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TITLE:	Combating Recalcitrant Cancer Types Through Rational Engineering of Antibody-Drug Conjugates
PRINCIPAL INVESTIGATOR:	Dr. James Dunleavey
CONTRACTING ORGANIZATION:	The Geneva Foundation, Tacoma, WA
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Combating Recalcitrant Cancer Types Through Rational Engineering of Antibody-Drug Conjugates

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6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development Command
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In aim 1 high-throughput genomics screening will be used to uncover genes that, when overexpressed, induce CD276 expression in cells. In aim 2, we will develop next generation CD276 ADCs with enhanced cell killing activity and an improved therapeutic index. Enhancements will be introduced to our ADC to prevent Fc receptor binding, without decreasing ADC stability. Site-directed conjugation will also be used to create ADCs that are less prone to aggregation and more stable in the circulation. In aim 3, we will create a new amanitin-based ADC for targeted killing of pancreatic cancers that are intrinsically resistant to PBD. The new ADC will be engineered site specifically with additional modifications to improve ADC stability. The specificity of our improved ADCs will be rigorously monitored by comparing activity in CD276 wildtype (WT)and knockout (KO) cell lines and CD276 WT and KO mice.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	12	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

In aim 1 high-throughput genomics screening will be used to uncover genes that, when overexpressed, induce CD276 expression in cells. In aim 2, we will develop next generation CD276 ADCs with enhanced cell killing activity and an improved therapeutic index. Enhancements will be introduced to our ADC to prevent Fc receptor binding, without decreasing ADC stability. Site-directed conjugation will also be used to create ADCs that are less prone to aggregation and more stable in the circulation. In aim 3, we will create a new amanitin-based ADC for targeted killing of pancreatic cancers that are intrinsically resistant to PBD. The new ADC will be engineered site specifically with additional modifications to improve ADC stability. The specificity of our improved ADCs will be rigorously monitored by comparing activity in CD276 wildtype (WT) and knockout (KO) cell lines and CD276 WT and KO mice.

2. KEYWORD

Oncology, Cancer, Pancreatic Cancer

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific aim 1: Unravel the mechanism(s) governing CD276 upregulation in tumors.

Specific aim 2: Improve therapeutic index through rational reengineering of CD276-PBD immunoconjugates.

Specific aim 3: Generate a novel amanitin-linked CD276 ADC for treatment of recalcitrant tumors.

What was accomplished under these goals?

Specific aim 1: Engineered model cell lines for CRISPR library screen.

Specific aim 2: CD276-PBD immunoconjugates were re-engineered as outlined in proposal and found to be less toxic in mice than our previous ADCs. Toxicology studies are still in progress.

Specific aim 3: Amanitin-linked CD276 ADCs were created. However, they have not yet been tested in vivo.

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.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The award was terminated due to the post-doctoral fellow (PI of grant) leaving the laboratory.

4. **IMPACT:**

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

Nothing to Report.

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:

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

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

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

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)**

Nothing to Report.

- **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?

Name: Jim Dunleavey
Project Role: Post-doctoral fellow
Researcher Identifier (e.g. ORCID ID): N/A (post-doc has left NCI)
Nearest person month worked: 6

Contribution to Project: Dr. Dunleavey has worked on all the aims of the proposal.
Funding Support: This DOD

Name: Yang Feng
Project Role: Laboratory Technician
Researcher Identifier (e.g. ORCID ID): 001-0395-131 (EID)
Nearest person month worked: 2

Contribution to Project: Dr. Feng has been working on producing amanitin linked ADCs.
Funding Support: NIH

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

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES: