhibit T cell activation through nitrating LCK in mouse cancers. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(40), 10094–10099. https://doi.org/10.1073/pnas.1800695115
5. Choudhry, H., Helmi, N., Abdulaal, W. H., Zeyadi, M., Zamzami, M. A., Wu, W., Mahmoud, M. M., Warsi, M. K., Rasool, M., & Jamal, M. S. (2018). Prospects of IL-2 in Cancer Immunotherapy. *BioMed research international*, *2018*, 9056173. https://doi.org/10.1155/2018/9056173
6. Nagaraj, S., Schrum, A. G., Cho, H. I., Celis, E., & Gabrilovich, D. I. (2010). Mechanism of T Cell Tolerance Induced by Myeloid-Derived Suppressor Cells. *The Journal of Immunology*, *184*(6), 3106–3116. https://doi.org/10.4049/jimmunol.0902661

7. Law, A., Valdes-Mora, F., & Gallego-Ortega, D. (2020). Myeloid-Derived Suppressor Cells as a Therapeutic Target for Cancer. *Cells*, *9*(3), 561. https://doi.org/10.3390/cells9030561
8. Damuzzo, V., Pinton, L., Desantis, G., Solito, S., Marigo, I., Bronte, V., & Mandruzzato, S. (2015). Complexity and challenges in defining myeloid-derived suppressor cells. *Cytometry. Part B, Clinical cytometry*, *88*(2), 77–91. https://doi.org/10.1002/cyto.b.21206
9. Elkabets, M., Ribeiro, V. S., Dinarello, C. A., Ostrand-Rosenberg, S., Di Santo, J. P., Apte, R. N., & Vosshenrich, C. A. (2010). IL-1β regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function. *European journal of immunology*, *40*(12), 3347–3357. https://doi.org/10.1002/eji.201041037
10. Jorgovanovic, D., Song, M., Wang, L., & Zhang, Y. (2020). Roles of IFN-γ in tumor progression and regression: a review. *Biomarker Research*, *8*(1), 1–16. https://doi.org/10.1186/s40364-020-00228-x
11. Chan, L. C., Li, C. W., Xia, W., Hsu, J. M., Lee, H. H., Cha, J. H., Wang, H. L., Yang, W. H., Yen, E. Y., Chang, W. C., Zha, Z., Lim, S. O., Lai, Y. J., Liu, C., Liu, J., Dong, Q., Yang, Y., Sun, L., Wei, Y., . . . Hung, M. C. (2019). IL-6/JAK1 pathway drives PD-L1 Y112 phosphorylation to promote cancer immune evasion. *Journal of Clinical Investigation*, *129*(8), 3324–3338. https://doi.org/10.1172/jci126022
12. Green, M. R., Monti, S., Rodig, S. J., Juszczynski, P., Currie, T., O'Donnell, E., Chapuy, B., Takeyama, K., Neuberg, D., Golub, T. R., Kutok, J. L., & Shipp, M. A. (2010). Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and

primary mediastinal large B-cell lymphoma. *Blood*, *116*(17), 3268–3277. https://doi.org/10.1182/blood-2010-05-282780

13. Jiang, Y., & Zhan, H. (2020). Communication between EMT and PD-L1 signaling: New insights into tumor immune evasion. *Cancer Letters*, *468*, 72–81. https://doi.org/10.1016/j.canlet.2019.10.013
14. Green, M. R., Monti, S., Rodig, S. J., Juszczynski, P., Currie, T., O'Donnell, E., Chapuy, B., Takeyama, K., Neuberg, D., Golub, T. R., Kutok, J. L., & Shipp, M. A. (2010). Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood*, *116*(17), 3268–3277. https://doi.org/10.1182/blood-2010-05-282780
15. Santos Ramos, F., Wons, L., João Cavalli, I., & M.S.F. Ribeiro, E. (2017). Epithelial-mesenchymal transition in cancer: An overview. *Integrative Cancer Science and Therapeutics*, *4*(3), 1–5. https://doi.org/10.15761/icst.1000243
16. Romeo, E., Caserta, C. A., Rumio, C., & Marcucci, F. (2019). The Vicious Cross-Talk between Tumor Cells with an EMT Phenotype and Cells of the Immune System. *Cells*, *8*(5), 460. https://doi.org/10.3390/cells8050460
17. Chen, L., Xiong, Y., Li, J., Zheng, X., Zhou, Q., Turner, A., Wu, C., Lu, B., & Jiang, J. (2017). PD-L1 Expression Promotes Epithelial to Mesenchymal Transition in Human Esophageal Cancer. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology*, *42*(6), 2267–2280. https://doi.org/10.1159/000480000

18. Diskin, B., Adam, S., Cassini, M. F., Sanchez, G., Liria, M., Aykut, B., Buttar, C., Li, E., Sundberg, B., Salas, R. D., Chen, R., Wang, J., Kim, M., Farooq, M. S., Nguy, S., Fedele, C., Tang, K. H., Chen, T., Wang, W., . . . Miller, G. (2020). PD-L1 engagement on T cells promotes self-tolerance and suppression of neighboring macrophages and effector T cells in cancer. *Nature Immunology*, *21*(4), 442–454. https://doi.org/10.1038/s41590-020-0620-x

19. Martín-Ruiz, A., Fiuza-Luces, C., Martínez-Martínez, E., Arias, C. F., Gutiérrez, L., Ramírez, M., Martín-Acosta, P., Coronado, M. J., Lucia, A., & Provencio, M. (2020). Effects of anti-PD-1 immunotherapy on tumor regression: insights from a patient-derived xenograft model. *Scientific Reports*, *10*(1), 1. https://doi.org/10.1038/s41598-020-63796-w

20. Han, Y., Liu, D., & Li, L. (2020). PD-1/PD-L1 pathway: current researches in cancer. *American journal of cancer research*, *10*(3), 727–742.

21. Qin, W., Hu, L., Zhang, X., Jiang, S., Li, J., Zhang, Z., & Wang, X. (2019). The Diverse Function of PD-1/PD-L Pathway Beyond Cancer. *Frontiers in Immunology*, *10*, 12. https://doi.org/10.3389/fimmu.2019.02298

22. Qu, J., Mei, Q., Liu, L., Cheng, T., Wang, P., Chen, L., & Zhou, J. (2021). The progress and challenge of anti-PD-1/PD-L1 immunotherapy in treating non-small cell lung cancer. *Therapeutic Advances in Medical Oncology*. https://doi.org/10.1177/1758835921992968

23. Wolchok, J. D., & Saenger, Y. (2008). The Mechanism of Anti‐CTLA‐4 Activity and the Negative Regulation of T‐Cell Activation. *The Oncologist*, *13*(S4), 2–9. https://doi.org/10.1634/theoncologist.13-s4-2

24. Intlekofer, A. M., & Thompson, C. B. (2013). At the bench: preclinical rationale for CTLA-4 and PD-1 blockade as cancer immunotherapy. *Journal of leukocyte biology*, *94*(1), 25–39. https://doi.org/10.1189/jlb.1212621

25. Egen, J. G., Kuhns, M. S., & Allison, J. P. (2002). CTLA-4: new insights into its biological function and use in tumor immunotherapy. *Nature Immunology*, *3*(7), 611–618. https://doi.org/10.1038/ni0702-611

26. Han, Y., Chen, Z., Yang, Y., Jiang, Z., Gu, Y., Liu, Y., Lin, C., Pan, Z., Yu, Y., Jiang, M., Zhou, W., & Cao, X. (2013). Human CD14+CTLA-4+regulatory dendritic cells suppress T-cell response by cytotoxic T-lymphocyte antigen-4-dependent IL-10 and indoleamine-2,3-dioxygenase production in hepatocellular carcinoma. *Hepatology*, *59*(2), 567–579. https://doi.org/10.1002/hep.26694

27. Arce Vargas, F., Furness, A., Litchfield, K., Joshi, K., Rosenthal, R., Ghorani, E., Solomon, I., Lesko, M. H., Ruef, N., Roddie, C., Henry, J. Y., Spain, L., Ben Aissa, A., Georgiou, A., Wong, Y., Smith, M., Strauss, D., Hayes, A., Nicol, D., O'Brien, T., … Quezada, S. A. (2018). Fc Effector Function Contributes to the Activity of Human Anti-CTLA-4 Antibodies. *Cancer cell*, *33*(4), 649–663.e4. https://doi.org/10.1016/j.ccell.2018.02.010

28. Jia, XH., Geng, LY., Jiang, PP. *et al.* The biomarkers related to immune related adverse events caused by immune checkpoint inhibitors. *J Exp Clin Cancer Res* 39, 284 (2020).

29. Erfani, N., Mehrabadi, S. M., Ghayumi, M. A., Haghshenas, M. R., Mojtahedi, Z., Ghaderi, A., & Amani, D. (2012). Increase of regulatory T cells in metastatic stage and CTLA-4 over expression in lymphocytes of patients with non-small cell lung cancer (NSCLC). *Lung Cancer*, *77*(2), 306–311. https://doi.org/10.1016/j.lungcan.2012.04.011

30. Rowshanravan, B., Halliday, N., & Sansom, D. M. (2018). CTLA-4: a moving target in immunotherapy. *Blood*, *131*(1), 58–67. https://doi.org/10.1182/blood-2017-06-741033

31. Peggs, K. S., Quezada, S. A., Korman, A. J., & Allison, J. P. (2006). Principles and use of anti-CTLA4 antibody in human cancer immunotherapy. *Current Opinion in Immunology*, *18*(2), 206–213. https://doi.org/10.1016/j.coi.2006.01.011

32. Tivol, E. A., Borriello, F., Schweitzer, A. N., Lynch, W. P., Bluestone, J. A., & Sharpe, A. H. (1995). Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity*, *3*(5), 541–547. https://doi.org/10.1016/1074-7613(95)90125-6

33. Kuehn, H. S., Ouyang, W., Lo, B., Deenick, E. K., Niemela, J. E., Avery, D. T., Schickel, J. N., Tran, D. Q., Stoddard, J., Zhang, Y., Frucht, D. M., Dumitriu, B., Scheinberg, P., Folio, L. R., Frein, C. A., Price, S., Koh, C., Heller, T., Seroogy, C. M., Huttenlocher, A., … Uzel, G. (2014). Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science (New York, N.Y.)*, *345*(6204), 1623–1627. https://doi.org/10.1126/science.1255904