Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

Tumor regression and allograft rejection after anti-PD-1 in a patient on chronic immunosuppression

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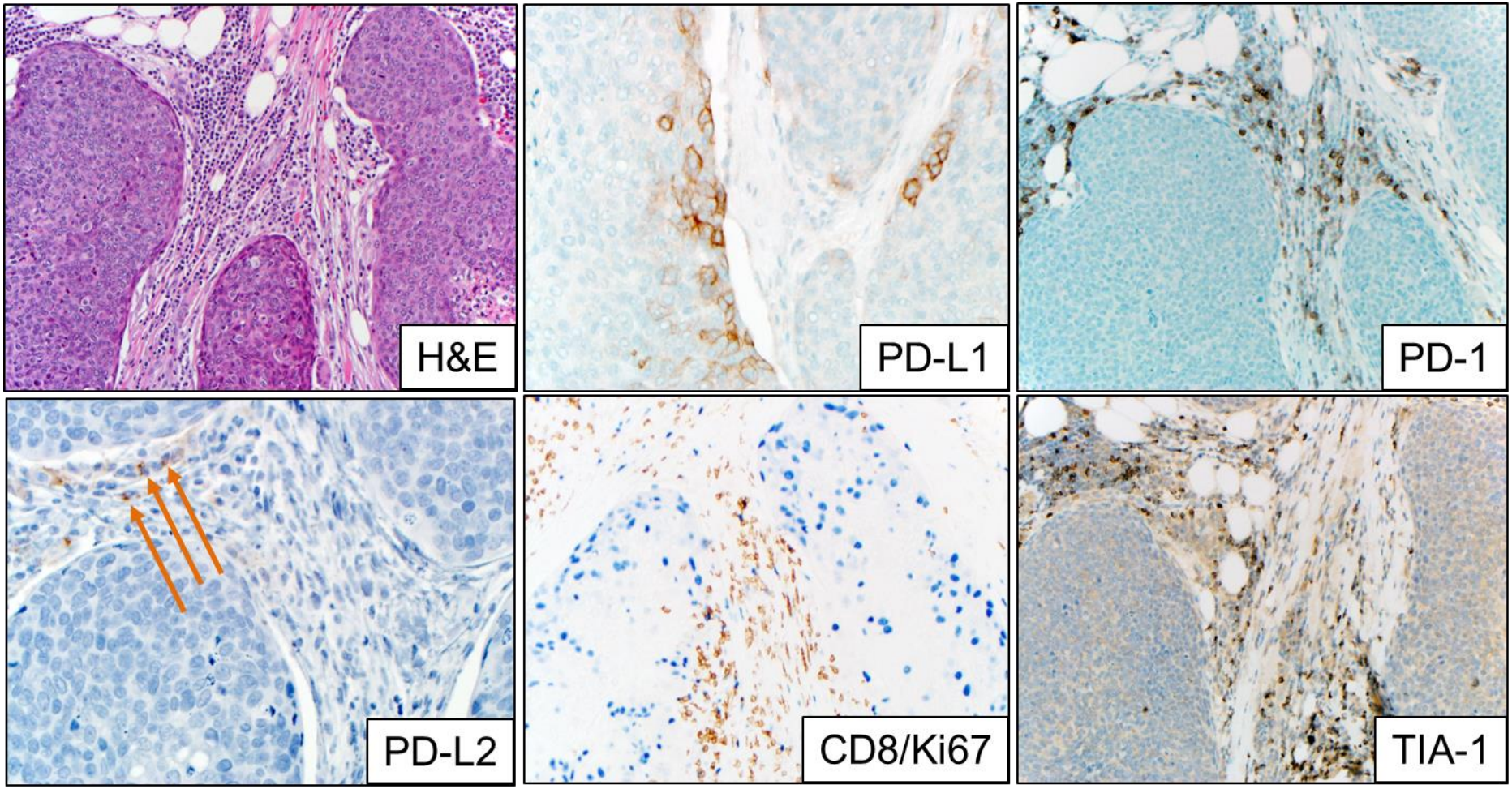
Additional information about methods used for evaluation of biological material

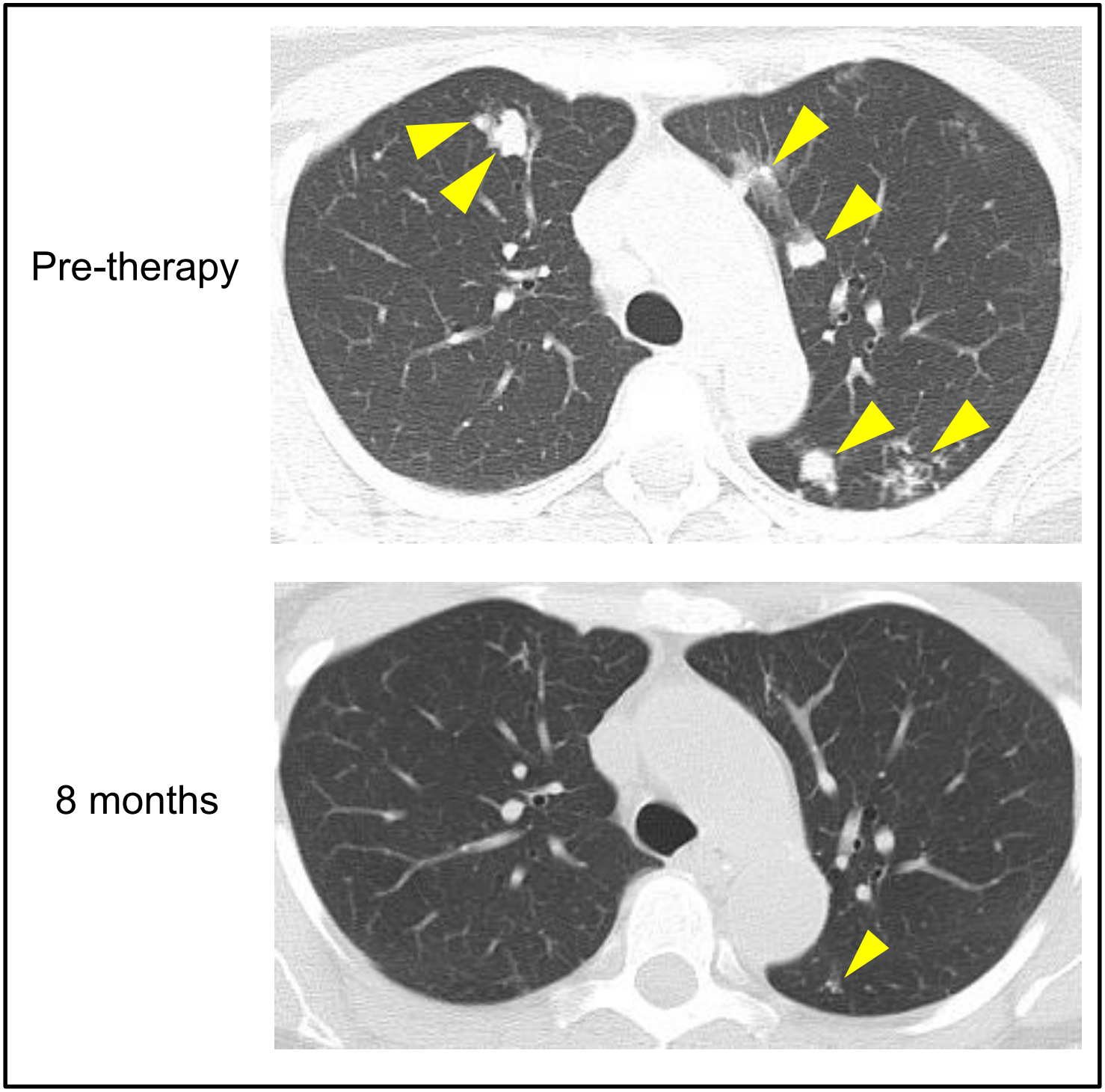
Evaluation of the patient's serum with multiplex bead assays was performed using the Luminex platform (Lifecodes class I and II ID panels, Immucor Gen-Probe, San Diego, CA and Single Antigen Beads, One Lambda, Canoga Park, CA). ELISA for angiotensin II type 1 receptor (AT1R) antibody was performed using EIA-AT1R (One Lambda, Canoga Park, CA). Additionally, flow cytometric crossmatches were performed against precursor endothelial cell targets obtained from two surrogate donors because the actual kidney donor was not available. Although the presence of endothelial antibodies cannot be entirely ruled out because it is unknown whether the surrogates carry the donor target antigens, results were negative.

Figure S1



Immunoarchitecture of pre-treatment cutaneous squamous cell carcinoma (cSCC) specimen. Histologic sections demonstrate an infiltrating cSCC with a vigorous immune infiltrate and immune checkpoint expression at the tumor-stroma interface. The cSCC expresses PD-L1 at the interface with the stroma and the host-immune response. PD-L1 expression is also seen on infiltrating macrophages and rare lymphocytes. The activated CD8+ T cell rich immune infiltrate demonstrates PD-1 on T cells and PD-L2 on myeloid cells (arrows). The infiltrating immune cells are predominantly CD8-positive and co-express Ki-67, consistent with an activated cytotoxic T cell phenotype; CD8 (brown chromagen) and Ki-67 (blue chromagen) double immunostain. TIA-1, indicative of cytotoxic activity, is expressed on CD8+ cells. (H&E, hematoxylin and eosin; TIA-1, T-cell intracytoplasmic antigen-1)

Figure S2



CT scans performed pre-therapy and 8 months after initiating pembrolizumab (anti-PD-1) demonstrate regression of cutaneous squamous cell carcinoma lung metastases in a kidney transplant recipient. Yellow arrowheads indicate sites of metastases.

Additional discussion

Adaptive immune responses to self and non-self antigens, including those against antigens in solid organ allografts, are largely regulated by a series of molecular signals that control T cell activation. Following the interaction between a T cell receptor and its cognate peptide (signal 1), a co-stimulatory signal (signal 2) is required to trigger T cell proliferation, acquisition of effector function, and migration to sites of antigen expression.[1] The prototypical immune checkpoint molecule, cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) is expressed on T cells, and functions by binding to the co-stimulatory ligands B7-1 and B7-2 with higher affinity than their co-stimulatory receptor, CD28, effectively hijacking signal 2 and attenuating T cell function. In normal hosts, immune checkpoints like CTLA-4 prevent immune activation against self-antigens that might otherwise lead to autoimmunity. PD-1, a second immune checkpoint, is also important in maintaining self-tolerance as well as mitigating collateral damage of tissues involved in active immune responses. In general, CTLA-4 likely operates in lymph nodes, where it functions by modulating activation during the initial (priming) phase of an immune response. PD-1, by contrast, likely functions within peripheral tissues and, in the case of cancer, within the tumor microenvironment, during the effector phase of a T cell response.[2-4] Although clinical trials testing drugs targeting the CTLA-4 and PD-1 pathways have demonstrated remarkable anti-tumor activity of these agents, they have excluded patients on chronic immunosuppressive drug regimens, both for autoimmune disease and solid organ transplants.

The first report of a solid organ transplant recipient (SOTR) receiving immune checkpoint blockade therapy involved two patients with metastatic melanoma who had previously undergone kidney transplantation. Renal allografts from both patients appeared to have been unaffected by administration of ipilimumab (anti-CTLA-4).[5] Both patients experienced an anti-tumor response to therapy. Similarly, allograft tolerance remained intact in two patients who had undergone liver transplantation and received ipilimumab for metastatic melanoma.[6, 7]

In contrast, pembrolizumab (anti-PD-1) likely contributed to allograft rejection in the current report. T cell-mediated rejection (TCMR) occurs rarely >10 years post-transplant.[8] In addition, the severity of the TCMR suggests that it was triggered by PD-1 blockade rather than discontinuation of immunosuppression. Although preclinical studies have suggested the importance of PD-1 and its ligands in influencing allograft rejection[9, 10], our case is the first to directly demonstrate the relevance of the PD-1 pathway in maintaining adaptive tolerance to solid organ transplants in humans.[11-13] These findings underscore the functional differences between CTLA-4 and PD-1, the latter more heavily influencing immunomodulation within peripheral tissues.

Our patient's explanted allograft demonstrated changes consistent with advanced cell mediated rejection. **(Figure 1)** The lack of C4d staining in the peritubular capillaries as well as the lack of HLA antibody suggest an absence of anti-donor humoral activity and argues against a component of antibody-mediated rejection. Although no histological or serological samples were available from the time at which allograft dysfunction was initially detected, if donor-specific antibodies were present at the time of rejection, it is likely that the inflammation associated with the surgical nephrectomy would have resulted in an increase in antibody affinity as well as the persistence of antibodies associated with rejection.[14] Taken together, our findings do not support a substantial humoral component of graft rejection in this case.

Our data further suggest that, in the setting of immune checkpoint blockade, allograft destruction is likely mediated by T cells. The relative roles of PD-1 and CTLA-4 in this process remain unclear; however, the lack of organ rejection in a small number of anti-CTLA-4 treated patients compared with the organ rejection seen in this anti-PD-1 treated patient further emphasize the difference between these two checkpoints in immune regulation. The differing roles of the CTLA-4 and PD-1 pathways in transplant tolerance has potential relevance to clinical management of melanomas in SOTR patients. Both anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab and pembrolizomab) are FDA-approved for treatment of advanced melanoma. The higher response rate and lower toxicity of anti-PD-1 relative to anti-CTLA-4 underpins a shift toward anti-PD-1 treatment of melanoma in the first line. However, we propose that anti-CTLA-4 should be considered before anti-PD-1 for treatment of melanoma in SOTRs because of the different effects on adaptive transplant tolerance.

In conclusion, this case demonstrates a critical role for the PD-1 pathway both in malignancies arising in the setting of long-term immunosuppression and in maintaining adaptive immune tolerance to transplanted organs. Importantly, these findings further suggest that PD-1 pathway agonists might be useful in the prevention of allograft rejection. This patient's robust anti-tumor response as well as her acute allograft rejection illustrate the complexity of the interactions between immune checkpoint molecules, alloantigens, and cancer neoantigens, thus making clinical outcomes to various immune activators difficult to predict.

Because advanced cSCC and other cancers often arise in patients with immunologic comorbidities (e.g., SOTR), further study into the contributions of various immunoregulatory molecules is necessary in order to better understand how to selectively activate anti-tumor immunity while minimizing immune-related toxicities.

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