

AWARD NUMBER: W81XWH-14-1-0324

TITLE: GrB-TWEAK: A Potential Novel Biologic for NSCLC Therapy

PRINCIPAL INVESTIGATOR: Jeffrey A. Winkles, Ph.D.

CONTRACTING ORGANIZATION: University of Maryland, Baltimore
Baltimore, MD 21201

REPORT DATE: July 2017

TYPE OF REPORT: Addendum to Final

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE July 2017		2. REPORT TYPE Addendum to Final		3. DATES COVERED 1 Sept 2016 - 30 Apr 2017	
4. TITLE AND SUBTITLE GrB-TWEAK: A Potential Novel Biologic for NSCLC Therapy				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-1-0324	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jeffrey A. Winkles, Ph.D. E-Mail: jwinkles@som.umaryland.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Maryland, Baltimore Baltimore, MD 21201				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel 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 My laboratory studies the cytokine named TWEAK and its cell surface receptor named Fn14 and their role in cancer biology. We reported previously that Fn14 is expressed at low levels in normal lung tissue but highly expressed in many non-small cell lung cancers (NSCLCs). Additionally, in collaboration with Dr. Rosenblum's research group we have successfully developed several Fn14-targeted fusion proteins that exhibit cytotoxic activity on cancer cells <i>in vitro</i> and <i>in vivo</i> . In this Lung Cancer Idea Award application we proposed to test the effects of these fusion proteins, and in particular the GrB-Fc-IT4 construct, on NSCLC cell growth in vitro (Aim 1) and in vivo (Aim 2). During year 1, we were able to demonstrate that two different Fn14-targeted proteins that use granzyme B (GrB) as the cell killing agent (TWEAK-GrB, GrB-Fc-IT4) exhibit pro-apoptotic activity when added to numerous Fn14-positive NSCLC cell lines. During year 2, we conducted additional work to inform the design of the in vivo studies. During our no-cost extension, we worked with Dr. Berens group to test whether the GrB-Fc-IT4 construct could inhibit lung cancer PDX growth in vivo. This data is described in this FINAL REPORT ADDENDUM.					
15. SUBJECT TERMS TWEAK, Fn14, targeted therapy, granzyme B, lung cancer					
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	UU	5	19b. TELEPHONE NUMBER (include area code)

FINAL REPORT ADDENDUM

1. Accomplishments:

During this no-cost extension period we were able to test whether our therapeutic construct GrB-Fc-IT4 could inhibit NSCLC tumor growth *in vivo* using the NSCLC patient-derived Fn14-positive cell line M2010-1005. The M2010-1005 line was established from a NSCLC patient with a tumor harboring the EGFR^{del747-752}-activating mutation and, as expected (Whitsett et al., *American Journal of Pathology* 181:111-120), Fn14 expression is robust in this line (Fig. 1A). M2010-1005 maintains histological (Fig. 1B) and genetic (unpublished data) characteristics of the original patient tumor during *in vivo* passaging.

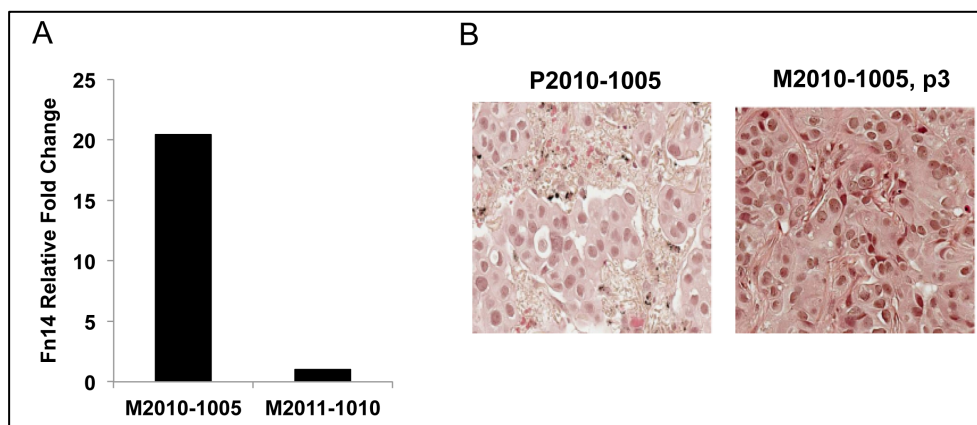


Figure 1. Properties of the patient-derived NSCLC explant M2010-1005. **A.** Fn14 mRNA expression levels in two patient-derived NSCLC explants was assessed by real-time PCR. Relative fold change calculated by $\Delta\Delta C_t$ method with histone as a reference gene is shown. **B.** Histological characteristics of the P2010-1005 patient tumor and the M2010-1005 explant at *in vivo* passage number 3 (H & E staining). Genetic characteristics of the P2010-1005 tumor, as analyzed by array CGH, are also maintained during *in vivo* passaging (data not shown).

Animal experiments to test GrB-Fc-IT4 antitumor efficacy were conducted via subcontract with Dr. Michael Berens at TGen (Phoenix, AZ) following approval by the Institutional Animal Care and Use Committee guidelines of St. Joseph's Hospital and Medical Center. Studies were carried out in nu/nu athymic mice (Jackson Labs) maintained under pathogen-free conditions and a 12-hour light/12-hour dark cycle. Fresh M2010-1005 tumor tissue originally resected from a patient at the time of surgery, with informed, written patient consent, was implanted subcutaneously (s.c.) into the flanks of 6-week-old nu/nu mice. Xenografts were allowed to grow to approximately 200 mm³. Animals were grouped randomly (6 mice per group) and treated with vehicle (sterile PBS) or GrB-Fc-IT4 (20 mg/kg) every other day over a ten day period for a total dose of 100 mg/kg via tail vein injection. Tumor size was measured 2-3 times per week by caliper measurements and volumes were calculated using the following formula: $V = (a \times b^2) / 2$, where 'a' is the largest dimension and 'b' the smallest. Tumor growth in GrB-Fc-IT4-treated animals was compared to vehicle-treated mice. We found that GrB-Fc-IT4 was able to inhibit tumor growth in this lung cancer PDX model (Fig. 2).

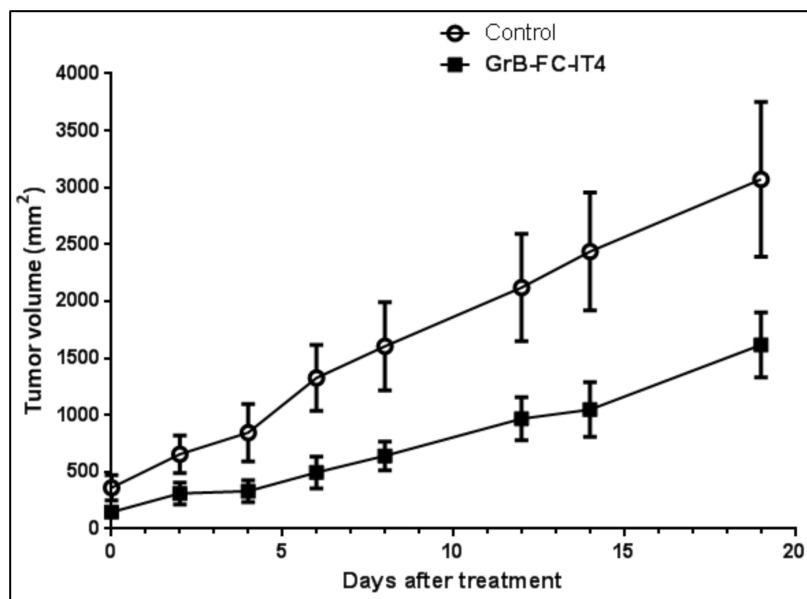


Figure 2. Antitumor efficacy of GrB-Fc-IT4 protein in M2010-1005 NSCLC PDX model. M2010-1005 tumor tissue was injected subcutaneously into nude mice and tumors were allowed to grow to ~200 mm³ in volume. Groups (n=6) were injected (iv, tail vein) with either saline (control) or GrB-Fc-IT4 at 20 mg/kg every other day over a ten day period for a total dose of 100 mg/kg. Tumor size was assessed by direct caliper measurements and tumor volumes calculated. Efficacy data is plotted as mean tumor volume +/- SEM.

In summary, in this Lung Cancer Idea Award application we proposed to test the effects of new Fn14-targeted fusion proteins, and in particular the GrB-Fc-IT4 construct, on NSCLC cell growth *in vitro* (Aim 1) and *in vivo* (Aim 2). We have accomplished these goals and more research is in progress. Notably, one of my collaborators on this project, Dr. Michael Rosenblum, has received financial support to begin work that we hope will move the GrB-Fc-IT4 therapeutic agent into a Phase 1 clinical trial (see abstract mentioned below).

2. Products (Reportable Outcomes from No-Cost Extension):

We have submitted an abstract describing the *in vivo* results for presentation at a scientific meeting. The meeting information and abstract is on the next page.

Targeted Granzyme B Immunotherapy: A Novel Approach Delivering GrB to Fn14-Positive Solid Tumors

¹Alvarez-Cienfuegos A, ¹Cheung LH, ¹Mohamedali KA, ²Winkles JA, ³Inge LJ, ³Whitsett TG, ⁴DePalatis LR and ¹Rosenblum MG, ¹Immunopharmacology and Targeted Therapy Laboratory, MD Anderson Cancer Center, Houston, TX 77030, ²Department of Surgery and Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21701, ³Norton Thoracic Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ 85013 and ⁴Mirata Biopharma, Spring, TX 77373

All immune effector cells including engineered T-cells and other immunotherapeutic approaches rely on the delivery of the serine protease granzyme B (GrB) to the target cells resulting in a potent cytotoxic (apoptotic) effect that has been well characterized. We have developed a series of novel, completely human constructs for targeted, tumor antigen-mediated delivery of GrB without the need for effector cells. These bivalent constructs can be targeted to cells by incorporating scFv proteins that bind cell-surface antigens. A series of GrB-based constructs are included in our Targeted GrB Immunotherapy (TGI) platform. Our initial focus for further development is GrB-Fc-IT4 (MRT-101), a dimeric, bivalent construct with a high molecular weight (160 kDa) that binds the TWEAK receptor fibroblast growth factor-inducible protein 14 (Fn14). This receptor is highly over-expressed in many solid tumor types. The construct was expressed in HEK293E suspension cells, harvested under serum-free conditions and purified to homogeneity. The specific activity of the GrB moiety, as assessed by cleavage of a synthetic chromogenic GrB substrate, was comparable to natural GrB. Against a panel of >40 human cancer cell lines expressing Fn14, MRT-101 showed high affinity and selective cytotoxicity in the nanomolar range (IC₅₀ ranged from 4 to 284 nM) and was 2-100X more potent than free GrB. Mechanistic studies demonstrated that MRT-101 activated caspase cascades and cytochrome C related pro-apoptotic mechanisms consistent with the known intracellular functions of GrB. Pharmacokinetic studies in mice revealed that the MRT-101 fusion protein exhibited a bi-exponential clearance from plasma with a rapid initial clearance ($t_{1/2\alpha} = 0.36$ hours) followed by a prolonged terminal-phase plasma half-life ($t_{1/2\beta} = 35$ hours). Toxicity studies in mice demonstrated that the MTD exceeds a cumulative dose of 100 mg/kg. Athymic nude mice bearing MDA-MB-231 orthotopic breast tumor xenografts were intravenously treated with saline or MRT-101 construct (Qod X 5). On average, tumors from saline-treated mice grew ~10-fold over 40 days. In contrast, tumors from mice treated with MRT-101 showed no growth over this period. Moreover, 3/5 mice treated with MRT-101 had complete tumor regression lasting beyond day 80 (end of the study). A second study in NSG-SCID mice bearing well-developed MDA-MB-231 orthotopic tumors showed significant tumor growth inhibition by MRT-101 in both intravenous and intraperitoneal treatment groups. Mice bearing subcutaneous lung adenocarcinoma PDX tumors treated with MRT-101 also demonstrated >50% tumor suppression vs saline controls. Overall, treatment with MRT-101 was well-tolerated (no loss of body weight) and demonstrated significant anti-tumor efficacy against multiple tumor types. These studies represent the characterization of a new class of targeted immunotherapeutics containing a synthetic GrB payload identical to that of immune effector cells. The MRT-101 fusion construct is currently undergoing IND-enabling studies and GMP manufacturing in advance of projected Phase 1 clinical trials. Research conducted, in part, by the Clayton Foundation for Research.