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14. ABSTRACT

This pilot proposal stems from our discovery that novel small molecule that interact with the retinoblastoma (Rb) protein domain C, AP-3-84, can induce myeloid-preferential apoptosis. Our data indicate that treatment with AP-3-84 can lead to myeloid cell depletion (in *ex vivo* cultures of human myeloid cells derived from ovarian tumors, as well as in a murine *in vivo* model). We will test the hypothesis that treatment with Rb modulators AP-3-84 will result in ovarian tumor regression and/or improved survival by increasing myeloid cell death and increasing anti-tumor cell-mediated responses. Specific aims will address: (1) To establish the impact of exposure to AP-3-84 on gene expression, viability and function of myeloid and lymphoid cell subsets isolated from fresh human ovarian cancer tissue. (2) A-To define the efficacy of treatment with AP-3-84 in achieving tissue and tumor myeloid cell depletion (TAM, MSDCs, rDCs) at different points of ovarian tumor progression in murine models and its effect on overall survival. B-in the same model, to assess the changes in anti-tumor cellular immunity induced by AP-3-84 treatment. The proposed studies seeks to establish that the Rb protein can serve as a new molecular target in immunosuppressive myeloid cells in ovarian cancer models, and that its blockage results in myeloid cell death within the tumor microenvironment, leading to a decrease in local immunosuppression, and enhanced T-cell control.

15. SUBJECT TERMS: NONE LISTED

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Table of Contents

1. INTRODUCTION:

Ovarian cancer is the most lethal gynecologic cancer in the Western world and has an incidence of approximately 22,200 women/year, 60% of the patients being diagnosed after the disease has spread outside of the ovaries, which is associated with a dismal 5-year survival rate of 28.9%. 5-year survival rates have improved little in the last 30 years. Poor clinical prognosis is associated with an increase in tumor infiltration of myeloid cells including tumor-associated macrophages (TAMs). In this study, we seek to develop the first small molecule therapeutics to reduce TAMs *in vivo* by targeting the retinoblastoma protein (Rb), a transcription factor crucial for myeloid cell phenotype, function and survival, yet without dampening T-cell from mediating subsequent tumor control. We hypothesize treatment with Rb modulators AP-3-84 will result in ovarian tumor regression and improved survival by increasing myeloid cell death particularly TAMs and increasing anti-tumor T-cell cellular responses. First, we establish the impact of AP-3-84 treatment on viability and function of purified myeloid and lymphoid cells isolated from human or murine ovarian cancer tissue. With this aim, we also will examine on the gene expression difference of cells which demonstrate different sensitivity to the compound. Secondly, we will define efficacy of treatment with AP-3-84 in achieving tissue and tumor myeloid cell depletion and the impact of myeloid cell depletion on T-cell cell activation, priming, infiltration, and consequently in altering tumor burden and animal survival. Third, we will identify the molecular mechanism of action (MOA) of AP-3-84. Use of Rb knockout mice are expected to be used to facilitate this MOA studies. Lastly, with information obtained from the above aims, we will try to combine the compound with other drugs such as check point inhibitors (anti-PD-L1/anti-PD-1) and chemotherapeutics.

2. KEYWORDS:

Ovarian Cancer, Rb/Retinoblastoma Protein, Apoptosis, Cell Death, Tumor Microenvironment (TME), Tumor Associated Macrophages (TAM), myeloid cells, CDK4/CDK6

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. The major goal for Aim 1 within the first 12 months (*Major Tasks and Substasks progress report/completion in first 12 months highlighted in yellow below*) is to determine the activity of AP-3-84 in myeloid and lymphoid cells *ex vivo* isolated from human ovarian cancer tissues.

Major Task 1: 25% Completed: Direct apoptosis effects on myeloid cells within human ovarian tumors	**Target Month**
Subtask 1: Local IRB Approval; **100% Completed by 3 months as proposed.**	1-3

Subtask 2: Establishing direct apoptosis effects on myeloid cells within human ovarian tumors; **50% Completion as of 12 months**	3-12
Subtask 3: Establishing direct apoptosis effects on myeloid cells within human ovarian tumors (13-24 samples).	13-22
Milestone Achieved	22

Major Task 2 – 30% Completed: Gene expression/protein change in myeloid and lymphoid cells after Rb inhibition by compound AP-3-84 as compared to carrier control.	
Subtask 1: Establishing gene expression change in myeloid and lymphoid cells after Rb modulation by compound AP-3-84 as compared to carrier control. (1 sample); **100% Completed**	10
Subtask 2: Completion of 4 added samples	12-16
Subtask 3: Bioinformatic Analysis	17-22
Milestone(s) Achieved:	23

Aim 2. The major goal for Aim 2 is to define the efficacy of AP-3-84 on tumor myeloid cell depletion at different points of ovarian cancer progression *in vivo* and impact on tumor burden and survival of the animal model.

Major Task 1 – 100% Completed: Differences between AP-3-84 therapy and carrier control in decreasing ovarian tumor burden.	
IACUC Approval: **100% Completed**	1-3
Subtask 1: First Experiment; **100% Completed**	3
Subtask 2: Second Experiment;**100% Completed**	10
Milestone Achieved: **100% Completed**	10

Major Task 2 – 100% Completed (ahead of schedule): Survival after treatment with Rb modulator and change in the distribution of myeloid and T-cell subsets in vivo.	
Subtask 1: Collection of data (time points); **100% Completed**	10
Milestone(s) Data analysis achieved:	10 (Originally 18)

Major Task 3 – 85% Completed: Direct tissue measurements after Rb modulator to assess if acute depletion of myeloid cells in the tumor microenvironment is matched with increased T-cell infiltrates.	**Months**
Subtask 1: Collection of data (time points): **100% Completed**	6-10
Milestone(s) Data analysis achieved	15

Major Task 4 – 50% Completed: After survival following treatment with Rb modulator therapy determine if mice can prevent tumor recurrence and/or T-cells protect against additional tumor challenge?	

Subtask 1: Per survival outcome of Aim 2-Major Task 1/First Experiment; **100% Completed**	6-9
Subtask 2: Subtask 1: Per survival outcome of Aim 2-Major Task 1/Second Experiment	13-15
Milestone(s) Achieved:	18
Major Task 5 – 0% Completed: Final results and manuscript Preparation	
Final data analysis and manuscript preparation	22-24
Milestone completed	24

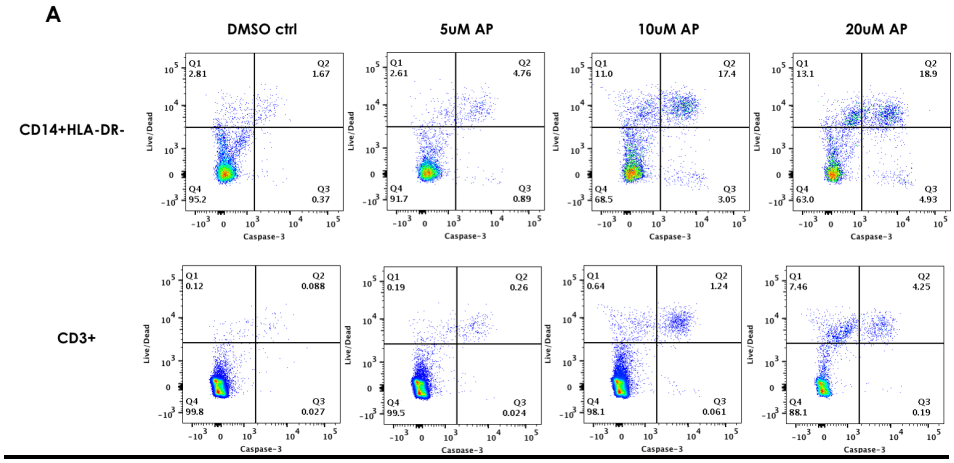
What was accomplished under these goals?

For AIM 1 (refer above to Major Tasks and Subtasks):

Completion of Regulatory Approvals. An IRB protocol collaborating with Christiana Care Health System was established and approved in July 2019. Clinical samples started to be received in January 2020. (Major Task 1, Subtask 1).

1) ***Establishing direct apoptosis effects on myeloid cells within human ovarian tumors***

Analysis of blood monocytes (CD14+HLA-DR-) and ascites macrophages (CD163+CD68+) cells following analysis by flowcytometry were detected to be sensitive to induced apoptosis by exposure to AP-3-84 whereas CD3+ T cells had a lower apoptosis response. Data documenting this observation and intended to be supported by added samples in year 2 is shown in Figure 1.



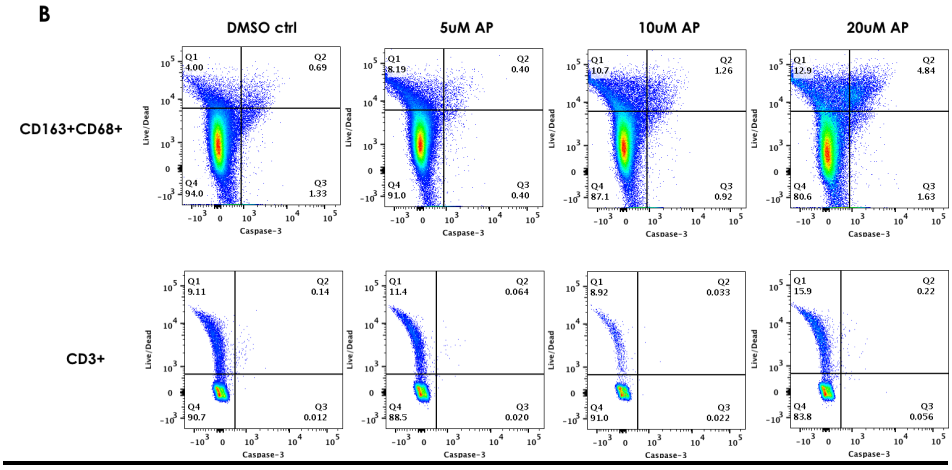


Figure 1. AP-3-84 preferentially induces apoptosis in monocytic myeloid suppressor cells and macrophages. (**A**) PBMCs isolated from ovarian cancer patient were cultured in complete RPMI media treated with or without AP-3-84 for around 24h. Surface staining and intracellular staining were performed to assess apoptosis. (**B**) Ascites cells enriched from ovarian cancer patient were cultured in complete RPMI media treated with or without AP-3-84 for around 24h. Surface staining and intracellular staining were performed to assess apoptosis.

For AIM 2 (refer above to Major Tasks and Subtasks):

1) ***Small molecule AP-3-84 induces apoptosis in murine myeloid cells from ovarian tumor-bearing mice ex vivo*** Compound AP-3-84 (Figure 2A) was discovered as an antagonist to E7 interactions with Rb through a proposed mechanism of displacing E7 from the Rb B-box domain and restoring the binding capacity of Rb with E2F1 to achieve proliferation inhibition. The binding biochemical property is shown in Figure 2B. However, the E7-negative cells also showed effect when exposed to the compound, an apoptosis inducing effect rather than proliferation inhibition. Both tumor cells and 3% thioglycolate induced macrophages demonstrated apoptosis effect as shown in Figure 2C. This apoptosis effect was also found in ascites cells collected from mice bearing stage III ovarian cancer as shown in Figure 2D. In addition, ascites macrophages were noted to be more sensitive to the compound than the associated tumor cells.

(Next Page) Figure 2. AP-3-84 induction of myeloid cell apoptosis in tumor-bearing mice.
(A) Chemical structure of AP-3-84. (**B**) IC50 values for AP-3-84 in the onco-viral protein present system. (**C**) Mouse triple negative breast cancer cells A7C11 were cultured with AP-3-84 treatment for 4h. Mouse 3% Thioglycollate Induced macrophages were cultured with AP-3-84 treatment overnight. Apoptosis was assessed through flow cytometry. (**D**) Ascites cells from stage III ID8 mouse ovarian cancer model were treated with AP-3-84 for 36h. Apoptosis was induced in both tumor and macrophages.

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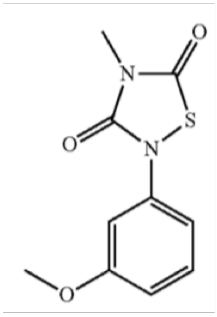


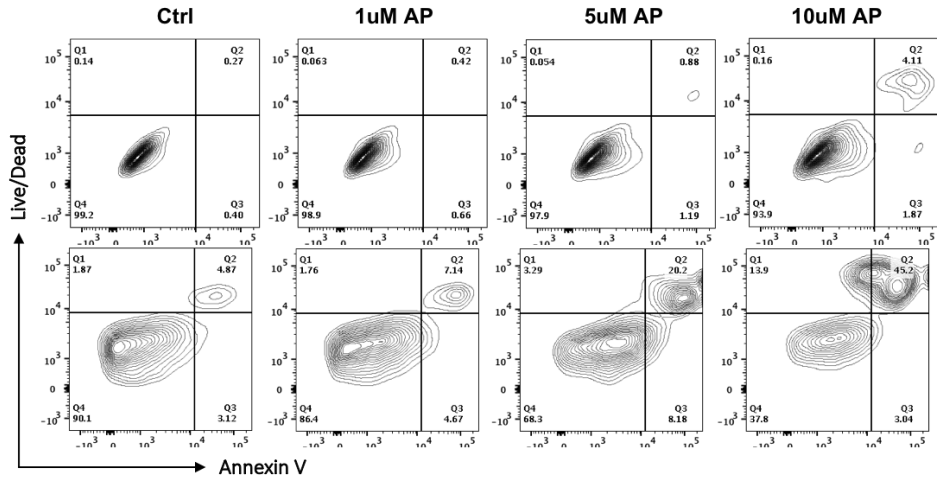
B

TABLE 1

IC50 values for AP-3-84 inhibiting HPV-E7-mediated disruption of pRb/E2F and disrupting pRb/viral Oncoprotein complexes

	16E7 (500nM)	1AE7 (500nM)	E1A (100nM)	K_D (uM)
pRb/E2F	0.34 ± 1.9	3.5 ± 1.7	3.2 ± 2.7	0.815 ± 0.070
pRb	0.29 ± 1.7	4.0 ± 2.5	2.8 ± 2.1	

C



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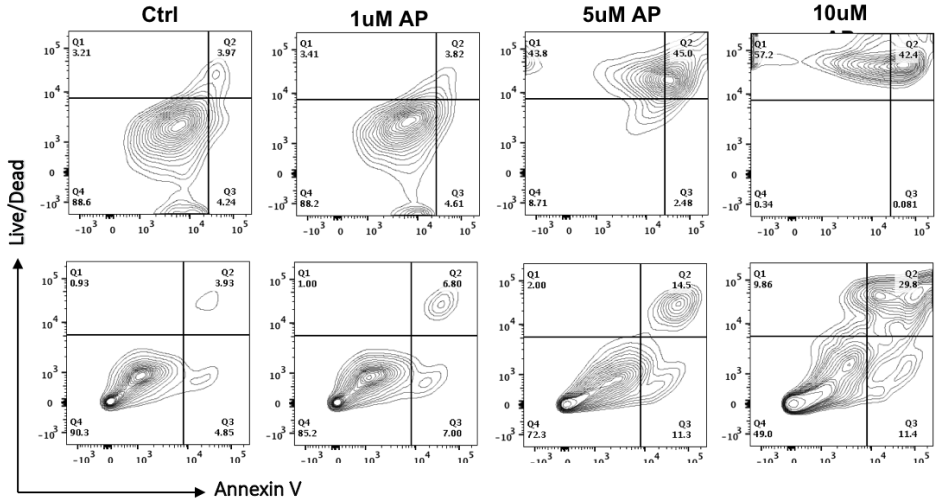


2) ***<u>AP-3-84 activity is not redundant with CDK4/CDK6 inhibitor Palbociclib and is a potentially safe drug candidate.</u>***

Palbociclib is a CDK4/CDK6 inhibitor and targets the same pathway that interacts with Rb. We established the mechanism of action (MOA) of AP-3-84 and palbociclib is different in that palbociclib inhibit cell proliferation whereas AP-3-84 induced apoptosis (Figure 3A). Another difference is that AP-3-84 showed less direct cancer cell cytotoxicity effect than palbociclib (Figure 3B). Moreover, we found the two compounds target different cell populations. AP-3-84 was found to be sensitive in inducing apoptosis of spleen monocytes whereas palbociclib induced apoptosis in neutrophils though such apoptosis effects were not observed in cancer cells (Figure 3C). In line with this finding, AP-3-84 demonstrated apoptosis inducing effect on ascites macrophages from mouse bearing ovarian cancer, whereas palbociclib did not (Figure 3D). SafetyScreen 44 assay (Cerep44), evaluating off-target interactions by AP-3-84 was completed (Figure 3E).

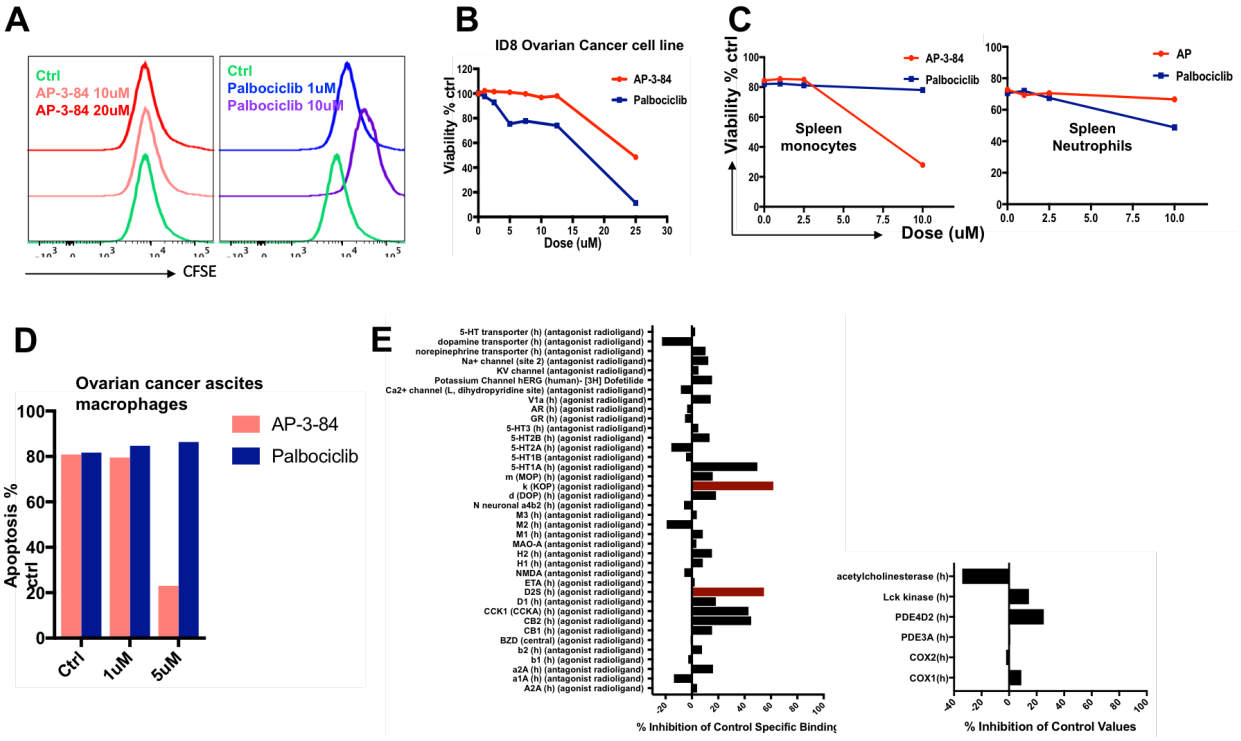


Figure 3. AP-3-84 is distinct from Palbociclib mechanism of action and has a favorable Cerep44 profile. (A) CFSE proliferation inhibition assay with AP-3-84 or palbociclib on Mouse triple negative breast cancer cells A7C11 cells. (B) Cancer cell cytotoxicity assay by MTS on mouse ovarian cancer ID8 cells treated with AP-3-84 or palbociclib for 72h. (C) splenocytes isolated from 6-8 weeks-old mouse were cultured with AP-3-84 or palbociclib treatment for 4h. Apoptosis was assessed by flow cytometry. (D) mouse ascites cells from ID8 ovarian cancer-bearing mouse were cultured in complete RPMI 1640 media with AP-3-84 or palbociclib treatment for 72h. Apoptosis was assessed by flow cytometry. (E) SafetyScreen 44 assay (Cerep44) greater than 90% inhibition was found and only two receptors were found to be inhibited more than 50%.

3) ***Macrophages targeted by AP-3-84 in tumor microenvironment (TME) of stage III mouse ovarian cancer model are confirmed as immunosuppressive.***

To recapitulate human metastatic ovarian cancer, we established a stage III mouse ovarian cancer model by injecting 2M ID8 mouse ovarian cancer cells to the peritoneal cavity (Figure 4A). This model generates ascites as advanced human ovarian cancer does. The onset and progression of ascites was shown to be corelated with poor prognosis. We propose, as reported by other researchers, studying the ascites is of utmost importance for advanced mouse ovarian cancer models. Immunosuppressive markers such as PD-L1 and Arginase were abundantly detected in the ascites macrophages (Figure 4A). immunosuppressive cytokines IL-10 and TNF-a was found to be secreted by macrophages (Figure 4A). Ascites macrophages increased as tumor progressed (Figure 4B). Consistently, macrophage amount negatively correlated with tumor burden and positively correlated with inhibitory T cells (Figure 4C and 4D). Results confirmed ascites macrophages are immunosuppressive in ovarian cancer progression, providing the rationale of targeting macrophage depletion.

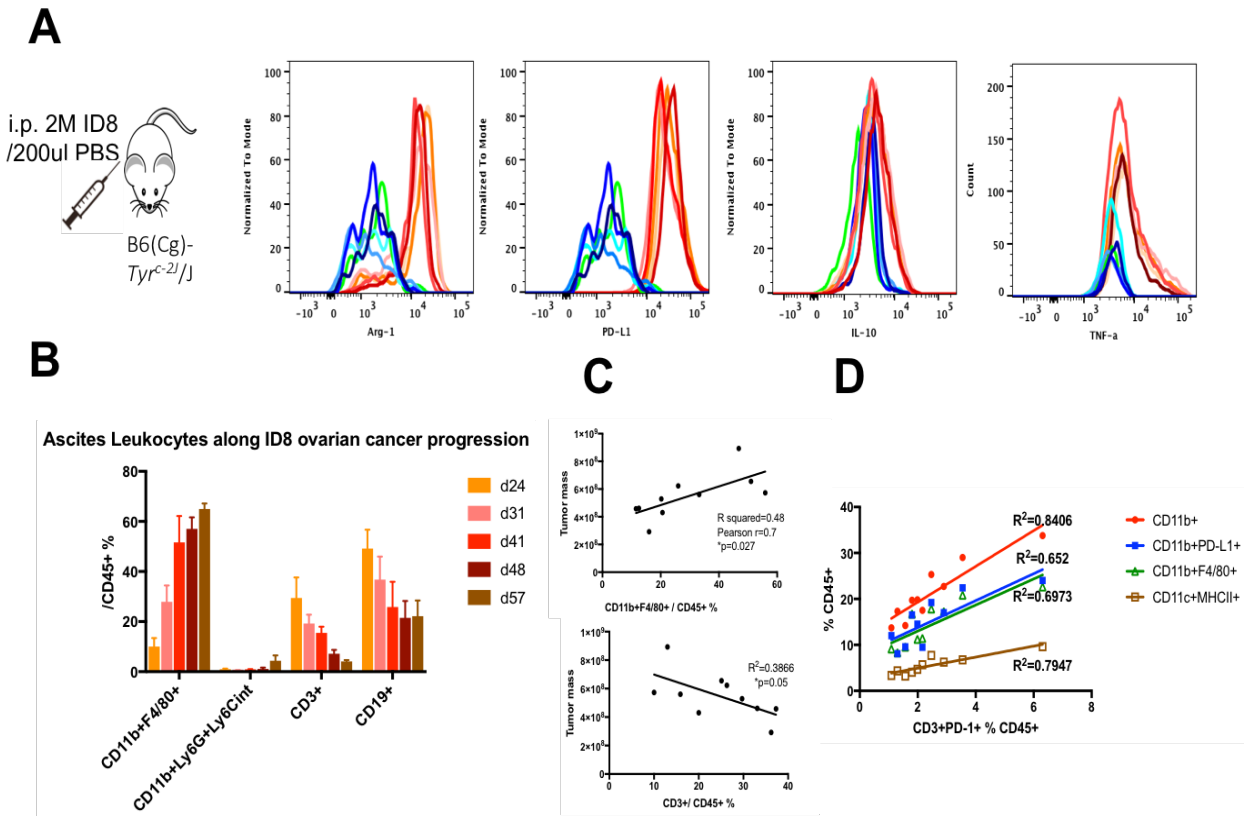
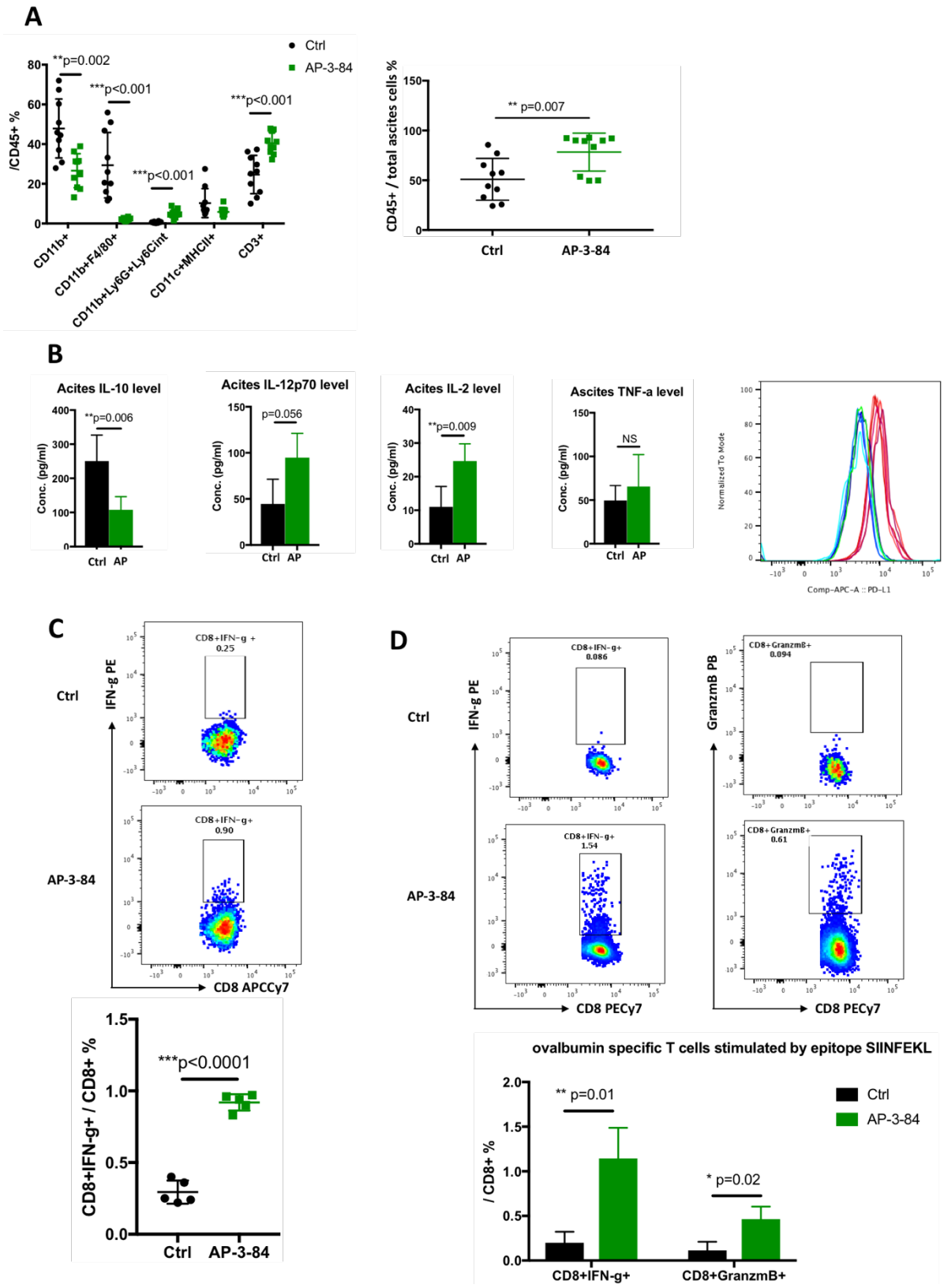


Figure 4. Increase in Immunosuppressive macrophages upon ovarian tumor progression. (**A**) Stage III ID8 mouse ovarian cancer model and immunosuppressive markers and cytokines of ascites macrophages. (B) Ascites macrophages declined along tumor progression. (C) ascites macrophages positively corelates with tumor burden whereas T cells the opposite. (D) ascites macrophages or myeloid cells negatively corelates with inhibitory T cells.

4) ***AP-3-84 depletes macrophages from the tumor microenvironment (TME) and changes the inflammatory profile of TME in Ovarian Cancer***

AP-3-84 or vehicle control was administered at 10mg/kg of mouse weight consecutively for 7 days starting at day 26 after tumor inoculation. We found the AP-3-84 treatment significantly decreased frequency of macrophages in the ascites of tumor-bearing mice. Overall myeloid cells (CD45+11b+ cells) were significantly decreased. Frequency of CD3+ T cells was increased whereas CD11c+MHCII+ DCs were not detected to change (Figure 5A). Through counting cells from ascites of both control and treatment group, we found a significant increase of CD3+ T cell (CD4+ and CD8+) infiltrates in addition to the frequency increase of CD3+ T cells (data not shown). Immunosuppressive cytokines and markers were decreased after the treatment (Figure 5B). Frequency of CD8+IFN-g+ clones were found to be significantly increased in the treatment group (Figure 5C). To assess the tumor antigen specific immunity, we also established the ID8-ova mouse ovarian cancer model by injecting 2M ID8 cells transfected with ovalbumin to the peritoneal cavity. At terminal point of the study, spleen from both groups were harvested and splenocytes were cultured ex vivo with ovalbumin epitope SIINFEKL and Golgistop. We found CD8+ cytotoxic effector clones CD8+GranzmB+ and CD8+IFN-g+ were significantly increased in the treatment group (Figure 5D). To summarize, we found Rb modulator AP-3-84 depleted immunosuppressive macrophages in the TME of stage III ovarian cancer, causing an enhanced T cell infiltration, changed the immunosuppressive TME to a more immunostimulatory TME, and promoted T cell mediated anti-cancer immunity locally and systematically.

(Next page) Figure 5. AP-3-84 depleted immunosuppressive macrophages in the TME of stage III ovarian cancer resulting in enhanced anti-tumor T cell infiltration and changed the immunosuppressive TME to a more immunostimulatory TME. (**A)** Ascites was collected at day 36 from Stage III ID8 mouse ovarian cancer model for profile of immune cell subsets by staining with surface markers followed by flow cytometry. (B) Ascites supernatant was harvested after centrifugation. Cytokine level from each mice group was examined with V-PLEX Proinflammatory Panel 1 Mouse Kit from MSD (Meso Scale Diagnostics). (C) Ascites was collected from stage III mouse ovarian cancer model after 7X treatment at day 34. After red blood cell lysis, ascites cells were cultured in complete RPMI 1640 media supplemented with Golgistop for 6h. Frequency of cytotoxic IFN-g+ CD8 effector clones was determined. (D) Mice bearing ID8-ova tumor cells were treated with vehicle control (5% DMSO/5% Tween-80) or AP-3-84 at 10mg/kg of mice weight. The treatment was continued daily for 7 days. Mice were euthanized at terminal point and splenocytes were collected and cultured 4h with GolgiStop and 1ug/ml Ovalbumin epitope SIINFEKL. Ovalbumin antigen specific CD8+IFN-g+ and CD8+GranzmB+ effector clones were assessed after surface and intracellular staining followed by flow cytometry detection.


A
Ctrl
AP-3-84
**p=0.002
***p<0.001
***p<0.001
***p<0.001
/CD45+ %
CD11b+
CD11b+F4/80+
CD11b+Ly6G+Ly6Cint
CD11c+MHCII+
CD3+
** p=0.007
CD45+ / total ascites cells %
Ctrl
AP-3-84
B
Acites IL-10 level
**p=0.006
Conc. (pg/ml)
Ctrl AP
Acites IL-12p70 level
p=0.056
Conc. (pg/ml)
Ctrl AP
Acites IL-2 level
**p=0.009
Conc. (pg/ml)
Ctrl AP
Ascites TNF-a level
NS
Conc. (pg/ml)
Ctrl AP
Normalized To Mode
Comp-APC-A :: PD-L1
C
Ctrl
AP-3-84
IFN-g PE
CD8+IFN-g + 0.25
CD8+IFN-g+ 0.90
CD8 APCCy7
***p<0.0001
CD8+IFN-g+ / CD8+ %
Ctrl
AP-3-84
D
Ctrl
AP-3-84
IFN-g PE
CD8+IFN-g+ 0.086
CD8+IFN-g+ 1.54
CD8 PECy7
GranzmB PB
CD8+GranzmB+ 0.094
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** p=0.01
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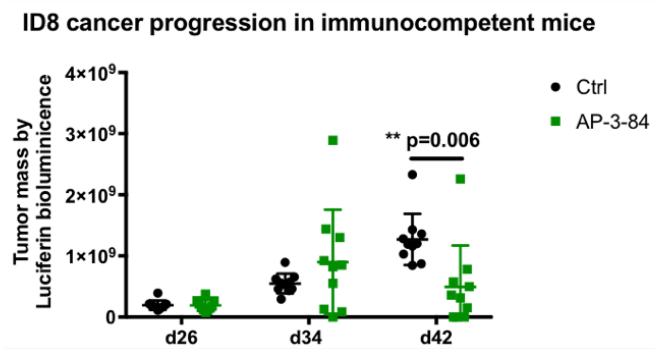
AP-3-84
CD8+IFN-g+
CD8+GranzmB+

5) ***AP-3-84 treatment significantly delayed ovarian cancer progression in immunocompetent mice but not in immunodeficient NSG mice***

The Stage III ID8 ovarian cancer model was used to assess the in vivo effect of AP-3-84 which was administered to mice at 10mg/kg of mice weight daily for 7 days consecutively. By the end of the treatment at day 42, we did not see an immediate tumor burden change between the control and treatment group (Figure 5A). However, one week after the treatment completion, there was a significant tumor burden drop comparing control and treatment groups which was a dramatic visual difference seen from the IVIS images (Figure 5A). We also examined the amount of tumor cells in the ascites through flow cytometry because the tumor cells are GFP transfected and auto-fluorescent. It was found the CD45-GFP+ cells in treatment group were significantly reduced (Figure 5A). Thus we conclude that AP-3-84 significantly delayed ovarian cancer progression. To investigate whether this in vivo anti-tumor effect was mediated through the enhanced anti-tumor immunity locally and systematically. We established the same model using NSG mice and treated the mice using the same regimen as that for the immunocompetent mice. Interestingly, there was no tumor burden change comparing the control and treated mice (Figure 5B). Therefore, AP-3-84 delayed tumor progression through the improved immunostimulatory immune system rendering it a promising immunotherapeutic candidate by targeting Rb, a novel immuno-target.

(Next Page) Figure 5 AP-3-84 decreases ovarian tumor burden in intact but not immunodeficient NSG mice. (**A**) Stage III ID8 mouse ovarian cancer model was established by injection of 2M ID8 luciferin transfected cells in PBS to mouse peritoneal cavity at day 0. Mice were treated with vehicle control (5% DMSO/5% Tween-80) or AP-3-84 at 10mg/kg of mice weight daily for 7 days (n=10). At day 34 and day 42, tumor burden from all groups of mice was assessed. Visual difference demonstrated by IVIS images are representative ones of 5 mice (one cage) from control or treated group respectively. At day 42, ascites cells were harvested for assessment of GFP+CD45- tumor cells in both groups by flow cytometry. (**B**) Stage III ID8 mouse ovarian cancer model was established by injection of 2M ID8 luciferin transfected cells in PBS to NSG mouse peritoneal cavity at day 0. At day 24, mice were treated with vehicle control (5% DMSO/5% Tween-80) or AP-3-84 at 10mg/kg of mice weight daily for 7 days (n=10). At day 31 and day 36, tumor burden from all groups of mice was assessed.

A


ID8 cancer progression in immunocompetent mice
Tumor mass by Luciferin bioluminicence
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2×10^9
1×10^9
0
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AP-3-84
d26
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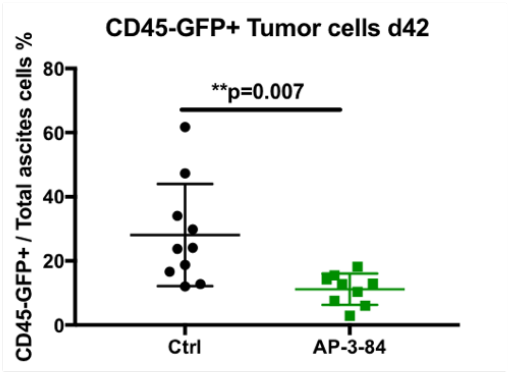
CD45-GFP+ Tumor cells d42
CD45-GFP+ / Total ascites cells %
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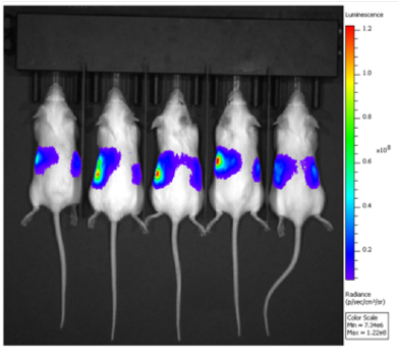
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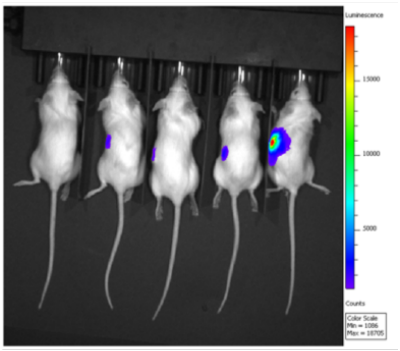
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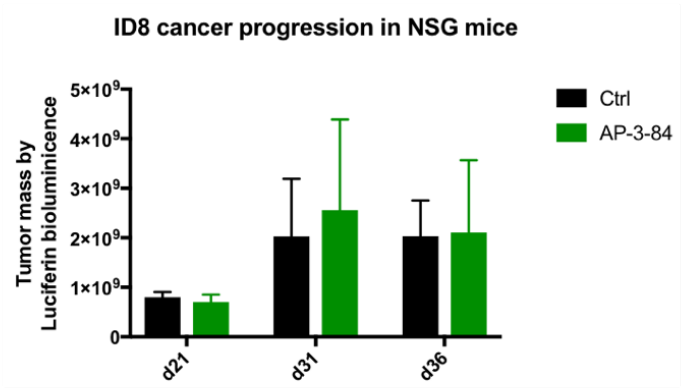
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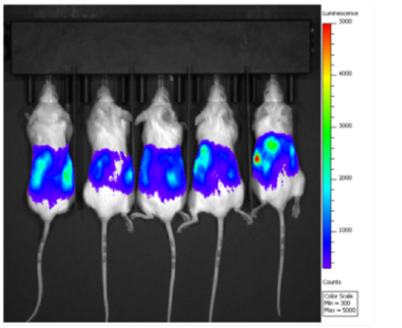
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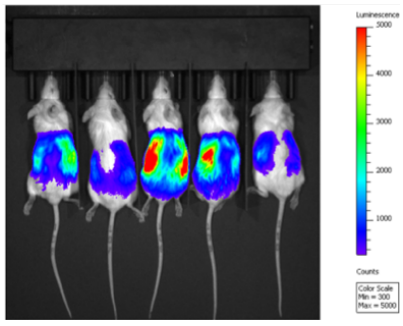
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What opportunities for training and professional development has the project provided?

Nothing to report

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?

For Aim 1, we plan to receive more clinical samples to complete Major Task 1 (Subtasks 2 and 3) and Major Task 2 (subtask 2 and 3) in order to assess the effect on apoptosis, function and gene change of human myeloid and lymphoid cells.

For Aim 2, we are planning to dissect the molecular and cellular mechanism of our compound in inducing macrophages apoptosis in vivo and evidence for resistance to rechallenge (Major Task 4, Subtask 2).

We plan to complete final data analysis and manuscript preparation for submission (Major Task 5).

4. IMPACT

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

Data collected to date in first year of award supports our central initial hypothesis that treatment with Rb modulators AP-3-84 can result in ovarian tumor regression and/or improved survival by increasing myeloid cell death and increasing anti-tumor immune-mediated responses. The ultimate impact of this study will be the introduction of targeting the Rb protein as a **new first-in-class molecular target** in immunosuppressive myeloid cells in achieving control of ovarian cancer

by promoting myeloid cell death within the tumor microenvironment, leading to a decrease in local immunosuppression, and enhanced immune-mediated cellular control.

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

Actual past problem/resolution in year 1. The combination of a delay in completion of regulatory approvals to receive first human surgical samples together with the replacement of Dr. Perales-Puchalt (scientist supported by award) by Dr. Muthumani, and the need to refine the process for identifying and transfer of samples in real-time within hours of collection (same day) lead to a delay in the anticipated rate of completion for Major Task 1, Subtask 2 otherwise projected to be completed by month 12 of award. No anticipated delays in the rate of samples to be analyzed in year 2 is expected.

Anticipated future problem in year 2. The COVID-19 pandemic (starting in end of first year of award) has resulted in a shutdown of clinical and research operations per state and federal mandates for stay-at-home and social distancing. It is expected that progress on year 2 of award will be delayed by at least a quarter on account of COVID-19.

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

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Luis Montaner
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Luis Montaner has supervised completion of all aims.
Funding Support:	This award

Name:	Xue Yang, PhD
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Dr. Xue Yang performed experiments as described by aims.
Funding Support:	This award

Name:	Kar Muthumani, PhD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Muthumani replaced effort for Dr. Alfredo Perales-Puchalt
Funding Support:	This award

Name:	Devivasha Bordoloi, PhD
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project:	Dr. Bordoloi performed experiments as described in aims.
Funding Support:	This award

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

Alfredo Perales-Puchalt left the Wistar Institute in 2019 at which time Dr. Muthumani replaced his effort due to his comparable experience in cancer immunobiology. Dr. Bordoloi joined as added effort to provide added support for molecular biology techniques.

What other organizations were involved as partners?

Christiana Care Health System (CCHS)

Location of Organization: Delaware

Partner's contribution to the project (identify one or more)

- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)

8. SPECIAL REPORTING REQUIREMENTS

Not Applicable

9. APPENDICES:

Quad Chart.

Development of Novel Small-Molecule Rb Protein Modulator for Ovarian Cancer Immunotherapy
OC180193
W81XWH-19-1-0092

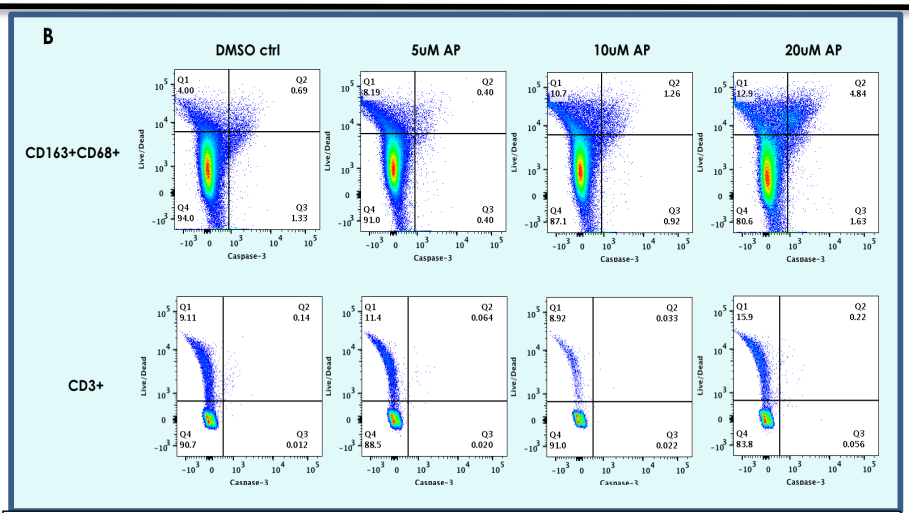
PI: Luis J. Montaner, Ph.D. **Org:** The Wistar Institute of Anatomy and Biology **Award Amount:** $444,334

Study/Product Aim(s)

- Specific Aim 1 – Establish the impact of exposure to AP3-84 on gene expression, viability, and function
- Specific Aim 2 - Defining the efficacy of treatment with AP-3-84 in achieve tissue and tumor myeloid cell depletion

Approach

We hypothesize that treatment with Rb modulators of AP-3-84 will result in ovarian tumor regression and improved survival by increasing myeloid cell death. We will establish the impact of AP-3-84 treatment on viability and function of purified myeloid and lymphoid cells from human or murine ovarian cancer tissue; define efficacy of treatment with AP-3-84 in achieving tissue and tumor myeloid cell depletion and the impact of myeloid cell depletion on T-cell cell activation, priming, infiltration, and altering tumor burden; identify molecular mechanism of action of AP-3-84; potentially combine compound with other drugs such as check point inhibitors or chemotherapeutics.



Accomplishment: Ascites cells enriched from ovarian cancer patient were cultured in complete RPMI media treated with or without AP-3-84 for around 24h. Surface staining and intracellular staining were performed to assess apoptosis with preferential apoptosis noted in myeloid cells.

Timeline and Cost

Activities CY	19	20
Task 1 Direct apoptosis effects		
Task 2 Gene expression change		
Task 3 In vivo Anti-tumor AP-3-84		
Task 4 In vivo Cell Apoptosis & Immune Control		
Estimated Budget ($K)	**$152,451**	**$444,334**

Updated: March 31, 2020

Goals/Milestones CY19

***Specific Aim 1* Major Task 1 – Direct apoptosis effects**

Local IRB and DoD HRPO approval

Establish direct apoptosis effects on myeloid cells

***Specific Aim 1* Major Task 2 – Gene expression change**

Establishing gene expression: myeloid and lymphoid cells after Rb modulation

***Specific Aim 2* Major Task 1-3 – in vivo AP-3-84 therapy**

Local IACUC and DoD ACURO approval

First experiment – determine efficacy of AP-3-84 treatment in achieving tissue and tumor myeloid cell depletion

Goals/Milestones CY20

Completion of Aim 1 Task 1, Aim 2 Task , and Manuscript Publication

Comments/Challenges/Issues/Concerns

- Major Task 1 delay, COVID-19 pandemic impact on timeline to completion.

Budget Expenditure to Date

Projected Expenditure: $221,255 (Direct and Indirect)

Actual Expenditure: $152,451 (Direct and Indirect)