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14. ABSTRACT

Immunotherapies largely ineffective in uveal melanoma (UM) and pancreatic ductal adenocarcinoma (PDAC). The mechanisms underlying poor response to immunotherapy in UM and PDAC are unclear. Our preliminary analysis showed that A2AR and CD73 were overexpressed in UM and PDAC and associated with poor survival. CD73 and A2AR are crucial factors in the immunosuppressive adenosine pathway. A major gap lies in our knowledge of the role of the adenosine pathway driving immune suppression in UM and PDAC. In this proposal, we hypothesize that the CD73-Adenosine-A2AR axis represents a stress-induced immunosuppressive mechanism in UM and PDAC. The overall goal of this proposal is to analyze the functional roles of CD73 and A2AR in the immunosuppressive microenvironment of UM and PDAC. Furthermore, we will develop a new strategy combining CD73/A2AR inhibitors with checkpoint inhibitors to inhibit UM and PDAC tumor growths. This preclinical translational research will help to establish CD73 and A2AR as novel biomarkers and immunotherapy targets for metastatic UM and PDAC.

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1. INTRODUCTION:

Immunotherapies are largely ineffective in uveal melanoma (UM) and pancreatic ductal adenocarcinoma (PDAC). The mechanisms underlying poor response to immunotherapy in UM and PDAC are unclear. Our preliminary analysis showed that A2AR and CD73 were overexpressed in UM and PDAC and associated with poor survival. CD73 and A2AR are crucial factors in the immunosuppressive adenosine pathway. A major gap lies in our knowledge of the role of the adenosine pathway in driving immune suppression in UM and PDAC. In this proposal, we hypothesize that the CD73-Adenosine-A2AR axis represents a stress-induced immunosuppressive mechanism in UM and PDAC. We will conduct a series of studies to systematically characterize CD73 and A2AR-related immune signatures in UM and PDAC tumors and release adenosine-driven immunosuppression by targeting CD73 and A2AR. The overall goal of this proposal is to analyze the functional roles of CD73 and A2AR in the immunosuppressive microenvironment of UM and PDAC. Furthermore, we will develop a new strategy combining CD73/A2AR inhibitors with checkpoint inhibitors to inhibit UM and PDAC tumor growths. This preclinical translational research will help to establish CD73 and A2AR as novel biomarkers and immunotherapy targets for metastatic UM and PDAC.

2. KEYWORDS:

Uveal melanoma (UM), Pancreatic ductal adenocarcinoma (PDAC), Immunotherapy, Immunosuppression, Adenosine pathway, CD73, A2AR, Checkpoint inhibitor.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals for this project are outlined below.

Specific Aim 1: To Quantify and evaluate CD73 and A2AR expressions and their clinical relevance in uveal melanoma (UM) and pancreatic ductal adenocarcinoma (PDAC) tumors.

Major Task 1: To characterize profiles of CD73 and A2AR in UM and PDAC tumors.

Major Task 2: To characterize the immune infiltrates and their correlations with CD73 and A2AR in UM and PDAC tumors.

All the experiments have been completed for Specific Aim 1. (100% complete in September 2022)

We are conducting the data analysis and statistical quantification of each marker, which are expected to be completed by January 31, 2023. We aim to publish manuscripts to report the finding of Specific aim 1 in June 2023.

Specific Aim 2: To enhance immune response in UM and PDAC tumors by small inhibitors targeting CD73 and A2AR, and further examine their anti-tumor effects in combination with CPIs.

Major Task 1: To examine the effects of CD73 and A2AR inhibitors on the growths of UM and PDAC cells *in vitro*.

50% of the experiments have been completed.
The *in vitro* experiments under normoxic conditions have been completed for Major Task 2.
The *in vitro* experiments under hypoxia conditions are currently pending due to delays in the parts to fully set up the hypoxia incubator through the vendor (Eppendorf) in our lab. We expect to finish the rest of the hypoxic experiments in February 2023.

Major Task 2: To examine the efficacy of checkpoint inhibitors (CPIs, anti-PD-1, or anti-CTLA4 drug) with CD73/A2AR inhibitors on antitumor immune cell response, especially for cytotoxic T lymphocytes, *in vitro*.

We have established GFP-stable pancreatic cancer cell lines, Panc-1 and MIA Paca-2, for *in vitro* T cell cytotoxic assays of this Major Task 2. We are currently constructing GFP-stable uveal melanoma cell lines (MP38 and 92.1), which are needed for the *in vitro* T cell cytotoxic assays.
About 15% of experiments have been completed to establish necessary cell lines for Major Task 2 of Specific Aim 2. We expect to finish all the experiments of this Task 2 in May 2023.

Major Task 3: To test the efficacy of combining CPIs (anti- PD-1 or anti-CTLA4 drug) with blockade of adenosine signaling (co-inhibition of CD73 and A2AR) on antitumor immune response in humanized mouse models of UM and PDAC.

We have established luciferase stable pancreatic cancer cell lines (Panc-1 and MIA Paca-2) and uveal melanoma cell lines (92.1 and 39) for *in vivo* xenograft studies of this Major Task 2. These Luc-stable cell lines have been successfully tested to establish pancreatic tumors or uveal melanoma in NSG mice. About 30% of experiments have been completed to establish necessary Luc-stable cell lines for Major Task 3 of Specific Aim 3. We expect to finish all the experiments of this Task 3 in August 2023.

What was accomplished under these goals?

Due to some obstacles related to the Pandemic on the establishment of my laboratories and the supplies of research equipment and materials in the last two years, we requested a no-cost extension (NCE) to complete all the proposed studies till 10/31/2023. We truly appreciated the approval of the NCE and the support from the managing and scientific teams at DOD. The complete closure of UTEP and its facilities started on March 16, 2020, in response to the COVID-19 Pandemic. Also, we slowly got back into the laboratory in the Spring of 2021. We still encountered another complete closure of laboratories and facilities in our department in November and December 2021 due to the outbreak of COVID-19 infections at our School. Our laboratory has been completely shut down for almost two months. We have made no progress on the stated goals of the grant during that period, nor have we incurred any expenditures on the grant during that time. The Pandemic also affected us to order and obtain some research supplies on time, especially for several CD73 and A2AR drugs/inhibitors, the hypoxia incubator, tumor tissues, and cancer cell lines. We had to wait more than six months to get

the hypoxia incubator after ordering through the vendor at Fisher Scientific due to the backorder. Also, we had to submit various documents requesting the uveal melanoma tumor tissue arrays (TMAs) from MD Anderson Cancer, which was taken up more than eight months for MD Anderson to release the TMAs to our studies of Specific Aim 1

Although with all these obstacles, we were hitting some very important milestones on the project. We have now finished all the experiments for Specific Aim 1. We have completed the staining of all the markers for uveal melanoma and pancreatic cancer TMAs. We are currently analyzing the data and characterizing the immune profiles relevant to the adenosine pathway in TMAs.

For Aim 2, Dr. Mariana Grigoruta (postdoctoral fellow) finished the training for generating mouse xenografts bearing tumors of uveal melanoma and pancreatic cancer. We also established and optimized the T cell cytotoxic assay research protocol using the Lionheart FX Automated Microscopes System (BioTek). Moreover, we have successfully established GFP-stable pancreatic cell lines (Panc-1 and MIA Paca-2), luciferase-stable pancreatic cancer cell lines (Panc-1 and MIA Paca-2), and luciferase-stable uveal melanoma cell lines (MP38 and 92.1).
All these cell lines are necessary tools for all the proposed experiments of Major Tasks 2 & 3 in Specific Aim 2. Moreover, we confirmed that those luciferase PDAC and UM cell lines could be used to establish tumors in NSG mice. We have established a new IRB protocol (1677978-1) and a new IACUC protocol (A-202007-2) for this project, which were approved by UTEP, HRPO (E00922.1b), and ACURO.

The progress to date is summarized below, and we detailed where we are behind in the timeline and when we plan to complete tasks.

Aim 1. To Quantify and evaluate CD73 and A2AR expressions and their clinical relevance in uveal melanoma (UM) and pancreatic ductal adenocarcinoma (PDAC) tumors.
Major Task 1: To characterize profiles of CD73 and A2AR in UM and PDAC tumors (IHC).
Major Task 2: To characterize the immune infiltrates and their correlations with CD73 and A2AR in UM and PDAC tumors (qMIF).

We have finished the immunohistochemistry (IHC) and quantitative multiplex immunofluorescence (qmIF) staining of two panels of immune markers on pancreatic cancer and uveal melanoma tissue microarrays (TMAs). These markers are CD4, CD8, CD56, CD73, A2AR, FOXP3, CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI. Two pancreatic cancer tumor TMAs (#27020111 and # 7020110, Biochanin) include a total of 150 cases. Two uveal melanoma TMAs (MD Anderson Cancer Center) include 62 cases.

We identified the CD73 antibody (CST, 13160) and the A2AR antibodies (Abcam, ab3461) as primary antibodies for IHC and qmIF staining of UM and PDAC TMAs. As shown in Figure 1A, we successfully detected CD73 and A2AR expressions in formalin-fixed paraffin-embedded (FFPE) melanoma tissues at a 1:100 dilution. We were able to quantify the IHC staining of A2AR for patients' tumors from these two UM TMAs (62 cases). There were 36 UM patients' tumors that showed positive staining for A2AR (78.3%), and 10 UM patients' tumors were negative for A2AR staining (21,7%) (Figure 1B). Moreover, 24 UM patients' tumors showed moderated or intensive staining of

A2AR (52.2%). These data further confirmed that the majority of UM tumors expressed high levels of A2AR.

Due to the correlation of immune infiltrate with tumor response to anti-CD73 or anti-A2AR therapy in several tumor models, we proposed to quantitatively analyze the immune infiltrate in patients' tumor samples by phenotype and function. After antibody optimization, we conducted qmIF staining of CD4, CD8, CD56, CD73, A2AR, FOXP3, CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI in the FFPE UM and PDAC TMAs. The same FFPE TMAs were used for qmIF staining. Ki-67 will be used as the cancer cell marker, and Glut1 will be applied as the hypoxic marker in our qMIF panels. The experimental conditions for qMIF staining of CD73 and A2AR were optimized in human melanoma tissues. The CD73 antibody with 1:400 titration and the A2AR antibody with 1:100 titration showed good staining intensity for qmIF (Figure 2 & 3).

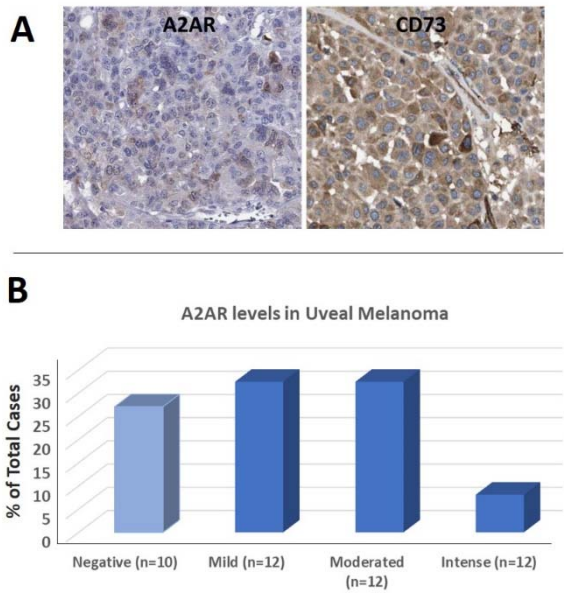


Figure 1. (A) Representative IHC staining of A2AR and CD73 in UM tumors. Positive staining in UM tumor cells was presented in brow chromogen. Magnification 20X. (B) Quantification of A2AR in UM tumors as negative, mild, moderated, and intense staining.

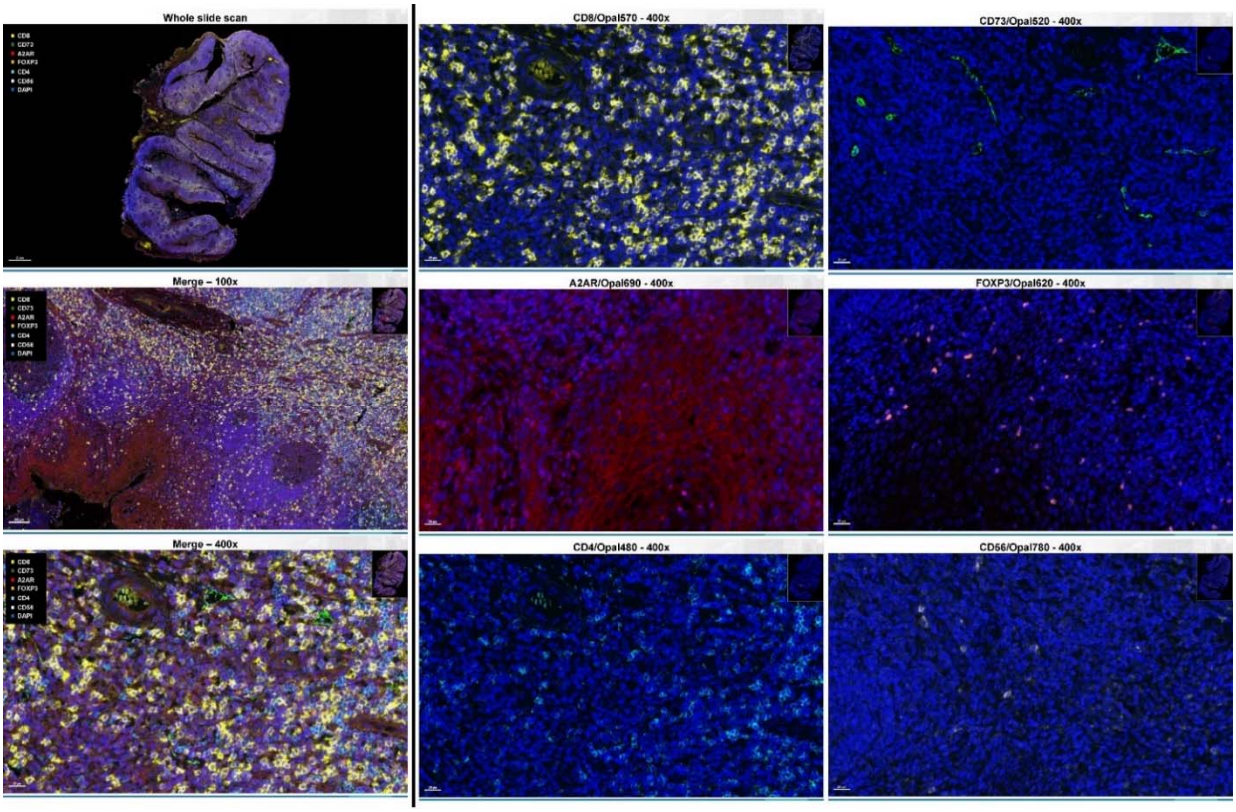


Figure 2. The optimization of multiplex immunofluorescence staining for CD8, CD73, A2AR, FOXP3, CD4, CD56, and DAPI in FFEP tonsil tissue. The whole slide/tissue scans were shown in the left panel. The individual immunofluorescence staining of each maker was shown in the right panel along with DAPI. The color for each maker was indicated on the right top of the whole slide scan.

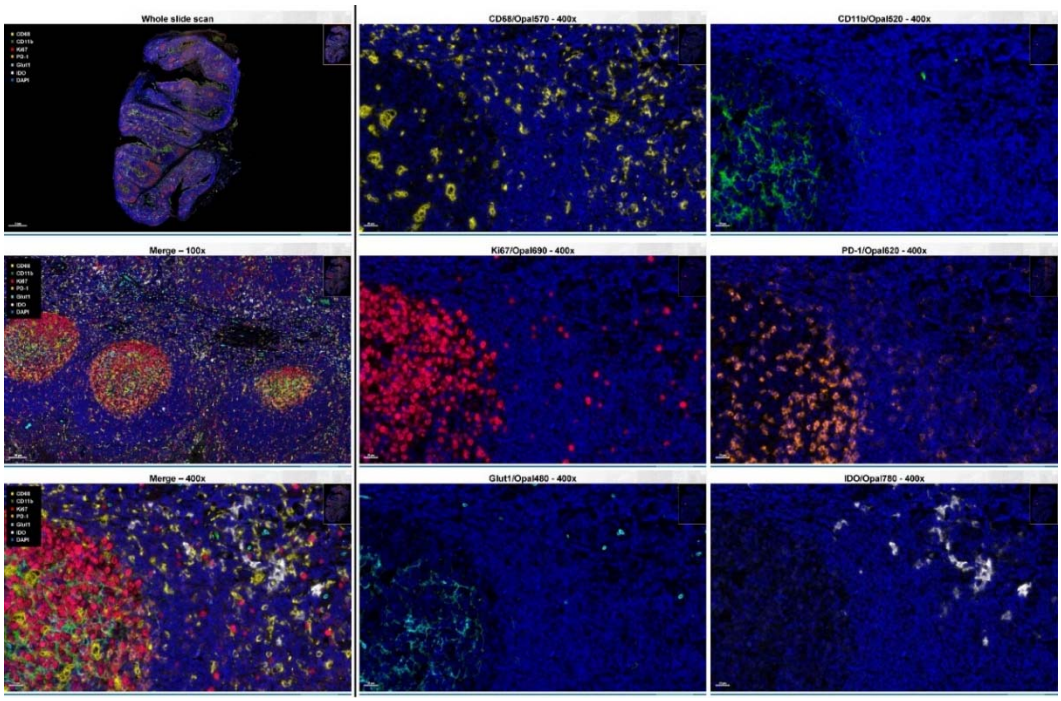


Figure 3. The optimization of multiplex immunofluorescence staining for CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI in FFEP tonsil tissue. The whole slide/tissue scans were shown in the left panel. The individual immunofluorescence staining of each maker was shown in the right panel along with DAPI. The color for each maker was indicated on the right top of the whole slide scan.

The complete qmIF for CD8, CD73, A2AR, FOXP3, CD4, CD56, and DAPI in each TMA was shown in the following Figures 4-7.

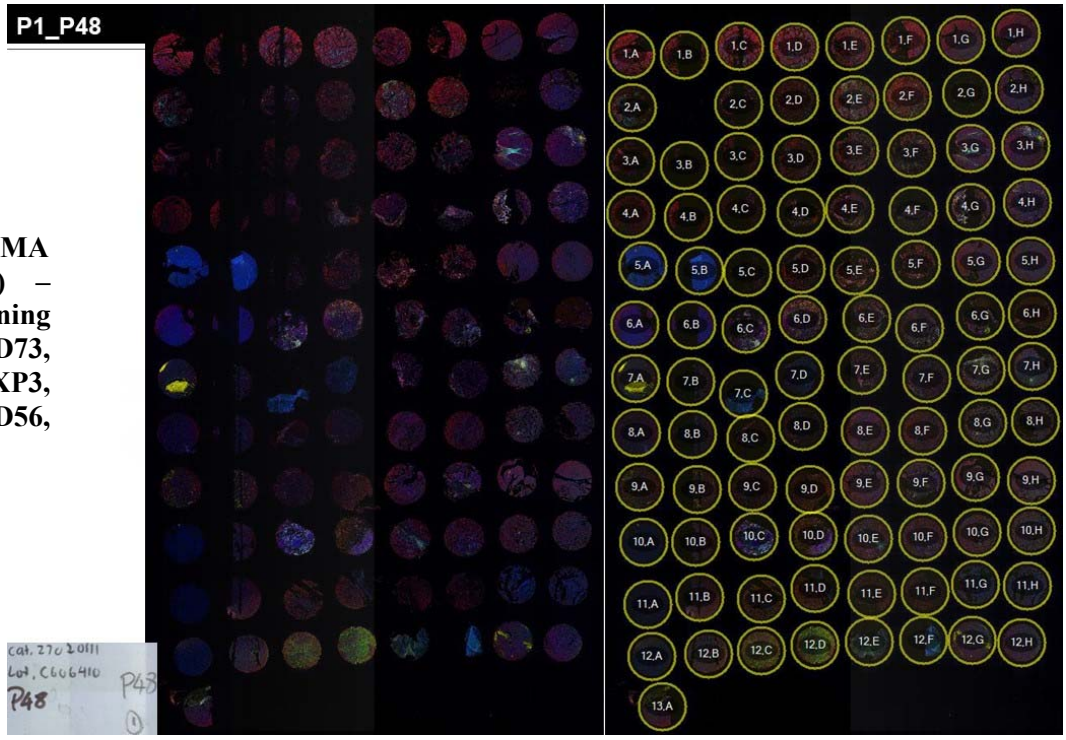


Figure 4: PDAC #1 TMA (48 Cases) – qmIF staining of CD8, CD73, A2AR, FOXP3, CD4, CD56, and DAPI.

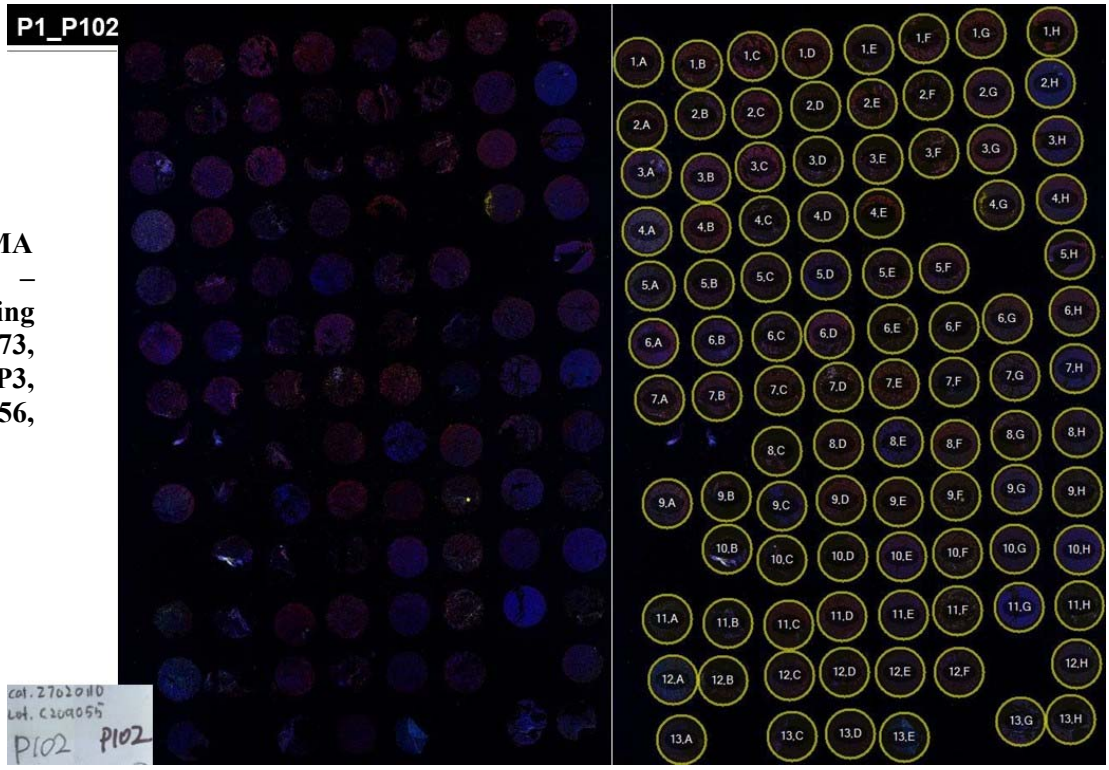


Figure 5:
PDAC #2 TMA (102 Cases) – qmIF staining of CD8, CD73, A2AR, FOXP3, CD4, CD56, and DAPI.

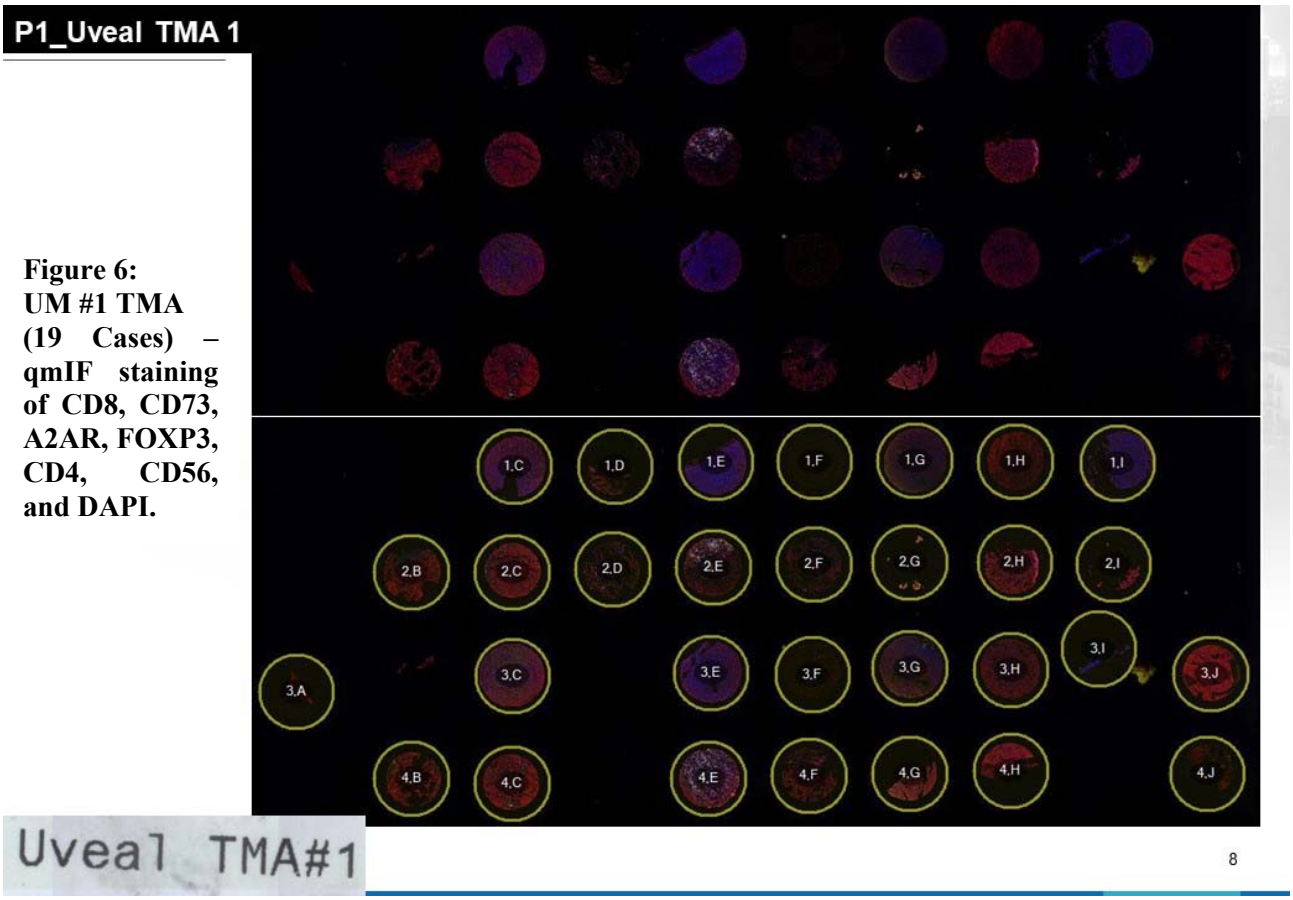


Figure 6:
UM #1 TMA (19 Cases) – qmIF staining of CD8, CD73, A2AR, FOXP3, CD4, CD56, and DAPI.

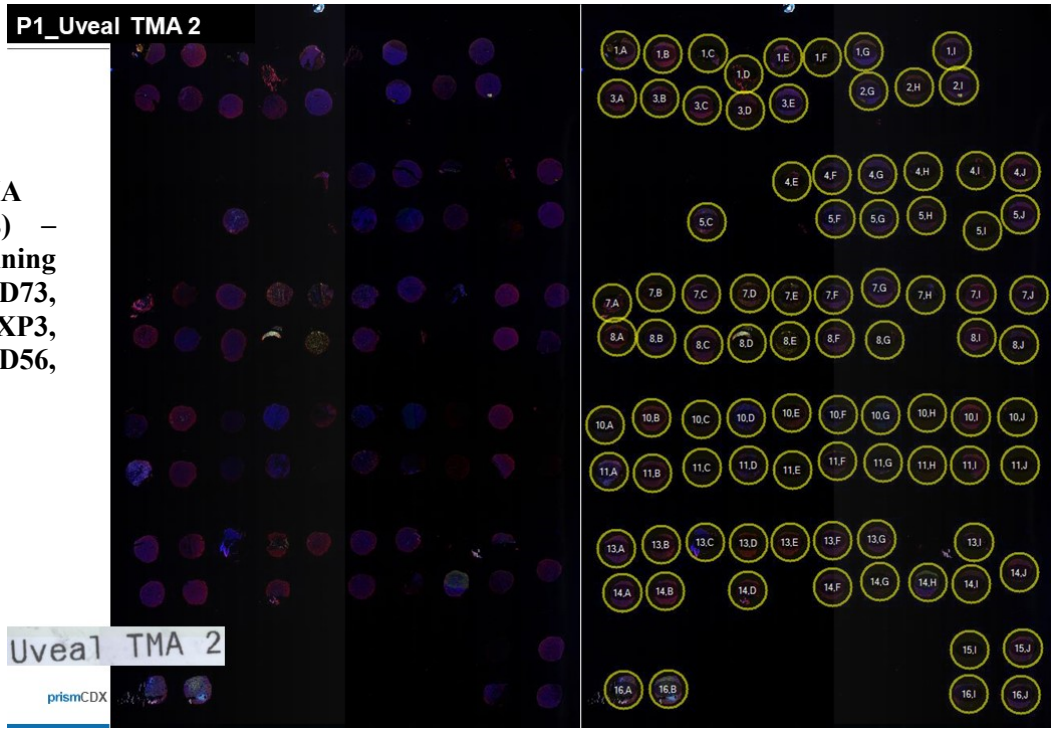


Figure 7: UM #2 TMA (43 Cases) – qmIF staining of CD8, CD73, A2AR, FOXP3, CD4, CD56, and DAPI.

The complete qmIF for CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI in each TMA was shown in the following Figures 8-11.

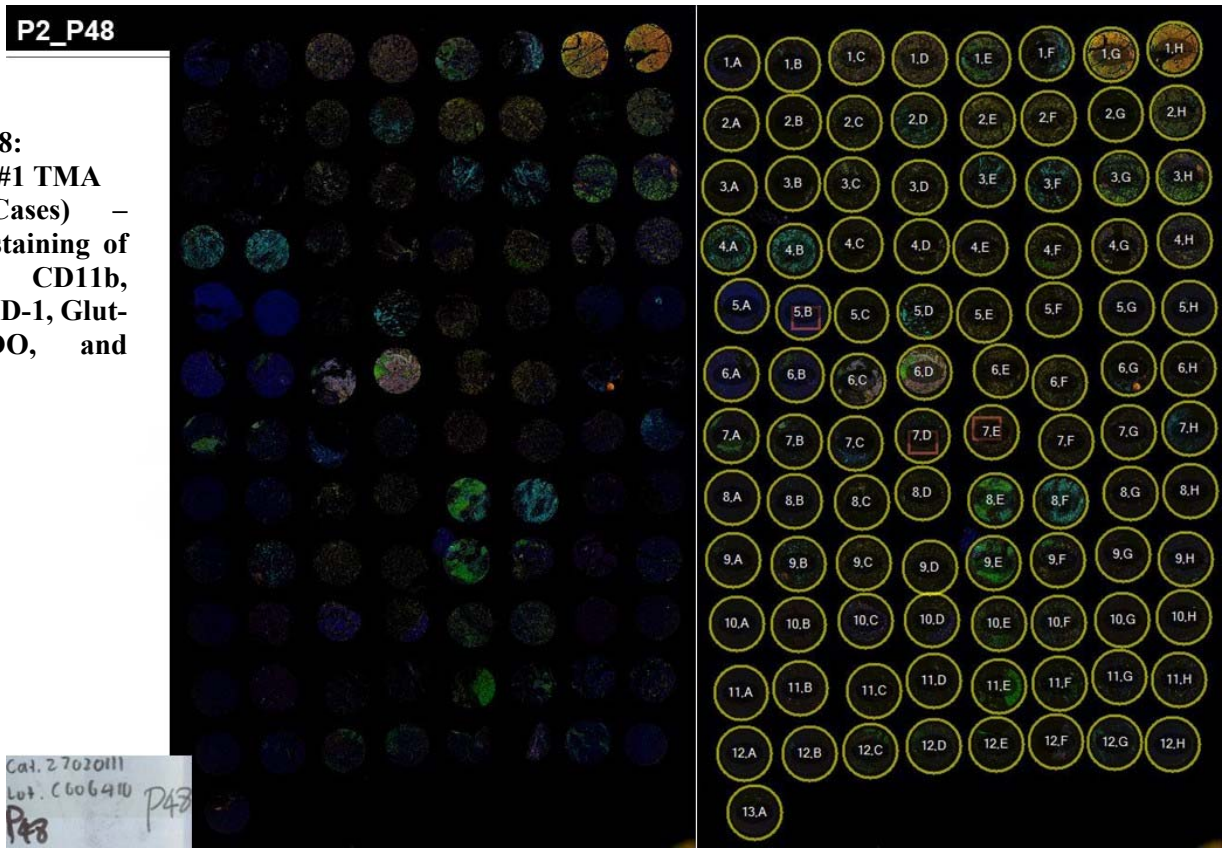


Figure 8: PDAC #1 TMA (48 Cases) – qmIF staining of CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI.

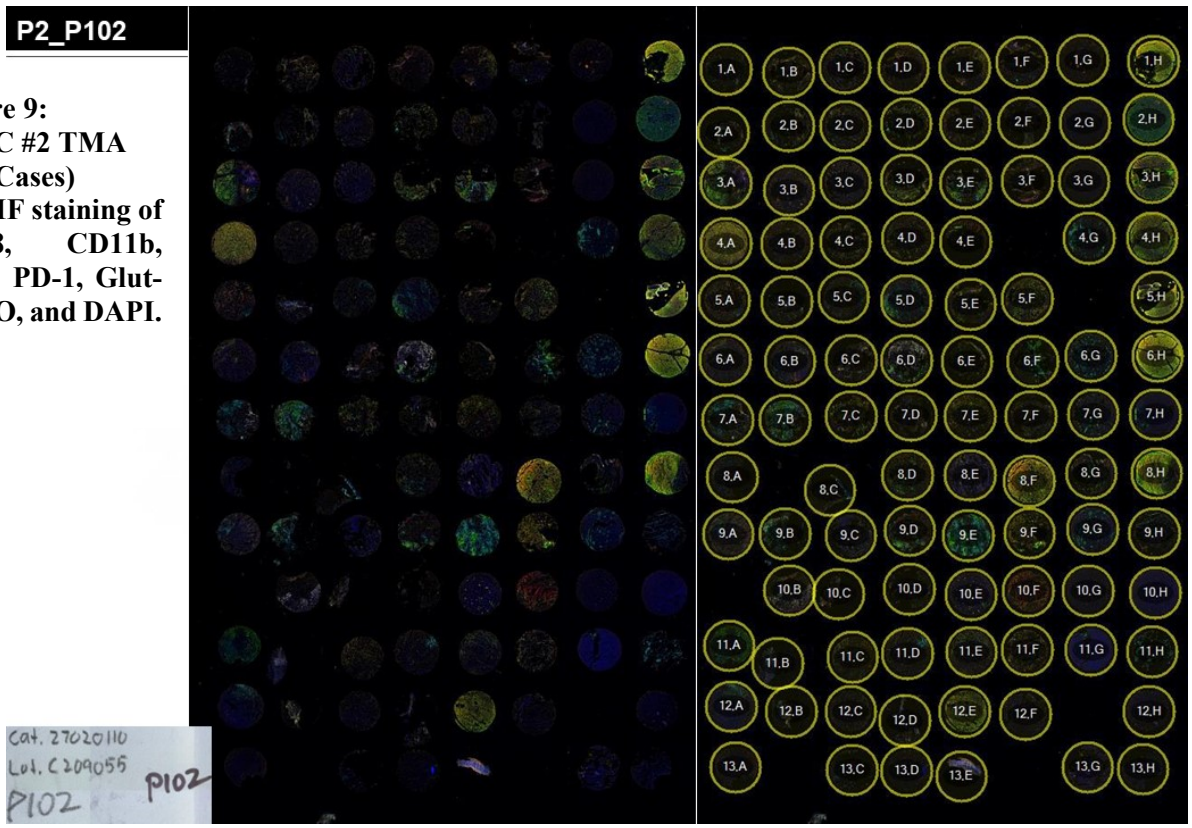


Figure 9:
PDAC #2 TMA (102 Cases) – qmIF staining of CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI.

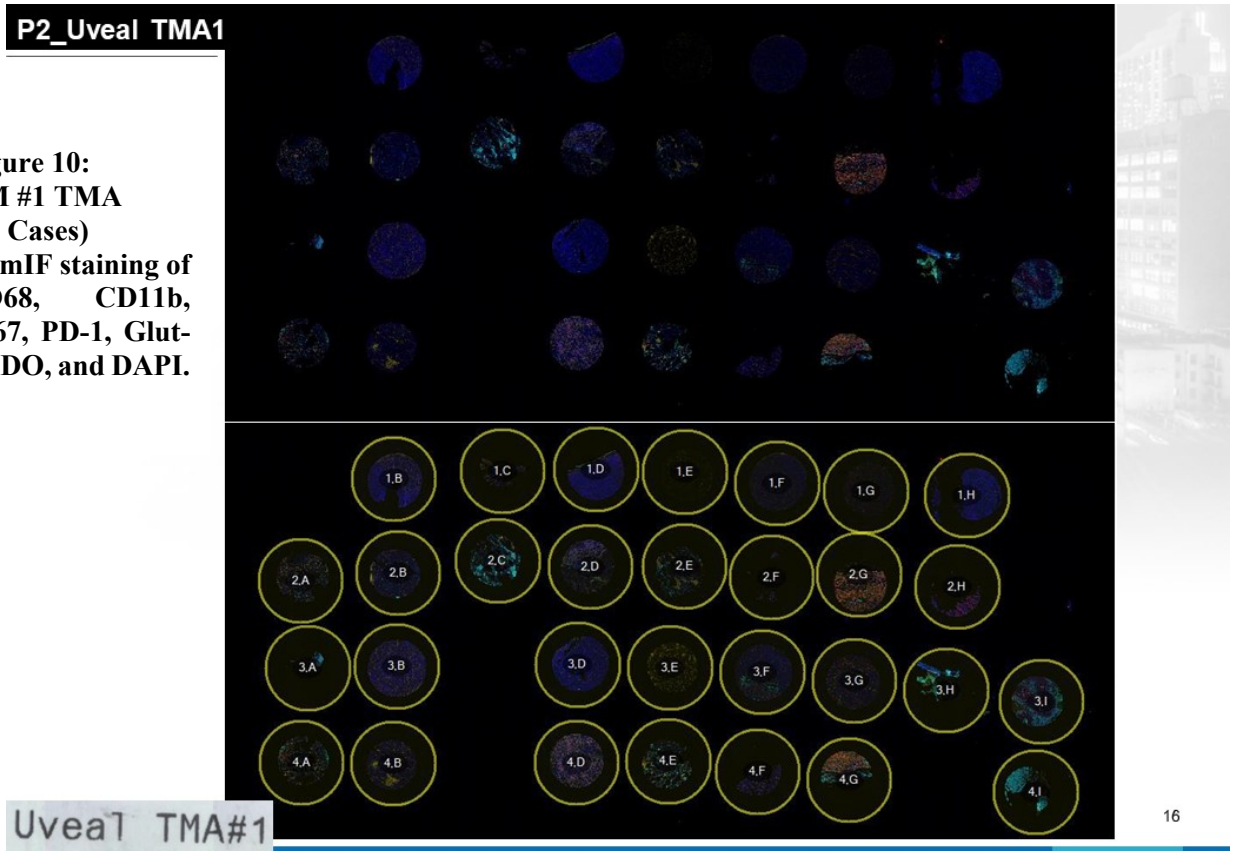


Figure 10:
UM #1 TMA (19 Cases) – qmIF staining of CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI.

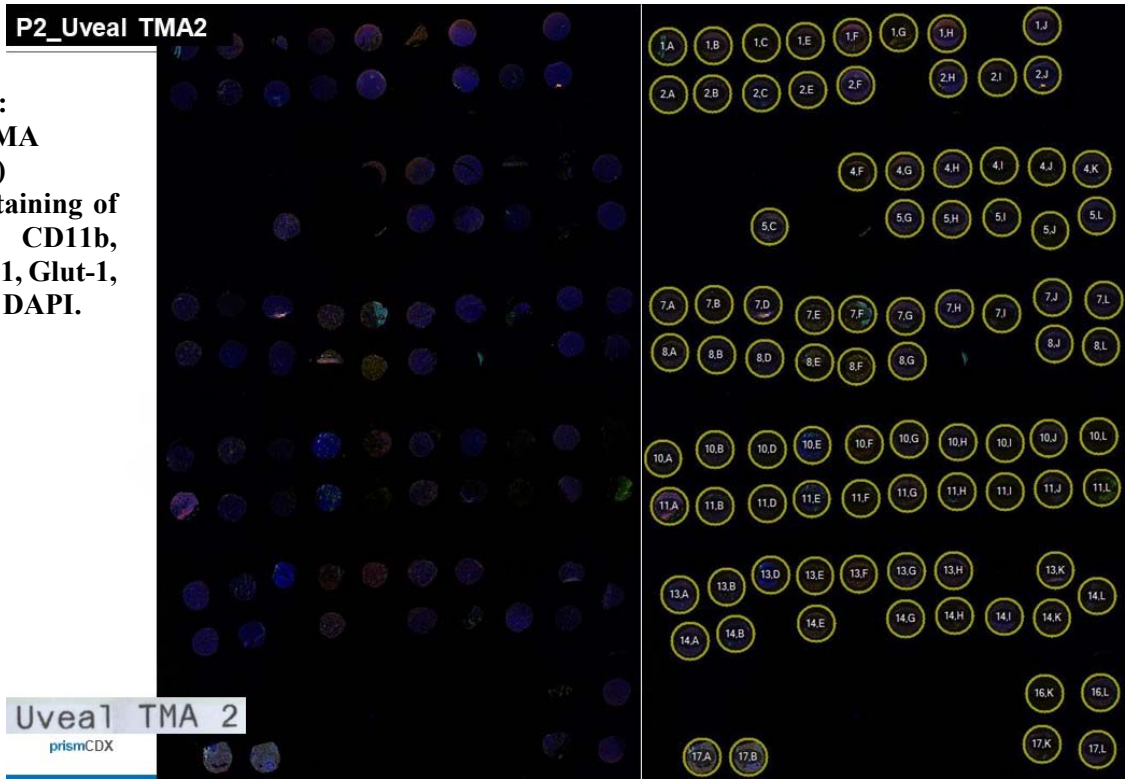


Figure 11:
UM #2 TMA (43 Cases) – qmIF staining of CD68, CD11b, Ki67, PD-1, Glut-1, IDO, and DAPI.

All the experiments have been completed for Specific Aim 1. (100% complete in September 2022)
We are conducting the data analysis and statistical quantification of each marker, which are expected to be completed by January 31, 2023. We aim to publish manuscripts to report the finding of Specific aim 1 in June 2023.

Aim 2: To enhance the immune response in UM and PDAC tumors by small inhibitors targeting CD73 and A2AR, and further examine their anti-tumor effects in combination with CPIs.

Due to the Pandemic, several of our orders of lab supplies, some A2AR and CD73 inhibitors, and the hypoxia incubator from the commercial vendors were delayed. The Pandemic also impeded the recruitment of the postdoctoral fellow for this project.
It is also challenging to recruit a postdoc for this project. Dr. Mariana Grigoruta joined our team after seven months after this project's starting date (11/1/2020). She is currently one of the main researchers conducting the experiments of Aim 2.

Major Task 1: To examine the effects of CD73 and A2AR inhibitors on the growths of UM and PDAC cells in vitro.

We conducted a series of MTT assays to examine the effects of various CD73 and A2AR inhibitors on the growths of UM and PDAC cells in vitro under normoxic conditions, including CPI-444 (A2AR antagonist), istradefylline (A2AR antagonist), AZD4635 (HTL1071, A2AR Antagonist), LY-3475070 (CD73 Inhibitor), and α,β-methylene ADP (APCP, CD73 inhibitor). Four UM cell lines

(MEL285, 92.1, MP38, and MP65) and two PDAC cell lines (Panc 08.13 and Mia Paca-2) were treated with various inhibitors for 72 hours (**Figures 12 and 13**).

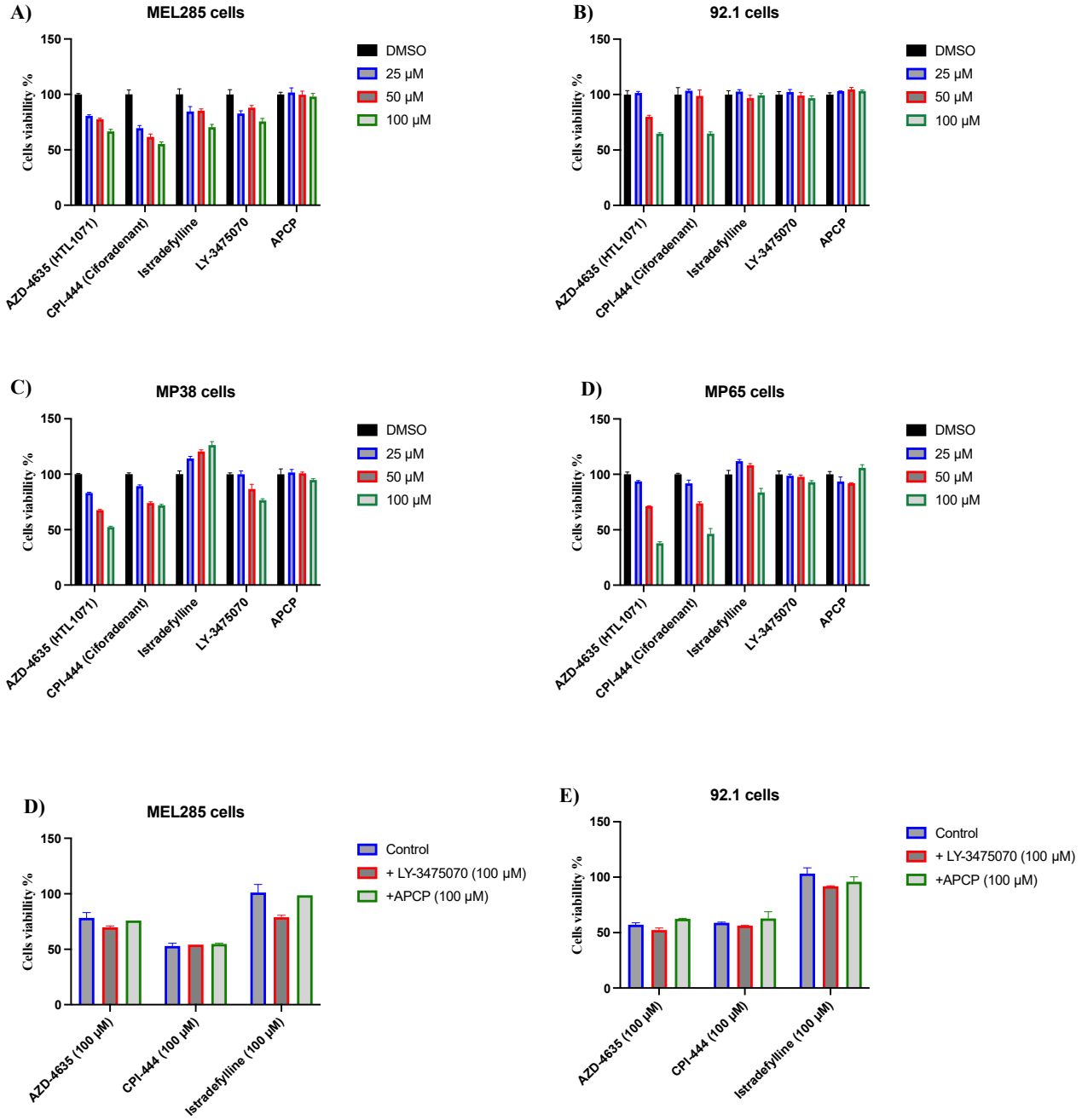


Figure 12. The effects of various CD73 and A2AR inhibitors on the growth of UM cell lines (MEL285, 92.1, MP38, and MP65) under normoxic conditions. D) & E) The combinational treatments of CD73 inhibitors and A2AR inhibitors were evaluated in UM cell lines, MEL85 and 92. After 72 h of culture, cell survival was determined via MTT assay. The percent cell survival in each treatment group was calculated relative to cells treated with medium only under the same conditions. Each experiment was carried out three times, and the means were presented here.

Under normoxic conditions, both CPI-444 and AZD4635 showed dose-dependent inhibition of the growths of all four UM cell lines, MEL285, 92.1, MP38, and MP65. 100 µM of AZD 4635 inhibits 40% - 70% growths of four UM cell lines (Figures 12 A-D). 100 µM of CPI-444 inhibits 30% - 50%

growths of four UM cell lines. The treatment of istradefylline, LY-3475070, and APCP did not show any inhibitory effects on the growths of 92.1 and MP65 cell lines (Figures 12 B and D). The treatments of istradefylline and LY-3475070 slightly inhibit the growth of MEL285 cells (~20%) at 100 µM (Figure 12A). LY-3475070 also showed slightly inhibitory effects on the growth of MP38 cells (~20%) at 100 µM (Figure 12C). However, the combinational treatments of CD73 and A2AR inhibitors did not show any synergistic inhibitory effects on the growths of MEL285 and 92.1 cell lines (Figures 12 E and F).

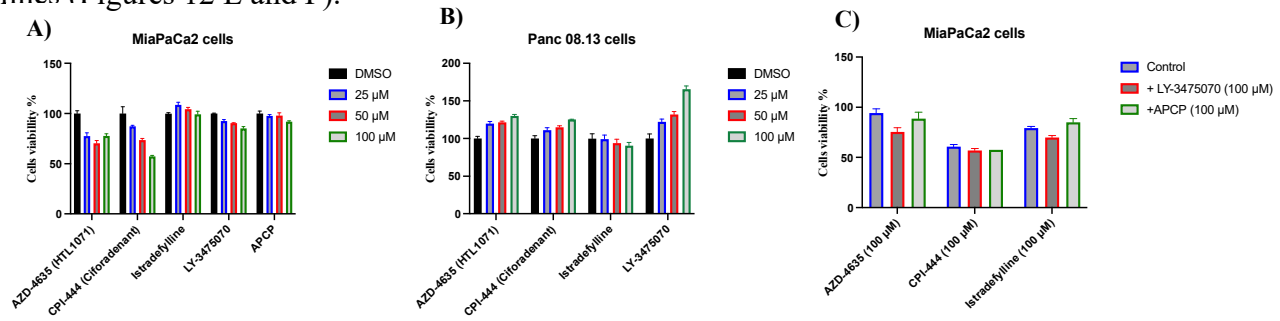


Figure 13. The effects of various CD73 and A2AR inhibitors on the growth of PDAC cell lines (MIA Paca-2 and Panc 08.13) under normoxic conditions. C) The combinational treatments of CD73 inhibitors and A2AR inhibitors were evaluated in the PDAC cell line, MIA Paca-2. After 72 h of culture, cell survival was determined via MTT assay. The percent cell survival in each treatment group was calculated relative to cells treated with medium only under the same conditions. Each experiment was carried out three times, and the means were presented here.

Under normoxic conditions, both CPI-444, and AZD4635 showed inhibitory effects on the growths of two PDAC cell lines, MIA Paca-2 and Panc 08.13 (Figure 13A). 100 µM of AZD 4635 inhibited 30% - 50% growths of MIA Paca-2 cells (Figure 13A). The treatment of AZD4635, istradefylline, LY-3475070, and APCP did not show any inhibitory effects on the growth of Panc 08.13 cells (Figure 13B). Moreover, the combinational treatments of CD73 and A2AR inhibitors did not show any synergistic inhibitory effects on the growths of MIA Paca-2 cells (Figure 13C).

With the support of the UTEP Startup Grant, I ordered one Galaxy 48R Hypoxia Incubator. However, this incubator has been on backorder, which is currently set up in our lab. The *in vitro* experiments under hypoxia conditions of this Task are currently pending due to delays in the parts to fully set up the hypoxia incubator through the vendor (Eppendorf) in our lab. We expect to finish the rest of the hypoxic experiments in February 2023.

The expression levels (mRNA and protein) of CD73 and A2AR in listed PDAC and UM cell lines will be examined under hypoxic and normoxic conditions in various UM and PDAC cell lines by western blot and real-time PCR. We plan to complete these works in the following 2-3 months. The data derived from this Major Task will guide us to select suitable UM and PDAC cell lines for the studies in Major Tasks 2 & 3 of Aim 2.

Task 2: To examine the efficacy of checkpoint inhibitors (CPIs, anti-PD-1 or anti-CTLA4 drug) with CD73/A2AR inhibitors on antitumor immune cell response, especially for cytotoxic T lymphocytes, in vitro.

To conduct the T-cell cytotoxic assays proposed in this Task, we ordered and set up the Lionheart FX Automated Microscopes System (BioTek) in my laboratory, which was supported by the School of

Pharmacy Startup fund. By applying this equipment, we built a T-cell cytotoxicity assay platform at UTEP.

About 15% of experiments have been completed to establish necessary cell lines for Major Task 2 of Specific Aim 2. We have established GFP-stable pancreatic cancer cell lines, Panc-1 and MIA Paca-2, for in vitro T cell cytotoxic assays of this Major Task 2. We are currently constructing GFP-stable uveal melanoma cell lines (MP38 and 92.1), which are needed for the in vitro T cell cytotoxic assays. We expect to finish all the experiments of this Task 2 in May 2023.

One of the goals of this Task is to evaluate the combination of CD73/A2AR inhibitors with immune checkpoint inhibitors. Therefore, for selecting suitable UM and PDAC cell lines for T cell cytotoxic assays, we examined the effects of IFN-γ on the expression of PD-L1 in PDAC cell lines (MIA Paca-2 and Panc 08.13), UM cell lines (MEL285, 92.1, MP38, and MP65), and the cutaneous melanoma cell line (SB2). As shown in Figure 14, the treatment of IFN-γ stimulates PD-L1 expression in MIA Paca-2, Panc 08.13, MEL285, 92.1, MP38, and SB2 cell lines. Therefore, these cell lines could be good candidates to be applied in T cell cytotoxic assays to evaluate the combination of CD73/A2AR inhibitors with immune checkpoint inhibitors.

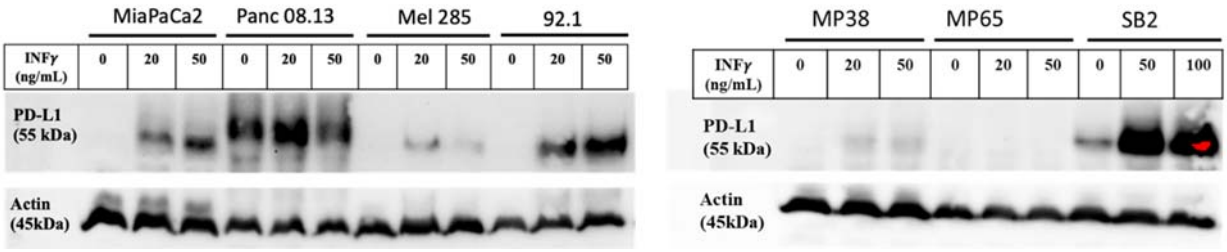


Figure 14. The effects of IFN-γ on the expression of PD-L1 in PDAC cell lines (MIA Paca-2 and Panc 08.13), UM cell lines (MEL285, 92.1, MP38, and MP65), and the cutaneous melanoma cell line (SB2). Western blots of PD-L1 and Actin were shown.

Task 3: To test the efficacy of combining CPIs (anti- PD-1 or anti-CTLA4 drug) with blockade of adenosine signaling (co-inhibition of CD73 and A2AR) on antitumor immune response in humanized mouse models of UM and PDAC.

We have established luciferase stable pancreatic cancer cell lines (Panc-1 and MIA Paca-2) and uveal melanoma cell lines (92.1, MM28, and 39) for *in vivo* xenograft studies of this Major Task 2. In collaboration with Dr. Chandrani Chattopadhyay's team at MD Anderson cancer center, we successfully established pancreatic tumors or uveal melanoma in NSG mice by applying these Luc-stable cell lines. As shown in Figure 15, the UM tumors developed by luciferase stable 92.1 and MM28 cells were harvested from NSG mouse xenografts.

About 30% of experiments have been completed to establish necessary Luc-stable cell lines for Major Task 3 of Specific Aim 3. We expect to finish all the experiments of this Task 3 in August 2023.

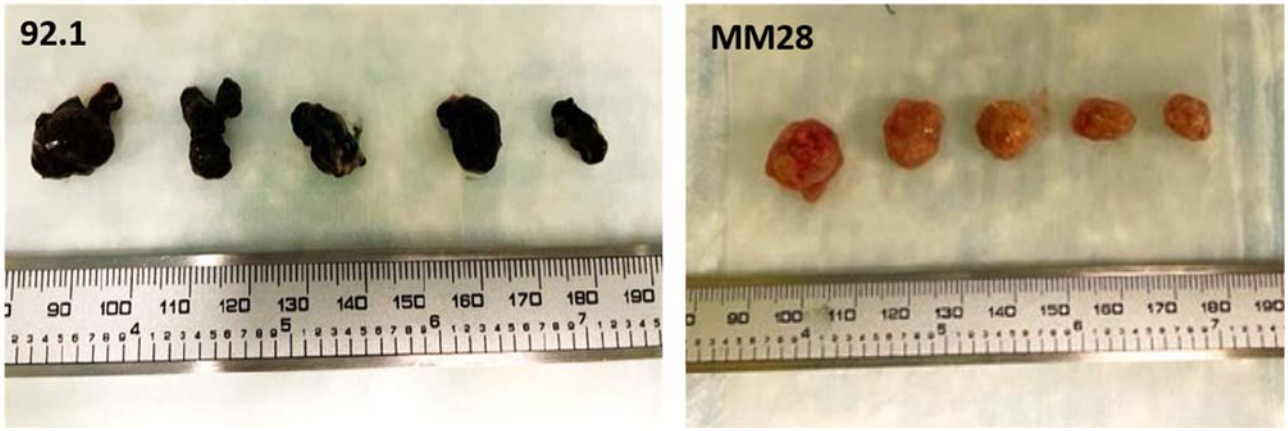


Figure 15. Subcutaneous tumors grown in NSG mice with luciferase stable UM cell lines (92.1 and MM28). The tumors were harvested from five xenografts in each group.

What opportunities for training and professional development has the project provided?

Dr. Mariana Grigoruta was recruited as a postdoctoral fellow to join us at UTEP on 6/7/2021. She is one of the main researchers for this project. Also, this project provides a great platform to train Dr. Grigoruta in cancer pharmacology, cell and molecular biology, and translation research.

Jordan Winfield, a P3 Pharmacy Student at UTEP, is also joining our research team and participating in some research experiments for this project. Through this project, Jordan receives training in translational cancer research and learns various molecular biological techniques.

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

University of Texas at El Paso Site (Dr. Yong Qin, PI):

Specific Aim 1:
All the experiments have been completed for Specific Aim 1. (100% complete in September 2022) We are conducting the data analysis and statistical quantification of each marker, which are expected to be completed by January 31, 2023. We aim to publish manuscripts to report the finding of Specific aim 1 in June 2023.

Specific Aim 2:
The *in vitro* experiments under hypoxia conditions are currently pending due to delays in the parts to fully set up the hypoxia incubator through the vendor (Eppendorf) in our lab. We expect to finish the rest of the hypoxic experiments in February 2023.

We are currently constructing GFP-stable UM cell lines (MP38 and 92.1), which are needed for the in vitro T cell cytotoxic assays. We expect to establish 2-3 GFP-stable UM cell lines by February 2022. About 15% of experiments have been completed to establish necessary cell lines for Major Task 2 of Specific Aim 2. We expect to finish all proposed T cell cytotoxic assays proposed in this Task in May 2023.

We also established luciferase stable pancreatic cancer cell lines (Panc-1 and MIA Paca-2) and uveal melanoma cell lines (92.1, MM28, and 39) for in vivo xenograft studies of this Major Task 2. These Luc-stable cell lines have been successfully tested to establish pancreatic tumors or uveal melanoma in NSG mice. About 30% of experiments have been completed to establish necessary Luc-stable cell lines for Major Task 3 of Specific Aim 3. We expect to finish all the in vivo experiments of this Task 3 in August 2023.

Our team will work towards examining the antitumor effects of small inhibitors targeting CD73 and A2AR in UM and PDAC tumors by applying humanized mouse models of UM and PDAC. We expect to determine whether the drugs targeting adenosine pathways could enhance the immune response in UM and PDAC tumors combined with checkpoint inhibitors based on proposed in vitro and in vivo models.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

As detailed above, we got about nine months behind in our timeline, given the delay in postdoc recruitment and setting up a couple of important pieces of crucial equipment through vendors. The studies were slowed by the need to order molecules from a wide variety of obscure international vendors. Currently, these obstacles have been solved and will not change our approach. We have most of the necessary equipment, cell lines, and materials to finish the remaining tasks in the NCE period.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to report

  Books or other non-periodical, one-time publications.

  Nothing to report

  Other publications, conference papers and presentations.

  4th Annual 20/20 Borderland Vision Symposium, invited speaker, Saturday, Feb. 5, 2022
  Presentation title: Immune Profile and Immunosuppressive mechanism of Uveal Melanoma

- **Website(s) or other Internet site(s)**
  Nothing to report

- **Technologies or techniques**

  Nothing to report

- **Inventions, patent applications, and/or licenses**

  Nothing to report

- **Other Products**

  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

University of Texas at El Paso (Dr. Qin, PI):

Name:	Yong Qin
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-4743-938x
Nearest person month worked:	2 months
Contribution to Project:	Dr. Qin provided oversight of the project, provided guidance and consultation to Mariana Grigoruta, and assisted with the analysis and interpretation of data.
Funding Support:	This project only

Name:	Mariana Grigoruta
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12 months
Contribution to Project:	Dr. Grigoruta will perform biomarker analyses of UM and PDAC tumor samples and pharmacological studies of CD73/A2AR inhibitors and immune checkpoint inhibitors in UM and PDAC. She will oversee tissue acquisition and

	storage and data management, conduct experimentation, and participate in data analysis and manuscript preparation.
Funding Support:	This project only

MD Anderson Cancer Center (Co-investigators):

Name:	Sapna Patel
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.48 months
Contribution to Project:	Dr. Patel will identify and acquire UM tumor tissues from appropriate patients via biopsy or surgical resection from the Melanoma Tissue Bank at MD Anderson Cancer Center and facilitate the transfer of samples from institutional banks to the research lab. She works directly with the laboratory investigators for all needed problem solving, review of plans, and data analysis.
Funding Support:	This project only

Name:	Jason Roszik
Project Role:	Co-investigator (Biostatistician)
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.36 months
Contribution to Project:	Dr. Roszik will assist with the integration, visualization, and analysis of data, especially in statistical analyses.
Funding Support:	This project only

Name:	Phyu Aung
Project Role:	Co-investigator (Pathologist)
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.36 months
Contribution to Project:	Dr. Aung will perform quality control for acquired tissues and will facilitate the transfer of specimens to the research lab. She will also provide support for IHC experimentation, digital capture, and tissue processing and analysis of in vivo studies.
Funding Support:	This project only

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

University of Texas at El Paso (Dr. Qin, PI): Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

This report is a single PI (Dr. Qin) project and was prepared by Dr. Qin.

COLLABORATIVE AWARDS:

Nothing to report

QUAD CHARTS:

Nothing to report

9. APPENDICES:

Presentation slides, 4th Annual 20/20 Borderland Vision Symposium, invited speaker, Saturday, Feb. 5, 2022, Immune Profile and Immunosuppressive mechanism of Uveal Melanoma

Immune Profile and Immunosuppressive mechanism of Uveal Melanoma

Yong Qin, PhD

Assistant Professor

Department of Pharmaceutical Sciences

The School of Pharmacy

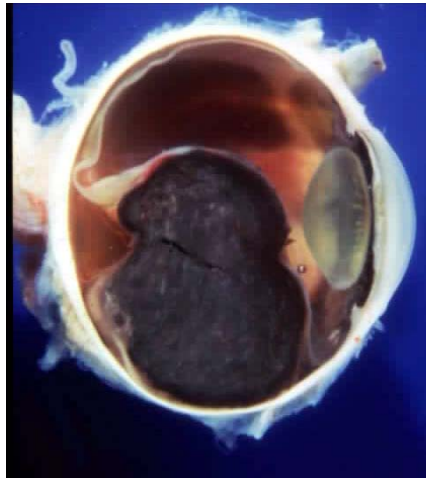
The University of Texas

Immunotherapy in Metastatic Uveal Melanoma

- **Uveal melanoma (UM) is a rare subtype of primary eye melanoma in adults, which derives from uveal melanocyte. No effective systemic treatment currently exist for metastatic uveal melanoma.**
- **It is noteworthy that the low response rates to immune checkpoint inhibitors in UM sharply contrast with promising response rates in cutaneous melanoma (CM, skin melanoma) patients. The majority of metastatic patients fail to respond to immune checkpoint inhibitors in limited clinical trials.**
- **There is a major gap in our understanding of the mechanism that suppresses UM response to immune therapy. Insights into the immune profile of UM are critically needed.**

Tumor type	Drug(s)	Patient number	Study information	Trial Results		
				PFS (PR+CR)	OS	RR
Uveal Melanoma	Ipilimumab	39	A multicenter, retrospective analysis of clinical activity of ipilimumab for metastatic uveal melanoma from 4 hospitals in the United States and Europe	N/A	Median = 9.6 months (3 mg/kg ipilimumab)	2.6% (week 12)
Uveal Melanoma	Ipilimumab	22	Ipilimumab in pretreated metastastic uveal melanoma patients	Median = 2.9 months	Median = 5.2 months	4.5%
Uveal Melanoma	Ipilimumab	82	Efficacy and safety of ipilimumab in patients with pre-treated, uveal melanoma	Median = 3.6 months	Median = 6.0 months	5%
Uveal Melanoma	Ipilimumab	13	Ipilimumab in pretreated patients with metastatic uveal melanoma	N/A	Median = 36 weeks	0
Uveal Melanoma	Ipilimumab	53	Phase II DeCOG-Study of ipilimumab in pretreated and treatment-Naïve patients with metastatic uveal melanoma	Median = 2.8 months	Median = 6.8 months	0
Uveal Melanoma	Pembrolizumab (38 patietns) Nivolumab (16 patients) Atezolizumab (2 patients)	56	Clinical Outcomes in Metastatic Uveal Melanoma Treated With PD-1 and PD-L1 Antibodies	Median = 2.6 months	Median = 7.6 months	3.6%
Uveal Melanoma	Nivolumab or Pembrolizumab	17	Anti-PD1 treatment in metastatic uveal melanoma in the Netherlands	Median = 2.3 months	Median = 9.6 months	0
Uveal Melanoma	Pembrolizumab	25	Clinical activity and safety of Pembrolizumab in Ipilimumab pre-treated patients with uveal melanoma	Median = 3.0 months	N/A	8%

PFS: Progression-free survival; OS: Overall survival; RR: Response rate; PR: Partial Response; CR: Complete Response; N/A: not available.



Rapisuwon S., et al. 2019, Cutaneous Melanoma (Book Chapter), Springer Nature.

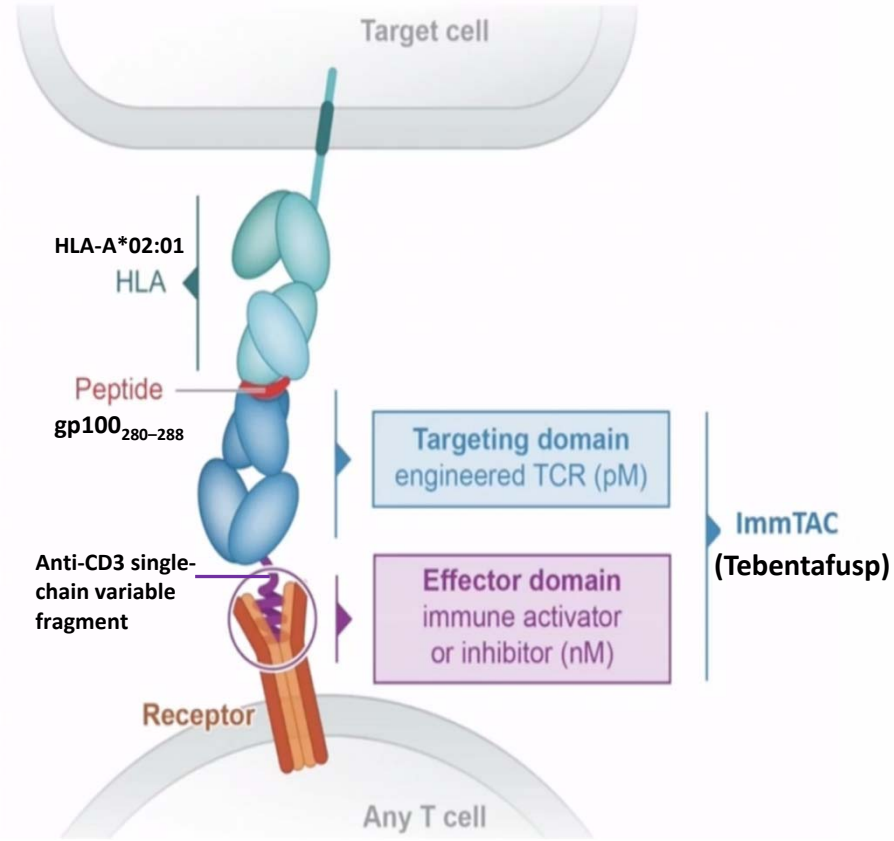
Tebentafusp Improves Survival in Advanced Uveal Melanoma

←AACR Annual Meeting 2021 Online Proceedings and Itinerary Planner Home

Session CTPL01 - Phase III Clinical Trials: Dedicated to the Memory of José Baselga

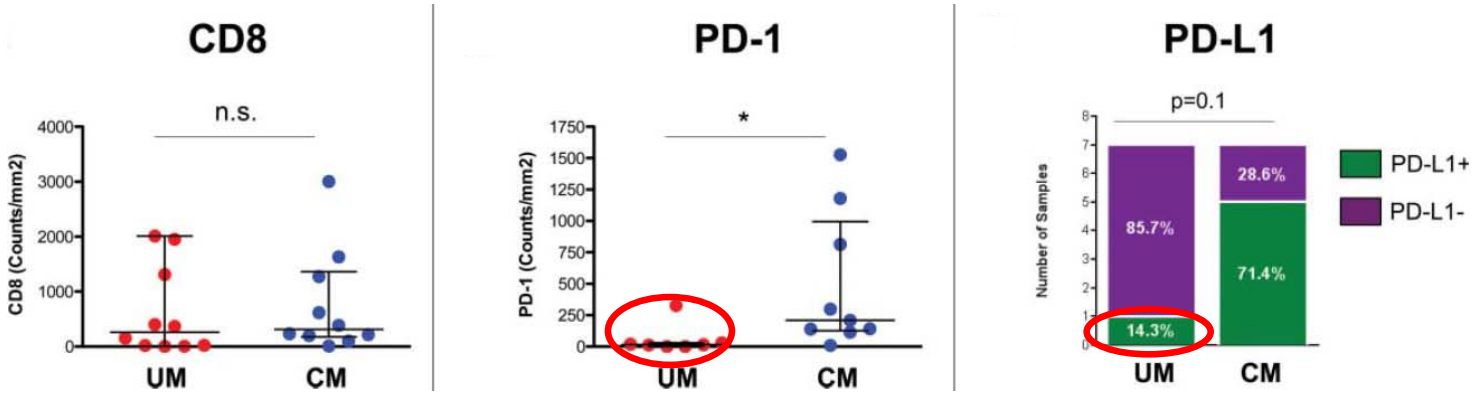
CT002 - Phase 3 randomized trial comparing tebentafusp with investigator's choice in first line metastatic uveal melanoma

- The median overall survival was **21.7** months for patients receiving tebentafusp versus **16** months for patients in the control group, the researchers found.
- This is the first clinical trial to report an improvement in overall survival for patients with metastatic uveal melanoma.
- A limitation is that only about 50% of Caucasian individuals are HLA-A*02:01 positive. Based on its MoA, tebentafusp is not effective in HLA-A*02:01-negative patients.
- Response rates according to RECIST were **9%** with tebentafusp (one complete response, 22 partial responses) and 5% with investigator's choice. The disease control rate including patients with stable disease at 12 weeks was 46% and 27%, respectively.



gynecologic-cancers.blogspot.com

Comparison of immune infiltrates in metastatic UM and CM tumors



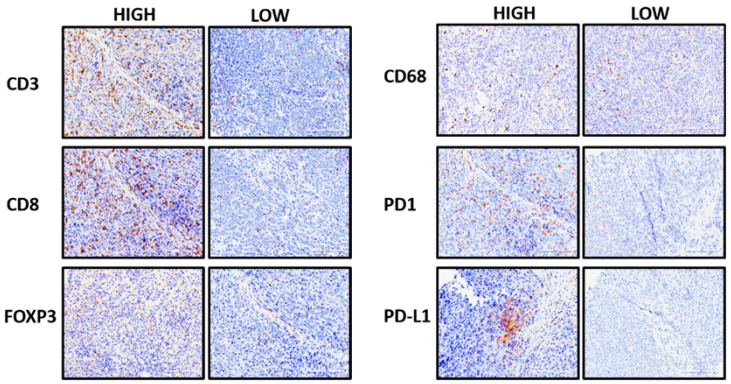
$CD8^+$ infiltrates were observed in UM metastatic tumors.

Overall $CD8^+$ T cell infiltration was similar across metastatic UMs and CMs (median infiltrate: UM 260.7 $CD8^+/mm^2$ vs. CM 311.0 $CD8^+/mm^2$).

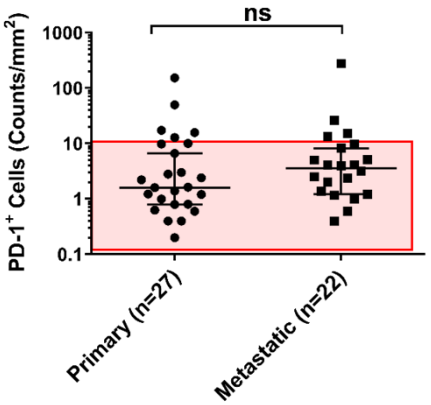
Although PD-1 and PD-L1 were detectable in UM metastases, their expression levels were significantly lower than those observed in CM metastases.

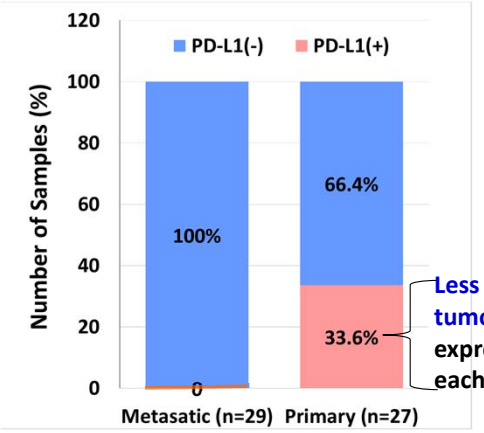
Qin et al. Oncoimmunology. 2017 May 8;6(6):e1321187.

Comparison of immune infiltrate in UM primary and metastatic tumors



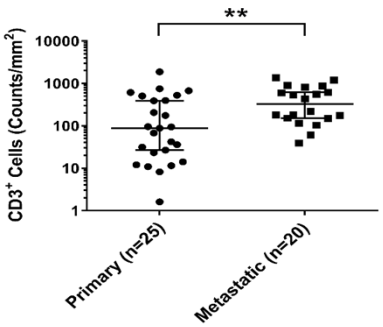
27 primary tumors vs 29 metastases (treatment-naïve)

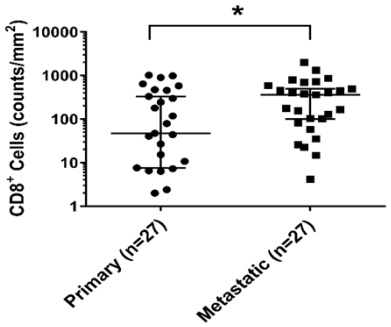


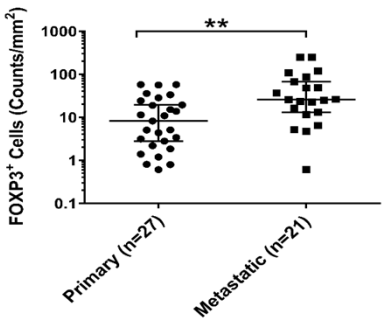


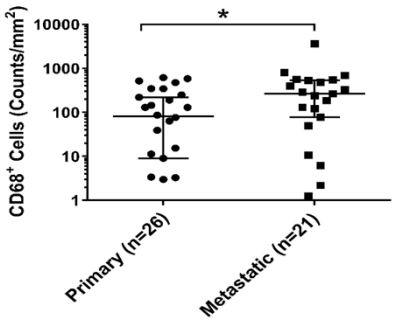
Less than 1% of tumor cells expressed PD-L1 in each tumor sample.

- CD3+, CD8+, CD68+, and FOXP3+ infiltrates were observed in all tested primary and metastatic UM tumors.
- There is no difference of PD-1+ infiltrates levels between UM primary and untreated metastatic tumors. The levels of PD-1+ infiltrates were very low in most of tested UM tumors (< 10 infiltrates /mm^2).
- All 29 treatment-naïve metastatic UM tumors were negative for PD-L1. 66.4 % of tested primary tumors are negative for PD-L1 expression via IHC. For those 33.6% of PD-L1+ primary tumors, less than 1% of tumor cells expressed PD-L1 in each tumor sample.





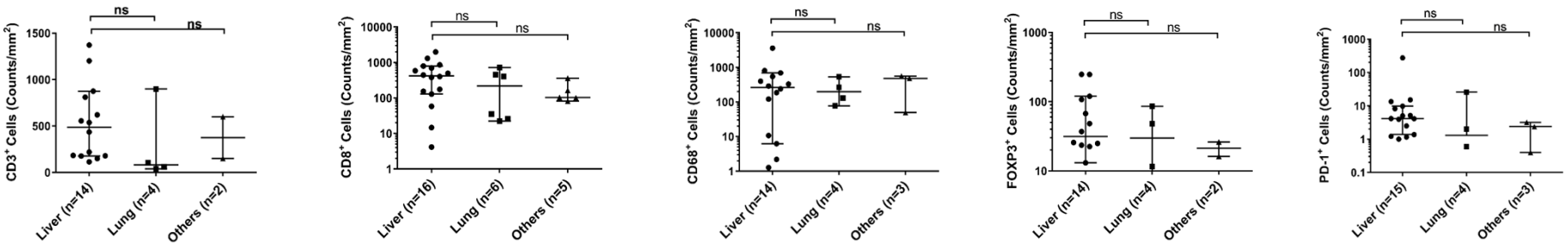




Compared to primary tumors, treatment-naïve metastatic UM showed significantly higher levels of CD3+, CD8+, CD68+, and FOXP3+ infiltrates.

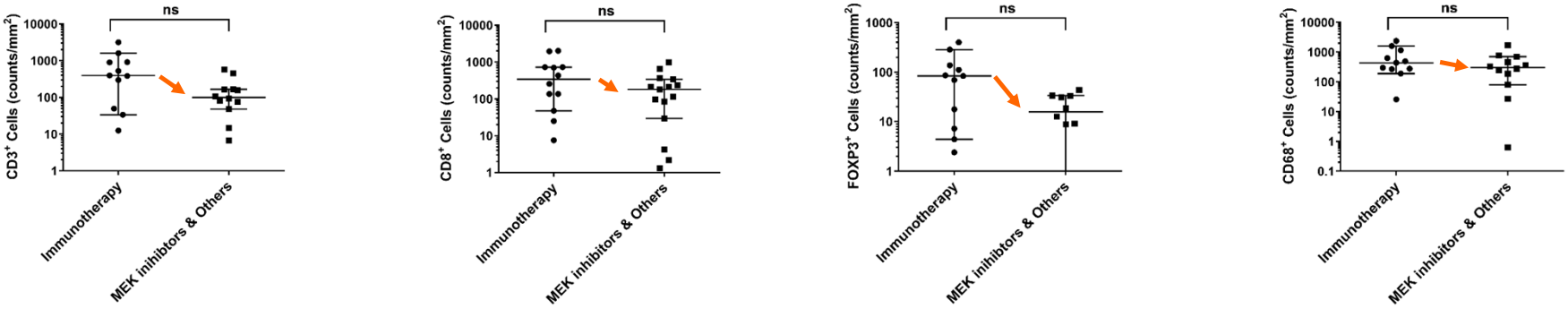
J Immunother Cancer. 2020 Nov;8(2):e000960.

Comparison of immune infiltrates in UM metastases by organ sites



In 29 treatment-naïve metastatic tumors, 62% were from liver metastases, and 38% of tumors were metastases from lung, soft tissues, and other sites. The levels of all tested immune markers were similar across treatment-naïve metastatic tissues from different organ sites.

Immune infiltrates in longitudinal tumor samples of UM patients on systematic therapies



There was no difference for infiltrating CD3+, CD8+, CD68+, PD-1+, and FOXP3+ cells between post-treatment samples of immunotherapy and MEKi.

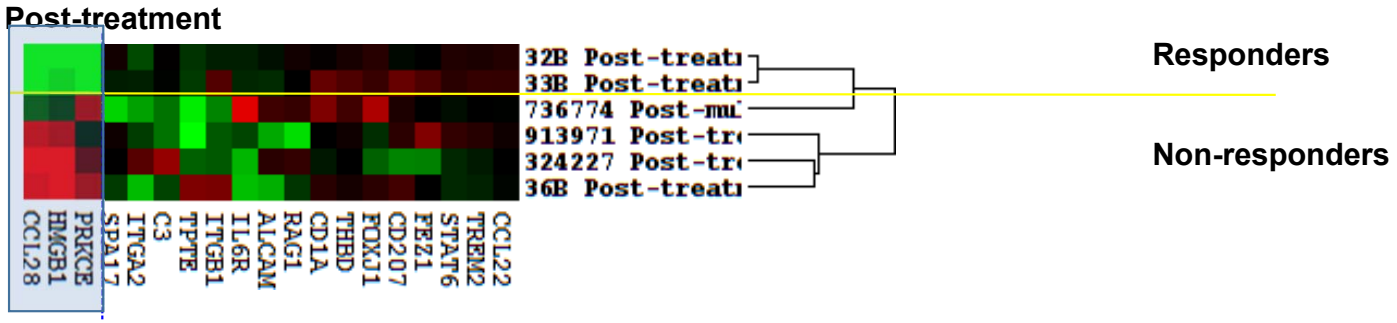
However, the metastatic tumor samples from patients post or on immunotherapy show a trend of higher CD3+, CD8+, CD68+, and FOXP3+ cells compared to tumors collected from patients post or on MEKi and other therapies.

J Immunother Cancer. 2020 Nov;8(2):e000960.

Gene signature of UM responding to immunotherapy

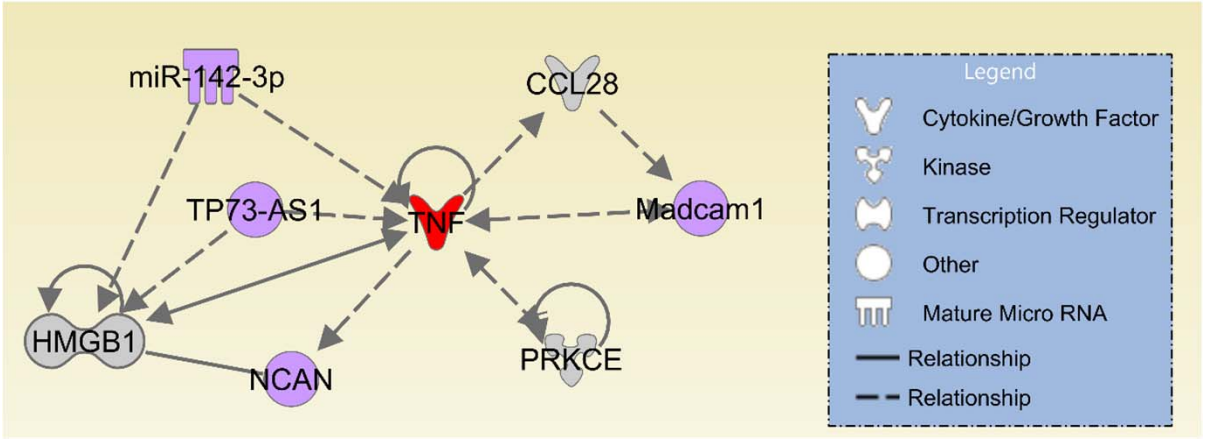
Matching metastatic tumors (pre-treatment and post-treatment of immunotherapy) from 6 UM patients (responders n=4 and non-responders n=2) ; 32 (iPi/nivo); 33 (OX40/41BB co-stimulation)
NanoString analysis (770 immune-relevant genes)

A set of three genes were differentially expressed between the responding and non-responding post-treatment tumors ($p \leq 0.05$).



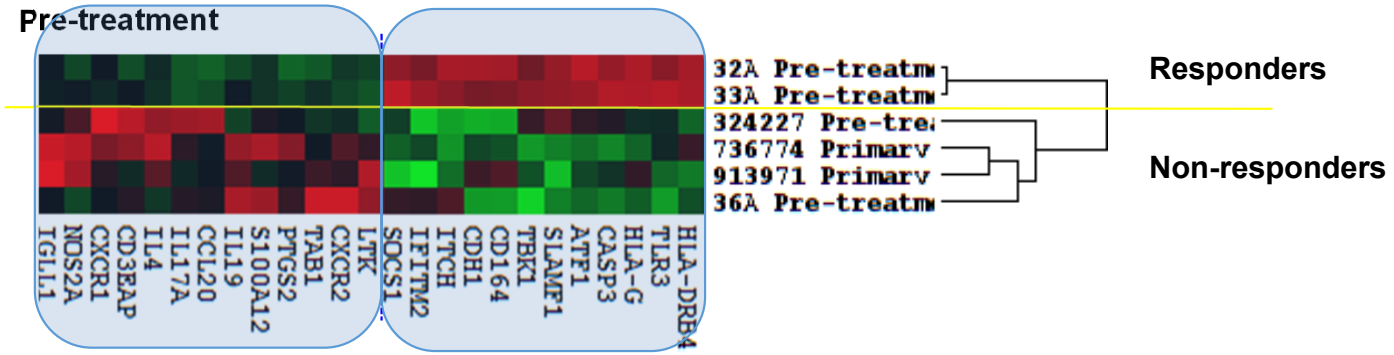
Functional network analysis by Ingenuity Pathway Analysis (IPA) reveals that TNF is the major upstream regulator for the expression of HMGB1, PRKCE, and CCL28, which are significantly expressed at a higher level in post-treatment tumors of non-responders to immunotherapy.

Enrichment of TNF signature in post-treatment tumors of non-responders.

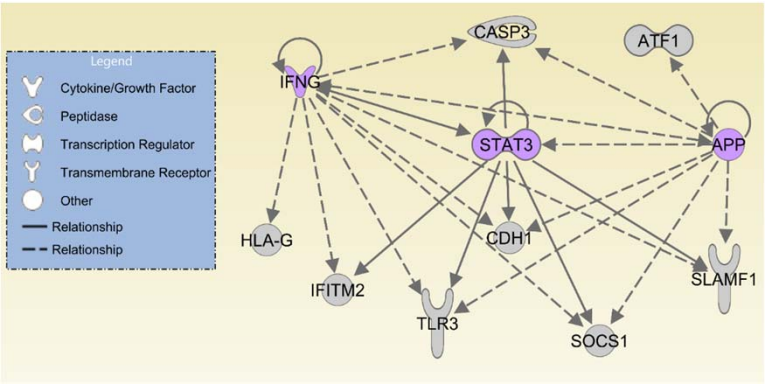


J Immunother Cancer. 2020 Nov;8(2):e000960.

Gene signature of UM responding to immunotherapy

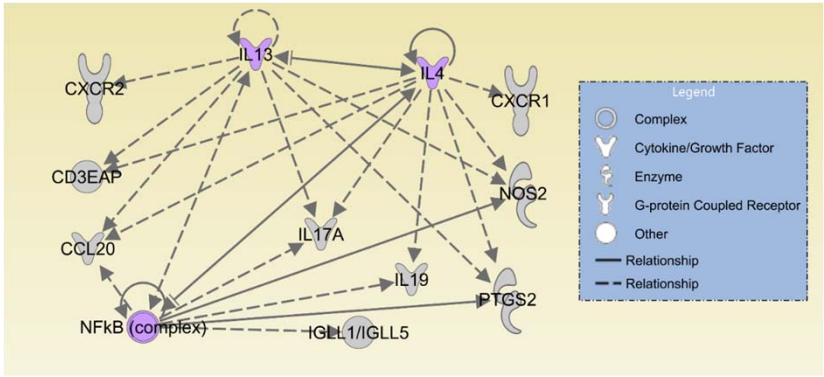


NanoString analysis of pre-treatment UM tumors of responders (n=4) and non-responders (n=2) to immunotherapy.
32 (iPi/nivo); 33 (OX40/41BB co-stimulation)
Two sets of genes were differentially expressed between the responding and non-responding pretreatment tumors ($p \leq 0.05$).



Functional network analysis by IPA reveals that IFN-γ is the major upstream regulator for the expression of a group of genes (CASP3, CDH1, HLA-G, IFITM2, SLAMF1, SOCS1, TLR3, and ATF1), which are significantly expressed at a higher level in pretreatment tumors of responders to immunotherapy.

Enrichment of IFN-γ signature in pre-treatment tumors of responders.



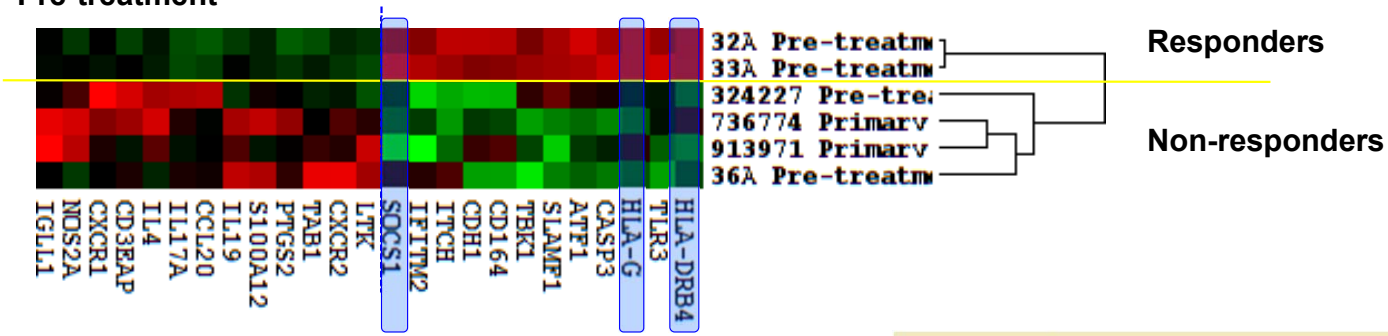
Functional network analysis by IPA reveals that proinflammatory NF-kB, IL-4, and IL-13 are major upstream regulators for the expression of a group of genes (CXCR1, CXCR2, PTGS2, NOS2A, IL4, IL17A, IL19, CCL20, IGLL1, LTK, TAB1, S100A12, and CD3EAP), which are significantly expressed at a higher level in pretreatment tumors of non-responders to immunotherapy.

Enrichment of proinflammatory signature in pretreatment tumors of non-responders.

J Immunother Cancer. 2020 Nov;8(2):e000960.

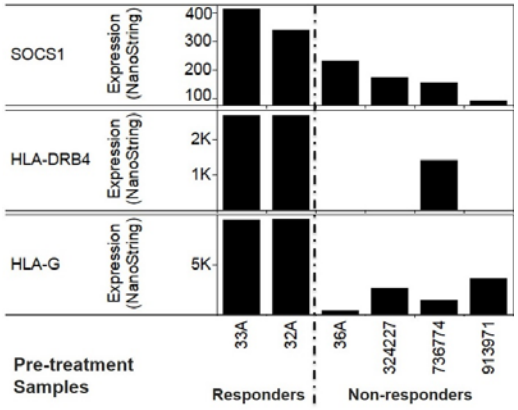
Role of SOCS1 in immune regulation

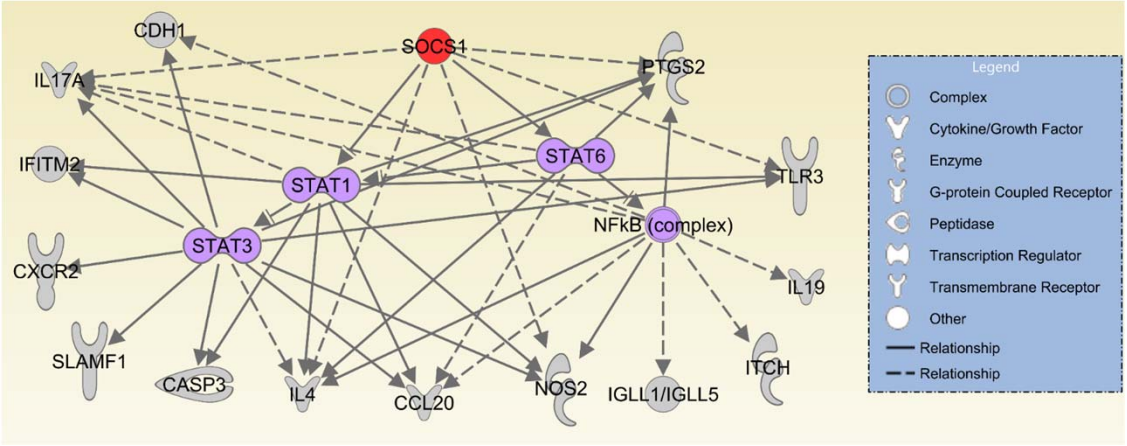
Pre-treatment



Responders

Non-responders





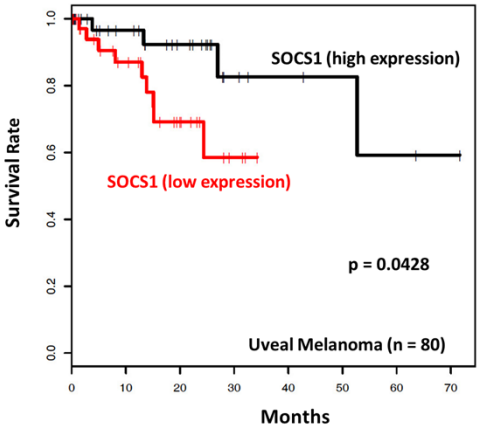
The mRNA levels of suppressor of cytokine signaling 1 (SOCS1) and major histocompatibility complex (MHC) molecules, HLA-G and HLA-DRB4, were significantly higher in pretreatment tumors of responders compared to non-responders.

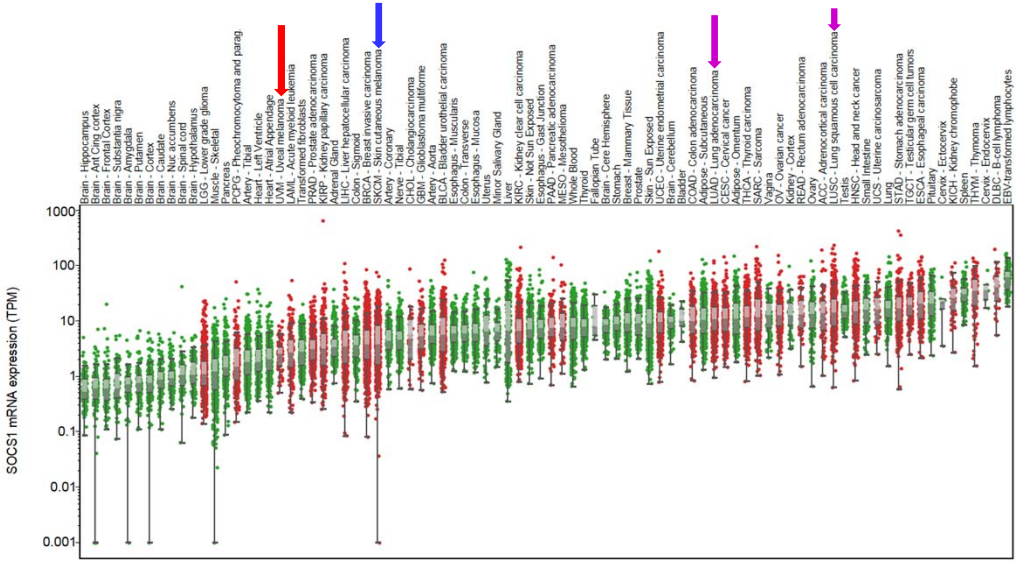
IPA study revealed that SOCS1 is a master upstream regulator for most of these genes identified in our NanoString analysis.

Two potential signaling pathways were identified to play crucial roles in SOCS1 regulating these genes, including SOCS1/STAT1/STAT3 and SOCS1/STAT6/NF-kB axes.

J Immunother Cancer. 2020 Nov;8(2):e000960.

SOCS1 is a key gene involved in immune response in UM tumors





Gene_1	Gene_2	Spearman Rho	p-value
SOCS1	HLA-A	0.411087662	0.000151916
SOCS1	HLA-B	0.322222233	0.003558534
SOCS1	HLA-C	0.416385382	0.000122208
SOCS1	HLA-DMA	0.322456628	0.003533064
SOCS1	HLA-DMB	0.356704175	0.001163051
SOCS1	HLA-DOA	0.397702754	0.000259111
SOCS1	HLA-DOB	0.293287873	0.008283405
SOCS1	HLA-DPA1	0.368026257	0.00078332
SOCS1	HLA-DPB1	0.365002334	0.000871762
SOCS1	HLA-DPB2	0.357601017	0.001127789
SOCS1	HLA-DQA1	0.31701827	0.004167862
SOCS1	HLA-DQA2	0.200164095	0.075041885
SOCS1	HLA-DQB1	0.329653084	0.002826087
SOCS1	HLA-DQB2	0.357949644	0.001114345
SOCS1	HLA-DRA	0.367557436	0.000796474
SOCS1	HLA-DRB1	0.365260184	0.000863881
SOCS1	HLA-DRB5	0.248640418	0.026151334
SOCS1	HLA-DRB6	0.286404133	0.010007918
SOCS1	HLA-E	0.262376934	0.018713555
SOCS1	HLA-F	0.312494129	0.004771237
SOCS1	HLA-G	0.28628692	0.010039812
SOCS1	HLA-H	0.402086258	0.000218081
SOCS1	HLA-J	0.19507736	0.082906384
SOCS1	HLA-L	0.216010317	0.054300283
SOCS1	IFNG	0.301690549	0.006535861
SOCS1	SOCS1	1	0

There was a significant survival disadvantage for patients whose tumors lacked SOCS1 expression (< median) compared to those with high SOCS1 expression (> median) based on TCGA data of 80 UM tumors.

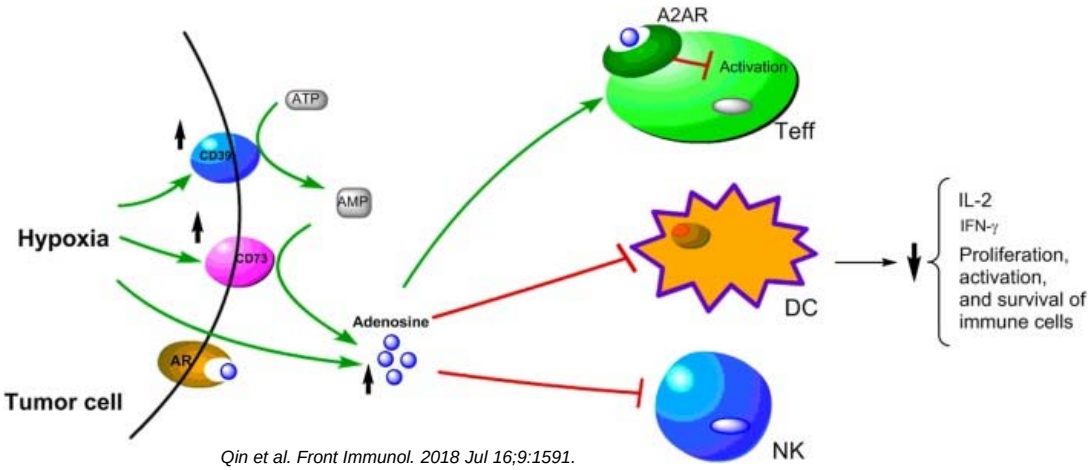
UM tumors express significant lower levels of SOCS1 compared to various tumors (red) and normal tissues (green) based on TCGA and GTEx data. UM (Red arrow), CM (blue arrow), Lung cancers (purple arrows)

Several studies have shown that SOCS1 regulated MHC I and II molecules' levels in fibroblast and tumor cells [PMID: 12391216 & 12670405]. SOCS1 mRNA levels were significantly positively correlated with the levels of most dateable HLA I & II molecules in UM tumors. Additionally, there is no SOCS1 mutation found in UM tumors from TCGA data.

Together, these data indicate that SOCS1 is a crucial regulator in UM immune microenvironment and MHC expression. Low-level expressions of SOCS1 and MHC genes (HLA-G and HLA-DRB4) in UM tumors may be correlated with innate resistance to immunotherapy.

Manuscript under preparation

Targeting a stress-derived immunosuppressive adenosine pathway in tumors resistant to checkpoint inhibitors. (DOD Idea Award, PI: Yong Qin)

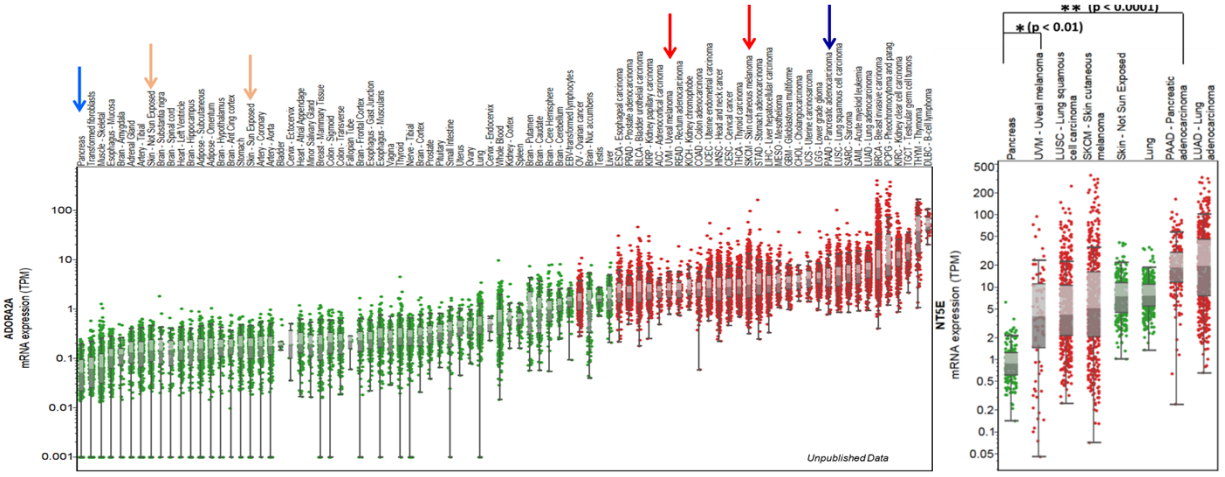


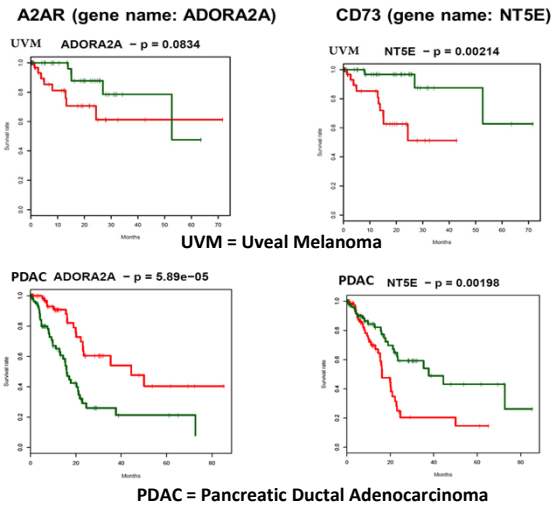
Qin et al. Front Immunol. 2018 Jul 16;9:1591.

The CD39–CD73-adenosine signaling represents an important pathway to generate extracellular adenosine. The adenosine accumulated in the tumor microenvironment acts as a negative regulator for both the activation and effector phases of the anti-tumor T cell response.

A2AR and CD73 were upregulated in various tumors including PDAC and UM and correlated with patients' survival.

Current project: 1) To characterize immune profiles related to A2AR and CD73 in UM and PDAC. 2) To enhance anticancer immunity of checkpoint inhibitors by targeting CD73 and A2AR2.





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