INTRODUCTION

Immune checkpoint inhibitors (ICIs), represented by anti-programmed cell death 1 (PD-1)/PD-1 ligand 1 (PD-L1) monoclonal antibodies (mAbs) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) mAbs, have been shown to be effective to a variety of cancers, including melanoma, non–small cell lung cancer, renal cell carcinoma, head and neck cancer, and gastrointestinal cancer.1–4 However, the efficacy of ICI monotherapy is limited, and the detailed mechanisms are not fully understood, necessitating more basic and clinical researches.5

In the tumor microenvironment (TME), T cells, especially CD8+ T cells, which reportedly attack cancer cells via recognition of cancer antigens in the context of major histocompatibility complex (MHC) molecules, play a crucial role in antitumor immunity.5–7 ICIs exert antitumor effects by inhibiting T-cell suppressive signals and subsequently activating T-cell–mediated cytotoxicity.5–7 Somatic mutation-derived neoantigens, which can be recognized as non-self antigens, reportedly induce strong immune responses similar to those induced by foreign antigens such as virus antigens.8,9 Thus, neoantigens are believed to induce an inflamed TME characterized by high CD8+ T-cell infiltration, which is very important for the ICI response, and the number of neoantigens is reportedly correlated with the degree of inflammation in the TME.10–13 In addition, CD8+ T-cell–mediated cytotoxicity reportedly induces PD-L1 expression via the interferon-gamma (IFN-γ) signaling pathway, and the induced...
PD-L1 suppresses CD8+ T cells by binding to PD-1. Therefore, PD-L1 expression, CD8+ T-cell infiltration, and tumor mutational burden (TMB) are candidate biomarkers for ICI therapy with close relations to each other. However, there are substantial conflicting data, and none of these biomarker candidates seems to be a sufficient predictor of ICI response.

On the other hand, tumors can modify the TME to one that benefits themselves through the process of tumor immune editing. Tumor cells can evade antitumor immunity through various mechanisms even during ICI treatment, such as loss of MHC and impaired IFN-γ signaling, leading to resistance to ICIs. Elucidation of primary and acquired resistance mechanisms to ICIs is needed to develop predictive biomarkers for ICIs and/or novel therapies with higher efficacy. Although many studies have shown some representative mechanisms, the complex nature of the mechanisms makes it difficult to be understood sufficiently.

While there are various complicated resistance mechanisms, most of them are related to the T-cell activation process because T-cell activation is essential for ICI efficacy. For example, MHC loss inhibits T-cell activation following the loss of antigen presentation. Reduced chemokines induce a noninflamed TME leading to ICI resistance. Suppressive immune checkpoint molecules and immune-suppressive cells inhibit the effector functions of T cells. In addition, abnormalities in the IFN-γ signaling pathway are involved in many of these mechanisms. In this review, we summarize mechanisms of resistance to ICIs related to the process of T-cell activation and novel therapies to overcome them.

2 | THE PROCESS OF T-CELL ACTIVATION AND THE EFFECTS OF IMMUNE CHECKPOINT MOLECULES

When CD8+ T cells are depleted in mouse models, tumors grow rapidly, and ICIs are completely ineffective. In addition, an inflamed TME characterized by high CD8+ T-cell infiltration is reportedly associated with ICI response. Thus, CD8+ T cells are essential for antitumor immunity, and ICIs exert antitumor effects by activating these T cells. The process in which T-cell activation leads to killing cancer cells is summarized in seven steps (Figure 1): (1) cancer antigen release from cancer cells, (2) presentation of cancer antigens via MHC molecules by antigen-presenting cells (APCs), (3) T-cell activation via recognition of cancer antigen in the context of MHC molecules (priming phase), (4) migration and (5) infiltration of activated T cells into the TME, (6) T-cell recognition of cancer antigen presented in the context of MHC molecules of cancer cells, and (7) killing cancer cells by activated T cells (effector phase). During these processes, immune checkpoint molecules modulate the activity of T cells in steps 3 (priming phase) and 7 (effector phase).

Immune checkpoint molecules, which are mainly expressed on the T-cell surface, control T-cell activation by binding to their ligands on APCs and/or cancer cells at the time of T-cell recognition of cancer antigens. Each immune checkpoint molecule acts through different mechanisms, with some demonstrating inhibitory and others stimulatory effects on T-cell activation (Figure 2).

CTLA-4 and PD-1 are both inhibitory immune checkpoint molecules, and mAbs against them are used clinically (anti-CTLA-4 and anti-PD-1/PD-L1 mAbs, respectively). T-cell receptor (TCR) stimulation with antigen recognition is essential for T-cell activation, and costimulation is also necessary. A representative type of costimulation is the CD28 signaling pathway, which promotes T-cell activation mainly by binding to CD80 or CD86. CTLA-4 suppresses T-cell activation by binding tightly to CD80/CD86, inhibiting CD28/CD80/CD86-mediated costimulation. In addition, regulatory T cells (Treg cells), one of the representative immune-suppressive cells, highly express CTLA-4 on their surface, and Treg cell–expressing CTLA-4 depletes CD80/CD86 on APCs by trogocytosis, leading to suppression of APCs. As CD80 and CD86 are mainly expressed in APCs, anti-CTLA-4 mAbs are expected to work mainly at the T-cell priming phase (step 3). On the other hand, PD-1 expressed on T cells binds to PD-L1 or PD-L2, and PD-1/PD-L1/PD-L2 mediates inhibitory signals by suppressing TCR signaling pathways. Anti-PD-1/PD-L1 mAbs are considered to work mainly at the effector phase (step 7), whereas PD-L1/PD-L2 are expressed on both tumor cells and APCs. Furthermore, it has been recently reported that PD-1 also exhibits its inhibitory effect by suppressing CD28 signaling.

In addition to PD-1 and CTLA-4, many other immune checkpoint molecules regulate T-cell activation. Coinhibitory immune checkpoint molecules include TIM-3, LAG-3, and TIGIT. On the other hand, costimulatory immune checkpoint molecules that activate immune responses by binding their ligands, like CD28 binds CD80/CD86, include OX40, GITR, 4-1BB, and ICOS.

3 | MECHANISMS OF RESISTANCE TO ICIS

As mentioned above, the process of T-cell activation is essential for the antitumor effects of ICIs. In cases of resistance, many factors inhibit the T-cell activation process, which can be classified into three main mechanisms (Figure 1), though there seems to be an overlap among them. The first category is “resistance mechanisms related to antigen recognition,” inhibiting steps 1-3 and 6; the second is “resistance mechanisms related to T-cell migration and/or infiltration,” inhibiting steps 4 and 5. The third category is the “resistance mechanisms related to effector functions of T cells,” inhibiting step 7. As these resistance mechanisms can induce both initial and acquired resistance, we will explain them together.

4 | RESISTANCE MECHANISMS RELATED TO ANTIGEN RECOGNITION

At the start of T-cell–mediated antitumor immunity, APCs present cancer antigens on their MHC. T cells with specific TCRs recognize the antigen-MHC complex on APCs and are stimulated via the TCR
signaling pathway, which induces subsequent T-cell activation. In addition, activated T cells recognize the cancer antigen-MHC complex on cancer cells in the TME, leading to killing cancer cells. Impairment of any part of the antigen recognition processes can lead to the loss of antitumor immunity. The factors related to these resistance mechanisms include (1) cancer cell antigen loss and (2) reduced antigen presentation.

### 4.1 Cancer cell antigen loss

Cancer antigens cause specific antitumor immunity mediated by antigen-specific T-cell activation, and the loss of these antigens abolishes specific T-cell cytotoxicity. In particular, neoantigens, derived from somatic mutations that alter amino acid sequences, are the most characteristic tumor-specific antigens, leading to strong T-cell activation as non-self antigens.\(^{11-14}\) Previous reports have shown that ICIs are highly effective for patients with high TMBs.\(^ {11-14,47,48}\) In contrast, some patients with low neoantigen levels are primarily resistant to ICIs, and some patients who respond initially acquire resistance to ICIs with the disappearance of neoantigens.\(^ {11-14,49,50}\)

### 4.2 Reduced antigen presentation

If any defects in the antigen presentation process occur, ICIs can be ineffective. One such mechanism is dysfunction of APCs. To escape antitumor immunity, cancer cells suppress the antigen-presenting activity of APCs through various mechanisms. They inhibit APC recruitment into the TME and/or maturation by activating the WNT/\(\beta\)-catenin signaling pathway or secretion of prostaglandin E2 (PGE2) or vascular endothelial growth factor (VEGF).\(^ {51-55}\)

Another mechanism is the loss of MHC expression by cancer cells. MHC class I (MHC-I) on cancer cells is an essential molecule for CD8\(^+\) T cells to recognize cancer antigens.\(^ {23,24}\) It has been reported that loss of heterozygosity in MHC-I genes is associated with immune escape in cancer.\(^ {56,57}\) which leads to ICI resistance.\(^ {58}\) We recently reported that MHC-I mutations accumulate in microsatellite instability-high tumors, suggesting that the accumulation of MHC-I mutations is an important immune evasion mechanism.\(^ {59}\) Other reports in clinical settings showed that loss or decreased expression of \(\beta\)-2 macroglobulin (B2M), one of the components of MHC-I, resulted in defects in antigen presentation and ICI resistance.\(^ {59,60}\) We also reported a case of melanoma with ICI resistance due to the loss of
Furthermore, downregulation of MHC expression is caused by epigenetic abnormalities such as EZH2 gene mutation and histone deacetylase abnormalities. Currently, there are attempts to develop drugs that upregulate MHC. One possible approach is to upregulate MHC-I by inhibiting TRAF3, which negatively regulates the NF-κB pathway. We also revealed the importance of MHC class II (MHC-II)-mediated cytotoxic CD4+ T cells in antitumor immunity even against MHC-I-negative tumors and suggested them as a potential therapeutic target.

5 | RESISTANCE MECHANISMS RELATED TO T-CELL MIGRATION AND/OR INFILTRATION

T cells need to migrate and infiltrate into the TME to attack cancer cells directly. Several kinds of chemokines, such as CXCL9, CXCL10, CXCL11, and CCL5, are necessary for T-cell migration and infiltration. Cancer cells and surrounding immune cells produce these chemokines, and disruption of the chemokine production process induces ICI resistance. Therefore, abnormalities in the IFN-γ signaling pathway, which is related to the production of these chemokines, inhibit T-cell migration and infiltration into the TME.

Several signaling pathways related to carcinogenesis, such as the WNT/β-catenin, PTEN, LKB1, and EGFR pathways, may also contribute to ICI resistance by suppressing the production of such chemokines. In addition, epigenetic changes have also been reported to downregulate these chemokines, contributing to resistance to ICIs.

Vascular endothelial growth factor, which directly affects immune cells, as mentioned above, also prevents T-cell migration and infiltration by inhibiting adhesion between T cells and vascular endothelial cells and suppressing the production of chemokines, such as CXCL10 and CXCL11. In clinical settings, VEGF inhibitors show efficacy against several types of cancer and are more effective when combined with ICIs. We also reported the case of an ICI-resistant patient who was successfully treated with a VEGF inhibitor.

In addition to VEGF, TGF-β also has immune-suppressive effects. In addition to directly suppressing T cells, TGF-β prevents T-cell infiltration by inducing activation of cancer-associated fibroblasts (CAFs) and affects CXCR3 expression on T cells, which interferes with chemokine-induced migration. Although it has not yet been clinically applied, combination therapy with a TGF-β-blocking drug is now being developed.

6 | RESISTANCE MECHANISMS RELATED TO EFFECTOR FUNCTIONS OF T CELLS

This section summarizes some of the significant immunomodulatory factors, including immune-suppressive cells and other suppressive immune checkpoint molecules, which lead to inadequate
effector functions of T cells in the TME. The IFN-γ, secreted mainly from activated T cells, plays an essential role in antitumor immunity. It has been reported that genetic mutations in JAK cause abnormal IFN-γ signaling, leading to resistance to ICIs.\textsuperscript{31,32} Cytotoxic T cells exhibit their effector functions via IFN-γ by inhibiting cell growth and inducing apoptosis.\textsuperscript{64} However, as mentioned above, the resistance due to defects in IFN-γ signaling could be mainly caused by decreased MHC-I.

### 6.1 | Other suppressive immune checkpoint molecules

PD-1, which interacts with PD-L1, is primarily expressed following the activation of T cells and suppresses T-cell effector function, causing T cells to fall into a progressive dysfunctional state called exhaustion.\textsuperscript{27} In the TME, not all T cells attack cancer cells, as there is a subset of bystander T cells.\textsuperscript{80,81} Among them, exhausted T cells in the TME directly attack cancer cells, and ICIs exhibit efficacy by reactivating them.\textsuperscript{81,82}

Among exhausted T cells, PD-1\textsuperscript{low}CXCR5\textsuperscript{+}TCF1\textsuperscript{+} progenitor-exhausted T cells are expected to be reactivated by anti-PD-1/PD-L1 mAbs. In contrast, PD-1\textsuperscript{high}CXCR5\textsuperscript{−}TCF1\textsuperscript{−} terminally differentiated exhausted T cells are considered to be dysfunctional and not able to be reactivated (Figure 3).\textsuperscript{83,84} These terminally differentiated exhausted T cells, which express not only PD-1 but also other inhibitory immune checkpoint molecules, such as LAG-3, TIM-3, and TIGIT, are not fully reactivated by blocking only PD-1, resulting in resistance (Figures 2 and 3).\textsuperscript{83-85} A clinical trial of combination therapy with inhibitors of PD-1 and these immune checkpoint molecules is now underway.

Using clinical samples and mouse models, we also demonstrated that the TIGIT/CD155 interaction is another mechanism by which melanoma with a high TMB and an inflamed TME can become resistant to therapy, including cases of both primary and acquired resistance.\textsuperscript{86} A clinical trial of anti-TIGIT mAbs combined with anti-PD-L1 mAbs for non-small cell lung cancer has been performed, and the combination showed possible efficacy against PD-L1-high tumors.\textsuperscript{87} Another inhibitory molecule, LAG-3, inhibits T-cell activation by binding to MHC-II (Figure 2). We reported that CD4+ T cells in the TME of MHC-II\textsuperscript{+} tumors express high LAG-3 levels and that combination therapy with anti-PD-1 and anti-LAG-3 mAbs increases CD4+ T-cell-mediated antitumor immunity.\textsuperscript{68} Indeed, anti-LAG-3 mAbs have already shown efficacy in combination with anti-PD-1 mAbs against malignant melanoma.\textsuperscript{88}

### 6.2 | Immunosuppressive cells

The ICI resistance mechanisms mediated by various immune-suppressive cells, such as Treg cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and CAFs, have been reported mainly in mouse models. Therefore, treatment to deplete these cells has been being developed.\textsuperscript{28-30} These immune-suppressive cells also inhibit the antigen presentation of APCs, suggesting that the early-phase T-cell activation process may be impaired by these suppressive cells. In addition, these cells secrete various immune-suppressive factors, such as VEGF and TGF-β.\textsuperscript{73,78,89,90}

Treg cells are known to suppress effector T cells and APCs by various mechanisms, such as consuming IL-2 and binding CD80/CD86 via CTLA-4.\textsuperscript{28} We reported that Treg cells in the TME had high PD-1 expression and that anti-PD-1 mAbs activated PD-1+ Treg cells as well as PD-1+CD8+ T cells, which contributed to ICI resistance.\textsuperscript{33} Moreover, we also found that hyperprogressive disease can be caused by PD-1+ Treg cells in the TME activated by anti-PD-1 mAbs.\textsuperscript{91} In contrast, anti-CTLA-4 mAbs are thought to have inhibitory effects on Treg cells, but even when combined with anti-PD-1 mAbs, approximately 40%-50% of treated patients are resistant.\textsuperscript{3-5} Further investigations are needed to clarify the effects of ICIs on Treg cells.

**Figure 3**  The progressive process of T-cell exhaustion. Among exhausted T cells, PD-1\textsuperscript{low}CXCR5\textsuperscript{+}TCF1\textsuperscript{+} progenitor-exhausted T cells are expected to be reactivated by anti-PD-1/PD-L1 mAbs. In contrast, PD-1\textsuperscript{high}CXCR5\textsuperscript{−}TCF1\textsuperscript{−} terminally differentiated exhausted T cells are considered dysfunctional and incapable of being reactivated. LAG-3, lymphocyte activation gene 3; PD-1, programmed cell death 1; TCF-1, T-cell factor 1; TIM-3, T-cell membrane protein 3. This figure was created with BioRender.com
Vascular endothelial growth factor has been reported to induce activation of immune-suppressive cells such as Treg cells, TAMs, and MDSCs and is also secreted by these immune-suppressive cells. VEGF inhibitors are expected to improve the TME by increasing cytotoxic T cells and decreasing suppressor cells. In a phase 2 trial, the combination of a VEGF inhibitor with an ICI in renal cell carcinoma was more effective in TAM-abundant cancers than TAM-deficient cancers.

**6.3 | Metabolism in the TME**

The TME is known as a hypoxic and low-glucose environment. In such an environment, the lack of glucose impairs T-cell activation, limits antitumor immunity, and induces resistance to ICIs. VEGF is known to establish a hypoxic TME, which is expected to be improved by VEGF inhibitors. On the other hand, lactate, abundant in the low-glucose TME, was recently reported to promote PD-1 expression in Treg cells and contribute to ICI resistance. In addition, abnormalities of several genes, such as PI3K, LKB1, and MYC, which are involved in cancer metabolism, can also contribute to resistance. Attempts are being made to modulate metabolism with drugs to increase antitumor immunity.

Amino acids play an essential role in T-cell activation. Serine and arginine are important amino acids for effector T-cell expansion and antitumor immunity. The enzyme IDO, which converts tryptophan to the immunosuppressive molecule kynurenine, has been found to be involved in ICI resistance. Although combination therapy with IDO inhibitors and anti-PD-1/PD-L1 mAbs was expected to be efficacious, clinical trials have failed to meet their primary endpoint. We speculate that the investigators should select an appropriate population based on any biomarkers.

Extracellular adenosine binds to its receptors on T cells and suppresses their function via the subsequent elevation of intracellular cAMP. Adenosine is produced from ATP through surface CD39 and CD73. Thus, CD73 and CD39 can suppress antitumor immunity, resulting in resistance to ICIs. Promising drugs that inhibit the binding of adenosine to its receptor have been developed, and clinical trials are underway for combination therapy with PD-1 inhibitors. The efficacy of anti-CD39/CD73 antibodies has also been reported at the preclinical stage, and the results of clinical trials are awaited.

**7 | OTHER RESISTANCE MECHANISMS**

In some patients who are resistant to anti-PD-L1 mAbs, PD-L1 splicing variants are secreted, working as "decoys" of the mAbs. ICI-neutralizing antibodies can also be produced in resistant patients, which can be related to resistance. Secreted factors, such as PD-L1 splicing variants and ICI-neutralizing antibodies, can disturb ICIs themselves, leading to ICI resistance.

Recently, the relationships between the intestinal microbiota and systemic immune responses and autoimmune diseases have been noted. Metabolites from the microbiota have also been reported to influence immune responses. The intestinal microbiota also affects antitumor immunity, and it has been pointed out that it may be involved in the response to ICIs. The combination of fecal transplantation and ICIs has already been applied in practice, and some reports indicate that fecal transplants from responders to ICIs can induce ICI responses. The detailed mechanism, including the relationships with resistance, is still unclear, so further research is expected.

**8 | CONCLUSION**

Immune checkpoint inhibitors have undoubtedly shifted the cancer therapy paradigm. However, tumors induce a TME that benefits themselves by various mechanisms, and there are still many challenges to be overcome. This review summarizes ICI resistance mechanisms related to T-cell activation process in three main categories. It is not always possible to identify a single mechanism, such as abnormalities in the IFN-γ signaling pathway. Various mechanisms are involved in a complex manner, suggesting that resistance occurs via a complex set of processes. However, as ICIs basically exert their effects by activating T cells, many resistance mechanisms can be attributed to the inhibition of T-cell activation. To overcome resistance, it is important to promote T-cell activation, and various attempts are being made, including combination therapy. In addition, cell therapy has been proven to be effective in ICI-resistant hematological tumors, including malignant lymphoma and leukemia, suggesting the possibility of response even in noninflamed tumors.

Therefore, cell therapy is expected to be applied in solid tumors in the future.

Cancer immunity has been studied mainly in mouse models. However, the mechanisms of resistance to ICIs in clinical settings seem to be very complicated, as we have summarized. Therefore, it can be difficult to understand the detailed mechanisms only in mouse models. We believe it is essential to elucidate the TME using human clinical samples to understand and overcome resistance mechanisms. We should promote translational research with both bedside-to-bench approaches and bench-to-bedside approaches.

**ACKNOWLEDGMENTS**

This study was supported by Grants-in-Aid for Scientific Research (B, 20H03694) from the Japan Society for the Promotion of Science (JSPS); the Fusion Oriented Research for Disruptive Science and Technology (21-211033868) from the Japan Science and Technology Agency (JST); the Project for Cancer Research and Therapeutic Evolution (18cm0106340h0001 and 21cm0106383); and the Practical Research for Innovative Cancer Control (19ck0106521h0001 and 22ama22103h0001) from the Japan Agency for Medical Research and Development (AMED).
CONFLICT OF INTEREST
Y. T. is a current editorial board member of Cancer Science. Y. T. received research grants and honoraria from Ono Pharmaceutical and Bristol-Myers Squibb; research grants from KOTAI Biotechnologies, Daiichi-Sankyo, and KORTUC; and honoraria from Chugai Pharmaceutical and MSD outside this work.

ORCID
Yosuke Togashi https://orcid.org/0000-0001-9910-0164

REFERENCES
1. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375:1823-1833.
2. Ferris RL, Blumenschein G, Fayette J, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med. 2016;375:1856-1867.
3. Motzer RJ, Nani NM, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;377:1277-1290.
4. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N Engl J Med. 2015;373:23-34.
5. Hellmann MD, Paz-Ares L, Bernabe Caro R, et al. Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. N Engl J Med. 2019;381:2020-2031.
6. Kang YK, Boku N, Satoh T, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390:2461-2471.
7. Zhou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. Sci Transl Med. 2016;8:328rv4.
8. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515:568-571.
9. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515:563-567.
10. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348:69-74.
11. Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature. 2012;482:400-404.
12. Ribas NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124-128.
13. Rooney Michael S, Shukla Sachet A, Wu Catherine J, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015;160:48-61.
14. Hacohen N, Fritsch EF, Carter TA, Lander ES, Wu CJ. Getting personal with neoantigen-based therapeutic cancer vaccines. Cancer Immunol Res. 2013;1:11-15.
15. Sugiyama E, Togashi Y, Takeuchi Y, et al. Blockade of EGFR improves responsiveness to PD-1 blockade in EGFR-mutated non-small cell lung cancer. Sci Immunol. 2020;5:eaav3937.
16. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer. 2016;16:275-287.
17. McGra1l DJ, Pilié PG, Rashid NU, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. Ann Oncol. 2021;32:661-672. doi:10.1016/j.annonc.2021.02.006
18. Voorwerk L, Slagter M, Horlings HM, et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. Nat Med. 2019;25:920-928.
19. Rizvi H, Sanchez-Vega F, La K, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. J Clin Oncol. 2018;36:633-641.
20. Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity. 2004;21:137-148.
21. Sharma P, Hu-Lieskovsk S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168:707-723.
22. Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. Nat Rev Immunol. 2020;20:25-39.
23. Inozume T, Yaguchi T, Ariausy R, et al. Analysis of the tumor reactivity of tumor-infiltrating lymphocytes in a metastatic melanoma lesion that lost major histocompatibility complex class I expression after anti-PD-1 therapy. J Invest Dermatol. 2019;139:1490-1496.
24. Kawazu M, Ueno T, Saeki K, et al. HLA class I analysis provides insight into the genetic and epigenetic background of immune evasion in colorectal cancer with high microsatellite instability. Gastroenterology. 2022;162:799-812.
25. Peng D, Kryczek I, Nagarsheth N, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature. 2015;527:249-253.
26. Kumagai S, Koyama S, Itahashi K, et al. Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments. Cancer Cell. 2022;40:201-218.e209.
27. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15:486-499.
28. Togashi Y, Hitata K, Nishikawa H. Regulatory T cells in cancer immunosuppression – implications for anticancer therapy. Nat Rev Clin Oncol. 2019;16:356-371.
29. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour- associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol. 2017;14:399-416.
30. Sahai E, Astsatsavuro I, Cukierman E, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20:174-186.
31. Shin DS, Zaretsky JM, Escuin-Ordinas H, et al. Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. Cancer Discov. 2017;7:188-201.
32. Zaretsky JM, García-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med. 2016;375:819-829.
33. Kumagai S, Togashi Y, Kamada T, et al. The PD-1 expression balance between effector and regulatory T cells predicts the clinical efficacy of PD-1 blockade therapies. Nat Immunol. 2020;21:1346-1358.
34. Chen Daniel S, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39:1-10.
35. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359:1350-1355.
36. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252-264.
37. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat Rev Immunol. 2013;13:227-242.
38. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone J. CD28 costimulation: from mechanism to therapy. Immunity. 2016;44:973-988.
53. Bonavita E, Bromley CP, Jonsson G, et al. Antagonistic inflammatory phenotypes dictate tumor fate and response to immune checkpoint blockade. J Exp Med. 2012;209:1201-1217.

51. Spranger S, Bao R, Gajewski TF. Melanoma- intrinsic resistance mechanisms in cancer immunotherapy. Int J Clin Oncol. 2020;25:810-817.

46. Schoenfeld AJ, Hellmann MD. Acquired resistance to immune checkpoint inhibitors in metastatic melanoma. Clin Cancer Res. 2018;24:1260-1270.

45. Kawakami Y, Ohta S, Sayem MA, Tsukamoto N, Yaguchi T. Immune-activator is a target for immune evasion in cancer. Blood Adv. 2020;4:4069-4082.

44. Gide TN, Wilmott JS, Scolyer RA, Long GV. Primary and acquired resistance to immune checkpoint inhibitors in non-small cell lung cancer. Cancer Discov. 2017;7:644-659.

43. Kraehenbuehl L, Weng C-H, Eghbali S, Wolchok JD, Merghoub T. Enhancing immunotherapy in cancer by targeting emerging immunomodulatory pathways. Nat Rev Clin Oncol. 2022;19:37-50.

42. Ruiz de Galarreta M, Bresnahan E, Molina- Sánchez P, et al. Catenin activation promotes immune escape and resistance to anti-CD1-therapy in hepatocellular carcinoma. Cancer Discov. 2019;9:1124-1141.

41. Hui E, Cheung J, Zhu J, et al. T cell costimulatory receptor expression potentiates immune checkpoint blockade. Cancer Discov. 2021;11:1524-1541.

40. Yokosuka T, Takamatsu M, Kobayashi-Imanishi W, Hashimoto-Tane A, Azuma M, Saito T. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J Exp Med. 2020;219:1201-1217.

39. Yokosuka T, Kobayashi W, Takamatsu M, et al. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. Immunity. 2010;33:326-339.

38. Shukla SA, Rooney MS, Rajasagi M, et al. Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes. Nat Biotechnol. 2015;33:1152-1158.

37. McGranahan N, Rosenthal R, Hiley CT, et al. Allele-specific HLA loss and immune escape in lung cancer evolution. Cell. 2017;171:1259-1271. e1211.

36. Lee JH, Shklovskaya E, Lim SY, et al. Transcriptional downregulation of MHC class I and melanoma de- differentitation in resistance to PD-1 inhibition. Nat Commun. 2020;11:1897.

35. Sade-Feldman M, Jiao YJ, Chen JH, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. Nat Commun. 2017;8:1136.

34. Gettinger S, Choi J, Hastings K, et al. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. Cancer Discov. 2017;7:1420-1435.

33. Yokosuka T, Kobayashi W, Takamatsu M, et al. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. Immunity. 2010;33:326-339.

32. Shukla SA, Rooney MS, Rajasagi M, et al. Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes. Nat Biotechnol. 2015;33:1152-1158.
83. Philip M, Schietinger A. Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections. *Curr Opin Immunol*. 2019;58:98-103.

84. Henning AN, Roychoudhuri R, Restifo NP. Epigenetic control of CD8(+) T cell differentiation. *Nat Rev Immunol*. 2018;18:340-356.

85. Koyama S, Akbay EA, Li YY, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Commun*. 2016;7:10501.

86. Kawashima S, Inozume T, Kawazu M, et al. TIGIT/CD155 axis mediates resistance to immunotherapy in patients with melanoma with the inflamed tumor microenvironment. *J Immunother Cancer*. 2021;9:e003134.

87. Rodríguez-Abreau D, Johnson ML, Hussein MA, et al. Primary analysis of a randomized, double-blind, phase II study of the anti-TIGIT antibody tiragolumab (tira) plus atezolizumab (atezo) versus placebo plus atezo as first-line (1L) treatment in patients with PD-L1-selected NSCLC (CITYSCAPE). *J Clin Oncol*. 2020;38:9503.

88. Lipson EJ, Tawbi HA-H, Schadendorf D, et al. Relatlimab (RELA) plus nivolumab (NIVO) versus NIVO in first-line advanced melanoma: primary phase III results from RELATIVITY-047 (CA224-047). *J Clin Oncol*. 2021;39:9503.

89. Tada Y, Yoshida H, Kotani D, et al. Targeting VEGFR2 with ramucirumab strongly impacts effector/ activated regulatory T cells and CD8(+) T cells in the tumor microenvironment. *J Immunother Cancer*. 2018;6:106.

90. Higflill SL, Cui Y, Giles AJ, et al. Disruption of CXCR2-mediated resistance to immunotherapy in patients with melanoma. *Cancer Sci*. 2016;4:237-267.

91. Kamada T, Yoshida H, Kai C, et al. PD-1+ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc Natl Acad Sci U S A*. 2019;116:9999-10008.

92. McDermott DF, Huseni MA, Atkins MB, et al. Clinical activity and efficacy of PD-1 blockade therapy in patients with metastatic melanoma. *J Immunother Cancer*. 2019;7:e003134.

93. Fong L, Hotson A, Powderly JD, et al. Adenosine 2A receptor blockade as an immunotherapy for treatment-refractory renal cell cancer. *Cancer Discov*. 2020;10:40-53.

94. Perrot I, Michaud HA, Giraudon-Paoli M, et al. Blocking antibodies targeting the CD39/CD73 immunosuppressive pathway unleash immune responses in combination cancer therapies. *Cell Rep*. 2019;27:2411-2425. e2419.

95. Allard D, Allard B, Stagg J. On the mechanism of anti-CD39 immune checkpoint therapy. *J Immunother Cancer*. 2020;8:e000186.

96. Kverneland AH, Enevold C, Donia M, Nielsen CH. Development of anti-drug antibodies is associated with shortened survival in patients with metastatic melanoma treated with ipilimumab. *Onco Targets Ther*. 2018;7:e142674.

97. Enrico D, Paci A, Chupat N, Karamouza E, Besse B. Antidrug antibodies against immune checkpoint blockers: impairment of drug efficacy or indication of immune activation? *Clin Cancer Res*. 2020;26:787-792.

98. Ruff WE, Greiling TM, Kriegel MA. Host-microbiota interactions in immune-mediated diseases. *Nat Rev Microbiol*. 2020;18:521-538.

99. Roos MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol*. 2016;16:341-352.

100. Vétiouz M, Pitt JM, Daillère R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*. 2015;350:1079-1084.

101. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. 2018;359:91-97.

102. Matson V, Fessler J, Bao R, et al. The commensal microbiome is associated with response to checkpoint immunotherapy in melanoma patients. *Science*. 2018;359:104-108.

103. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*. 2018;359:97-103.

104. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. *Cancer Cell*. 2018;33:570-580.

105. Davar D, Dzutsev AK, McCulloch JA, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*. 2021;371:595-602.

106. Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory metastatic melanoma patients. *Science*. 2021;371:602-609.

107. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer*. 2021;21:145-161.

108. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377:2531-2544.

109. Schuster SJ, Svboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med*. 2017;377:2545-2554.
124. Grosser R, Cherkassky L, Chintala N, Adusumilli PS. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. *Cancer Cell*. 2019;36:471-482.

125. Qi C, Gong J, Li J, et al. Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. *Nat Med*. 2022;28:1189-1198. doi:10.1038/s41591-022-01800-8

**How to cite this article:** Nagasaki J, Ishino T, Togashi Y. Mechanisms of resistance to immune checkpoint inhibitors. *Cancer Sci*. 2022;113:3303-3312. doi: [10.1111/cas.15497](https://doi.org/10.1111/cas.15497)