AWARD NUMBER: **W81XWH-12-1-0534**

TITLE: Cell-Penetrating Bispecific Antibodies for Targeting Oncogenic Transcription Factors in Advanced Prostate Cancer

PRINCIPAL INVESTIGATOR: **Michael Lilly, MD**

CONTRACTING ORGANIZATION: **Medical University of South Carolina**
Charleston, SC 29425-8080

REPORT DATE: December 2016

TYPE OF REPORT: **Final**

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2016	Final	30Sep2012 - 29Sep2016

4. TITLE AND SUBTITLE
Cell-Penetrating Bispecific Antibodies for Targeting Oncogenic Transcription Factors in Advanced Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH- 12-1-0534

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Michael Lilly, MD
Richard Weisbart, MD

E-Mail: lillym@musc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Medical University of South Carolina
Charleston, SC 29425

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
The goal of these studies is to develop and validate cell-penetrating bi-specific antibodies as an agent that can selectively inhibit the function of intracellular proteins. We have developed 3E10-AR441 bi-specific antibody to inhibit the function both ligand-dependent and independent forms of the androgen receptor (AR), key drivers of prostate carcinogenesis.

15. SUBJECT TERMS
Nothing 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			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	12	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION

This report covers research activities supported by DOD contract W81XWH-12-1-0534, entitled Cell-penetrating bispecific antibodies for targeting oncogenic transcription factors in advanced prostate cancer. The research is a collaborative effort between Michael Lilly, MD (Principal Investigator) and Richard Weisbart (Co-investigator). Dr. Lilly is based at the Hollings Cancer Center, Medical University of South Carolina, while Dr. Weisbart is at the Sepulveda VA, affiliated with the University of California Los Angeles. This contract was activated at MUSC on October 1, 2012. The reporting period is therefore from 10/1/2015 through 9/30/2016. Work under this contract began in Dr. Lilly's laboratory about November 1, 2012. Dr. Weisbart's group has worked from December, 2012, following the completion of a subcontract agreement between MUSC and Dr. Weisbart's institution.

Three specific aims were proposed. In the first, Dr. Weisbart's group would produce a prototype bispecific antibody targeting the androgen receptor (AR) and optimize its structure and production. The second specific aim, to be carried out by Dr. Lilly's laboratory, focuses on the biochemical and biologic properties of the bispecific antibody, through the use of biochemical and biologic assay systems. The final specific aim was tol examine the ability of the bispecific antibody to perturb the growth of prostate cancer cells in murine models. Additional studies would characterize PK/PD parameters of the antibody. Substantial progress has been made in the first two aims. Work on the third specific aim has been delayed because of the tenuous supply of bispecific antibody. The issue was discussed at length with DOD/CDMRP staff and a modified SOW was accepted in early 2015, limiting the animal work to an initial PK/PD experiment. Final DOD/CDMRP animal use approval was obtained in June, 2015. This work was performed during a NCE ending 9/30/2016, and is reported here. We have also performed an additional cell growth inhibition experiment using a cell system that demonstrates testosterone-dependent growth, and experiments to determine the feasibility of producing 3E10-AR441 in a mammalian expression system.

2. KEYWORDS

Prostate cancer, antibody, bispecific, androgen receptor, castration-resistant

3. ACCOMPLISHMENTS

The proposed activities are contained within the modified statement of work, which is immediately below *in italics*. For each task annotations and supporting data are provided further below in the lettered paragraphs.

Month 1-34: Specific Aim #1 (Weisbart Lab).

Month 1-6 *Prepare cDNA for scFv-AR441 antibody; test for binding to AR by immunoblot; compare binding affinity with that of intact AR44a antibody.*

See sections A, B, J. These activities were 100% completed in the proposed time.

Month 5-19 *Construct cDNA for bispecific scFv-3E10-AR441; optimize sequence; optimize production parameters*

See sections C, D, O. These activities were 100% completed in the proposed time.

Months 9-34 *Produce bispecific antibodies needed for specific aims #2, 3*

See section D. These activities were 100% completed in the proposed time.

Deliverables: *--Optimized, active scFv-3E10-AR441, inactive versions scFv-3E10(R95Q)-AR441 and scFv-3E10-(mut)AR441, up to 20mg of each, purified, endotoxin-free, and at least 1mg/mL concentration*
--data documenting the testing and optimization of above bispecific antibodies

Optimized antibodies, primarily 3E10-AR441 and 3E10-MycHis-AR441, plus negative control 3E10scFv were produced in adequate quantity and purity.

Month 1-16 Specific Aim #2 (Lilly Lab).

Month 1-8 Mutagenesis of pCMhAR plasmid to produce T877A, exon 3 splice variants, and E231G mutants; testing by transient transfection for expression of expected protein; creation of BPH1/ARELuc and BPH1/neo cell lines; introduction of WT and mutant AR cDNAs in BPH1 background cell lines; standardization of assays for AR effects.

The pCMhAR plasmid was mutagenized to produce a Q680X variant, with a premature stop codon. We decided not to study the E231G mutation since current genomic profiles show it is extremely rare in clinical prostate cancer. The splice variant plasmids were obtained from a colleague. These were introduced transiently or stably into LNCaP and HEK cells. It was not possible to obtain stable transfectants in BPH1 background. See G, H, I, J, K. These studies were 100% completed by 36 months.

Month 9-16 Determine if scFv-3E10-AR441 bispecific antibodies inhibit the biologic and biochemical effects of WT and mutant ARs in BPH1 cellular background.

See G, H, I, J, N. We used the LNCaP subline C4-2 for growth experiments since it did demonstrate androgen-stimulated growth and enzalutamide-mediated growth inhibition, and thus active proliferative AR signaling. The biochemical studies were 100% completed by the proposed time. The biologic studies were 100% completed by 40 months, extending into the NCE.

Deliverables: --data documenting the biochemical and biologic phenotypes of WT and mutant AR signaling in BPH1 cells
--data documenting the effects of active and negative bispecific antibodies on androgen-dependent genomic, non-genomic, and biologic signaling via WT and mutant ARs
--plasmids and cells lines characterized as described in research plan.

Month 15-34: Specific Aim #3 (Lilly Lab).

Month 15-20 Develop EIA assay to measure scFv-3E10-AR441 antibody in biological and clinical specimens

See section K. These studies were 100% completed by the proposed time.

Month 19-36 Pharmacokinetic studies of single intravenous injection (10 Spraque Dawley rats)

See section M. These activities were 100% completed by 40 months, extending into the NCE.

Months 34-36: All Laboratories

Data analysis and manuscript preparation

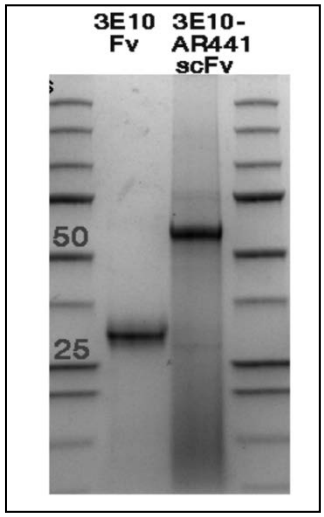
A manuscript has been submitted for publication.

WEISBART LABORATORY

A. Production of 3E10-AR441 bispecific antibody (Fig. 1). Key studies focused on the production of a bispecific single-chain (sc) Fv fragment consisting of the Fv fragment of mAb 3E10, an intracellular transporter and mAb AR441, an antibody that binds the androgen receptor. The molecular fusion protein was constructed and shown to be correct by sequencing and characterized by SDS-PAGE. As shown in Fig. 1, the fusion protein is twice the size of the 3E10 Fv alone as expected. An SDS gel shown is stained with Gel-Code Blue.

B. Binding of bispecific antibody to denatured target protein. To determine if 3E10-AR441 BiAb was able to bind the androgen receptor, an immunoblot was done with lysate from LNCaP cells containing the androgen receptor (not shown). The 3E10-AR441 bound the 110kd androgen receptor as shown by comparison with reactivity of AR441 hybridoma monoclonal antibody.

Fig. 1. Recombinant Antibodies for project



C. Studies of alternate linkers to enhance binding affinities. Although our initial construct penetrated prostate cancer cells *in vitro*, bound to the androgen receptor and was cytotoxic, two issues remained that needed improvement. 1. AR441 Fv has lower affinity for the androgen receptor compared to the original monoclonal antibody. 2. The yield of 3E10-AR441 produced in *Pichia pastoris* is low. The binding affinity of the Fv is largely determined by the linker between VL an VH which is responsible for bringing the two chains in close apposition to form an antigen binding site. The low affinity Fv was the result of using the standard (GGGGS)3 linker. Therefore, we constructed and produced AR441 Fv with different linkers that have been cited in the literature. We identified one linker that increased affinity of binding of AR441 Fv 4-fold by independent evaluation of binding affinity in Dr. Lilly's laboratory. We then produced a new construct of 3E10-AR441 with the higher affinity AR441. This was desibnated 3E10-Myc-His-AR441.

D. Production of bispecific antibodies. The second issue is the ability to produce sufficient recombinant protein for studies both *in vitro* and *in vivo*. Currently we are able to produce only 1 mg of 3E10-AR441 from 1 liter of *Pichia* supernatant. We recently compared two methods of producing 3E10-AR441 as a secreted protein in *Pichia pastoris* : a. the standard method of growing yeast in glycerol and then inducing production of protein with methanol, and b. simultaneous growth of *Pichia* and synthesis of protein in the presence of methanol without glycerol. The latter method is borrowed from industry where large yields of protein have been observed. This latter method was found to work well in our laboratory with other bispecific scFv antibodies containing 3E10 Fv, but was unsuccessful for 3E10-AR441. This result suggests that AR441 is toxic. In addition to toxicity, the conformation of the bispecific antibody influences its ability to be secreted. The conformation of the antibody complex is determined to a great extent by the linker used to connect the two Fv fragments (3E10 Fv and AR441 Fv). We completed the process of producing different constructs of 3E10-AR441 with different linkers in an attempt to increase yield of protein, but the improvement in overall yield of active antibody was minimal. Dr. Weisbart's group did however continue to produce both antibody forms in sufficient quantity to complete the proposed studies in the revised SOW.

LILLY LABORATORY

E. Subcellular localization of bispecific antibody. LNCaP cells were incubated with the scFv 3E10 antibody, the bispecific 3E10-AR441 antibody or control medium containing FCS, x 18hr. The cells were then fixed with MeOH, and stained with secondary reagent (anti-MYC-FITC conjugate). The cells were then examined by confocal microscopy. There was no staining of the cells initially labelled with medium alone. However, cells treated with either scFv 3E10, or with the 3E10-AR441 bispecific antibody had definite nuclear staining. The concentration of the bispecific was about half that of the scFv, leading to paler staining. These data demonstrate that the scFv 3E10 and its bioconjugates can penetrate prostate cancer cells and localize in the nucleus.

F. Direct binding of bispecific antibody to its molecular target, AR. To demonstrate directly that 3E10-AR441 can bind to WT and mutant ARs, we have examined the ability of 3E10-AR441 to bind to AR in native conformation in an immunoprecipitation format. Test antibodies were mixed with a lysate of LNCaP and other prostate cancer cells as a source for AR. The lysate was prepared with non-denaturing detergents. Immune complexes were recovered with Protein L-agarose, which binds to conserved domains of K light chains found both in native antibodies and most scFv antibodies. The complexes were then recovered from solid phase with SDS, and detected by immunoblotting, using anti-AR and other antibodies.

In general the parental MoAb AR441 and the bispecific 3E10-AR441 antibodies were able to bind to both wild type AR and ligand-binding-domain-deficient splice variants reported from 22Rv1 and VCaP cells (not shown). Interestingly the scFv AR441 alone was unable to bind to the native conformation of AR in a precipitation assay. This suggests that the whole bispecific antibody develops specific conformations that are not present in the separate component scFvs.

G. Development of cell lines stably expressing WT and mutant ARs. To most precisely catalogue the AR forms that react with 3E10-AR441 we will still need to express these individually in mammalian cells that lack substantial amounts of endogenous AR. Since BPH1 cells were not satisfactory we prepared stable transfectants of HEK cells for this purpose. HEK/AR WT, HEK/ARv3, HEK/ARv7, and HEK/AR Q640X cell lines were prepared and characterized. These were used as a source for pure AR proteins for antibody binding assays. LNCaP cells were transiently transfected with the ARv7 and other plasmids for biochemical assays of antibody inhibition of AR signaling.

H. Inhibition of AR genomic signaling by bispecific antibody. A key goal of specific aim #2 was to demonstrate that the 3E10-AR441 antibody can engage its target (AR) and interrupt AR-dependent signaling and effects. During the 01 year we developed a ARE-dependent luciferase reporter gene system in LNCaP cells (LNCaP/ARELuc), and used it to show that 3E10-AR441 can disrupt genomic AR signaling. During the 02 year we expanded these studies to show that the bispecific antibody can also disrupt AR signaling through the endogenous PSA promoter. For these studies LNCaP cells were treated with DHT, with or without 3E10-AR441 antibody or control antibodies. Changes in the levels of PSA mRNA were measured by real time PCR. The bispecific antibody completely prevented the ligand-dependent increase in PSA mRNA.

I. Inhibition of AR non-genomic signaling by bispecific antibody. AR can transduce signals in the cell membrane and cytoplasm immediately following ligand binding. These non-genomic signals do not require nuclear translocation and gene transcription. Non-genomic signaling can lead to release in calcium from intracellular pools within seconds of ligand application. 3E10-AR441 dramatically reduces DHT-dependent calcium release in LNCaP cells. The 3E10 scFv alone did not significantly impair ligand-dependent calcium release (fig. 2).

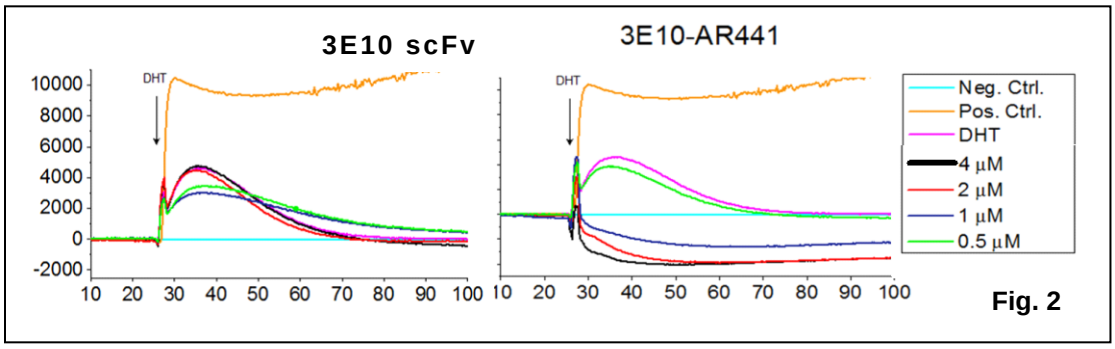
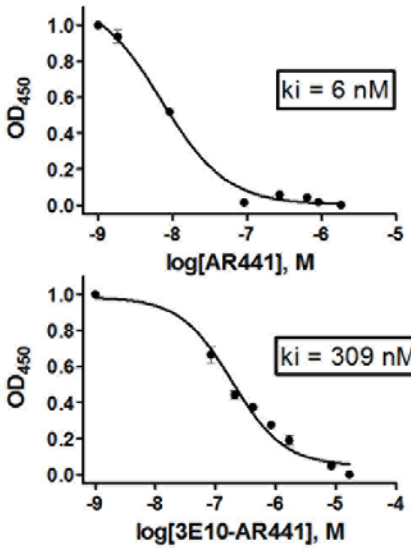


Fig. 2

J. Binding affinity of bispecific antibody 3E10-AR441 and 3E10-Myc-His-AR441. We have continued to use our sandwich ELISA-based assay to measure the binding affinity of newer versions of 3E10-AR441 to WT and variant AR proteins (fig. 3). We have now produced a comprehensive catalog of binding affinities for the original AR441 mAb, our initial 3E10-AR441 bispecific Ab, the AR441 scFv alone, and a new version of the bispecific, 3E10-Myc-His-AR441. This latter protein was produced by Dr. Weisbart to see if the antigen binding affinity would be enhanced. The changes included moving the Myc, His tags from the C-termiinus to the linker region between the 2 scFvs. In addition the ordering of the VH and VK sequences was reversed in the AR441scFv motif. We demonstrated that the binding affinity increased from about 271nM to 49nM, more than a 5-fold improvement (Fig. 3). Unfortunately the 3E10-Myc-His-AR441 protein was still not produced well in a *Pichia* system.



	AR441scFv Ki (nM)	AR441 Ki (nM)	3E10-AR441 Ki (nM)	3E10-Myc-His-AR441 Ki (nM)
LNCap	2438	6	309	45
HEK/AR WT	N/A	29	260	100
HEK/ARQ640X	N/A	23	119	33
HEK/ARv1	N/A	21	379	150
HEK/ARv7	N/A	21	307	69
HEK/AR2b	N/A	16	295	85

Fig. 3. Binding affinity of antibody variants against WT and mutant, splice variant AR expressed in LNCaP or transiently-transfected HEK293 cells.

An interesting observation was that the binding affinity of the parental mAb AR441 to WT AR from LNCaP cells was higher by a factor of 5 than its binding to WT AR produced in transiently transfected HEK293 cells. This likely reflects subtle conformational changes produced by several known AR binding proteins present in LNCaP cells, but possibly missing in HEK293 cells.

K. Development of a sandwich ELISA to detect 3E10-containing antibodies in biologic samples. In order to proceed to PK/PD studies in rodents, we proposed to develop an assay capable of detecting very small amounts of 3E10-AR441 in biologic specimens. During the 02 year we prepared a polyclonal rabbit antibody against the 3E10 scFv antibody, to allow us to measure not just 3E10-AR441, but any monovalent or bivalent antibody containing the 3E10 scFv. During the 03 year we have succeeded in formulating a useful ELISA. This was accomplished by using the rabbit polyclonal anti-scFv 3E10 for both the capture and detection antibodies. Since this polyclonal antibody reacts with multiple epitopes on the 35Kd antigen, it is likely that multiple "pairs" of antibody are present, allowing the same antibody to serve both roles. The detection antibody was biotinylated, and a streptavidin-HRP reagent was used as the secondary detection agent. This ELISA proved to be sensitive and facile to use. An unexpected problem however was that the anti-3E10 antibody cross-reacts with normal mouse serum proteins, presumably *kappa* light chains. This required that we do all PK/PD studies in rats (see below).

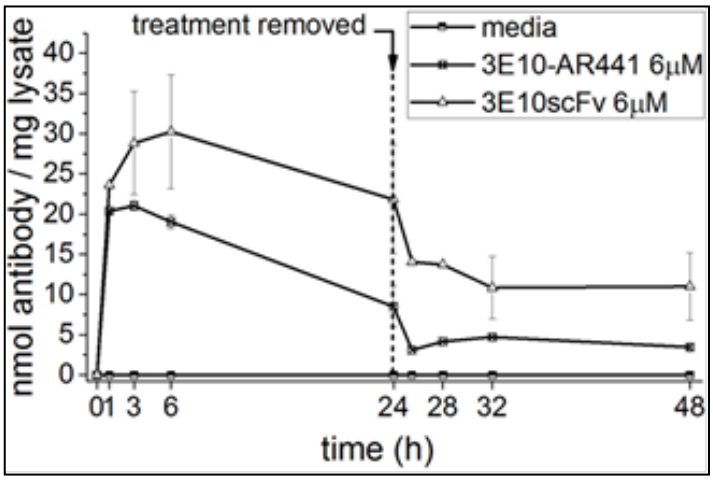


Fig.4. Cell-associated antibody after addition (0-24hr) or removal (24-48hr) of 3E10scFv, or 3E10-AR441 biAb.

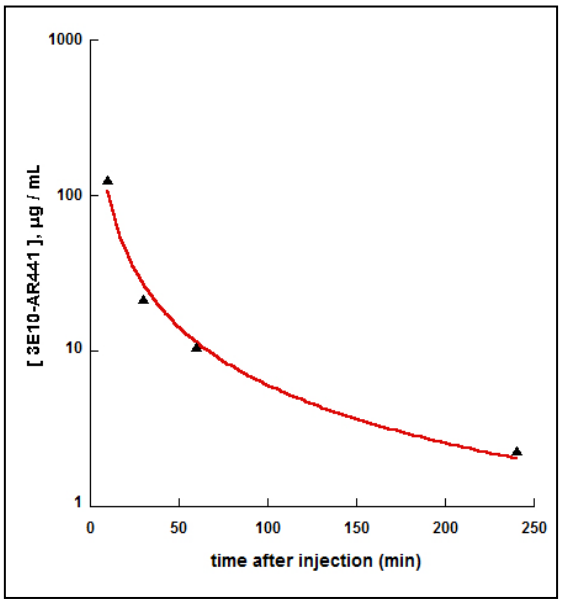
We have used our ELISA assay to quantitatively describe the ingress and egress of 3E10-AR441 and the 3E10scFv alone, in and out of LNCaP cells in culture (Fig. 4). Data shown in previous reports using confocal microscopy demonstrated that cell-associated antibodies are almost entirely in the nucleus (not shown). Uptake of either antibody occurred rapidly, and was maximum between 1 and 3hrs after antibody addition. When antibody was removed from the culture medium a substantial quantity of the cell associated antibody was lost, appearing into the fresh medium rapidly. However between 25-50% of the initial cell-associated antibody remained, and there was little or no change over the subsequent 24hrs. It is likely that this stable, cell-associated antibody is bound to DNA through the 3E10scFv motif. There may be continuing interaction with nuclear AR by these antibody molecules "trapped" in the cell nucleus. The 3E10 scFv

protein was taken up more rapidly, and remained in the cells at higher levels, than did the 3E10-AR441 bispecific antibody.

L. Demonstration that scFv AR441 sequences prevent secretion of fusion proteins in yeast. We have used a yeast display platform to directly demonstrate that sequences present in the scFv AR441 motif of the 3E10-AR441 bispecific antibody limit secretion of the protein from yeast.

M. Pharmacokinetics of 3E10-AR441 bispecific antibody after IV injection. We used rats for this study since our anti-3E10 antibody cross reacted with mouse immunoglobulin light chains. Ten six-week old Sprague-Dawley rats were injected with 0.5mg/animal of 3E10-AR441 bispecific antibody intravenously. Serum was collected for analysis before, and at 10 minutes, 30 minutes, 60 minutes, and 240 minutes after injection (Fig. 5). The elimination of the antibody showed a biphasic curve, with an extremely short initial phase (half-life 30 minutes) and a longer secondary phase (half-life around 4hrs). Each point is the mean of levels from 2 animals.

Fig. 5. Serum concentration of 3E10-AR441 following intravenous administration



N. Inhibition of growth of C4-2 prostate cancer cells. Work during years 01-03 showed that the 3E10-AR441 bispecific antibody would inhibit both genomic and non-genomic AR signaling. To see if this activity would be associated with a significant biological effect (inhibition of AR-dependent growth) we tested the antibodies against C4-2 cells. We initially had to determine relevant conditions for demonstrating testosterone-stimulated growth. We cultured the C4-2 cells at low density (initially 400-800 cells/well of a 24-well plate) in phenol red-free medium and charcoal-stripped serum. Each component of this culture system was necessary. DHT at 0.5-1nM final concentration stimulated cell growth while higher and lower concentrations either had no effect, or markedly inhibited growth. Cultures were maintained with growth regulators for 14 days, with a refeeding (including test agents) at day 7. Figure 6 shows a typical experiment.

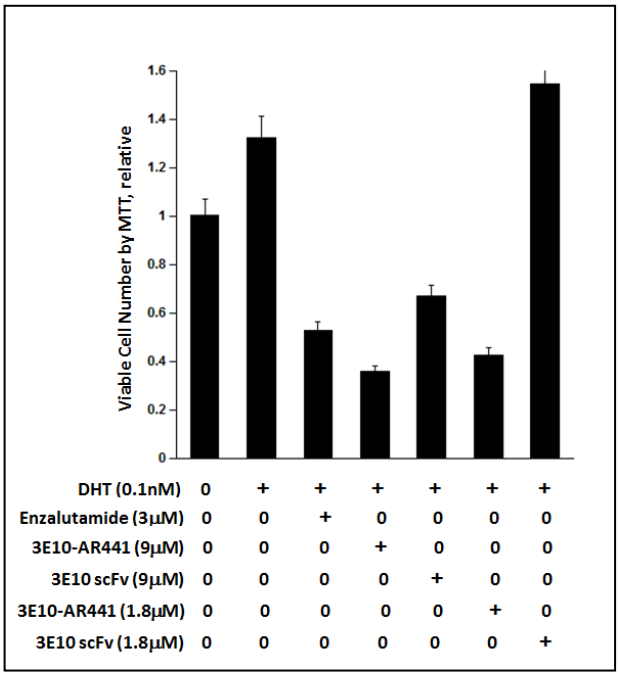


Fig. 6. Inhibition of day 14 growth of C4-2cells

O. Production of 3E10-AR441 bispecific antibody in mammalian expression system. Because of the very low amount of bispecific antibody produced in a yeast system, we explored the utility of a mammalian cell expression system. We ligated the cDNA for the 3E10-AR441 antibody into a mammalian expression vector with an albumin leader peptide and CMV promoter (Sigma). Sequencing of the insert confirmed the correct sequence. The plasmid was transfected into CHO cells and HEK293 cells. The culture medium from the transfected cells was examined over a 5 day period for the presence of the secreted bispecific antibody. This was detected by both immunoblotting and ELISA. The antibody was not identified in the medium or cells from the

transfected CHO cell cultures. HEK293 cells secreted small amounts of the antibody, <100mcg/L after 5 days of culture.

Training Opportunities. Some of the work performed by the Lilly Laboratory was carried out by Nancy Goicochea, PhD, as part of a post-doctoral fellowship funded from another source.

Dissemination of Results. The bulk of the data (not including PK, cell growth experiments) were presented as a poster at AACR 2015. Dr. Goicochea was the first author on the presentation. She is also the first author on the manuscript that is now under review.

Activities for next year. Nothing to report.

4. IMPACT

Impact of discipline. The prototype cell-penetrating bispecific antibody functioned as planned by binding with relatively high affinity to a wide spectrum of clinically relevant AR species. Further it inhibited the biochemical and biologic function of the target molecule. These are important observations to support the validity of the approach.

Unfortunately the future of this technology is uncertain due to the unexpected death of Dr. Richard Weisbart, the developer of the 3E10 antibody, in November, 2016. Access to his critical reagents and methods is therefore uncertain.

Impact on other disciplines, technology transfer, and society. Nothing to report.

5. PROBLEMS OR CHANGES IN APPROACH

Several problems became apparent during the studies.

1. *Production of the bispecific antibodies*. There were persistent problems with production and stability of the 3E10-AR441 bispecific antibodies. Only minimal improvements in yield were obtained by changing the order of the heavy and light chain motifs, and the composition of the linkers. In addition, switching to a mammalian expression system was unhelpful. The project was continually plagued by limited supply of antibody, which ultimately limited the number of animal experiments.

2. *Cell Models.* We originally proposed to produce test cell lines that were stably transduced with WT, mutant ARs in BPH1 cells. This proved impossible. We therefore used LNCaP cells (or its C4-2 subline) for transient or stable transfection with WT and mutant ARs. We feel this was successful.

3. *Animal models.* We had originally planned to use mice with PCa xenografts. This proved to be impractical because of the very limited supply of antibody. In addition our sandwich ELISA for measuring the bispecific antibody in biologic fluids could not be used in mice. We were therefore limited to a small pharmacokinetic experiment using young rats as a substitute. This was successful in demonstrating that 3E10-AR441 also showed the extremely rapid elimination from serum described for other scFv BiAbs that are not cell penetrating.

6. PRODUCTS

The basic data developed during this project were described in a poster at AACR 2015:

Nancy L. Goicochea, Maria Garnovskaya, Mary Blanton, Grace Chan, Richard Weisbart, Michael Lilly. Cell-penetrating bispecific antibodies for targeting androgen receptor signaling in advanced prostate cancer. [abstract]. In: Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18-22; Philadelphia, PA. Philadelphia (PA): AACR; *Cancer Res* 2015;75(15 Suppl):Abstract nr 642.

The data were commented on favorably in a recent review of N-terminal AR-targeting molecules:

Antonarakis ES, Chandhasin C, Osbourne E, Luo J, Sadar MD, Perabo F. Targeting the N-Terminal Domain of the Androgen Receptor: A New Approach for the Treatment of Advanced Prostate Cancer. *Oncologist*. 2016 Dec;21(12):1427-1435.

Other deliverables include:

- Purified anti-3E10 antibody, both native and biotinylated, for constitution of the sandwich ELISA
- Data books containing the original source studies
- 3E10scFv antibody, approximately 8mg
- AACR poster 2015

7. PARTICIPANTS AND COLLABORATING INSTITUTIONS

This project was a collaborative effort between Dr. Lilly (Hollings Cancer Center, Medical University of South Carolina) and Dr. Weisbart (Sepulveda VAMC, via Sepulveda Research Corporation). The following personnel were supported by this award:

Table 1. Personnel supported by award

name	title	institution	% effort	months
Richard Weisbart, MD	*Principal Investigator*	*Sepulveda Res Corp*	0	12
			12.5	12
			14	12
			14	6
Grace Chan	*Research biologist*	*Sepulveda Res Corp*	100	4
Michael Lilly, MD	*Principal Investigator*	*Hollings Cancer Ctr, MUSC*	4	36
Mary Blanton	*Research Specialist II*	*Hollings Cancer Ctr, MUSC*	50	21
			65	10
Mary Berkaw	*Research Specialist II*	*Hollings Cancer Ctr, MUSC*	50	3

8. CONCLUSIONS

- The bispecific 3E10-AR441 antibody has performed as expected in tissue culture experiments, by entering cells, translocating to the nucleus, and blocking androgen-dependent signaling through the AR. The antibody binds to both wild-type and LBD-mutant ARs, demonstrating robust target engagement.
- The binding affinity of the 3E10-AR441 is probably several-fold lower than that of the parental monoclonal AR441. This may require much larger amounts of antibody than expected for a significant *in vivo* biologic effect.
- Further protein engineering of the 3E10-AR441 antibody is probably needed to 1) enhance the binding affinity to the target protein, and 2) to improve yields from the current yeast expression system
- The binding affinity of the 3E10-AR441 is probably several-fold lower than that of the parental monoclonal AR441. This may require much larger amounts of antibody than expected for a significant *in vivo* biologic effect.
- Further protein engineering of the 3E10-AR441 antibody is probably needed to 1) enhance the binding affinity to the target protein, and 2) to improve yields from the current yeast expression system.
- The bispecific antibody can inhibit the proliferation of androgen-dependent prostate cancer cells.
- Improvements in the production of the antibody are needed to make further animal studies possible.

- The studies accomplished to date by both the Lilly and Weisbart laboratories represent a substantial completion of the propose studies for months 1-36 as outlined in the approved modified Statement of Work.

REFERENCES

none

APPENDICES

Cancer Research

search

Advanced Search

Home About Articles For Authors Alerts

Experimental and Molecular Therapeutics

Abstract 642: Cell-penetrating bispecific antibodies for targeting androgen receptor signaling in advanced prostate cancer

Nancy L. Golcochea, Maria Garnovskaya, Mary Blanton, Grace Chan, Richard Weisbart, and Michael Lilly

DOI: 10.1158/1538-7445.AM2015-642 Published 1 August 2015

Article Info & Metrics

Proceedings: AACR 106th Annual Meeting 2015; April 18-22, 2015; Philadelphia, PA

Abstract

Androgen receptor (AR) plays a critical role in the development and progression of prostate cancer (PCa). Current therapies target the ligand-binding domain (LBD) of AR by inhibiting production or binding of ligand. In castration-resistant PCa (CRPC), AR isoforms lacking the LBD are highly expressed, resulting in constitutive, ligand-independent AR signaling. Therapies to block ligand-independent AR signaling have not been introduced into clinical use. We have developed bispecific antibodies (bsAbs) that penetrate PCa cells and bind to the N-terminal domain of the AR, inhibiting AR signaling. The 3E10-AR441 bsAb was engineered by connecting two single-chain variable fragments (scFv) with a linker. One half of the bsAb molecule is scFv 3E10, derived from a lupus anti-DNA antibody. This module enters cells through the ENT2 nucleoside salvage receptor and locates in the nucleus. The other half is an scFv based on the anti-AR monoclonal antibody AR441. The 3E10-AR441 bsAb is expressed in yeast as a single recombinant protein. Treatment of LNCaP with bsAb resulted in nuclear accumulation of the antibody, visualized by confocal microscopy. The 3E10-AR441 bsAb also engaged its target under denaturing and non-denaturing conditions. The bsAb could detect AR when used to probe an AR immunoblot. It could also immunoprecipitate WT AR, as well as mutant/variant AR lacking the LBD (AR(Q640X), Arv7). The scFv 3E10 alone did not bind to AR. BsAb binding affinity to AR was assessed using a competitive sandwich-type ELISA. An anti-AR antibody-coated microtiter plate was used to capture WT AR or mutant/variant AR from cell lysates. AR441-HRP antibody conjugate was mixed with increasing concentrations of 3E10-AR441 or AR441 as competitors. Binding affinity of 3E10-AR441 to WT AR (180nM) was higher than that of the parental AR441 MoAb (7nM). Affinity of bsAb was 3 fold higher to WT AR than to an AR(Q640X) mutant. The bsAb blocked genomic AR signaling in LNCaP cells, as measured by dihydrotestosterone (DHT) activation of both artificial (ARE-luciferase) and endogenous (PSA) reporters. The magnitude of inhibition was similar to that seen with enzalutamide at 5 μM. The 3E10 scFv alone had minimal effect on reporter gene expression. Non-genomic AR signaling was measured by calcium release upon DHT treatment. Calcium5 dye was added to LNCap treated with variable doses of bsAb or 3E10. The fluorescence intensity is proportional to the amount of calcium released. 3E10-AR441 blocked DHT-induced release of calcium while 3E10 did not. Current work is focused on the design of 3E10-AR441 derivatives with enhanced binding affinity towards AR mutants lacking the LBD. 3E10-AR441 bsAb is an attractive therapeutic agent due to its ability to inhibit AR function in a ligand-independent manner.

Citation Format: Nancy L. Golcochea, Maria Garnovskaya, Mary Blanton, Grace Chan, Richard Weisbart, Michael Lilly. Cell-penetrating bispecific antibodies for targeting androgen receptor signaling in advanced prostate cancer. [abstract]. In: Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18-22; Philadelphia, PA. Philadelphia (PA): AACR; Cancer Res 2015;75(15 Suppl):Abstract nr 642. doi:10.1158/1538-7445.AM2015-642

August 2015
Volume 75, Issue 15
Supplement
Table of Contents
Index by author

Search this issue

Sign up for alerts

Request Permissions
patientACCESS
Article Alerts
Email Article
Citation Tools

Share

Like 0

G+1 0

Jump to section
Article
Info & Metrics

Related Articles
No related articles found.
Google Scholar

Cited By...

More in this TOC Section