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TITLE: Immunotherapy Targeting Stromal CD5L in Ovarian Cancer

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CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center

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14. ABSTRACT

Although the FDA approved anti-angiogenesis therapies, such as bevacizumab, for treatment in upfront and relapsed ovarian cancers, development of adaptive resistance to such therapy remains a major clinical barrier. To date, only a portion of the molecular mechanisms underlying drug resistance to anti-VEGF antibody therapy like bevacizumab have been studied. The proposed work aims to investigate the therapeutic potential for a novel monoclonal antibody, rAb-anti-CD5L to overcome adaptive resistance in ovarian cancer during application of anti-VEGF drugs. We also aim to characterize the roles for CD5L and its endothelial-specific receptors in mediating development of resistance to anti-VEGF antibody therapeutics in endothelial cells, and the mechanisms of rAb-anti-CD5L in interfering with such activities. The ***short-term goal*** of this proposal is to understand mechanisms of action of rAb-anti-CD5L in targeting stromal CD5L in ovarian tumors with anti-VEGF-therapy resistance. We expect this work will lead to a new therapeutic approach for ovarian cancer patients. Overall, our proposal is highly translational and has profound implications for developing a novel, antibody-based therapy for ovarian cancer. Thus, this proposal is directly responsive to the Program Announcement for the Investigator-Initiated Research Award from DOD-OCRP, in that we will identify novel approaches for overcoming adaptive resistance to anti-VEGF therapy in ovarian cancer. Further clinical development of rAb-anti-CD5L will require formal safety studies (GLP regulations); we are well poised to carry out such work and have extensive experience in drug development. Moreover, the MoonShot Program for Ovarian Cancer at MD Anderson will provide clinical resources for us to formally test the utility of novel drugs following induction with bevacizumab-based therapy.

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

Introduction

Anti-angiogenesis therapy using the monoclonal anti-VEGF antibody bevacizumab is an efficacious treatment of advanced ovarian cancer in either the maintenance or recurrent setting – for which it has FDA approval. Unfortunately, the majority of women treated with this drug will eventually develop resistance, leading to subsequent recurrence or progression of disease. Therefore, new and effective approaches are needed to avoid or reverse adaptive resistance to bevacizumab treatment. Previous unpublished work from our laboratory identified markedly elevated levels of CD5L in tumor endothelial cells related to emergence of adaptive resistance to anti-VEGF therapy. ***Our central hypothesis is that that in addition to interfering with the known effects of CD5L on macrophages, rAb-anti-CD5L will overcome adaptive resistance to anti-VEGF therapy by targeting tumor vasculature.*** This hypothesis is based on our findings that decreasing the activity of CD5L – whether from an mRNA or protein level – leads to improved sensitivity to anti-VEGF therapy. Our team includes leading experts in ovarian cancer biology, angiogenesis pathways, developmental therapeutics, and the care of women with ovarian cancer. Furthermore, our laboratory has published multiple previous studies involving the complete derivation of cellular pathways and the phenotypic effect of pathway disruptions using *in vivo* animal models. Therefore, these factors have placed us in an excellent position to carry out the proposed study In this proposal, we will test our central hypothesis under the following aims:

Specific Aims: **#1**: **Evaluate the therapeutic efficacy of rAb-anti-CD5L in overcoming adaptive resistance to anti-VEGF therapy using cell-based and patient-derived xenograft orthotopic ovarian cancer models**; We will use our established syngeneic and orthotopic (e.g., ID8) mouse models of ovarian cancer and ovarian cancer patient-derived xenograft models. The effects of rAb-anti-CD5L will be tested in these ovarian cancer models, as well as those with adaptive resistance to AVA therapy. Renal and liver toxicities of the rAb-anti-CD5L antibody will be addressed by measuring the creatinine and liver function tests pre- and post-treatment. The stromal effects of rAb-anti-CD5L will be assessed with immunohistologic analysis in the resulting tumors.

Specific Aims: **#2**: **Determine the mechanisms by which rAb-anti-CD5L reduces resistance to anti-VEGF therapy in endothelial cells.** ***Study Design***: We will first functionally characterize the endothelial-specific, cell-membrane receptors binding to stromal CD5L and mediating the angiogenic signaling. Second, we will determine the potential factors/mechanisms for upregulating CD5L in addition to PPAR-γ, an upstream factors of CD5L promoting AVA resistance and hypoxia. Thirdly, we will also perform protein profiling using reverse phase protein arrays (RPPA) and angiogenic array to identify downstream factors for rAb-anti-CD5L treatment in endothelial cells with bevacizumab resistance. Lastly, we will investigate the mechanical effect of rAb-anti-CD5L on autophagy and angiogenic properties in endothelial cells.

Keywords

Ovarian cancer, CD5L, therapeutic antibody, adaptive resistance

Accomplishments

Aim 1: Evaluate the therapeutic efficacy of rAb-anti-CD5L in overcoming adaptive resistance to anti-VEGF therapy using cell-based and patient-derived xenograft orthotopic ovarian cancer models.

Major task 1a: Investigate the effect of rAb-anti-CD5L using syngeneic and orthotopic nude mouse models of ovarian cancer with adaptive resistant to AVA therapy.

- *Subtask 1a-1: Submit documents for local IACUC and IRB review;*

We have completed the submission of institution's IACUC approval for performing an array of animal experiments for using orthotopic, syngeneic ovarian cancer cell-based models, and high-grade ovarian cancer patient xenograft derived models for testing our rAb-anti-CD5L antibodies R35 (intraperitoneal or subcutaneous tumor injection). IACUC protocol (00001029-RN03) has been approved at MD Anderson Cancer Center at 2/11/2021.

The protocol was approved by Animal Care and Use Review Office (ACURO) at March, 2021. We have received exempt from HRPO approval or exempt from human subjects/HAS related studies on April 17, 2021.

- *Subtask 1a-2: Investigate the effect of rAb-anti-CD5L using syngeneic and orthotopic nude mouse models of ovarian cancer with adaptive resistant to AVA therapy.*

Given our preliminary data showed that AVA resistance is mediated, in part, by overexpression of CD5L, we have tested an antibody to specifically target CD5L, named as rAb-anti-CD5L. First, we selected a large panel (>350 binding hits) of anti-CD5L monoclonal antibodies through the following two methods: (1) screening single B cells isolated from CD5L antigen–immunized rabbit, and (2) panning human antibody phage display libraries. We have selected 10 antibodies for further evaluation based on *in vitro* characterization of binding affinity (Kd), CD5L mouse cross reactivity, and binding epitopes. Among them, the clone R35 was selected based on its significant effect in binding affinity from the *in vitro* surface plasmon resonance (SPR) kinetic analysis (Fig 1).

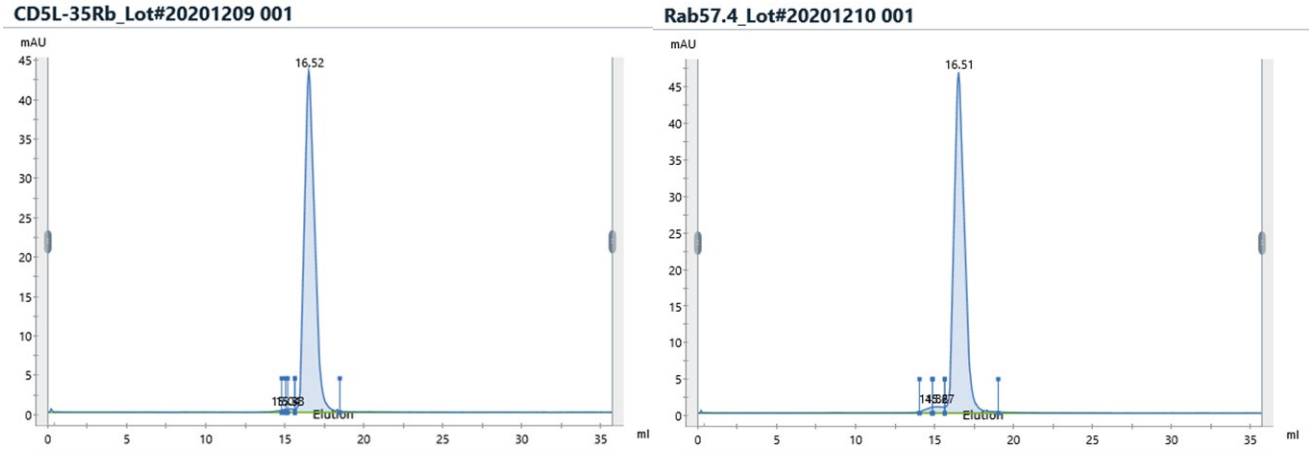
As proposed, our collaboration Drs. Ningyan Zhang and An Zhiqiang at the University of Texas Health Science Center at Houston have prepared 100mg of CD5L mAb (R-#35). The SPR K_{on}/K_{off} values and the purification of the rAb-anti-CD5L(CD5L-35Rb) and control antibody Rab57.was performed by liquid chromatography (AKTA-FPLC). (Fig 1). Sufficient amount (for both Aim#1 & Aim#2) of rAb-anti-CD5L(CD5L-35Rb) and control antibody Rab57.4 (~100mg) with high binding affinity to the ligand and high purity (>90% Eur) have been accomplished.

Figure 1. Evaluation of the purified CD5L-35Rb and control antibody Rab57.4. (A) Lot information for two antibodies; (B) Purity of two antibodies; (C) Binding activity.

A.

#	Name	Total mg	Delivery info						
			Lot #	Tubes	Conc. mg/ml	Amount mg	ml/tube	Buffer	Sterile	Endotoxin EU/mg IgG
1	CD5L-35Rb	89.05	Lot#20201209	5.00	17.81	89.05	1.0	PBS	Yes	5.1
2	Rab57.4	82.28	Lot#20201210	4.00	20.57	82.28	1.0	PBS	181217	4.0

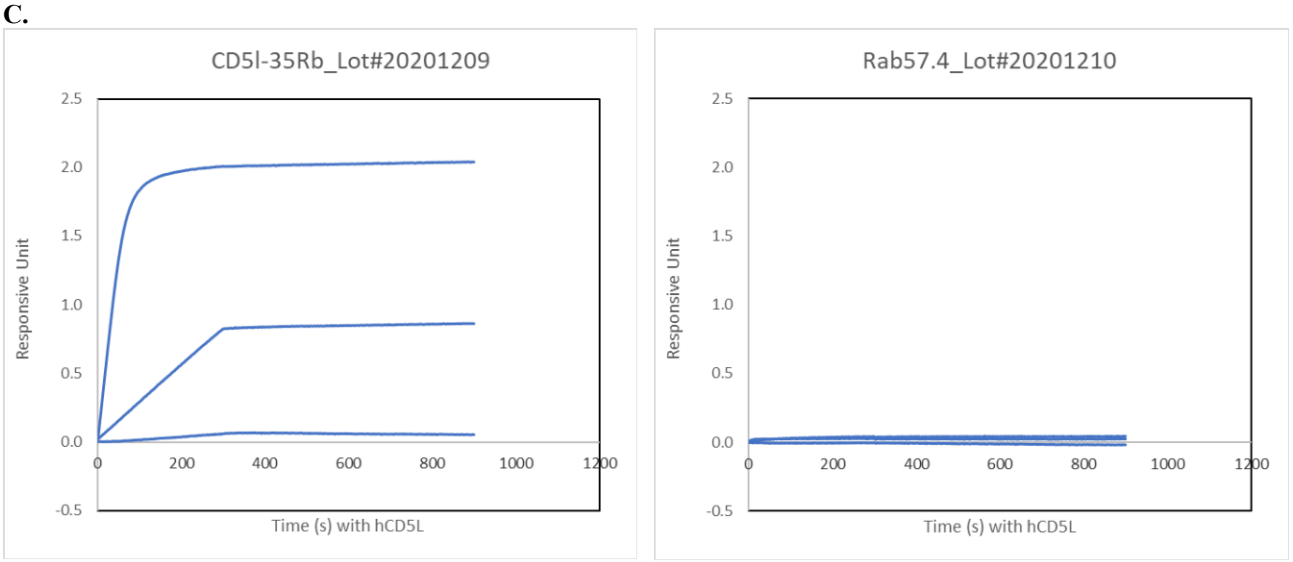
B.



Name	Batch #	Peak	Retention (ml)	Area %
CD5L-35Rb	Lot#20201209	Peak A	15.040	0.27
		Peak B	15.326	0.64
		Peak C	16.517	99.09

Name	Batch #	Peak	Retention (ml)	Area %
Rab57.4	Lot#20201210	Peak A	14.862	1.20
		Peak B	15.274	1.89
		Peak C	16.512	96.92

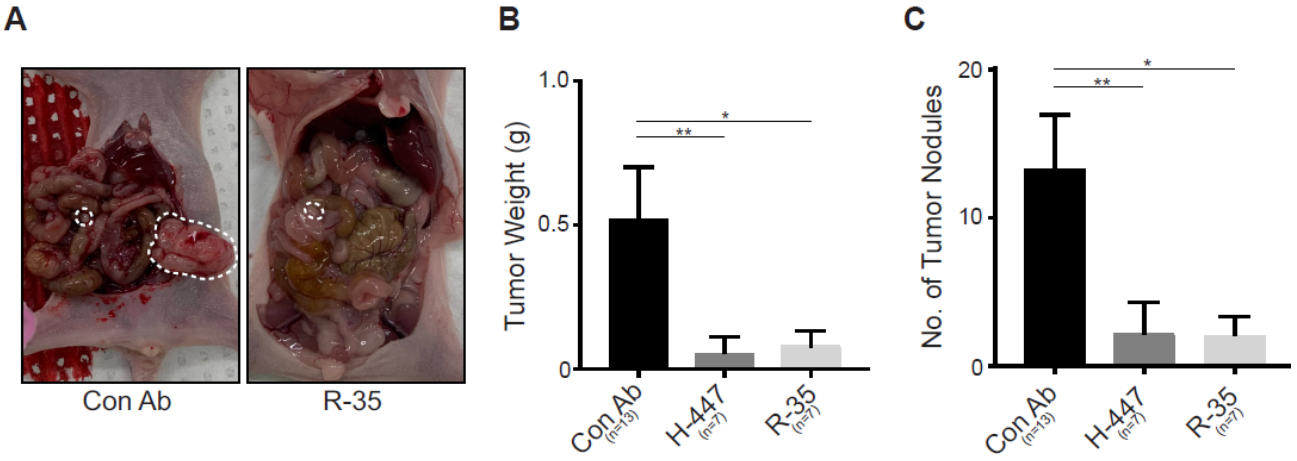
C.



KD (M)	kon(1/Ms)	kdis(1/s)	Full X^2	Full R^2
<1.0E-12	2.10E+05	<1.0E-07	1.4488	0.9992

We then tested the CD5L-35Rb and control antibody Rab57.4 antibodies *in vivo* with use of an orthotopic ovarian cancer mouse xenograft tumor model (SKOV3ip1).(Fig 2). Mice treated with the isotype control antibody had a larger tumor burden than did mice treated with an effective anti-CD5L antibody (Fig. 2A). Treatment with the two effective anti-CD5L antibodies resulted in significantly lower tumor weight and fewer tumor nodules compared with the control antibody (Fig. 2B, C). In particular, treatment with the R-35 antibody resulted in significantly fewer tumor blood vessels than did treatment with control antibody (Fig. 2D).

Figure 2. CD5L targeted antibodies rAb-anti-CD5L(CD5L-35Rb) exhibit antitumor and antiangiogenic effects.
(A) Photographs of representative mice of control antibody and anti-CD5L antibody (rAb-anti-CD5L(CD5L-35Rb) treated groups. Mice were treated intraperitoneally with either PBS or rAb-anti-CD5L(CD5L-35Rb) (10 mg/kg) starting on Day 8 after tumor injection until Day 35. **(B, C)** Tumor weight (B) and number of tumor nodules (C).



Ongoing experiments:

To establish the syngeneic and orthotopic nude mouse models of ovarian cancer with adaptive resistant to AVA therapy, we have submitted the proposal titled as "Effect of CD5L inhibition on growth and survival of ovarian/endometrial cancer models" to Genentech, Inc and requested anti-human VEGF-A antibody Avastin and the anti-human/mouse VEGF-A antibody B20 4.1.1. This MTA has been processed and approved at July 2021.

We are currently in the process to establish syngeneic and orthotopic nude mouse models of ovarian cancer with adaptive resistant to AVA therapy.

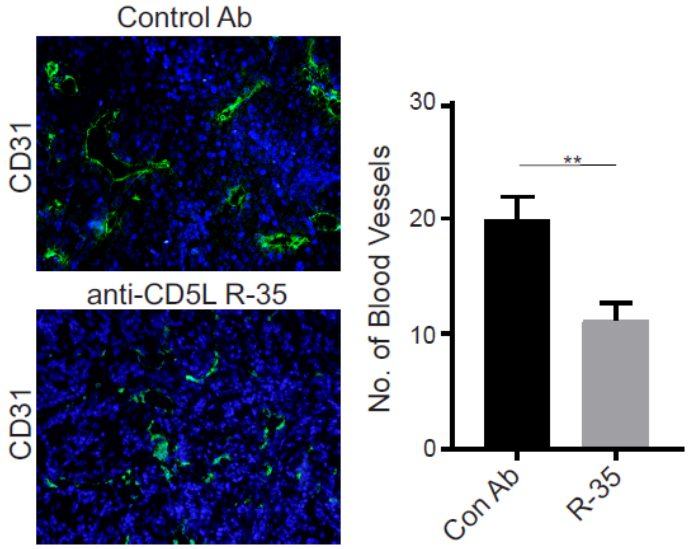
- *Subtask 1a-3: Investigate the effect of rAb-anti-CD5L in orthotopic mouse models of OVCA-432-luciferase and OVCA8-luciferase, and using athymic nude mice with adaptive resistance to B20.*

We plan to establish the orthotopic nude mouse models of ovarian cancer (OVCA-432-luciferase and OVCA8-luciferase) with adaptive resistance to B20 anti-VEGF-A antibody, in parallel with syngeneic models.

Major task 1b: Determine the stromal effects of rAb-anti-CD5L in the AVA-resistant tumors.

- Subtask 1b-1: Test the hypoxia effect in *rAb-anti-CD5L* treated orthotopic and syngeneic ovarian tumors with adaptive resistance to AVA therapy.
  We will perform this experiment at the end of *Subtask 1a-2 &3.*
- Subtask 1b-2: immunohistochemical (IHC) analysis using antibodies for proliferation (Ki67), angiogenesis represented by microvascular density (MVD) with CD31, and apoptosis (TUNEL);
  In the orthotopic SKOV3 model, we have observed that treatment with the R-35 antibody resulted in significantly fewer tumor blood vessels than did treatment with control antibody (Fig. 3).

Figure 3. Treatment of CD5L targeted antibodies rAb-anti-CD5L(CD5L-35Rb) resulted in significantly fewer tumor blood vessels than did treatment with control antibody. CD31 immunofluorescence staining of tumors from control versus anti-CD5L antibody-treated groups. For statistical analysis, five randomly selected tumors per group were stained, and five random fields per tumor were scored.



- Subtask 1b-3: Compare the expression of CD5L/CD36 axis on macrophage using F4/80 co-staining with flow cytometry;
  We will perform this experiment at the end of Subtask 1a-2 &3.
- Subtask 1b-4: Profile other immune cell populations (MDSCs, DC, T-regs, CD4/8 cells, and NK cells) by co-immunofluorescence staining and by CyTOF system in the resulting tumors.
  We will perform this experiment at the end of *Subtask 1a-2 &3.*

Major task 1c: Determine the effects of rAb-anti-CD5L in the AVA-resistant PDX models from high-grade serous ovarian cancer (HGSC) patients.

This experiment is currently under preparation.

Impact

Although a portion of the molecular pathway leading to anti-VEGF resistance has been reported by literature, our laboratory is committed to mechanistically characterize the molecular

pathways underlying the development of adaptive resistance to anti-angiogenic therapy, as well as to develop the novel therapeutic strategies for the treatment of ovarian cancer with such resistances. Our proposed experiments below will lead to have a complete understanding of all upstream and downstream components of CD5L as well as its associated receptor(s). Most importantly, our proposal plans to generate and validate the first monoclonal antibody against CD5L designed to reverse adaptive resistance to anti-VEGF therapy. The implications of this on ovarian cancer treatment are substantial and would result in a profound change in the way ovarian cancer patients are treated.

Changes/Problems
Nothing to report.

Products
Related publications/manuscripts from this grant:

Peer-reviewed Publications:

1. **Wen Y*** (*Corresponding author), Wang Y, Chelariu- Raicu A, Stur E, Liu Y, Corvigno S, Bartsch FJ, Redfern L, Zand B, Kang Y, Liu JS, Baggerly K, Sood AK "Blockade of the short-form of prolactin receptor induces FOXO3a/EIF-4EBP1–mediated cell death in uterine cancer" ***Molecular Cancer Therapeutics***, 2020 Jul 31: PMID: 32737156

Manuscripts in revision

2. **Wen Y*** (***Corresponding author), Chelariu- Raicu A, Stur W, Nick AM, Jiang D, Chen X, Lingegowda-Selanere M, Lopez-Berestein G, HungMC, Sood AK "Endothelial p130cas confers resistance to anti-angiogenesis therapy" Cell Reports- **In revision** (CELL-REPORTS-D-20-03326-R3), July 07, 2021.
3. LaFargue CJ, Amero P, Noh K, Lingegowda SM, Lu C, **Wen Y**, Pradeep S, Wan Yh, Yoo W, Bayraktar E, Dasari SK, Vathipadiekal V, Chelariu-Raicu V, Roopaimoole R, Ku ZQ, Hui D, Xiong W, Choi HJ, Ali-Fehmi R, Birrer MJ, Hu W, Zhang NY, Lopez-Berestein G, Franciscis V, An ZQ, Sood AK "Overcoming Adaptive Resistance to Anti-VEGF Therapy by Targeting CD5L" Nature Communication- **In revision** (NCOMMS-20-20029-R2), May 2021

Participants & Other Collaborating Organizations

Drs. Ningyan Zhang and An Zhiqiang at the University of Texas Health Science Center at Houston

Special Reporting Requirements
Nothing to report.

Appendices
Nothing to report.