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14. ABSTRACT

Our studies have led to the establishment of a variant of the ID8 ovarian tumor model (ID8-VEGF) that has allowed us to develop important new insight into why subcutaneously implanted ID8 tumors regress over time. To this end, our new data is consistent with an early infiltration of activated CD8+ T-cells that contribute to controlling tumor growth, as depleting CD8+ T-cells slowed spontaneous regression and enhanced tumor size. Our data suggest for the first time that ID8 and SKOV-3 tumor cells can generate the low molecular weight RGDKGE collagen fragment themselves. These novel findings suggest the presence of an autocrine signaling mechanism that may regulate YAP activity in these tumor cells. Collectively, these observations provide evidence for a role for a hippo-dependent signaling mechanism by which the XL313 collagen fragment regulates ovarian tumor growth. Taken together, our data provides new mechanistic insight that may help optimize the use of Mab XL313 to treat ovarian cancer.

15. SUBJECT TERMS
Tumor Associated Macrophages Lymphocyte Activation Gene Programmed Cell Death Ligand 1. Extracellular Matrix Immune Checkpoint Inhibitors Integrins Ovarian Cancer T-Cells Collagen Monoclonal Antibodies

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1). Introduction:

Ovarian cancer continues to represent a major health problem for women in the United States and other countries. In fact, this disease continues to be a leading cause of cancer death among women with gynecologic malignancies. While progress continues to be made in developing new treatment strategies for some types of cancer, these clinical approaches have had only minimal if any impact on ovarian cancer. Thus, uncovering novel mechanisms that limit the efficacy of immunotherapy in ovarian cancer may lead to more effective treatment paradigms. Previously, we made the discovery that a highly conserved RGDKGE containing collagen fragment that binds to integrin receptor alpha-V beta-3, was generated and secreted by a subset of macrophages. A soluble form of this RGDKGE collagen fragment potently induced angiogenesis and inflammation in vivo. An antibody (Mab XL313) that selectively binds the RGDKGE collagen fragment, but not other RGD containing collagen fragments, nor to intact triple helical collagen, blocks binding of this collagen fragment to integrin alpha-V beta-3 expressing cells and inhibited angiogenesis and inflammation in vivo. Our new data now suggest that the RGDKGE collagen fragment may be generated within human ovarian tumors. In addition, cellular interactions with this endogenously generated collagen fragment may control expression of immune checkpoint molecules including LAG-3 and PD-L1. The current proposal was designed to test the central hypothesis that targeting the soluble RGDKGE collagen fragment may re-activate immune control of ovarian cancer and enhance the efficacy of immune checkpoint inhibitors by selectively disrupting a novel integrin-dependent signaling cascade.

2). Key Words:

1). Tumor Associated Macrophages
2). Yes-Associated Protein
3). Programmed Cell Death Ligand 1.
4). Extracellular Matrix
5). LAG-3
6). Integrins
7). Ovarian Cancer
8). LATS1/2
9). Collagen
10). Monoclonal Antibodies

3). Accomplishments:

Summary of major goals of project:

A). As outlined in aim-1, tasks 1 and 2, we have proposed to test the effects of blocking cellular interactions with the soluble RGDKGE collagen fragment on ovarian tumor growth. In addition, we will determine whether this novel treatment strategy selectively enhances the therapeutic response of ovarian cancer to immune checkpoint inhibitors.

B). As outlined in aim-2, tasks 3 through 5, we have proposed to determine how the soluble RGDKGE collagen fragment stimulates immunosuppression by activating a unique integrin signaling pathway in T-cells.

Specific Accomplishments for reporting period as they relate to the major goals described above.

We have made significant progress towards the overall goals of our project during the second funding period (April 2018 through April 2019). Due to significant and unexpected delays with the murine ID8 ovarian cancer model, several studies are still on going and a no-cost extension was granted for an additional 12-month period. In this regard, we provide below a detailed summary of our current research accomplishments as they pertain to the tasks outlined in the statement of work. Specifically, we have made substantial progress on both aims 1 and 2 and their associated tasks.

Aim 1 Tasks.

Establishment and characterization of a variant of the murine ID8 ovarian tumor model. Studies documented in our previous report surprisingly showed that while murine ID8 ovarian tumor cells injected subcutaneously into C57BL/6 mice did form tumors, their growth was quite slow and these tumors began to regress over time without treatment. These unexpected findings resulted in significant delays in carrying out several of our planned experiments. Given these unexpected issues we sought to establish a second ID8 ovarian tumor model by using murine ID8 tumor cells that overexpress the angiogenic growth factor VEGF (ID8-VEGF). As shown in figure 1A, injection of $10x10^6$ ID8-VEGF cells subcutaneously into C57BL/6 mice formed well defined growing ID8-VEGF tumors. Importantly, while these ID8-VEGF tumors also regressed over time beginning at approximately 5 days following tumor cell injection, the kinetics of the regression was such that this model could be used to carry out additional experiments. In similar studies, ID8-VEGF cells were injected I.P. in order to establish an intraperitoneal ascites tumor formation model. Beginning at approximately 4 weeks after I.P injection of ID8-VEGF cells, mice started to exhibit signs of ascites fluid formation and by 13-weeks mice clearly had formed ascites fluid as indicated by dramatically enlarged abdomens (Fig 1B). To confirm the formation of I.P tumors, mice were sacrificed and the abdominal cavity was dissected. As shown in figure 1C, numerous well-defined ID8-VEGF intraperitoneal tumors (black arrows) were observed throughout the abdominal cavity. These tumors were dissected and analyzed morphologically by H&E staining (figure 1D).

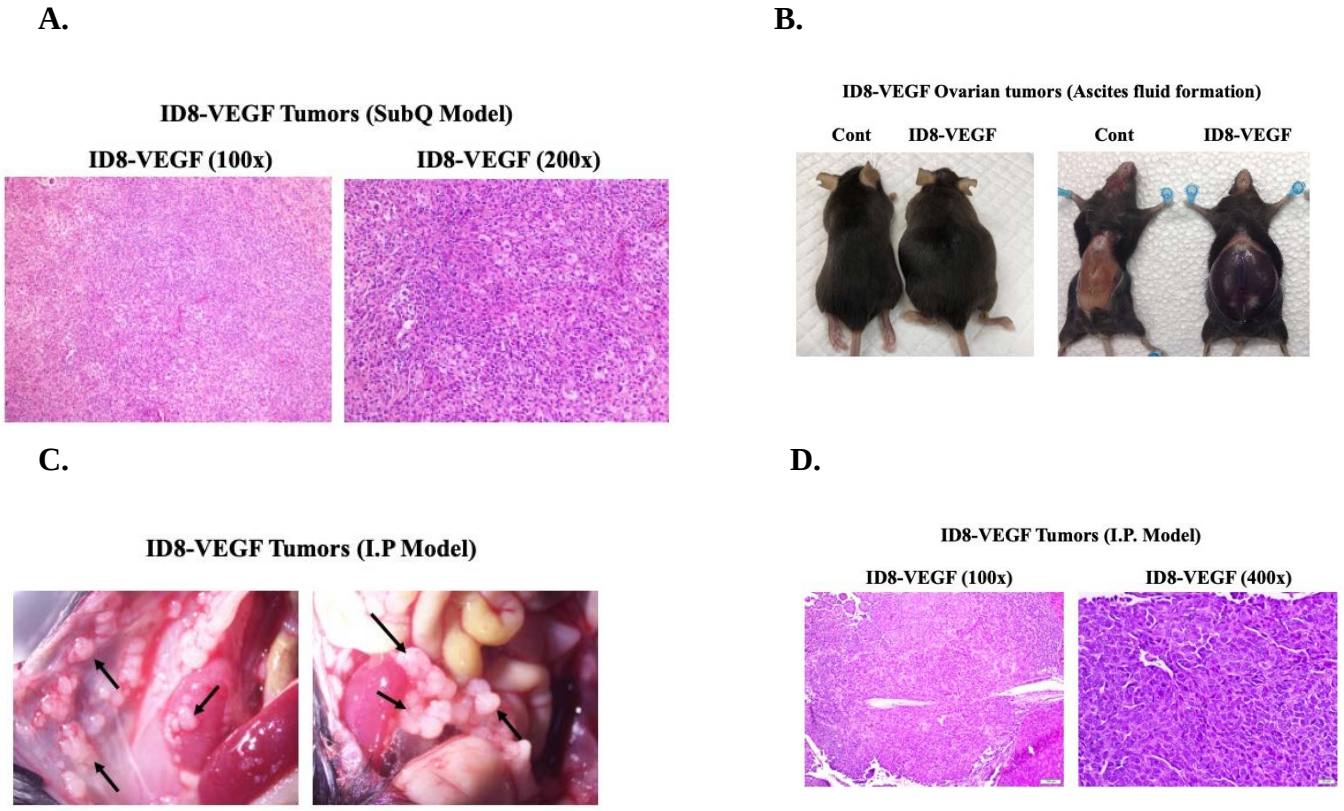


Figure 1. Characterization of a variant of the murine ID8 ovarian tumor model in C57BL/6 mice. Murine ID8-VEGF ovarian carcinoma cells ($10x10^6$/mouse) were injected subcutaneously (A) or intraperitoneally (B-D) in C57BL/6 mice. A). Representative examples of subcutaneous ID8-VEGF ovarian tumors stained by H&E on day 42. Photos taken at 100X and 200X. B). Representative examples of control non-injected or I.P injected mice with ID8-VEGF ovarian tumors (13 weeks). C). Representative examples of some of the I.P ID8-VEGF ovarian tumors (Arrows). Photos taken at 20X. D). Representative examples of ID8-VEGF ovarian tumors stained by H&E. Photos taken at a magnification of 100X and 400X.

Adaptive immune cells contribute to early regression of ID8-VEGF ovarian tumors in vivo. Our previous studies have documented that early spontaneous regression of ID8 ovarian tumors growing in C57BL/6 mice were associated with differential infiltration of immune cells including CD4+ and CD8+ T-

cell subsets. To examine whether the infiltration of these immune cells played a functional role in contributing to regression of ID8-VEGF tumors, we carried out an immune depletion study. Briefly, mice were pre-treated with non-specific control antibody or either anti-CD4 or anti-CD8 antibodies prior to injection with ID8-VEGF cells. To maintain immune depletion, mice were treated every 2 days with antibodies and tumor growth was monitored over a 21-day time course. At the end of the treatment period mice were sacrificed and single cell suspensions of spleen tissues were examined by flow cytometry to confirm immune depletion. As shown in figure 2A and B, anti-CD4 and anti-CD8 antibodies significantly depleted their corresponding immune cell populations in these mice, while control antibodies had no effect. Importantly, immune depleting CD8 positive T- cells slowed the regression of ID8-VEGF tumors, and by 21 days of growth the tumors from CD8 T-cell depleted mice were significantly larger than those tumors in controls depleted mice (Figure 2C). These data indicate that CD8+ T-cells play a functional role in the early regression of ID8-VEGF ovarian tumors in this model.

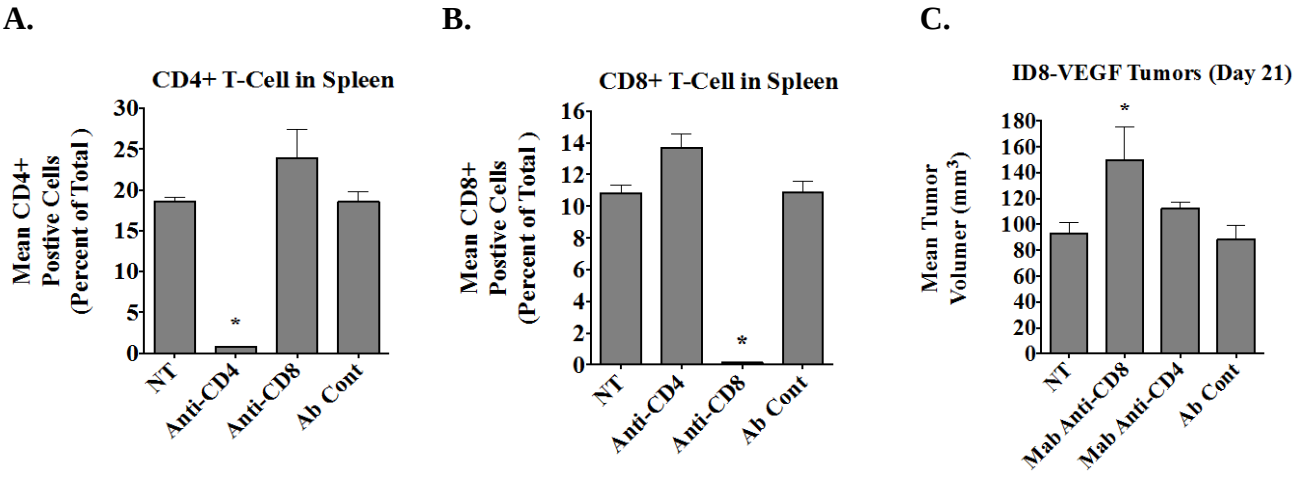


Figure 2. Adaptive immune cells contribute to early regression of ID8-VEGF ovarian tumors in vivo. C57BL/6 mice (N=4 per group) were pre-treated (i.p.) with anti-CD4, anti-CD8 or non-specific control antibody (Ab Cont) 24 hours prior to injection of tumor cells. Following injection of tumor cells, mice were further treated 3 x per week with the indicated antibodies for 21 days. Tumor size was monitored by caliper measurements. On day 21 mice were sacrificed and single cell suspensions prepared from spleens and the relative levels of CD4+ and CD8+ cells quantified by flow cytometery. A). Mean relative levels of CD4+ T-cells from immune depleted mice. B). Mean relative levels of CD8+ T-cells from immune depleted mice. C). Mean tumor size from immune depleted mice on day 21. Data bars indicate mean $\pm$ SE.

Mab XL313 inhibits ID8-VEGF ovarian tumors in vivo. Given our new studies, and the fact that we have now established the new ID8-VEGF ovarian tumor model, we sought to determine the effects of the anti-RGDKGE containing collagen fragment Mab XL313 on tumor growth in vivo. First, we sought to confirm that the ID8-VEGF tumor cells expressed the beta-3 integrin receptor that mediates interactions with the RGDKGE containing collagen fragment. As shown in figure 3A, beta-3 integrin could be readily detected in whole cell lysates of ID8-VEGF ovarian tumor cells as indicated by western blot. Next, we examined whether Mab XL313 directed to the RGDKGE collagen fragment could specifically block the ability of this collagen fragment to bind to ID8-VEGF tumor cells. To facilitate these studies, ID8-VEGF cells were allowed to bind to the RGDKGE containing collagen fragment in the presence of either a non-specific control antibody or Mab XL313. As shown in figure 3B, Mab XL13 significantly inhibited the binding of the RGDKGE containing collagen fragment to ID8-VEGF cells by over 90% as compared to non-specific control antibody. To confirm that the ID8-VEGF tumors growing in mice generate the RGDKGE-containing collagen fragment, we next examined the expression of the RGDKGE containing collagen fragment in ID8-VEGF tumors using Mab XL313. As shown in figure 3C, immunofluorescence staining analysis with Mab XL313 indicated clear expression of the RGDKGE containing collagen fragment (Red). Finally, we examined the effects of Mab XL313 on the growth of ID8-VEGF tumors in vivo. Briefly, mice were injected subcutaneously with ID8-VEGF ($10x10^6$) cells and tumors were allowed to form for either 5 days (Fig 3D) or 14 days (Fig 3E) prior to treatment (arrows). Next, mice were treated with the indicated antibodies

(100ug/injection) 2x per week. Treatment of mice with Mab XL313 significantly inhibited the growth of ID8-VEGF ovarian tumors by over 50% as compared to control antibody. Taken together, these studies indicate for the first time that selective targeting the RGDKGE containing collagen fragment can potently inhibit ID8-VEGF ovarian tumor growth in vivo.

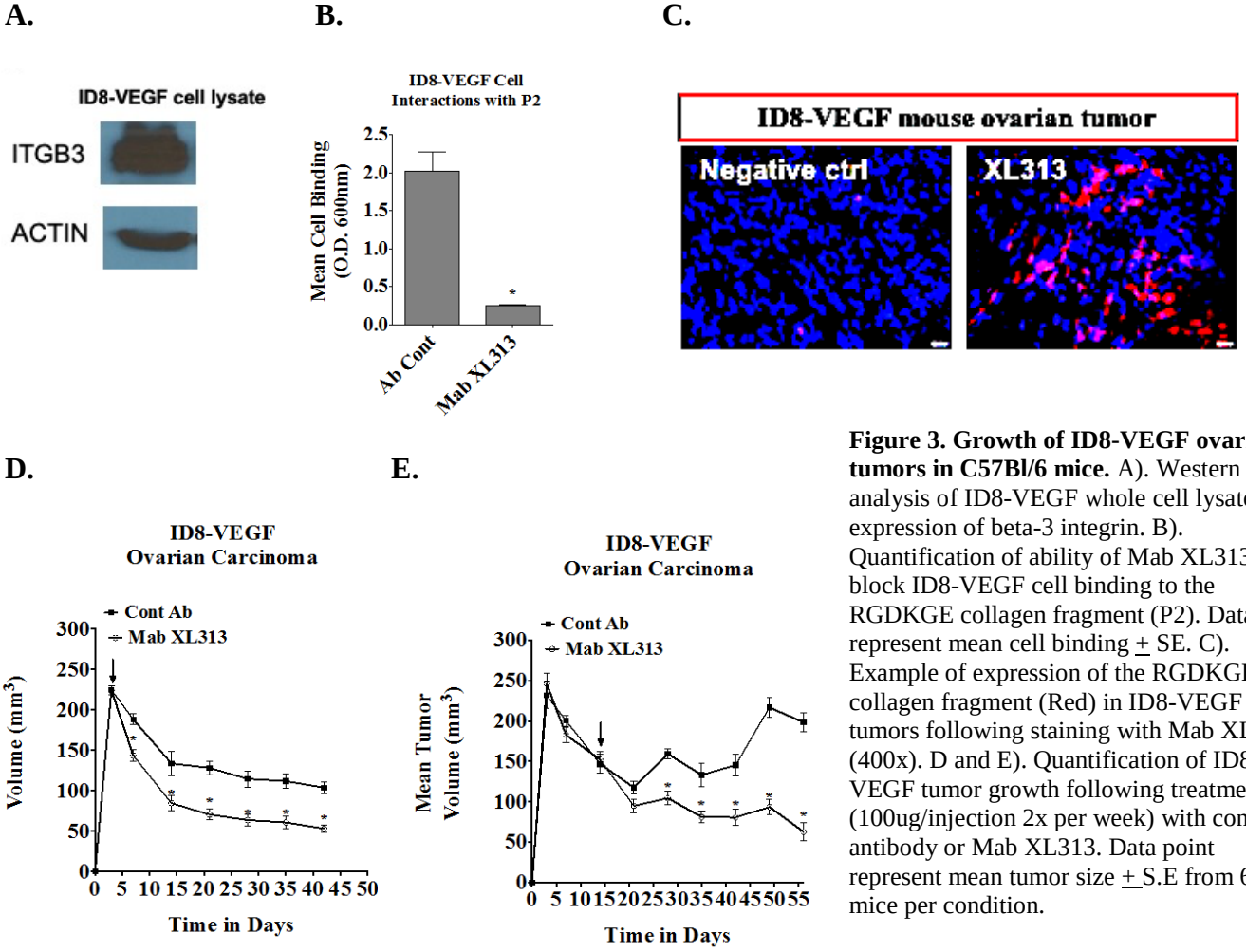
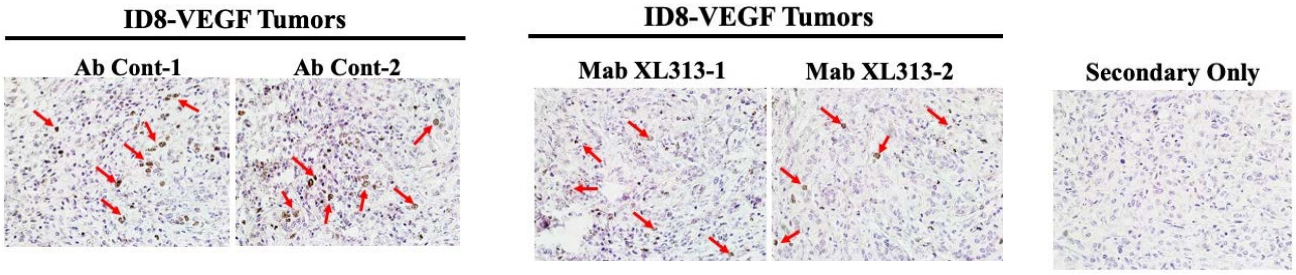


Figure 3. Growth of ID8-VEGF ovarian tumors in C57Bl/6 mice. A). Western blot analysis of ID8-VEGF whole cell lysate for expression of beta-3 integrin. B). Quantification of ability of Mab XL313 to block ID8-VEGF cell binding to the RGDKGE collagen fragment (P2). Data bars represent mean cell binding $\pm$ SE. C). Example of expression of the RGDKGE collagen fragment (Red) in ID8-VEGF tumors following staining with Mab XL313 (400x). D and E). Quantification of ID8-VEGF tumor growth following treatment (100ug/injection 2x per week) with control antibody or Mab XL313. Data point represent mean tumor size $\pm$ S.E from 6 to 8 mice per condition.

Alterations in proliferation and apoptosis markers in ID8-VEGF ovarian tumors in vivo. Given our studies indicating that targeting of the RGDKGE collagen fragment significantly inhibited ID8-VEGF tumor growth, we began to establish the experimental conditions needed to examine potential mechanisms by which Mab XL313 may inhibit tumor growth. In this regard, we first carried out a preliminary study to establish the working conditions to examine the expression of both proliferation (Ki67) and apoptosis (Caspase 3) markers in the ovarian tumors. Briefly, tumor sections from mice treated with control non-specific antibody or Mab XL313 were stained by immunohistochemistry for expression of Ki67 to help mark actively proliferating cells, and activated caspase 3 to help mark cells starting to undergo apoptosis. As shown in figure 4A, control treated ID8-VEGF tumors exhibited wide spread Ki67 staining (red arrows) indicating actively proliferating cells within these tumors. Interestingly, tumors from mice treated with Mab XL313 showed variable staining for Ki67 with a trend toward reduced levels. In similar studies, tumor sections from each group were analyzed for expression of Caspase-3. As shown in figure 4B, tumors from mice treated with non-specific control antibodies exhibited only a few scattered cells per field that stained positive for Caspase-3, consistent with relatively limited levels of apoptosis. While the relative levels of caspase-3 positive cells in tumors from mice treated with Mab XL313 were also limited and variable, a trend towards enhanced expression of caspase-3 was noted. Taken together, these studies suggest that a more in-depth analysis and quantification of the relative levels of proliferation and apoptosis within these treated tumors is warranted.

A.



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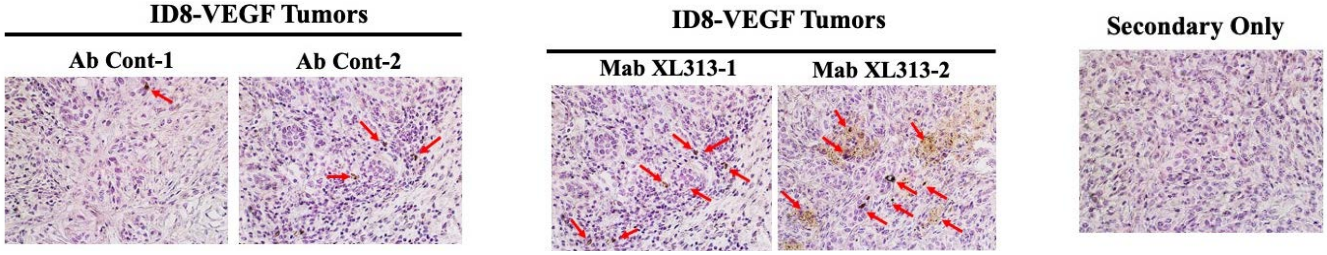


Figure 4. Detection of cellular proliferation and apoptosis in ID8-VEGF ovarian tumors in C57BL/6 mice. Murine ID8-VEGF ovarian tumors from mice treated with control antibody or Mab XL313 (N= 3 per group) were analyzed by immune-histochemistry for expression of the proliferation marker (Ki67) or apoptosis marker (Caspase-3). A). Examples of ID8-VEGF tumors from mice treated with control antibody (left panel) or Mab XL313 (right panel) stained for proliferation marker Ki67. B). Examples of ID8-VEGF tumors from mice treated with control antibody (left panel) or Mab XL313 (right panel) stained for apoptosis marker (Caspase-3). Photos taken at a magnification of 200X.

Aim-2 Tasks

Effects of anti-XL313 antibody on the levels of LAG-3 in bone marrow of mice with ID8-VEGF tumors. Our previous studies suggested that blocking immune cell interactions with the RGDKGE containing collagen fragment may alter the relative levels of the immune checkpoint molecule LAG-3. Therefore in an initial experiment, we sought to examine the levels of LAG-3 expressing cells in the bone marrow of mice with ID8-VEGF tumors following treatment with anti-XL313 antibody directed to the RGDKGE collagen fragment. Briefly, mice were injected with ID8-VEGF tumor cells as described above and tumors were allowed to grow for 5 days. The mice were next treated 2x per week with either control non-specific antibody or Mab XL313 as described above. At the end of the experiment (day 42) single cell suspensions were prepared from the bone marrow from the treated mice and analyzed for the relative levels of LAG-3 positive cells by flow cytometry. As shown in figure 5, significantly reduced levels of LAG-3 expressing cells were observed in the bone marrow of ID8-VEGF tumor bearing mice treated with Mab XL313 as compared to control antibody. These data are consistent with the possibility that blocking immune cell interactions with the RGDKGE collagen fragment may reduce the levels of LAG-3 in vivo.

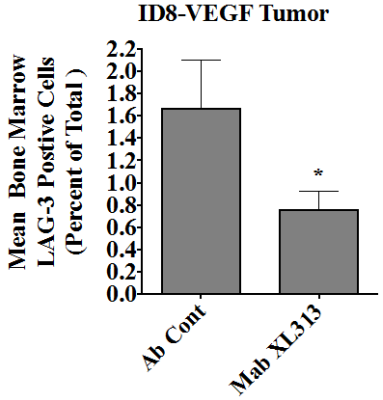


Figure 5. Effects of anti-XL313 Mab on the levels of LAG-3 in bone marrow of mice with ID8-VEGF tumors. Mice with ID8-VEGF ovarian tumors were treated (100ug/injection) 2x per week for 42 days with either non-specific control antibody (Ab Cont) or Mab XL313. Single cell suspensions of bone marrow cells isolated from each group was prepared and analyzed by flow cytometery. Data bars represent the mean relative percent of LAG-3 positive cells per group ± SE.

Effects of anti-CTLA-4 antibody on ID8-VEGF ovarian tumors in vivo. Given our studies suggesting that the murine immune system may impact ID8 tumor growth in vivo, we began to establish the working condition to assess the effects of blocking the immune checkpoint molecule CTLA-4 on ID8-VEGF tumor growth in vivo. Briefly, in an initial preliminary study we injected ID8-VEGF tumor cells I.P in C57BL/6 mice. Following a 4-week incubation period, mice were treated (100ug/mouse) 2x per week I.P with either non-specific control antibody or anti-CTLA-4 antibody. At the end of the 13-week treatment period, mice were sacrificed and intraperitoneal tumors dissected. As shown in figure 6A, as expected, multiple scattered ID8-VEGF tumors were readily detected within the mice (N=5 to 6 per condition). Quantification of the mean total tumor burden (wet weight) per mouse showed no significant change between experimental conditions. These data suggest that under these conditions, targeting the CTLA-4 immune checkpoint failed to impact ID8-VEGF tumor growth. Given these findings we are now in the process of testing anti-CTLA-4 antagonists along with Mab XL313 using the ID8-VEGF model.

A. **B.**

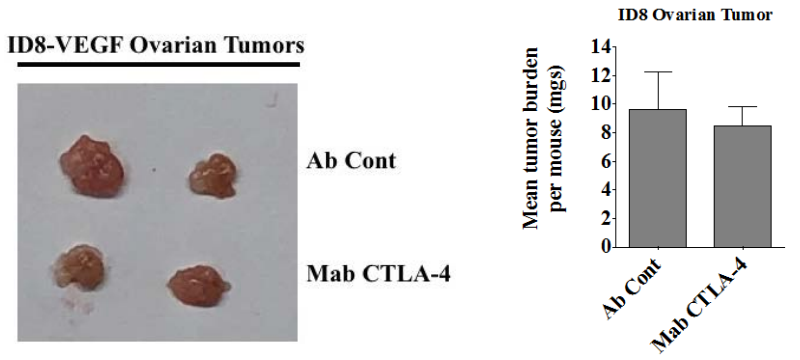


Figure 6. Effects of anti-CTLA-4 antibody on ID8-VEGF ovarian tumors in vivo. ID8-VEGF cells ($10x10^6$/mouse) were injected i.p. in C57BL/6 mice. Tumors were allowed to grow for 4-weeks. Mice were next treated 2x per week with non-specific control antibody or Mab XL313 (100ug/injection) for 13 weeks. A). Examples of intraperitoneal ID8-VEGF tumors from each condition. B). Quantification of mean total tumor burden per experimental condition $\pm$ S.E.

Aim-2 Tasks

Effects of ID8-VEGF cell binding to the RGDKGE collagen fragment P2 on YAP Activity. Previous studies suggest that the Yes Associated Protein (YAP) may play a role in regulating PD-L1 expression. In this regard, our previous studies suggest that endothelial cell interactions with the RGDKGE collagen fragment regulate the localization and activity of YAP. To further study possible mechanisms by which the XL313 antibody may impact ID8-VEGF tumor growth and PD-L1 expression, we examined the effects of ID8-VEGF cell interaction with the RGDKGE collagen fragment P2 on YAP activity by examining the relative levels of phosphorylated YAP. The ability of YAP to accumulate in the nucleus can be controlled in part by a Hippo-dependent phosphorylation event involving YAP phosphorylation on Serine 127, which restricts nuclear YAP accumulation. In this regard, we examined YAP phosphorylation following ID8-VEGF binding to the RGDKGE collagen fragment P2. As shown in figure 7A, reduced levels of phosphorylated YAP were detected in ID8-VEGF cells binding to the RGDKGE collagen fragment as compared to the control. Given these results, we next examined the relative levels of active nuclear YAP following ID8-VEGF binding to bind to RGDKGE collagen fragment P2 in the presence of Mab XL313 or control antibody. Consistent with the possibility that the RGDKGE collagen fragment P2 controls YAP activity, reduced levels of nuclear YAP were detected in ID8-VEGF cells following blocking binding the RGDKGE collagen fragment P2 with Mab XL313. Taken together, these data suggest for the first time that direct interactions of ID8-VEGF cells with the RGDKGE collagen fragment P2 controls YAP activation.

A. **B.**

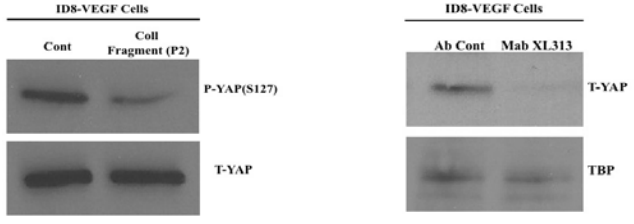


Figure 7. The RGDKGE containing collagen fragment P2 regulates YAP activity in ID8-VEGF cells. ID8-VEGF cells were allowed to bind the RGDKGE containing collagen fragment P2 for 1 hour in the presence of Mab XL313 or control non-specific antibody. Cytoplasmic and nuclear fractions were prepared. A). Western blot analysis of cytoplasmic fraction for phosphorylated YAP (P-YAP-S127) or total YAP (T-YAP). B). Western blot analysis of nuclear fraction for total YAP (T-YAP) or control TBP.

Effects of Mab XL313 on LATS1/2 phosphorylation in ID8-VEGF cells binding to the RGDKGE collagen fragment P2. Give our preliminary findings that suggest that ID8-VEGF cell binding to the RGDKGE collagen fragment P2 may regulate YAP phosphorylation, activation and nuclear accumulation, we sought to determine whether the ability of the RGDKGE collagen fragment to regulate YAP activity was associated with changes in hippo signaling given that the hippo effector kinase LATS1/2 is known to regulate phosphorylation of YAP on serine 127. To facilitate these studies we prepared whole cell lysates from ID8-VEGF cells attached to the RGDKGE collagen fragment P2 in the presence of Mab XL313 or the control antibody, and these lysates were analyzed by western blot for phosphorylated LAT1/2. As shown in figure 8, blocking ID8-VEGF binding to the RGDKGE collagen fragment P2 with Mab XL313 caused enhanced levels of phosphorylated LAT1/2 as compared to non-specific control antibody. These findings are consistent with the possibility that blocking the RGDKGE collagen fragment binding to ID8-VEGF cells may alter hippo signaling.

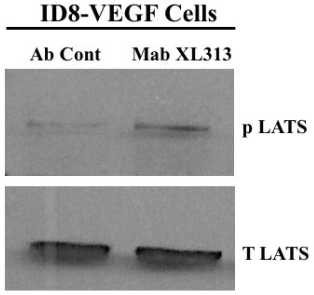
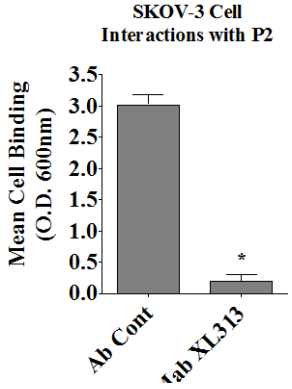


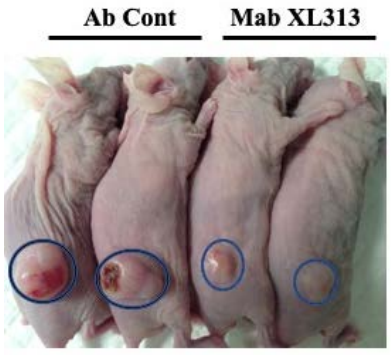
Figure 8. Effects of Mab XL313 on LATS1/2 phosphorylation in ID8-VEGF cells binding to the RGDKGE collagen fragment P2. ID8-VEGF cells were allowed to bind the RGDKGE containing collagen fragment P2 for 1 hour in the presence of Mab XL313 or control non-specific antibody and whole cell lysates were prepared. Western blot analysis for phosphorylated LATS1/2 or total LATS1/2.

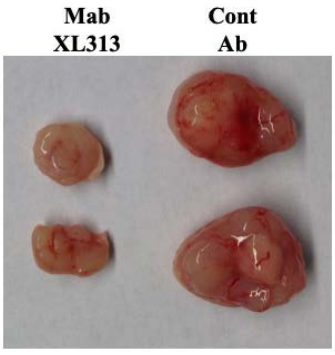
Aim-1 Tasks

Effects of Mab XL313 on human SKOV-3 ovarian tumor growth in vivo. We next examined the effects of Mab XL313 directed to the RGDKGE containing collagen fragment on human SKOV-3 tumor growth. First, we sought to examine whether Mab XL313 directed to the RGDKGE collagen fragment could block the ability of this collagen fragment to bind to SKOV-3 tumor cells. To facilitate these studies, SKOV-3 cells were allowed to bind to the RGDKGE containing collagen fragment in the presence of either a non-specific control antibody or Mab XL313. As shown in figure 9A, Mab XL313 significantly inhibited the binding of the RGDKGE containing collagen fragment to SKOV-3 cells by over 90% as compared to the control antibody. Given our previous studies indicating that SKOV-3 tumors growing in nude mice expressed the RGDKGE collagen fragment, we tested the effects of Mab XL313 on tumor growth in vivo. Briefly, mice were injected subcutaneously with SKOV-3 cells, and tumors were allowed to form for 3 days prior to treatment. Next, mice were treated 3x per week for 35 days. As shown in figures 9B-D, treatment of mice with Mab XL313 significantly ($P<0.05$) inhibited the growth of SKOV-3 ovarian tumors by approximately 50% as compared to the control antibody. Taken together, these studies indicate that Mab XL313 not only inhibits murine ID8-VEGF tumors, but also potently inhibits human SKOV-3 tumor growth as well.

A. **B.** **C.**







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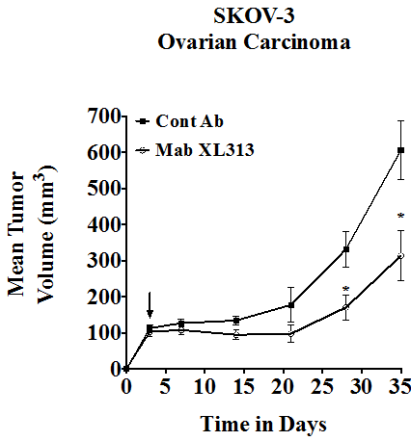

Figure 9. Effects of Mab XL313 on human SKOV-3 ovarian tumor growth in vivo. A). Human SKOV-3 cells were allowed to bind to the RGDKGE containing collagen fragment (P2) in the presence of non-specific control antibody (Ab Cont) or Mab XL313. Data bars indicate mean SKOV-3 cell binding to the RGDKGE containing collagen fragment (P2) under each condition ± S.E. B). Human SKOV-3 ovarian carcinoma cells ($4x10^6$/mouse) were injected subcutaneously in nude mice. SKOV-3 ovarian tumors were allowed to grow for 3 days then mice were treated 3x per week with 100ug per injection. Examples of tumors from each condition (blue circles). C). Examples of dissected tumors from each condition. D). Quantification of mean tumor size ± S.E from each experimental condition.

Altered levels of tumor blood vessels in SKOV-3 tumors following treatment with Mab XL313. Our current studies indicating that selective targeting of the RGDKGE collagen fragment significantly inhibited both ID8-VEGF and SKOV3 tumor growth in vivo, and preliminary evidence, suggest that selectively blocking cellular interactions with the RGDKGE containing collagen fragment may alter proliferation and or apoptosis in vivo. In addition, we sought to examine what effect targeting the RGDKGE containing collagen fragment might have on tumor angiogenesis. In this regard, our previous studies suggested that Mab XL313 could inhibit purified growth factor-induced angiogenesis using the chick CAM model. Therefore, we began to establish the working conditions to examine the effects of Mab XL313 on tumor angiogenesis. To facilitate these studies, we carried out an initial preliminary experiment and stained frozen sections of SKOV-3 ovarian tumors with anti-CD31. As shown in figure 10, tumors from mice treated with non-specific control antibody exhibited high levels of CD31 positive tumor vessels. Interestingly, while the relative levels of CD31-positive vessels in tumors from mice treated with Mab XL313 were variable, a trend towards reduced angiogenesis was noted. Taken together, these studies suggest that a more in-depth analysis and quantification of the relative levels of angiogenesis within these treated tumors is warranted and is currently underway.

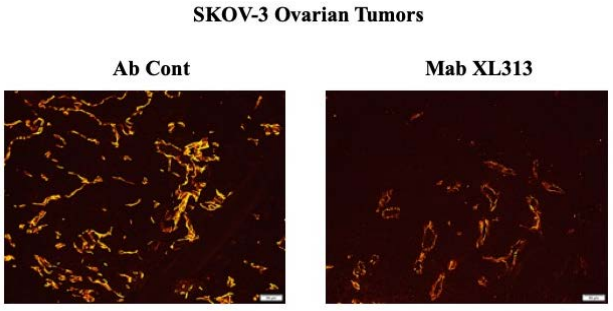

Figure 10. Altered levels of tumor blood vessels in SKOV-3 tumors following treatment with Mab XL313. Frozen sections of human SKOV-3 ovarian tumors from non-specific control (Ab Cont) or Mab XL313 treated mice from each experimental condition were analyzed by staining the tissues with the endothelial cell marker anti-CD-31 antibody (Red). Examples of tumor vessels from each experimental condition. Photos taken at a magnification of 200X.

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? Now that we have established a new variant of the ID8 tumor model (ID8-VEGF) we plan to use this model to finish the studies outlined in our proposal. Specifically, we plan to finish examining the effects of Mab XL313 alone and in combination with CTLA-4 on tumor growth in vivo. In addition, we plan to finish our studies to assess the potential cellular and molecular mechanisms by which Mab XL313 inhibits ovarian tumor growth, including quantifying proliferation, angiogenesis and apoptosis within the treated tumors. We plan to finish the remaining studies to examine the roles of mir-34a, c-Myc and P53 in regulating the ability of the RGDKGE collagen fragment to control PD-L1. Finally, we plan to use these new data as part of a new manuscript detailing the roles of the RGDKGE collagen fragment in regulating ovarian tumor growth. When taken together, if successful these studies should complete the overall goals of our project.

4). Impact.

A). What was the impact on the development of the principal disciplines of the project? Our new data detailed in the current report provides critical new immunological insight into why subcutaneously implanted ID8 ovarian tumors may regress over time. Specifically, our new data are consistent with an early infiltration of activated CD8+ T-cells that contribute to inhibiting tumor growth, as specifically depleting these T-cells resulted in slowing spontaneous regression and enhanced the overall size of these tumors as compared to the control. These studies may assist in the optimization and use of the ID8 model to study cellular and molecular mechanisms of ovarian tumor biology. In addition, our exciting new data also suggest, for the first time, that ID8 and SKOV-3 tumor cells can generate the RGDKGE collagen fragment and, importantly, can directly bind this collagen fragment, which was shown for the first time to regulate YAP activity in these tumor cells. These novel findings provide evidence for a possible mechanism by which Mab XL313 may inhibit tumor growth. Moreover, our new data also provide the first evidence that a unique collagen fragment may regulate YAP activity by a hippo-dependent mechanism. Taken together, our exciting new data provide new mechanistic insight that may help optimize the use of Mab XL313 to treat ovarian cancers.

B).What was the impact on other Disciplines? Nothing to report.

C). What was the impact on technology transfer? Nothing to report.

D). What was the impact on society beyond science and technology? Nothing to report

5. Changes or Problems. During the current reporting period, we made the surprising observations that while murine ID8 ovarian tumor cells injected subcutaneously into C57BL/6 mice did form tumors, their growth was quite slow and these tumors began to regress over time without treatment. These unexpected findings resulted in significant delay in carrying out several of our planned experiments. Given this delay, we requested a no-cost extension from the DoD which was recently approved. Moreover, these unexpected results made it difficult to carry out some of the tumor experiments using this murine cell line. In order to solve this problem, we began to establish an alternative ID8 cell line model with different growth characteristics. Specifically, we sought to establish a second ID8 ovarian tumor model by using murine ID8 tumor cells in which the cells overexpress the angiogenic growth factor VEGF (ID8-VEGF). In order to use this cell line in vivo we needed to amend our IACUC animal protocol. The amended protocol was approved by both the IACUC at Maine Medical Center as well as by the DoD. We now have established this alternative ID8-VEGF ovarian cancer model which should allow us to finish our tumor experiments outlined in the proposal.

6. Products:

A). Manuscripts: Nothing to report
B). Websites of Other Internet sites: Nothing to report.
C). Inventions, patent applications, and/or licenses: Nothing to report.
D). Other Products: Nothing to report.

7). Participants and other collaborating organizations.

Name	Peter Brooks
Project Role	Principal Investigator
Nearest Person-Month Worked	1.8 calendar months
Contribution to Project	Provides overall scientific direction, analysis, writes manuscripts, supervises Staff Scientist
Funding Support	

Name	Xianghua Han
Project Role	Staff Scientist
Nearest Person-Month Worked	6 calendar months
Contribution to Project	Performs cell biological, molecular and animal experiments
Funding Support	

Has there been a change in the active other support of the PD/PI or senior Key personnel since the last reporting period? No

What other organizations were involved as partners? None

Special Reporting requirements: N/A

Appendix: N/A