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TITLE: Single-Cell Analysis of LAG3 Checkpoint Inhibition in Metastatic Uveal Melanoma

PRINCIPAL INVESTIGATOR: Dr. Jose Lutzky

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6. AUTHOR(S)
Jose Lutzky, MD
Christina Decatur
James Dollar

E-Mail: jose.lutzky@med.miami.edu

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University of Miami
1320 S Dixie Hwy STE 650
Coral Gables, FL 33146-2919

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The most common cause of death from cancer is spread or metastasis to vital organs. Uveal melanoma (UM) is a highly aggressive eye cancer that metastasizes in up to 50% of patients, with a strong predilection for the liver. UM has a tendency to undergo early micrometastasis prior to treatment of the primary tumor, with later development of overt metastatic disease. However, the molecular mechanisms by which UM metastasizes remain poorly understood, and there are no cancer drugs available that are effective in treating metastatic UM. The proposed DOD Melanoma Research Translational Research Award will provide for the analysis of correlative biomarkers, which will be important to gain as much insights as possible into the tumor microenvironment, tumor responsiveness, and therapeutic prevention. The overall objective of our UM research program is to improve patient survival rates by identifying effective therapies based on new mechanistic insights into the tumor microenvironment. Our preliminary results have identified pathways that are upregulated or downregulated after treatment and evaluation of these changes can inform future clinical interventions. Deeper data analysis is underway to better understand the impact of these changes. We anticipate that the results of this work will lead to a paradigm shift in the management of metastatic UM.

15. SUBJECT TERMS
uveal melanoma, metastasis, single-cell RNA-sequencing, V(D)J, LAG3 inhibition, tumor microenvironment, immunotherapy, checkpoint inhibition, relatlimab

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1. INTRODUCTION:

Uveal melanoma (UM) is a highly metastatic cancer that, in contrast to cutaneous melanoma, is largely unresponsive to anti-CTLA4 and anti-PD1 checkpoint immunotherapy. In a recent study from our laboratory published in *Nature Communications*, we found using single-cell RNA-sequencing (scRNA-seq) of 11 UM samples that the predominant checkpoint marker on CD8+ T cells is LAG3, rather than CTLA4 or PD1. Further, we and other have shown using V(D)J repertoire analysis that clonally expanded T cells are present in the UM microenvironment, and that many of these T cells recognize melanoma-specific antigens. Taking together these exciting discoveries, we **hypothesize** that *checkpoint immunotherapy can be successful in metastatic UM by targeting LAG3 for inhibition*. Consequently, we have engaged with Bristol Myers Squibb and received approval for a phase II clinical trial to test their LAG3 inhibitor, relatlimab, in 27 patients with metastatic UM. Approval for the trial required that we co-treat with nivolumab, but since UM responds poorly to this agent, the main objective of the trial is to evaluate responses to relatlimab.

To gain new mechanistic insights into the tumor microenvironment and therapeutic response in UM, we **propose** to take advantage of this landmark clinical trial to conduct scRNA-seq and single-cell V(D)J profiling of metastatic tumor samples and circulating T cells before and after anti-LAG3/PD1 therapy. This approach will allow us to identify the immune cells in the tumor microenvironment and those trafficking to the tumor from the circulation that become activated and clonally expanded in patients with clinical response, compared to those without response. These analyses will allow us to correlate clinical and immune responses to therapy with the presence of known driver mutations in UM.

2. KEYWORDS:

uveal melanoma, metastasis, single-cell RNA-sequencing, LAG3 inhibition, tumor microenvironment, immunotherapy, checkpoint inhibition, relatlimab

3. ACCOMPLISHMENTS:

What were the major goals of the project?

To gain new mechanistic insights into the tumor microenvironment and therapeutic response in UM, we propose in this funding mechanism to take advantage of the opportunity provided by our landmark clinical trial to conduct scRNA-seq and single-cell V(D)J profiling of metastatic tumor samples and circulating T cells before and after anti-LAG3 therapy. Such a comprehensive analysis of the tumor microenvironment and circulating immune response to checkpoint immunotherapy has never been conducted in uveal melanoma.

Below are the major tasks and specific aims:

Major Task 1: Approval for human subjects' studies.
Percent completed: 100%

Specific Aim 1. Characterize the tumor microenvironment and circulating lymphocytes in metastatic UM at single-cell resolution.
Percent completed: 50%

- Major Task 1 (Aim 1A): Perform scRNA-seq of metastatic tumors to analyze gene expression in neoplastic and non-neoplastic cells.
- Major Task 2 (Aim 1B): Perform scRNA-seq on blood samples to analyze gene expression and and V(D)J repertoire in circulating B and T cells.

Specific Aim 2. Elucidate factors contributing to therapeutic resistance and prevention in metastatic UM at single-cell resolution.
Percent completed: 50%

- Major Task 1 (Aim 2A): Perform scRNA-seq of metastatic tumors to analyze gene expression in neoplastic and non-neoplastic cells.
- Major Task 2 (Aim 2B): Perform scRNA-seq on blood samples to analyze gene expression in peripheral T cells and determine V(D)J repertoire for clonal expansion of T cells.

Specific Aim 3. Identify clinical predictors of therapeutic response in metastatic UM using bulk V(D)J repertoire analysis of peripheral blood.
Percent completed: 10%

- Major Task 1 (Aim 3A): To assess predictors of therapeutic response, we will perform bulk V(D)J repertoire

analysis in blood samples collected prior to LAG3 and PD1 inhibitor therapy.

- Major Task 2 (Aim 3B): To assess indicators of therapeutic prevention, we will collect peripheral blood at 12 weeks after initiation of therapy and/or at disease progression.
- Major Task 3: Bioinformatic analysis.

What was accomplished under these goals?

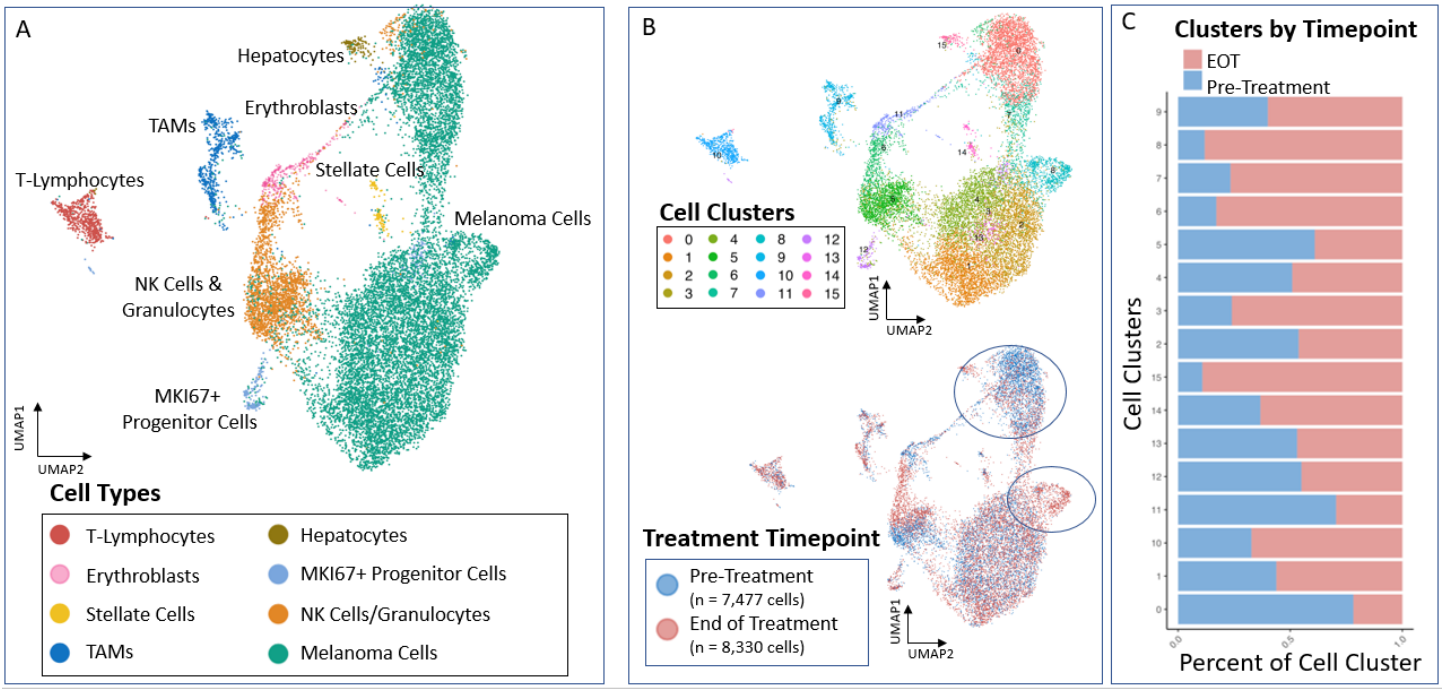
Specific Aim 1. Characterize the tumor microenvironment and circulating lymphocytes in metastatic UM at single-cell resolution.

Specific Aim 2. Elucidate factors contributing to therapeutic resistance and prevention in metastatic UM at single-cell resolution.

The samples were collected at the clinic and immediately processed into single cell suspensions then handed off to OGSR where single-cell libraries were generated and stored until sequenced. For the scRNA-seq in metastatic and blood samples as outline in Specific Aims 1 and 2, to ensure high quality results and cost efficiency, our protocol has several steps of quality assessment to ensure consistent control of data. During sorting of CD3+ T-cells from blood-derived PBMCs, non-viable cells are excluded from downstream application. Cell suspensions from blood derived CD3+ T-cells and tumor samples underwent a fluorescence cell viability assay to assess viability, requiring sample to be more than 80% viable before undergoing 10X single cell RNA sequencing (scRNAseq). Furthermore, tumor-derived cellular suspensions were assessed to ensure no contamination of RBCs and necrotic debris would jeopardize the samples. Following scRNAseq, captured cells are filtered based on their viability and RNA integrity, which is judged based on cellular percentage of mitochondrial genes, cellular RNA count, and RNA diversity.

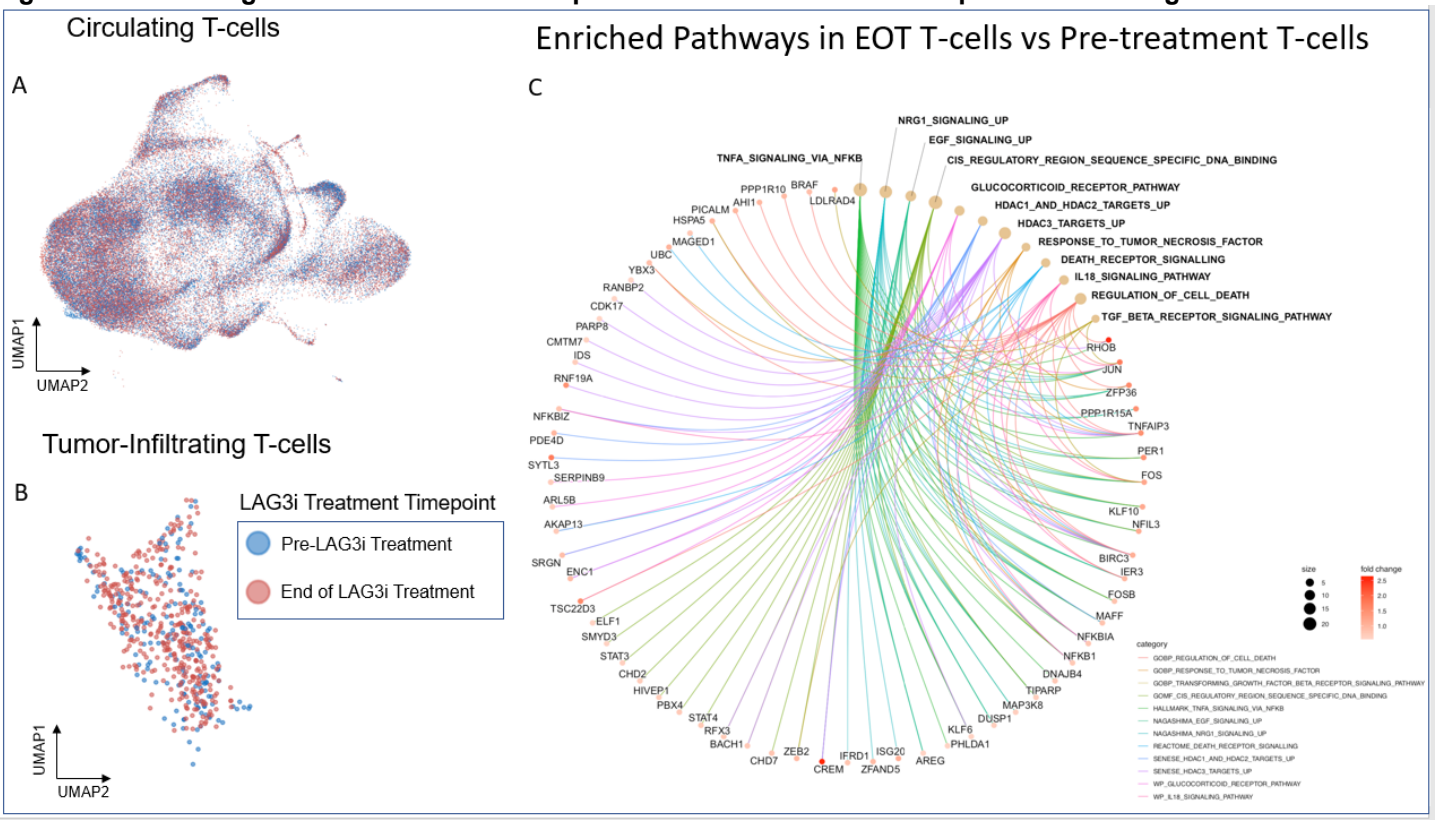
We have collected 8 metastatic tumor samples from 6 patients in which we were able to capture gene expression profiling at single-cell resolution (Figure 1A). To assess the sample output, the data was fully analyzed for 2 of the 6 patients in which both pre and end of treatment metastatic tumor samples have been collected. We were successfully able to obtain gene expression profiling of the metastatic tumor biopsies which shows treatment specific clustering in cells, indicating differential expression of genes with treatment (Figure 1B, 1C). Unexpectedly, it was revealed that so few cells were present to perform an adequate assessment of the V(D)J analysis, resulting in a low yield of cells (15,807 total) for 8 metastatic tumor samples. The data from the T-cells extracted from the tumor specimens did not produce adequate T-cell VDJ sequences to indicate this experiments were successful. Although the gene expression profiling at single-cell resolution was successful, it was decided that the poor output for the V(D)J analysis could not justify patient discomfort and sequencing costs, so we decided to forego the metastatic tumor biopsies and focus on the correlative blood collection.

Figure 1. LAG3i Single Cell Correlatives: Tumor Biopsies. A total of 15,807 cells were analyzed from 8 metastatic tumor samples from 6 patients.



To determine whether peripheral T cell expansion correlated with treatment response we performed single-cell mRNA and T-cell receptor (TCR) variable, diversity, and joining (V[D]J) sequencing pre-treatment and at treatment progression. 10 of the 21 patients have had both pre and end of treatment PBMC-derived CD3+ cells samples collected, processed, and subjected to scRNA-seq and scV(D)J sequencing, currently undergoing deeper analysis. Interestingly, we observed circulating T-cells present in the blood (Figure 2A), as well as tumor-infiltrating T-cells in the tumor (Figure 2B). We have identified genes differentially expressed in both circulating and tumor infiltrating T-cells (Figure 2C) that are enriched with treatment.

Figure 2. LAG3i Single Cell Correlatives: Comparison Metastatic Tumor sample vs Circulating Tumor Cells.



For the V(D)J analysis, only cells that were filtered through the Seurat gene expression pipeline were used for the VDJ analysis. Further analysis to determine which of these T-cells are specific to melanoma antigens vs. viral antigens is underway. A collaboration with the University of Helsinki to use the TCRGP algorithm ("https://github.com/emmijokinen/TCRGP") they developed has been established. Additional samples of pre- and end of treatment peripheral T-cell V(D)J data are awaiting to be sequenced. Study enrollment is underway, and samples are continuing to be collected.

In parallel, blood components (plasma and PBMCs) have been collected and continue to be collected and properly stored for future V(D)J bulk sequencing.

<u>Specific Aim 3. Identify clinical predictors of therapeutic response in metastatic UM using bulk V(D)J repertoire analysis of peripheral blood.</u>

Plasma is being collected, processed, and properly stored for future bulk sequencing.

In summary, we are on target with the specific aims outlined in this proposal within the proposed milestones. Interrogating the scRNA-seq and single-cell V(D)J profiling of metastatic tumor samples and circulating T cells before and after anti-LAG3 therapy, we strive to gain new mechanistic insights into the tumor microenvironment and therapeutic response in UM. We have identified genes differentially expressed in both circulating and tumor infiltrating T-cells that are enriched with treatment. Deeper analysis will shed light on such a comprehensive analysis of the tumor microenvironment and circulating immune response to checkpoint immunotherapy has never been conducted in uveal melanoma. These preliminary results

have identified pathways that are upregulated or downregulated after treatment and evaluation of these changes can inform future clinical interventions. Deeper data analysis is underway to better understand the impact of these changes.

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

Graduate student James Dollar, has attended the following webinars/conferences:

1) Novel, scalable, and cost-effective single-cell CRISPR screens (Webinar)- Sept 24, 2020.
2) Resolve Biological Complexity with Single Cell Solutions (Webinar)- October 22, 2020.
3) 10X Clinical Translational Research Network Roundtable (Virtual Conference)- October 28, 2020.
4) Single cell learning series: Considerations for sample prep (Webinar)- April 27, 2021.
5) Immunogenomic determinants of response and resistance to checkpoint blockade (Webinar)- May 11, 2022.

Christina Decatur is currently attending weekly sessions for 2022 Design and Management of Cancer Clinical Trials Course, from September 16, 2022, through October 21, 2022.

How were the results disseminated to communities of interest?

Results to do were presented to the scientific community through talks and posters at the following meetings:

1) Society for Immunotherapy of Cancer Annual Meeting. Virtual Meeting (2020).
2) American Society of Clinical Oncology Annual Meeting. Virtual Meeting (2021).
3) Society for Melanoma Research Congress. Edinburgh, Scotland (2022).

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

For the next reporting period, we plan to continue enrolling participants until enrollment goal has been met; continue collecting blood samples, sc-RNA sequencing, and analyzing data. We look forward to expanding our collaboration with the University of Helsinki to use their TCRGP algorithm ("https://github.com/emmijokinen/TCRGP") to interrogate the V(D)J data.

4. IMPACT:

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

These preliminary results have identified pathways that are upregulated or downregulated after treatment and evaluation of these changes can inform future clinical interventions. Deeper data analysis is underway to better understand the impact of these changes.

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
Nothing to report

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
Nothing to report.

Significant changes in use or care of human subjects
Nothing to report.

Significant changes in use or care of vertebrate animals
Not applicable.

Significant changes in use of biohazards and/or select agents
Not applicable.

6. PRODUCTS:.

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

The following posters/talks were given to the science community:

1) Lutzky J, Feun L, Harbour JW. A phase II study of nivolumab + BMS-986016 (relatlimab) in patients with metastatic uveal melanoma (UM) (CA224-094). Society for Immunotherapy of Cancer Annual Meeting. Virtual Meeting (2020).
2) Lutzky J, Feun L, Harbour JW. NCT04552223. A phase II study of nivolumab + BMS-986016 (relatlimab) in patients with metastatic uveal melanoma (UM) (CA224-094). American Society of Clinical Oncology Annual Meeting. Virtual Meeting (2021).
3) Lutzky J, Durante M, Feun LG, Hernandez-Aya L, Correa Z, Decatur C, King J, Dollar J, Bisbal-Loubriel E, Magallanes N, Reis I, Harbour JW. Phase 2 Trial of Nivolumab/relatlimab in Metastatic Uveal Melanoma. Society for Melanoma Research Congress. Edinburgh, Scotland (2022).

Journal publications.
Nothing to report.

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers and presentations.
Nothing to report.

- **Website(s) or other Internet site(s)**
  This clinical trial titled " Nivolumab Plus Relatlimab in Patients With Metastatic Uveal Melanoma" is listed on ClinicalTrials.gov
  https://clinicaltrials.gov/ct2/show/NCT04552223?cond=metastatic+melanoma&cntry=US&state=US%3AFL&city=Miami&draw=3&rank=20

- **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?

Jose Lutzky, MD – PI. In November 2021, Dr. Harbour requested the transfer of PI role to Dr. Lutzky, in which he has accepted and the DOD approved. No change.
James William Harbour, MD - Other Significant Contributor, accepted the position of Chair of Ophthalmology at University of Texas Southwestern (UTSW) Medical Center effective November 1, 2021. The DOD approved Dr. Harbour's request to remain on this project as Other Significant Contributor. Dr. Harbour holds an adjunct position at the University of Miami. No change.
James Dollar - No change
Christina Decatur - No change
Sarah Kurtenbach – Dr. Kurtenbach was on medical leave during this past year. As such, Anthony Cruz took over the process of collecting, processing, sorting, staining, and storing the samples for this project.
Anthony Cruz- Mr. Cruz provided coverage during Sarah Kurtenbach's absence on this project.

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

Yes, there has been a change in Dr. Lutzky's active other support since last reporting period. One clinical trial has been completed- four have been added.

Completed Research Support
Title: A Phase II Randomized, Open-label, Multi-center Study of the Safety and Efficacy of IMCgp100 Compared with Investigator's Choice in HLA-A*0201 Positive Patients with Previously Untreated Advanced Uveal Melanoma (IMCgp100-2020)
Time commitments: 10% effort, 48 calendar months
Supporting agency: Immunocore Limited
Procuring contracting/grants officer: N/A
Immunocore Limited
101 Park Drive, Milton Park Abingdon
Oxon OX14 4RY, England
Performance period: 06/01/18 - 05/31/21
Level of funding: Direct costs
Brief description:
Role: PI
Overlap: No scientific or budgetary overlap with the proposal.

Active Research Support
Title: Open Label, Multicenter Phase II Study of the C5a Antibody IFX-1 Alone or IFX-1 + Pembrolizumab in Patients With PD-1 or PD-L1 Resistant/Refractory Locally Advanced or Metastatic Cutaneous Squamous Cell Carcinoma (CSCC).
Time commitments: 1.20 calendar months
Supporting agency: InflaRx GmbH (IFX-1-P2.8)
Performance period: 05/18/2022 - 05/17/2026
Level of funding:
Brief description: Phase II Study of the C5a Antibody IFX-1 Alone or IFX-1 + Pembrolizumab
Role: PI
Overlap: No scientific or budgetary overlap with the proposal.

Title: A Phase 2, Open-label, Multicenter Study Evaluating the Safety and Efficacy of Autologous Tumor-infiltrating Lymphocytes (TILs) in Subjects with Advanced Melanoma (DELTA-1)
Time commitments: 1.20 calendar months
Supporting agency: Instil Bio (ITIL-168-101)
Performance period: 01/28/2022 - 02/16/2027
Level of funding:
Brief description: A Phase 2, Open-label, Multicenter Study Evaluating the Safety and Efficacy of Autologous Tumor-infiltrating Lymphocytes
Role: PI

Overlap: No scientific or budgetary overlap with the proposed proposal.

Title: An Open-Label, Dose-Escalation Phase 1b/2 Study to Evaluate the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Antitumor Activity of Modakafusp Alfa (TAK-573) as a Single Agent and in Combination with Pembrolizumab in Adult Patients With Ad
Time commitments: 1.20 calendar months
Supporting agency: Takeda Development Center Americas Inc (TAK-573-1001)
Performance period: 03/29/2022 - 03/28/2026
Level of funding:
Brief description: An Open-Label, Dose-Escalation Phase 1b/2 Study to Evaluate the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Antitumor Activity of Modakafusp Alfa (TAK-573)
Role: PI
Overlap: No scientific or budgetary overlap with the proposal.

Title: A Phase 1 Study of AGEN2373, an Anti-CD137 Monoclonal Antibody, as Monotherapy and in Combination with AGEN1181, an Fc-Engineered Anti-CTLA-4 Monoclonal Antibody, in Patients with Advanced Cancer
Time commitments: 1.20 calendar months
Supporting agency: Agenus Inc (C-1100-01)
Performance period: 03/29/2022 - 03/28/2026
Level of funding:
Brief description: A Phase 1 Study of AGEN2373, an Anti-CD137 Monoclonal Antibody, as Monotherapy and in Combination with AGEN1181, an Fc-Engineered Anti-CTLA-4 Monoclonal Antibody
Role: PI
Overlap: No scientific or budgetary overlap with the proposed MRP proposal.

What other organizations were involved as partners?

University of Helsinki
Helsinki, Finland
A collaboration with the University of Helsinki to use the TCRGP algorithm ("https://github.com/emmijokinen/TCRGP") they developed has been established. We plan to use their algorithm to interrogate the V(D)J data.

8) SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable.
QUAD CHARTS: Not applicable.

9) APPENDICES:

Abstracts

NCT04552223: A phase II study of nivolumab plus BMS-986016 (relatlimab) in patients with metastatic uveal melanoma (UM) (CA224-094). Abstract #335869

Jose Lutzky, Lynn G. Feun, Norma Magallanes, Deukwoo Kwon, J. William Harbour; University of Miami Sylvester Comprehensive Cancer Center, Miami, FL; Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL; Sylvester Comprehensive Cancer Center, Miami, FL; Bascom Palmer Eye Institute, Miami, FL

Background:
Uveal melanoma (UM) is a rare disease but 50% of patients will eventually develop metastatic disease, for which not effective therapy is available. Liver-directed therapies, immunotherapy, targeted therapy and chemotherapy have limited activity [1]. Lymphocyte activation gene 3(LAG-3) is an immune checkpoint receptor associated with decreased T-cell effector function and tumor escape. Preclinical models have shown that dual inhibition of LAG-3 and PD-1 blockade generates synergistic anti-tumor activity [2]. Recent preclinical data indicates that uveal melanoma CD8+ T cells express the checkpoint receptor LAG3 to a greater extent than PD1 or CTLA4 [3,4]. Therefore, LAG3 is a potential candidate for checkpoint inhibitor immunotherapy in UM. Relatlimab is a human LAG-3-specific antibody isolated from immunized transgenic mice which binds to a defined epitope on LAG-3 with high affinity and specificity and potently blocks the interaction of LAG-3 with its ligand, MHC Class II.

Methods:
This is an open-label, single arm, single site investigator-initiated phase II study, NCT04552223. Based on Simon two-stage minimax design, 13 patients will be enrolled in Stage 1. If at least one response is noted, the study will proceed to Stage 2 and enroll an additional 14 patients. The null hypothesis will be rejected if 4 or more responses are observed among 27 patients. This design achieves 5% type I error and 80% power when the true ORR is 20%. The trial opened to accrual in December 2020. As of February 15, 2021 four patients had been enrolled the first stage of accrual.
Main eligibility criteria include patients with biopsy proven metastatic uveal melanoma, previously untreated with PD-1, CTLA-4 and/or LAG-3 blocking antibodies and in good performance status.vEnrolled patients are treated in the outpatient setting. Nivolumab 480 mg is mixed in the same bag with relatlimab 160 mg and administered intravenously over 60 minutes every 4 weeks until disease progression or intolerable toxicity for up to 24 months.

The primary endpoint is best objective response rate (ORR). Secondary endpoints include disease control rate (DCR), progression-free survival (PFS), overall survival (OS), median duration of response (mDOR), and adverse events (AEs). Correlative studies will evaluate pre- and post-treatment characteristics of T cells in the tumor microenvironment and blood.

A phase II study of nivolumab + BMS-986016 (relatlimab) in patients with metastatic uveal melanoma(UM) (CA224-094).

Jose Lutzky[1], Lynn Feun[1], Norma Magallanes[1], Deukwoo Kwon[1], William J Harbour[2].
[1]University of Miami Sylvester Comprehensive Cancer Center, Miami, Florida
[2]Bascom Palmer Eye Institute, Miami, Florida
jxl810@med.miami.edu
dkwon@med.miami.edu
lfeun@med.miami.edu
nkm42@med.miami.edu
harbour@med.miami.edu

Keywords: uveal, melanoma, ocular, LAG3, nivolumab, immunotherapy, metastatic

Background: Fifty percent of patients with uveal melanoma (UM) develop metastatic disease, surviving 6–12 months from metastatic diagnosis. Liver-directed therapies, immunotherapy, targeted therapy and chemotherapy have limited activity. Lymphocyte activation gene 3(LAG-3) is an immune checkpoint receptor associated with decreased T-cell effector function and tumor escape. Preclinical models have shown that dual inhibition of LAG-3 and PD-1 blockade generates synergistic anti-tumor activity [1]. In uveal melanoma, CD8+ T cells express the checkpoint receptor LAG3 to a greater extent than PD1 or CTLA4 [2,3]. This recent discovery nominates LAG3 as a potential candidate for checkpoint inhibitor immunotherapy in UM.

Methods: This is an open-label, single arm, single site investigator-initiated phase II study. Based on Simon two-stage minimax design, 13 patients will be enrolled in Stage 1. If at least one response is noted, the study will proceed to Stage 2 and enroll additional 14 patients. The null hypothesis will be rejected if 4 or more responses are observed among 27 patients. This design achieves 5% type I error and 80% power when the true ORR is 20%.
Main eligibility criteria includes patients with biopsy proven metastatic uveal melanoma, previously untreated with PD-1, CTLA-4 and/or LAG-3 blocking antibodies and in good performance status.
Enrolled patients will be treated in the outpatient setting. Nivolumab 480 mg will be mixed in the same bag with relatlimab 160 mg and administered intravenously over 60 minutes every 4 weeks until disease progression or intolerable toxicity for up to 24 months.

The primary endpoint is best objective response rate (ORR). Secondary endpoints include disease control rate (DCR), progression-free survival (PFS), overall survival (OS), median duration of response (mDOR), and adverse events (AEs). Correlative studies will evaluate pre- and post-treatment characteristics of T cells in the tumor microenvironment and blood.

References:
Woo, S.R., Turnis, M.E., Goldberg, M.V., et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res., 2012. 72(4): p. 917-27.
Durante MA, Rodriguez DA, Kurtenbach S, et al. Single-cell analysis reveals new evolutionary complexity in uveal melanoma. Nat Commun. 2020;11(1):496. Published 2020 Jan 24. doi:10.1038/s41467-019-14256-1
Karlsson J, Nilsson LM, Mitra S, et al. Molecular profiling of driver events in metastatic uveal melanoma. Nat Commun. 2020;11(1):1894. Published 2020 Apr 20. doi:10.1038/s41467-020-15606-0

Phase 2 Trial of Nivolumab/relatlimab in Metastatic Uveal Melanoma.

Jose Lutzky[1], Michael Durante[2], Lynn G. Feun[1], Leonel Hernandez-Aya[1], Zelia Correa[3], Christina Decatur[3], Jeanelle King[1], James Dollar[3], Edda Bisbal-Loubriel[1], Norma Magallanes[1], Isildinha Reis[1], J. William Harbour[4].
[1]Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida, USA; [2]Department of Medicine, University of Miami Miller School of Medicine, Miami, Florida, USA; [3]Bascom Palmer Eye Institute, University of Miami, Miami, Florida, USA; [4]Department of Ophthalmology, University of Texas Southwestern, Dallas, Texas, USA.

Treatment options for metastatic uveal melanoma are limited. Lymphocyte activation gene 3 (LAG-3) is an immune checkpoint receptor associated with T cell dysfunction. Relatlimab is a human LAG-3 specific blocking antibody. Dual nivolumab and relatlimab blockade is approved in unresectable or metastatic melanoma. Uveal melanoma CD8+ T cells highly express LAG-3 (Durante et al. Nat Comm 2020). We are conducting a phase II single-institution trial with a Simon two-stage minimax design with 27 patients (13 in stage 1, 14 in stage 2) setting a 5% type I error and 80% power under true objective response rate (ORR) of 20%. The null hypothesis (ORR=5%) is rejected if 4 or more responses are observed in 27 patients. No prior PD-1, CTLA-4 and/or LAG-3 blocking antibody treatment was allowed. Primary endpoint is ORR. Secondary endpoints are disease control rate (DCR), progression-free survival (PFS), overall survival (OS), duration of response (DOR), and safety (AEs). We performed single-cell mRNA and T-cell receptor (TCR) variable, diversity, and joining (V[D]J) sequencing pre-treatment and at either progression or response. As of 5/1/2022, 20 patients were enrolled and treated with nivolumab 480 mg/relatlimab 160 mg IV q4wks to progression or intolerable toxicity. At a median follow -up of 6.2 months, the ORR in 18 evaluable patients was 11.1% (2/18). Stable disease (SD) and progressive disease (PD) were 33.3% (6/18) and 55.6% (10/18) respectively. All patients experienced treatment related adverse events (TRAE). Of 72 TRAEs, 69 (95.8%) were grade 1-2 and 3 (4.2%) were grade 3 (diarrhea, hyperglycemia and elevated lipase). The most common TRAEs were fatigue (15.3%), hypothyroidism (9.7%), transaminase elevation (8.3%), arthralgias (5.6%) and diarrhea (4.2%). Serious adverse reactions (SAE) were seen in 4 patients, all related to progressive disease. Results of secondary outcomes and correlative studies will be presented.