Dynamic Observation: Immune-Privileged Microenvironment Limited the Effectiveness of Immunotherapy in an Intraocular Metastasis Mouse Model

Tianchang Tao\textsuperscript{a, b, c}, Yang Liu\textsuperscript{d}, Jun Zhang\textsuperscript{e}, Lvzhen Huang\textsuperscript{a, b, c}, Ye Tao\textsuperscript{f}

\textsuperscript{a}Department of Ophthalmology, Peking University People’s Hospital Eye Diseases and Optometry Institute, Beijing, China; \textsuperscript{b}Beijing Key Laboratory of Diagnosis and Therapy of Retinal and Choroid Diseases, Beijing, China; \textsuperscript{c}College of Optometry, Peking University Health Science Center, Beijing, China; \textsuperscript{d}Department of Ophthalmology, The First Hospital of Lanzhou University, Lanzhou, China; \textsuperscript{e}Department of Internal Medicine, Division of Nephrology University of California, Davis, CA, USA; \textsuperscript{f}Department of Otolaryngology-Head and Neck Surgery, The First Affiliated Hospital of Anhui Medical University, Hefei, China

Keywords
Intraocular metastasis · Animal model · Immunotherapy · Ocular immune privilege · Blood-ocular barrier

Abstract

Introduction: Intraocular metastasis (IM) occurred in approximately 8–10% of patients with metastatic malignancy, for whom oncological immunotherapies showed poor visual potential. However, the mechanism for that inefficiency remains unclear and requires further exploration. Methods: We established a novel mouse model of IM by intracarotid injection of cutaneous melanoma cells. We investigated disease progression using ophthalmic and histological examinations. We used combined anti-PD-1 and anti-CTLA4 antibodies for immunotherapy and evaluated the therapeutic effects in the mouse model. In addition, we characterized the immune microenvironment of tumor-infiltrating CD8\textsuperscript{+} T by fluorescence staining and assessed their cytotoxicity by flow cytometry. Results: All mice presented IM in the left eye, while the right eye was healthy. Uveal tissues with rich vascularity (e.g., the iris, ciliary body, and choroid) initiated IM at an early stage, and IM development resulted in several secondary changes, including corneal swelling, retinal detachment, and intratumoral hemorrhage. Immunotherapy could inhibit IM and prolong the time to eye rupture but did not prevent rupture ending. This inefficiency might be attributed to ocular tissues specificities that inhibited CD8\textsuperscript{+} T-cell infiltration via PD-L1 expression. PD-L1\textsuperscript{low} corneal tissue resisted tumor invasion with high levels of CD8\textsuperscript{+} T-cell infiltration, whereas CD8\textsuperscript{+} T cells were deficient in PD-L1\textsuperscript{high} uveal metastasis. Furthermore, we found a significantly increased PD-1\textsuperscript{−/+} CD4\textsuperscript{+} and PD-1\textsuperscript{−/+} CD8\textsuperscript{+} T cells infiltrating the intratumoral hemorrhage area. Although these CD8\textsuperscript{+} T cells in the IM were not exhausted and had a higher capacity of cytotoxicity (higher interferon-\(\gamma\) ratio) than CD8\textsuperscript{+} T cells in the blood, FasL\textsuperscript{+} PD-L1\textsuperscript{+} ocular tissue can strongly inhibit these IM-infiltrating T cells. Conclusions: Immunotherapy can inhibit the disease progression of IM. Enhancing the effects of tumor-infiltrating CD8\textsuperscript{+} T cells should be one of the highest potentials to improve the visual potential.

Tianchang Tao and Yang Liu have contributed equally to this work.
Introduction

Approximately 8–10% of patients with metastatic malignancy have intraocular metastasis (IM) [1, 2]; the rich vascularity of the uveal tract, which enables metastatic cell seeding, could explain the high incidence of this condition [3]. The primary tumor sites mainly include the breast, lung, and skin, and IM can involve intraocular tissues such as the choroid, iris, and ciliary body [4], resulting in visual impairment and ocular pain [5]. However, clinical treatments for visual protection and eyeball preservation are difficult to develop [6], which may be attributed to the blood-ocular barriers (BOBs) and the intraocular immune-privileged microenvironment that prolong the acceptance of tumor allografts [7, 8].

Activation of therapeutic antitumor immunity may be a promising therapeutic strategy, and immune checkpoint blockade of the B7:CD28 family pathway has demonstrated strong potential against brain metastasis of cutaneous melanoma [9]. Blocking the B7:CD28 family can release T cells from an inhibitory state and activate the tumor-killing function of CD8+ T cells through secretion of granzyme B, perforin, and interferon-γ (IFN-γ) [10]. However, immune therapies for the checkpoint blockade of granzyme B, perforin, and interferon-γ (IFN-γ) [10]. However, immune therapies for the checkpoint blockade showed poor visual potential in the IM of cutaneous melanoma, and the outcomes of this ineffectiveness have not been thoroughly evaluated [11]. Further evaluation requires mirroring the intraocular melanoma process in animal models. Most models are generated using trans-scleral injection [12], and the tumor arising from this type of injection is usually a solitary expanding lesion with BOB disruption [13]. Therefore, the aims of this study were as follows: (1) establish a mouse model of IM by injecting cutaneous melanoma cells into the internal carotid artery (ICA) and observe the pathological progression; (2) assess the effects of immune checkpoint blockade on eyeball preservation; and (3) analyze the features of the immune-privileged microenvironment and identify its therapeutic potential.

Methods

Cell Lines

Since immunotherapy showed optimistic therapeutic effects in melanoma metastasis, we used the mouse melanoma cell line B16-F10 purchased from the National Infrastructure of Cell Line Resource and was authenticated by STR profiling. Melanoma cells were cultured in RPMI-1640 medium supplemented with HEPES, 1-glutamine, 10% fetal bovine serum, 1% nonessential amino acids, 1% sodium pyruvate solution, 1% MEM vitamin solution, and 1% antibiotic-antimycotic solution at 37°C in a humidified incubator with 5% CO2. During culture, cells were grown to 80% confluence before being harvested, washed with Hank’s solution, trypsinized, and harvested for experimental use.

Mice and Modeling of IM

This study obtained forty specific pathogen-free C57BL/6 mice (8 weeks old, male) from the Nanjing Model Animal Institute, and all animals were raised under the following conditions: room temperature, 55 ± 5% humidity, and a standard 12-h dark/12-h light cycle. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Peking University People’s Hospital.

We used isopentane gas inhalation for general anesthesia and injected melanoma cells into the ICA on the left side to establish a model of IM. We shaved the neck fur and used paper tape to fix the animals in a supine position on board. After fixing a mouse in position, we sterilized the neck and made a vertical incision in the skin at the center of the neck. The incision length was approximately 6–8 mm, and we separated the submandibular glands along the midline and exposed the left common carotid artery. Subsequently, we completed the surgery under a stereomicroscope (Olympus Z60) and injected melanoma cells (5 × 106 cells in 100 μL of PBS) into the ICA (detailed procedures in the related video and online suppl. Fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000524485). On the 3rd day post-ICA injection, we intraperitoneally administered anti-programmed death 1 receptor (PD-1) (RMP1-14), anti-CTLA-associated antigen 4 (CTLA4) (9D9), and IgG control (MPC11) antibodies from Bio-X-Cell to 20 mice (200 μg per mouse) every 2 days for a total of 6 injections to evaluate immunotherapy’s effect on eye protection [14, 15]. In addition, mice were euthanized when the eye ruptured during the disease progression.

Ophthalmic Examination in vivo

We examined the anterior and posterior segments with a slit-lamp biomicroscope (SL990; Costruzione Strumenti Oftalmici SRL, Florence, Italy) and an Optos scanning laser ophthalmoscope (P200T; Optos PLC, Dunfermline, UK) for color fundus photography (CFP). In addition, the examination was performed on the 7th and 14th days post-ICA injection, and the mice were anesthetized by isoflurane inhalation before examination.

Histopathologic Examination and Immunofluorescence

Reagents

Eyeballs were harvested on the 7th and 14th days postinjection. We fixed the samples with 4% paraformaldehyde and embedded them in paraffin. Five-micron-thick serial sections of the eyeballs were cut and stained with hematoxylin and eosin for routine microscopic examination to investigate intraocular tumor formation, tumor location, and tumor-infiltrating lymphocytes (TILs).

Immunohistochemistry and immunofluorescence experiments were carried out as described previously [16]. Sections from the eyeballs removed on the 14th day postinjection were stained with primary antibodies against FasL (Thermo, PA5-84227), PD-1 (Abcam, ab214421), PD-L1 (Abcam, ab213480), CD3 (Abcam, ab16669), and CD8 (Abcam, ab217344). Images were acquired using a Leica SP8 confocal laser scanning microscope (Leica Microsystems), and fluorescence images were viewed and analyzed (software: Case Viewer 2.0). We used ImageJ (Java 1.8) to assess the expression and count the cells, according to the criteria previously described [17].
Flow Cytometry and Lymphocytes Stimulation

The eyeballs containing IM were removed and dispersed into a single-cell suspension, and the erythrocytes of peripheral blood from mice were lysed with Tris-NH4Cl (0.83% in 0.01 M Tris-HCl). Blood lymphocytes and TILs cells were stimulated for 4 h with 30-ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma) and 1 μM ionomycin (Sigma) in the presence of 2.5 μg/mL monensin (eBioscience) as previously described [18]. After stimulation, cells were stained for surface markers, fixed and permeabilized with eBioscience FoxP3 fixation buffer according to the manufacturer’s instructions. The fixed cells were stained with antibodies to IFN-γ (XMG1.2, BioLegend), granzyme B (QA16A02, BioLegend), and TNF (MP6-XT22, BioLegend). The samples were then resuspended and analyzed with a flow cytometer (Navios, Beckman, Brea, CA, USA) using FlowJo software.

Statistical Analysis

We used SPSS (IBM SPSS software, version 22.0) for statistical analysis. Student’s t tests and χ² tests were used for subgroup comparisons. In addition, Kaplan-Meier curves were used to analyze survival. \( p < 0.05 \) (2-tailed) was set as the significance threshold \( \ast, p < 0.05; \ast\ast, p < 0.01; \ast\ast\ast, p < 0.001 \).

Results

Modeling Method and Physical Symptoms of IM

This mouse model mirrored the process of human IM, and ICA injection resulted in tumor cells spreading through the intracranial blood circulation to the ophthalmic arteries, thereby resulting in IM (detailed procedures are shown in the online supplementary Fig. S1; video). Since human IM has a higher incidence on the left side than on the right side, we used the left ICA to perform the injection. All mice demonstrated IM with physical signs and pathological symptoms in the left eyes, while all the right eyes on the opposite side were healthy (online suppl. Table S1). Slit-lamp biomicroscopy demonstrated tumor formation in the anterior chamber (AC), visualized as irregular white spots in the AC angle on the 7th day; on the 14th day, these tumor spots had grown to enlarged tumor masses that occupied the pupil, with tumor-associated vascularization and hemorrhages on the surface of the corneal limbus (Fig. 1a–d).

Histopathologic Characteristics at the Early and Advanced Stages of IM Development

We used histological examination (hematoxylin and eosin staining) to compare the healthy controls (right eyes) and the eyes with IM (left eyes). Intraocular uveal
Fig. 2. Histopathologic alterations in the early stage of IM. a The right eyes showed normal intraocular anatomical features, such as corneas (a1), iris (a2), ciliary bodies (a3), and retinas and choroids (a4), and the white arrow shows the RPE layer. b The left eye showed initial tumor formation on the 7th day postinjection as follows: the cornea had a normal thickness (b1), the tumor in the AC blocked the pupil (b2), the tumor in the PC arose from the ciliary body (b3), and one small tumor (white arrow) was identified on the optic nerve head (b4). RPE, retinal pigment epithelium; AC, anterior chamber; PC, posterior chamber.

Fig. 3. Histopathologic alterations in the advanced stage of IM. The development of IM progressed rapidly within 1 week. The tumor had occupied almost all intraocular spaces and invaded intraocular tissues, resulting in a series of secondary changes, such as the corneal swelling, lens dislocation, and RD. Specifically, the observations on those events on the 14th day were as follows: (a) the cornea was swollen (thickness 505.9 μm) with lymphocyte infiltration; (b) tumor invasion was observed on the cornea; (c) the tumor mass occupied both the AC and the PC, and the white arrow shows the iris; (d) a broken iris (white arrowhead) due to tumor invasion was observed in the chambers; (e) RD resulted in the layer of NSR moving forward; (f) the tumor grew between the RPE and sclera, which still integrated and resisted tumor invasion; (g) the tumor showed a small area of hemorrhage with lymphocyte infiltration; and the area of extraocular muscle was observed (h). RD, retinal detachment; AC, anterior chamber; PC, posterior chamber; RGC, retinal ganglion cell; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer.
tissues (e.g., the iris, ciliary body, and choroid) with rich vascularity demonstrated initial IM at an early stage (Fig. 2).

IM developed rapidly to the advanced stage within 1 week. The rapid development of IM resulted in secondary changes, such as corneal swelling, lens dislocation, retinal detachment (RD), and choroidal intratumoral hemorrhage (IH). In the anterior segment, the tumor occupied almost all fluid spaces of the AC and the posterior chamber and destroyed the iris and ciliary body; similarly, in the posterior segment, the choroidal tumor destroyed the choroidal vascular layer and pushed the retinal pigment epithelium (RPE) forward (Fig. 3).

Specifically, the destroyed tissues of the iridociliary area indicated broken blood-aqueous barriers (BABs) in the anterior segment. However, in the posterior segment, the RPE (outer blood-retinal barrier, oBRB) maintained its structural integrity during tumor progression, and the inner side of RPE was tumor free. These results suggested that the IM tumor colonization was located in the uveal capillaries that located outside the oBRB, and IM development can destroy the BAB, while the inner retinal tissue might be inviolated because the RPE maintained its integrity and resisted tumor invasion.

**Immunotherapy Can Alleviate IM but Does Not Prevent Final Eye Rupture**

The immune checkpoint blockade (PD-1 and CTLA4) can eliminate cutaneous melanoma cells [10]. Therefore, we used the combined immune checkpoint blockade (anti-CTLA4 and anti-PD-1 antibodies) to test the effects on intraocular cutaneous melanoma metastasis. We used eye rupture as the final event for eye preservation in the analysis.

In this study, we found that slit-lamp biomicroscopy and CFP demonstrated that immunotherapy inhibited
Fig. 5. T-cell infiltration and immune characteristics in the subareas of IM. a–d Corneal subarea: the cornea showed a significant increase in T-cell infiltration, and the infiltrating T cells were mainly CD8+ T cells (a) but not CD4+ T cells (b); most of these CD8+ T cells were PD-1 negative (c); a few corneal cells expressed PD-L1 (d). e–h Iridociliary area: the tumor occupied this area with little T-cell infiltration (e, f); some tumor cells expressed PD-1 (g); and the iris expressed a high level of PD-L1 (h). i–l The subarea of choroidal metastasis: RD resulted from the choroidal tumor, with few CD8+ T cells and a few CD4+ T cells infiltrating. m–p Subarea of IH in choroidal metastasis: IH resulted in increased T-cell infiltration (m), which consisted of both CD4+ and CD8+ T cells (n); and (o) these T cells were PD-1+; furthermore, the surrounding tissues of these T cells had a high level of expression of PD-L1. q CD8+ T-cell quantity comparison in subareas of the IM. r CD4+ T-cell quantity comparison in subareas of the IM. s PD-1 expression in subareas of the IM. t PD-L1 expression in the subareas of the IM. **, p < 0.01; ***, p < 0.001; ****, p < 0.0001. IH, intratumoral hemorrhage.
IM progression. Furthermore, immunotherapy could prolong the time to eye rupture. However, all eyes of the IM group underwent a tumor-associated rupture process (Fig. 4).

**T-Cell Infiltration and Immune Characteristics in the Subareas of IM**

The ineffectiveness of immune therapy for IM requires more specific evaluations of the subareas of the IM (corneal, iridociliary, and choroidal areas and the IH area). Therefore, we assessed the CD4+ and CD8-cell quantities and PD-1 and PD-L1 expression in these subareas of the IM.

The cornea demonstrated strong resistance to IM invasion with high levels of CD8+ T-cell infiltration, and these T cells were PD-1 negative, indicating a high potential tumor-killing ability. Furthermore, the PD-L1 ligand, expressed by corneal cells, could inhibit T-cell functions and had much lower expression than that in the other subareas (Fig. 5a–d). In contrast, the iridociliary area presented tumor-promoting characteristics. The iris and ciliary body tissues expressed high PD-L1 expression, and the AC and posterior chamber areas occupied by the tumor had little CD4+ or CD8+ T-cell infiltration (Fig. 5e–h). The high expression of PD-L1 and T-cell deficiency indicated an immune-privileged state in the iridociliary area. Similarly, the subarea of choroidal metastasis, located between the RPE and sclera, had scattered PD-1+ CD4+ T-cell infiltration and CD8+ T-cell deficiency (Fig. 5i–l).

Interestingly, the choroidal tissue had rich vascularity that resulted in not only rapid tumor development but also the constant appearance of IH, in which we found significantly increased PD-1+/− CD4+ and PD-1+/− CD8+ T cells, and these infiltrating T cells were surrounded by choroidal tissues with a high level of PD-L1 expression (Fig. 5m–p). This phenomenon indicated that IH allows T cells to enter the tumor and generate antitumor immunity; however, the PD-L1high choroidal tissues could inhibit PD-1+ T-cell function. We assessed these T-cell-associated immune checkpoint characteristics within different subareas of IM, and the comparisons of those characteristics confirmed that the corneal tissue expressed a low level of PD-L1 and resisted tumor invasion with high levels of CD8+ T cells, while CD8+ T cells were deficient in the uveal subareas, with high PD-L1 expression in the iridociliary and choroidal tissues (online supplementary Table S2; Fig. 5q–t).

**FasL+ Ocular Tissues Can Inhibit IM-Infiltrating CD8+ T Cells**

Ocular immune privilege was dependent on Fas (CD95)-Fas ligand (FasL)-mediated apoptosis and did not cause tissue damage in response to viral infections and other inflammatory diseases. However, whether FasL+ corneal and choroidal tissues inhibit cytotoxic CD8+ T cells during IM progression remained unknown. Therefore, we used fluorescent double staining of anti-FasL and anti-CD8 to assess the location of FasL+ corneal and choroidal tissue and CD8+ T cells. This finding showed that some CD8+ T cells were close to FasL+ cornea (Fig. 6a) and most CD8+ T cells were surrounded by FasL+ choroidal tissue (Fig. 6b). This close location suggests that
CD8⁺ T cells may be strongly inhibited by the FasL⁺ corneal and choroidal tissues surrounding them. However, a large proportion of the CD8⁺ T cells infiltrating the cornea were not inhibited by FasL⁺ cornea, indicating that the cornea has capacity of resistance to tumor invasion during IM progression.

**IM-Infiltrating CD8⁺ T Cells Were Not Exhausted but Possessed High Cytotoxicity**

Although IM-infiltrating CD8⁺ T cells were mostly inhibited by FasL⁺ PD-L1⁺ ocular tissues, identification of CD8⁺ T cells exhaustion could indicate the potential effect of immunotherapy. Therefore, we extracted these TILs and cultured them with stimulation, and the flow...
cytometry assay revealed that the IM-infiltrating CD8+ T cells were not only not exhausted, but also had a higher capacity of IFN-γ secretion than the CD8+ T cells in the blood (Fig. 7a–f).

Discussion

Uveal metastasis is the most common intraocular malignancy. This high incidence of this condition could be explained by the seed and soil hypothesis [19], which states that the tumor cells (seeds) and stromal environment (soil) participate in important molecular interactions and thereby initiate metastasis with tissue specificity [20].

The angiogenic privilege of the cornea might prevent tumor invasion and lead to a significant increase in CD8+ T-cell infiltration, which has further antitumor functions. A delicate balance between proangiogenic and antiangiogenic stimulation in the normal cornea maintains a relatively avascular state, which is essential for optical clarity. However, in this mouse model of IM, the cornea was swollen (∼600 μm) to approximately 5~6 times the standard corneal thickness (∼100 μm); this increased thickness and simultaneous corneal edema or opacity indicated the incidence of secondary glaucoma and intensive alterations in humoral immunity within the AC [21]. Furthermore, antigen presentation by major histocompatibility complex class I (MHC-I) and class II (MHC-II) molecules is a prerequisite for T-cell engagement and activation. Selective expression of MHC molecules on corneal epithelial cells (reduced MHC class I and no MHC class II expression) [22] could explain why the cornea showed significant infiltration of CD8+ T cells but not CD4+ T cells.

The BOBs did not limit the entry of the immunotherapy antibodies, but IM destroyed the structure of the BAB. BOBs can strictly limit inward crossing compounds with high specificity and selectivity, thereby representing the main obstacles in the systemic treatment of intraocular diseases. Furthermore, the BOB consists of the BAB and the BRB; the BAB is composed of the nonpigmented epithelium of ciliary body, the posterior iris epithelium, the endothelium of iris vessels, and the endothelium of Schlemm’s canal and the BRB is composed of two types of cells (e.g., retinal capillary endothelial cells, RPE cells) that constitute the inner blood-retinal barrier and oBRB, respectively [23]. In our study, the tumor cells injected into the ICA and its ophthalmic artery branch colonized the endings of arteriovenous circulation and the surface of the uveal vascular endothelial cells but were not found in inner area of RPE. Therefore, metastatic tumor colonization was outside the oBRB during IM progression, where antibodies can reach these TILs and thus allow them to perform their tumor-killing activities. Consistently, immunotherapy inhibited IM progression and prolonged the time to eye rupture.

Choroidal metastasis results in exudative RD between the neurosensory retina (NSR) and the RPE, similar to exudative RD in primary choroidal melanoma. Although both the NSR and the RPE are derived from the neuroectoderm that lines the optic vesicle during embryogenesis, there are no structural junctions between the NSR and RPE cells, and therefore, the forces of attachment between the NSR and RPE are weak. Thus, either mechanical forces or pathological alterations that can overwhelm this weak force could result in RD between the two layers [24]. Specifically, choroidal tumor progression can not only push the RPE forward but also obstruct the retinal or choroidal vessels, which supplies nutrition and oxygen and transfers metabolites for the RPE and NSR. Furthermore, vascular obstruction and tumor hypermetabolism disrupt the physiological equilibrium and result in a series of pathological alterations between the RPE and the NSR (e.g., ischemia and hypoxia, tumor-associated inflammation, tumor exudation).

The uveal tissues show a specificity that established an intraocular immune-privileged microenvironment with inhibited antitumor immunity. The uvea constructs the BAB with unique anatomical features and thereby forms an enclosed microenvironment in which sufficient immunosuppressive molecules (growth factors, cytokines, neuropeptides, and soluble receptors) can inhibit the activity of immune cells and thereby induce a systemic form of tolerance called AC-associated immune deviation [25]. Importantly, the iris pigment epithelium expressed a high level of CD86 (CTLA4 ligand) and thereby neutralized effector T cells and converted them into regulatory T cells (immune-inhibiting function) when these effector T cells passed through the iris pigment epithelium layer [26]. Furthermore, α-melanocyte-stimulating hormone (α-MSH) and transforming growth factor-β2 (TGF-β2), soluble in aqueous humor, can convert effector T cells into T regulatory cells [27].

Although immunotherapy can inhibit IM progression, immunotherapy cannot prevent eye rupture, and this ineffectiveness may be attributed to intraocular immune-privileged tissue specificities or immune checkpoint inhibitors (ICIs)-induced potential ocular adverse events. In this study, the uveal tissues (iris, ciliary body, and cho-
rroid), which were disrupted by tumor invasion, showed high PD-L1 expression, and their surrounding tumors had little CD8+ T-cell infiltration. The high PD-L1 expression in uveal tissues can compete with immunotherapy antibodies and thereby limit the therapeutic effects. The reason for PD-L1 expression remains unknown; however, a recent study revealed that IFN-γ, as a classic antitumor cytokine, can stimulate cell lines of uveal tissues to increase PD-L1 expression [28]. Besides, the FasL has been reported to mediate the interaction between the immune system and the intraocular tissues, which plays an essential role in maintaining the immune privilege [29, 30]. Hallermalm et al. [31] also found that FasL protected the intraocular melanoma cells from the cytotoxic lymphocyte killing by acting in an autocrine manner and defined a unique mechanism of tumor escaping from the immune surveillance; these findings indicate FasL might contribute to the ocular immune privilege of IM in this model. It is noteworthy that some patients who underwent treatment with ICIs might develop uveitis [32–34], which is shown to be significantly associated with melanoma and anti-PD-1 plus anti-CTLA-4 combination. Bomze et al. [33] suggested that ICIs could eliminate the inhibitory signal on T-cell activity to unleash autoimmune response, thereby causing adverse events on uveal tissues. In addition to the B7-H2 immune checkpoints family blockage, ocular tissues also express other inhibitory molecules that perform immune inhibitory function (e.g., FasL, IL-10, TGF-β) to attenuate the effects of ICIs. This might explain why anti-PD-1 and anti-CTLA-4 could not completely prevent IM in our study. Importantly, the intraocular tissue specificities that participate in immunity regulation are mostly unknown and require further mechanical studies to enhance the effects of immunotherapy.

Statement of Ethics
This study was approved by the Institutional Animal Care and Use Committee of Peking University People’s Hospital (Number: 2019PHE059).

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Funding Sources
This study was supported by the National Natural Science Foundation of China Grant (81670870, 82171128), the Science and Technology Innovation Project of Chinese Academy of Medical Sciences (2019-RC-HL-019), the National Key Research and Development Program of China (No. 2020YFC2008200), the China Postdoctoral Science Foundation (2019M652215), the Initial Research Fund of the First Affiliated Hospital of Anhui Medical University, the Fundamental Research Funds for the Central Universities (WK2070000149), the New Medicine Fund of the University of Science and Technology of China (WK2070000170), the Academic Fund from the Frist Hospital of Lanzhou University (Idyvn2018-22), and the Health Industry Management Project of Gansu Province (GSWSKY-2019-93).

Author Contributions
Ye Tao and Lvyzen Huang designed this study. Ye Tao and Tianchang Tao performed the experimental process. Jun Zhang analyzed the data. Tianchang Tao and Yang Liu wrote this article.

Data Availability Statement
All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.

References
1 Wickremasinghe S, Dansingani KK, Tranos P, Liyanage S, Jones A, Davey C. Ocular presentations of breast cancer. *Acta Ophthalmol Scand.* 2007;85(2):133–42.
2 Eliassi-Rad B, Albert DM, Green WR. Frequency of uveal metastases in patients dying of cancer in eye bank populations. Br J Ophthalmol. 1996;80(2):125–8.
3 Biscotti CV, Singh AD. Uveal metastases. *Monogr Clin Cytol.* 2012;21:17–30.
4 Shields CL, Shields JA, Gross NE, Schwartz GP, Lally SE. Survey of 520 eyes with uveal metastases. *Ophthalmology.* 1997;104(8):1265–76.
5 Konstantinidis L, Rospond-Kubiak I, Zeolite I, Heimann H, Groenewald C, Coupland SE, et al. Management of patients with uveal metastases at the Liverpool Ocular Oncology Centre. Br J Ophthalmol. 2014;98(1):92–8.
6 Mathis T, Jardel P, Loria O, Delaunay B, Nguyen AM, Lanza F, et al. New concepts in the diagnosis and management of choroidal metastases. *Prog Retin Eye Res.* 2019;68:144–76.
7 Niederkorn J, Streilein JW, Shadduck JA. Deviant immune responses to allogeneic tumors injected intracamerally and subcutaneously in mice. *Invest Ophthalmol Vis Sci.* 1981;20(3):355–63.
8 Streilein JW. Ocular immune privilege: therapeutic opportunities from an experiment of nature. *Nat Rev Immunol.* 2003;3(11):879–89.
9 Iorgulescu JB, Harary M, Zogg CK, Ligon KL, Reardon DA, Hodi FS, et al. Improved risk-adjusted survival for melanoma brain metastases in the era of checkpoint blockade immunotherapies: results from a national cohort. *Cancer Immunol Res.* 2018;6(9):1039–45.
10 Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science.* 2015;348(6230):74–80.
11 Francis JH, Berry D, Abramson DH, Barker CA, Bergstrom C, Demirci H, et al. Intravitreous cutaneous metastatic melanoma in the era of checkpoint inhibition: unmasking and masquerading. Ophthalmology. 2020;127(2):240–8.
12 Cao J, Jager MJ. Animal eye models for uveal melanoma. Ocul Oncol Pathol. 2015;1(3):141–50.
13 Richards JR, Yoo JH, Shin D, Odelberg SJ. Mouse models of uveal melanoma: strengths, weaknesses, and future directions. Pigment Cell Melanoma Res. 2020;33(2):264–78.
14 Taggart D, Andreou T, Scott KJ, Williams J, Rippaus N, Brownlie RJ, et al. Anti-PD-1/anti-CTLA-4 efficacy in melanoma brain metastases depends on extracranial disease and augmentation of CD8(+) T cell trafficking. Proc Natl Acad Sci U S A. 2018;115(7):e1540–9.
15 Allard B, Allard D, Stagg J. Methods to evaluate the antitumor activity of immune checkpoint inhibitors in Preclinical Studies. Methods Mol Biol. 2016;1458:159–77.
16 Wang W, Chan A, Qin Y, Kwong JMK, Caprioli J, Levinson R, et al. Programmed cell death-1 is expressed in large retinal ganglion cells and is upregulated after optic nerve crush. Exp Eye Res. 2015;140:1–9.
17 Tao Y, Gross N, Liu Y, Zhang L, Li G, Huang Z, et al. A high ratio of IL-12Rβ2-positive tumor-infiltrating lymphocytes indicates favorable prognosis in laryngeal cancer. Oral Oncol. 2017;74:148–56.
18 Zhang Q, Bi J, Zheng X, Chen Y, Wang H, Wu W, et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. Nat Immunol. 2018;19(7):723–32.
19 Fidler II, Poste G. The “seed and soil” hypothesis revisited. Lancet Oncol. 2008;9(8):808.
20 Fokas E, Engenhart-Cabillic R, Danilidis K, Rose F, An HX. Metastasis: the seed and soil theory gains identity. Cancer Metastasis Rev. 2007;26(3–4):705–15.
21 Stein-Streilein J, Streilein JW. Anterior chamber associated immune deviation (ACAID): regulation, biological relevance, and implications for therapy. Int Rev Immunol. 2002;21(2–3):123–52.
22 Nicholls SM, Bradley BB, Easty DL. Effect of mismatches for major histocompatibility complex and minor antigens on corneal graft rejection. Invest Ophthalmol Vis Sci. 1991;32(10):2729–34.
23 Cunha-Vaz J, Bernardes R, Lobo C. Blood-retinal barrier. Eur J Ophthalmol. 2011;21 Suppl 6:S3–9.
24 Ghazi NG, Green WR. Pathology and pathogenesis of retinal detachment. Eye. 2002;16(4):411–21.
25 Vendomele J, Khebizi Q, Fisson S. Cellular and molecular mechanisms of anterior chamber-associated immune deviation (ACAID): what we have learned from knockout mice. Front Immunol. 2017;8:1686.
26 Sugita S, Horie S, Nakamura O, Futagami Y, Takase H, Keino H, et al. Retinal pigment epithelium-derived CTLA-2a induces TGFβ-producing T regulatory cells. J Immunol. 2008;181(11):7525–36.
27 Taylor AW, Ng TF. Negative regulators that mediate ocular immune privilege. J Leukoc Biol. 2018;103(6):1179–87.
28 Yang W, Li H, Chen PW, Alizadeh H, He Y, Hogan RN, et al. PD-L1 expression on human ocular cells and its possible role in regulating immune-mediated ocular inflammation. Invest Ophthalmol Vis Sci. 2009;50(1):273–80.
29 Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. Science. 1995;270(5239):1189–92.
30 Sano Y, Sotozono C. Role of Fas ligand in ocular tissue. Cornea. 2002;21(2 Suppl 1):S30–2.
31 Hallermalm K, De Geer A, Kessling R, Levitsky V, Levitskaya J. Autocrine secretion of Fas ligand shields tumor cells from Fas-mediated killing by cytotoxic lymphocytes. Cancer Res. 2004;64(18):6775–82.
32 Sun MM, Levinson RM, Filipowicz A, Anesi S, Kaplan HI, Wang W, et al. Uveitis in patients treated with CTLA-4 and PD-1 checkpoint blockade inhibition. Ocul Immunol Inflamm. 2020;28(2):217–27.
33 Bomzé D, Meirson T, Hasan Ali O, Goldman A, Flatz L, Habot-Wilner Z. Ocular adverse events induced by immune checkpoint inhibitors: a comprehensive pharmacovigilance analysis. Ocul Immunol Inflamm. 2022;30(1):191–7.
34 Sun MM, Kelly SP, Mylavarapu BS AL, Holland GN, Coleman AL, Yu F, et al. Ophthalmic immune-related adverse events after anti-CTLA-4 or PD-1 therapy recorded in the American academy of ophthalmology intelligent research in sight registry. Ophthalmology. 2021;128(6):910–9.