AWARD NUMBER: W81XWH-22-1-0038

TITLE: Inhibition of RET Proto-Oncogene as Novel Immune-Based Strategy Against SCLC

PRINCIPAL INVESTIGATOR: Dinesh Ahirwar

CONTRACTING ORGANIZATION: Indian Institute of Technology Jodhpur
Rajasthan, India

REPORT DATE: MAY 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
MAY 2023	Annual	1APR2022 - 31MAR2023

4. TITLE AND SUBTITLE

Inhibition of RET Proto-Oncogene as Novel Immune-Based Strategy Against SCLC

5a. CONTRACT NUMBER
W81XWH-22-1-0038

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dinesh K. Ahirwar, PhD
Indian Institute of Technology Jodhpur

E-Mail:dineshahirwar@iitj.ac.in

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Indian Institute of Technology Jodhpur
NH 62, Karwar, Jodhpur
Rajasthan, India,

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Small cell lung cancer (SCLC) is the most aggressive subtype of lung cancer and is defined by neuroendocrine features, high rate of metastasis, and strong association with smoking. SCLC patient respond to chemotherapy at first, however, relapse quickly with acquired resistance to other chemotherapies. Even the immune checkpoint inhibitors have shown only marginal benefits in SCLC patients. Therefore, there is an utmost need to develop novel therapeutic agents against this deadly subtype of lung cancer. Screening of patient samples has identified mutation in REaranged during Transfection (RET) gene linked to SCLC pathogenesis. However, its clinical significance in SCLC has not been studied yet. Here, we are analyzing the ability of Pralsetinib, a highly potent and selective inhibitor of RET, to inhibit SCLC in pre-clinical mouse models of SCLC. RET signaling in monocytes has been shown to increase the expression of chemokine and cytokines associated with myeloid-derived suppressor cells (MDSCs). Tumors are known to recruit MDSCs to inhibit anti-tumor immune response and dampen immunotherapy response. We will also analyze the ability of Pralsetinib to activate anti-tumor immune responses and enhance the efficacy of immunotherapy against SCLC by suppressing MDSCs. Overall, these studies will help in establishing the preclinical significance of Pralsetinib against SCLC.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	5	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. **Introduction**

Among different types of lung cancer, SCLC is the most aggressive subtype with faster growth rate, late diagnosis with widespread metastasis and strong association with tobacco smoking. SCLC is one of the deadliest cancers with 5-year survival rate <5-8% and NIH-NCI has designated it as a recalcitrant disease due to its quick relapse after front-line therapy and acquired resistance to following therapies. Almost 95% SCLC patients do not contain immune cells, making them difficult to treat by immunotherapies that rely on immune cells activating. Indeed, recent clinical trial observed only marginal benefit from addition of anti-PD-L1 therapy to standard front-line chemotherapy. SCLC harbor immunosuppressive monocytes and macrophages in the TME that can suppress the recruitment and activity of anti-PD-L1 therapy induced immune T cells. Therefore, targeting immunosuppressive myeloid cells in the SCLC TME could be a promising strategy to enhance the therapeutic responses induced by anti-PD-L1 therapy against SCLC. RET has been shown to be expressed on human PBMCs, including myeloid monocytes. Treatment of PBMCs with RET ligand induces secretion of immunosuppressive molecules, including CXCL1, IL6 and IL8, which are known to suppress anti-tumor immune cells functions. Genomics analysis of SCLC patient tumors have identified mutations in RET gene activating its expression. However, the role of RET in creating immunosuppressive SCLC TME and thereby dampening anti-PD-L1 therapy responses has not been evaluated yet. We hypothesize that RET pharmacological inhibitor Pralsetinib can inhibit myeloid-derived suppressor cells (MDSCs) and can synergistically act with anti-PD-L1 therapy to enhance the anti-tumor immune responses against SCLC. Pralsetinib is an orally bioactive and FDA approved pharmacological inhibitor of RET that is being tested for different cancers in clinical trials. The overall objective of this proposal is to identify if Pralsetinib can act synergistically with anti-PD-L1 therapy to exponentially enhance the anti-tumor immune responses against SCLC by targeting MDSCs. In this proposal, we will analyze the clinical utility of Pralsetinib alone or in combination with anti-PD-L1 therapy against SCLC in pre-clinical humanized mouse model of patient-derived xenografts. We will also perform genomic and proteomic studies to identify novel signaling pathways involved in enhancing immune suppressive cells and reducing anti-tumor immune cells activity.

2. **Keywords:** Small Cell Lung Cancer, REaranged during Transfection gene, Metastasis, Pralsetinib, myeloid-derived suppressor cells,

3. **Accomplishments**

None

Completeness of each task in SOW: The research work has not been started yet due to the reasons stated above.

4. **Impact:** SCLC has been designated as a recalcitrant disease as it relapses quickly after first-line therapy with acquired resistance to multiple therapies. Hyperactive immunosuppressive mechanisms in SCLC tumors have been proposed as reason for limited response to anti-PD-L1 therapy observed in SCLC patients. Our studies defining the role of RET receptor on myeloid cells in promoting SCLC will significantly contribute to the understanding of molecular mechanisms involved in creation of immunosuppressive TME in SCLC. In addition, the clinical studies using humanized mice and PDX mouse models will establish the potential of Pralsetinib to rejuvenate anti-tumor T-cell responses activated by anti-PD-L1 therapy. These

studies will provide novel insights for future studies designing immune-based therapies for immune-inert SCLC tumors.

5. **Changes/Problems:** The proposed research work has not been started yet due to project transfer to an international location.

   This project was funded to me while I was appointed at the Ohio State University, Columbus, USA. I moved to India and requested to transfer the project from The Ohio State University, Columbus, Ohio, USA to Indian Institute of Technology Jodhpur, India in March 2022. The extensive paperwork requirement for international transfer delayed the start of project significantly.

   Although the project has been transferred to Indian Institute of Technology Jodhpur on 10th January 2023, the fund has not been released to us yet, due to unavailability of SAM.GOV unique entity identifier for our collaboration institute (All India Institute of Medical Sciences Bhopal, Madhya Pradesh, India). Therefore, the proposed work has not been started yet. We have obtained this information now and submitted it to USAMRAA office. This information has been accepted and validated at the USAMRAA office and a grant voucher has been provided to us.

   At present, we are in the process of submitting this grant voucher to WAWF for the payment. In addition, we have submitted a request for an additional 1 year no cost extension to our grant manager.

6. **Products**

   None

7. **Participants & Other Collaborating Organizations**

   Collaborator: Ashok Kumar, Associate Professor, Department of Biochemistry, All India Institute of Medical Sciences Bhopal, Madhya Pradesh, India.

8. **Special Reporting Requirements**

   None

9. **Appendices**

   None