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TITLE: Tumor metabolism as the Achilles' heel in prostate cancer

PRINCIPAL INVESTIGATOR: Nagalakshmi Nadiminty, PhD

CONTRACTING ORGANIZATION: The University of Toledo

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

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

The University of Toledo
Health Science Campus
3000 Arlington Avenue
Toledo, OH 43614

8. PERFORMING ORGANIZATION REPORT NUMBER

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

14. ABSTRACT

The hypothesis of this proposal was that the inhibition of monocarboxylate transporters (MCTs) can overcome resistance to enzalutamide in therapy-resistant prostate cancer cells. In advanced prostate cancer, glycolysis produces high levels of the toxic by-product lactate. Hence, prostate cancer cells upregulate the expression of MCTs to aid in lactate export. High levels of expression of MCTs have been associated with poor prognosis and biochemical failure in prostate cancer. Our findings so far show that the inhibition of MCT activity can suppress survival and proliferation of enzalutamide-resistant cells preferentially and can inhibit the growth of enzalutamide-resistant xenografts. In addition, we found that the combination of MCT inhibitors with enzalutamide reduced basal and compensatory glycolysis as well as extracellular acidification rates in enzalutamide-resistant prostate cancer cells. Once completed, our project may have an enormous impact on the future of prostate cancer research.

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			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

PCa energetic metabolism is unique. Normal prostate cells use glucose oxidation to synthesize and secrete citrate, resulting in incomplete Krebs cycle and minimal oxidative phosphorylation for energy production. In contrast, PCa cells do not secrete citrate, but reactivate the Krebs cycle as the energy source. The accumulation of the metabolic endproduct lactate in either case is toxic. In response, cancer cells upregulate the expression of monocarboxylate transporters (MCTs) to increase lactate efflux, thereby reducing extracellular acidification. Our preliminary data showed that: 1) the expression of MCTs is higher in PCa tissues and PCa cells resistant to enzalutamide; 2) MCT antagonists resensitize resistant cells to enzalutamide; and 3) the inhibition of MCTs has no significant effects on normal prostate cells. Hence, we hypothesized that the **inhibition of MCTs in therapy-resistant PCa cells may augment the efficacy of targeted therapeutics such as enzalutamide**.

Specific aims proposed:

1) Test the combination of MCT inhibitors with enzalutamide in PCa cells in vitro
2) Test the combination of MCT inhibitors with enzalutamide in PCa cells and xenografts in vivo
3) Analyze the mechanisms governing the synergism between MCT inhibitors and enzalutamide

We believe MCT inhibition would be a highly innovative strategy to overcome enzalutamide resistance in PCa.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Prostate cancer, enzalutamide, androgen receptor, monocarboxylate transporter, metabolism, glycolysis, resistance

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Research-Specific Tasks:

Specific Aim 1: To test the combination of MCT inhibitors with enzalutamide in PCa cells *in vitro*				
Major Task 1: To characterize the effects of MCT inhibitors in				

enzalutamide-resistant cells in vitro				
Subtask 1: Treat cell lines with AR-C155858, AZD3965, or syrosingopine either singly or in combination with enzalutamide and analyze Cell lines used: C4-2B parental, C4-2B-MDVR; 22Rv1 parental, 22Rv1-MDVR; VCaP parental, VCaP-MDVR	1-6	Sayani Bhattacharjee	Completed; see accomplishments	
Subtask 2: Confirm above effects with MCT shRNAs Cell lines used: C4-2B parental, C4-2B-MDVR; 22Rv1 parental, 22Rv1-MDVR; VCaP parental, VCaP-MDVR	7-12	Sayani Bhattacharjee	In progress	
Subtask 3: Explore structure-activity relationships to optimize effects of MCT inhibitors in collaboration with College of Pharmacy, UT	9-12	TBD	Not started due to unforeseen effects of the COVID-19 pandemic	
Subtask 4: Generate resistant cell lines and analyze relative activities of MCT inhibitors	10-14	Dr. Nadiminty, Sayani Bhattacharjee	Cell line generation in progress	
Milestone(s) Achieved: Characterization of the effects of MCT inhibitors in vitro				
Specific Aim 2: To test the combination of MCT inhibitors with enzalutamide in PCa cell xenografts and patient-derived xenografts *in vivo*				
Major Task 2: To characterize the effects of MCT inhibitors in enzalutamide-resistant cells and PDX models in vivo				
Subtask 1: Submit documents for ACURO approvals	8-12	Dr. Nadiminty	Completed; approval granted 04/13/2021	
Subtask 2: Generate xenografts of enzalutamide-resistant cells in SCID mice, treat with MCT inhibitors either singly or in combination with enzalutamide	12-16	Dr. Nadiminty, Sayani Bhattacharjee	Completed work with C4-2B and C4-2B-MDVR cells and AR-C155858 and AZD3965	

Cell lines used: C4-2B parental, C4-2B-MDVR; VCaP parental, VCaP-MDVR Animals requested: 352; Supplier: Charles River				
Subtask 3: Pharmacokinetic analyses of MCT inhibitors in SCID mice	15-18	Dr. Nadiminty, Dr. Sarver, Sayani Bhattacharjee	Planned in the next reporting period	
Subtask 4: Analyze the effects of MCT inhibitors in PDX models Animals requested: 120; Supplier: Living Tumor lab	15-21	Dr. Nadiminty, Dr. Petros, Sayani Bhattacharjee	Planned in the next reporting period	
Milestone(s) Achieved: Characterization of the effects of MCT inhibitors and their pharmacokinetic/toxicity profiles in vivo				
Specific Aim 3: Analyze the mechanisms governing the synergistic activity of MCT inhibitor and enzalutamide combination				
Major Task 3: Metabolomic analyses to analyze the pathways involved in enzalutamide resistance and the effects of MCT inhibitors				
Subtask 1: Analyze extracellular flux, glucose metabolism, intracellular lactate levels, cellular acidification, and cell death in cells treated with MCT inhibitors	21-24	Dr. Nadiminty, Sayani Bhattacharjee	Completed experiments with extracellular acidification, glycolysis, and cell death; see accomplishments	
Subtask 2: Perform LC-MS metabolomic analyses of cellular metabolite levels	24-30	Dr. Nadiminty, Dr. Matam Vijay Kumar, Sayani Bhattacharjee	Not started	
Subtask 3: Analyze results and repeat/optimize experiments, if necessary	30-34	Dr. Nadiminty, Sayani Bhattacharjee		

Major Task 4: Prepare data for publication and submit 1-2 manuscripts	30-36	Dr. Nadiminty, Sayani Bhattacharjee and/or Graduate student (TBD)		
Milestones achieved: 1-2 peer-reviewed journal articles				

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities:

a) To test the combination of MCT inhibitors in combination with enzalutamide in PCa cells in vitro

b) To generate shRNAs against MCTs and confirm that the observed effects are due to MCT expression

c) To submit IACUC protocols for institutional approval of animal work

d) To submit ACURO documents for in vivo animal work and obtain approval

e) To generate enzalutamide-resistant cell xenografts in mice and treat them with MCT inhibitors to analyze effects on tumor growth

f) Perform Seahorse-based Glycolytic stress or Glycolytic rate assays to measure levels of extracellular acidification, glycolysis, and cell death in PCa cells treated with MCT inhibitors singly or in combination with enzalutamide

2) Specific objectives:

a) We treated C4-2B parental, C4-2B-MDVR; or VCaP parental, VCaP-MDVR cells with AR-C155858, AZD3965, or syrosingopine singly or in combination with enzalutamide and tested cell survival and cell proliferation. Cell survival was measured using the Cell Viability Fluor kit (Promega) and cell proliferation was measured using the Cell-Titer One kit (Promega).

b) We obtained shRNAs against MCTs 1,2, and 4 from Open Biosystems (ThermoFisher). These shRNAs were propagated in bacteria to obtain larger quatities of plasmids. Next, we tested their knock down efficiency in PCa cells using qPCR and Western blotting. Work to stably transfect the shRNAs into enzalutamide-resistant cells is on-going.

c) We are in the process of generating MCT inhibitor-resistant cell lines. We have encountered problems with cell viability after longer term culture and the process is taking longer than expected.

d) We generated sub-cutaneous xenografts of C4-2B and C4-2B-MDVR cells in SCID mice (n=10/group). After the tumors were palpable, we divided them randomly into 6 groups and treated via oral gavage daily with 1) vehicle, 2) 25 mg/kg enzalutamide, 3) 10 mg/kg AR-C155858, 4) 50 mg/kg AZD3965, 5) Enza+AR-C155858, or 6) Enza+AZD3965. Syrosingopine was not included in this study due to the inability of the supplier to supply sufficient quantities of syrosingopine in time. We measured tumor growth twice a week and mouse weights twice a week. When the control tumors reached a size of 2 cm^3, the mice were euthanized, and tumor tissues were collected. Immunohistochemistry to assess levels of proliferation and apoptosis in the tumor tissues is on-going.

e) We performed the Glycolysis stress test (Agilent) using the Seahorse Extracellular Flux analyzer to measure the extracellular acidification rate (ECAR) in PCa cells treated with MCT inhibitors singly or in combination with enzalutamide. After completing the experiments, the company changed their guidance for the calculation of proton efflux rate (PER). Hence, we were advised to use the Glycolytic rate assay for the accurate measurement of glycolytic PER (glycoPER). Accordingly, the Glycolytic rate assay was performed for accurate measurement of glycoPER and ECAR and the results are reported below.

f) We are analyzing the significant results obtained so far and preparing a manuscript for publication.

3) Key Outcomes: (Please see pages at the end of this document for figures)

a) We found that the combination of MCT inhibitors with enzalutamide suppressed cell survival significantly in C4-2B-MDVR (Figs. 1-3) and VCaP-MDVR (Figs. 4-6) enzalutamide-resistant cells compared with parental cells.

b) Similarly, cell proliferation in C4-2B-MDVR and VCaP-MDVR cells was reduced significantly when treated with a combination of enzalutamide and MCT inhibitors (Fig. 7) compared with parental cells.

c) We have obtained approvals for animal work from the University of Toledo IACUC and the ACURO.

d) Growth of C4-2B-MDVR xenografts was suppressed significantly when treated with AR-C155858 or AZD3965 or their combinations with enzalutamide; interestingly, the MCT inhibitors or combinations with enzalutamide had little effect on C4-2B parental cell xenograft growth (Fig. 8-

9), indicating that enzalutamide-resistant xenografts are more sensitive to treatment with MCT inhibitors.

e) Glycolytic rate assays revealed that levels of basal glycolysis were similar in parental and enzalutamide-resistant cells (Figs. 10-11). We also found that basal as well as compensatory glycolysis rates were reduced more significantly in MDVR cells compared with parental cells (Figs. 10A-B & 11A-B). The combination of MCT inhibitors with enzalutamide virtually eliminated basal and compensatory glycolysis levels in both C4-2B-MDVR (Figs. 10A-B) and 22Rv1-MDVR (Figs. 11A-B). Similarly, as expected, extracellular acidification rates were reduced significantly in cells treated with MCT inhibitors (Figs. 10C & 11C). These results correlated with levels of cell death observed in the different treatment groups. Surprisingly, we found that enzalutamide also reduced extracellular acidification in PCa cells, which we will explore in the next reporting period.

f) **Conclusions**: Taken together, these results indicate that our hypothesis about the utility of MCT inhibitors in overcoming resistance to enzalutamide may be valid. Further work will shed light on the specific mechanisms underlying these observations.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

We plan to perform the pharmacokinetic analyses of MCT inhibitors and their combinations with enzalutamide in collaboration with Dr. Sarver. We plan to perform structure-activity relationship studies in collaboration with the Center for Drug Design at the University of Toledo. We plan to complete the in vitro studies with MCT shRNAs and resistant cells.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We have had problems with recruiting a post-doctoral fellow due to the COVID-19 pandemic. We hope to be able to recruit one during the next reporting period.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We have had problems with recruiting a post-doctoral fellow due to the COVID-19 pandemic. We hope to be able to recruit one during the next reporting period.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Sayani Bhattacharjee, Rebecca Wynn, Jonathan Doan, Tariq Shah, Puneet Sindhwani, Firas Petros, Nagalakshmi Nadiminty. (in preparation) Monocarboxylate transporter inhibition overcomes enzalutamide resistance in prostate cancer cells. (To be submitted)

- **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic*

information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1) Presented a poster at the Annual Meeting of the SBUR, 2020 (virtual meeting): MCT inhibition as a potential therapeutic strategy to target enzalutamide-resistant prostate cancer
2) Presented a poster at the Annual Meeting of the AACR, 2021 (virtual meeting): MCT inhibition as a potential therapeutic strategy to target enzalutamide-resistant prostate cancer

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

1) Name:	Nagalakshmi Nadiminty
Project Role:	PI
ORCID:	0000-0003-3408-3206
Nearest person months worked:	1
Contribution to project:	Dr. Nadiminty has performed work with vertebrate animals and standardized Seahorse assays.
Funding support:	College of Medicine and Life Sciences, University of Toledo
2) Name:	Sayani Bhattacharjee
Project Role:	Graduate Student
ORCID:	0000-0001-8781-5867
Nearest person months worked:	12
Contribution to project:	Ms. Bhattacharjee performed most of the in vitro work and assisted in the in vivo work.
Funding support:	None
3) Name:	Rebecca Wynn
Project Role:	Research Associate
ORCID:	0000-0002-2801-6798
Nearest person months worked:	3
Contribution to project:	Ms. Wynn assisted in in vivo work and acted as lab manager.
Funding support:	Department of Urology, COMLS, University of Toledo

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Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial

or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

All the following pages contain data and images that are unpublished. Please protect.

We treated C4-2B parental, C4-2B-MDVR; or VCaP parental, VCaP-MDVR; or 22Rv1 parental or 22Rv1-MDVR cells with AR-C155858, AZD3965, or syrosingopine either singly or in combination with enzalutamide. We found that the combination of MCT inhibitors with enzalutamide suppressed cell survival and cell proliferation significantly. We also found that MDVR cells are more sensitive to treatment with MCT inhibitors compared to the parental cells. In vivo mouse xenograft assays indicated that MCT inhibitors synergize with enzalutamide to reduce tumor growth kinetics. Seahorse-based Glycolytic rate assays indicated that the combination of enzalutamide and MCT inhibitors is much more effective in reducing extracellular acidification (ECAR) and compensatory glycolysis rates, especially in enzalutamide-resistant cells.

Fig. 1A

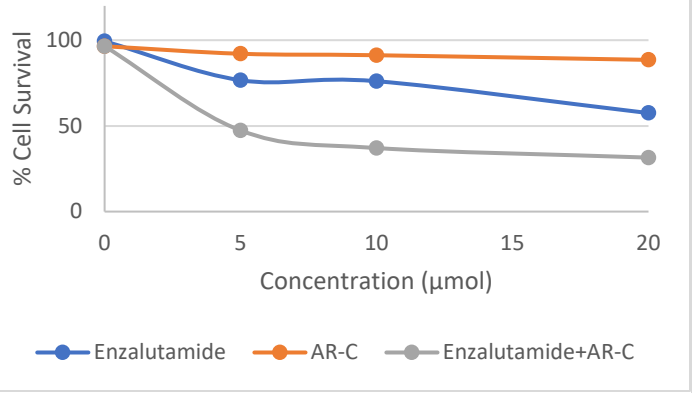

Fig. 1B

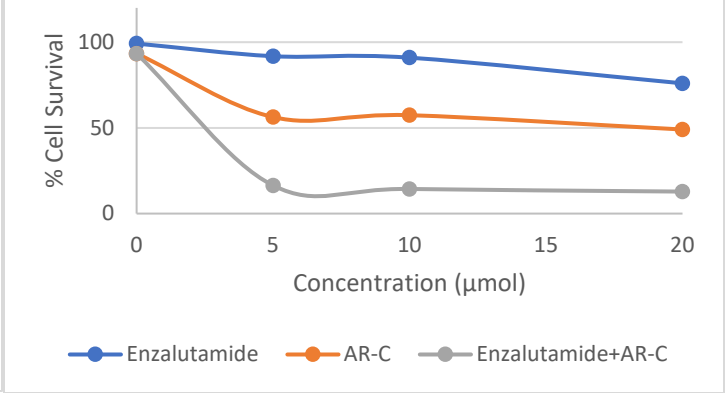

Fig. 2A

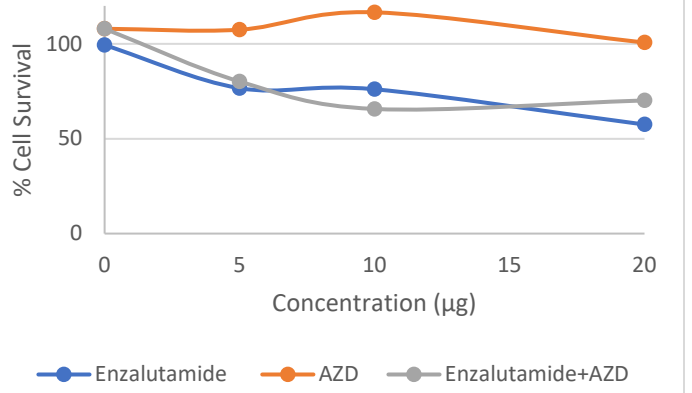

Fig. 2B

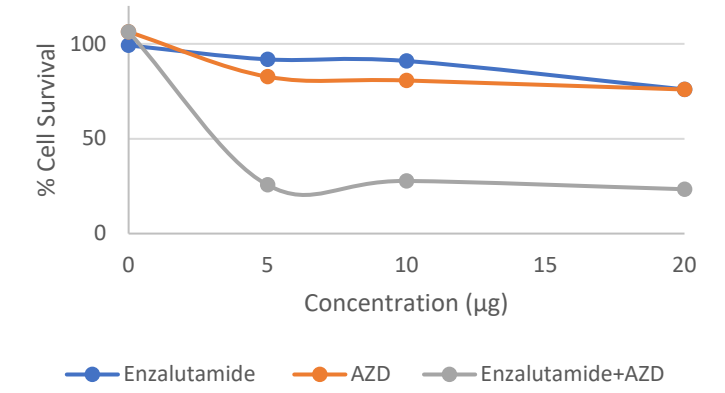

Fig. 3A

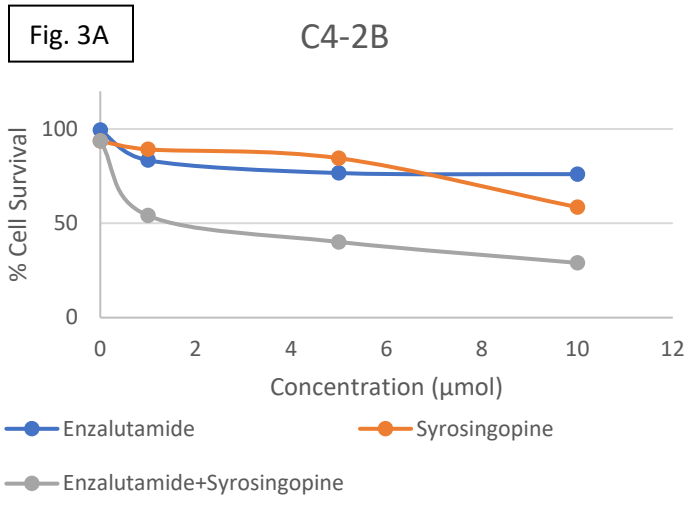

Fig. 3B

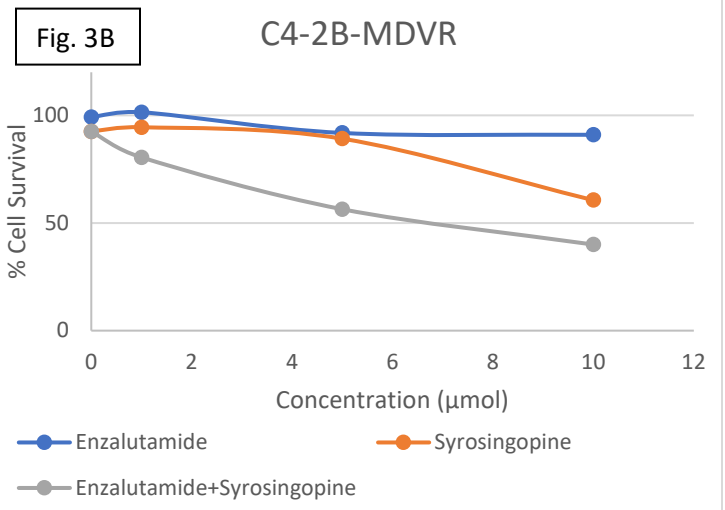

Fig. 4A

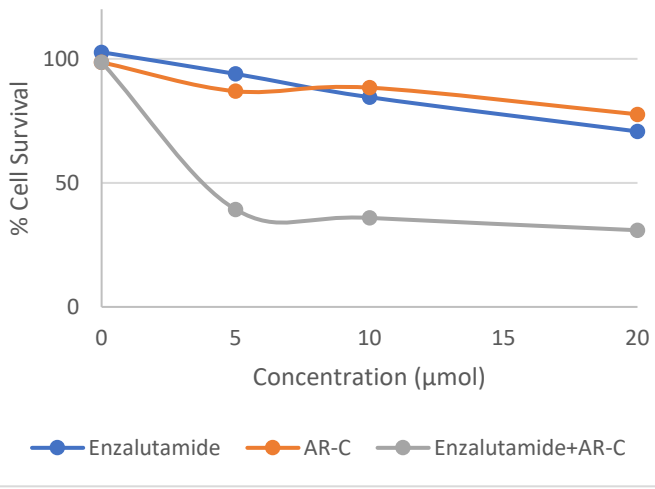

Fig. 4B

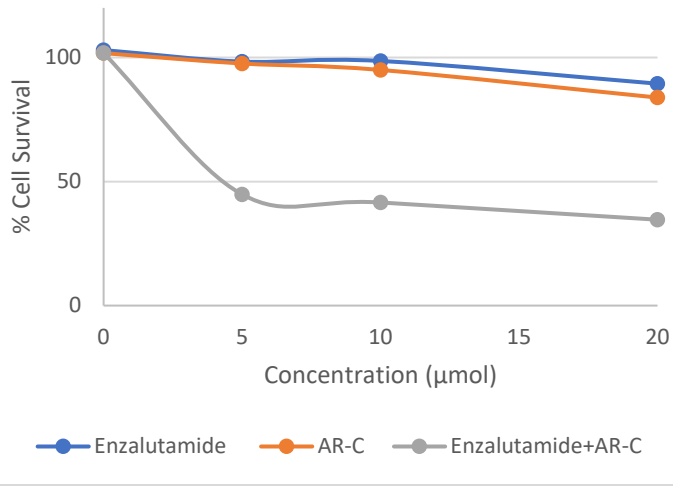

Fig. 5A

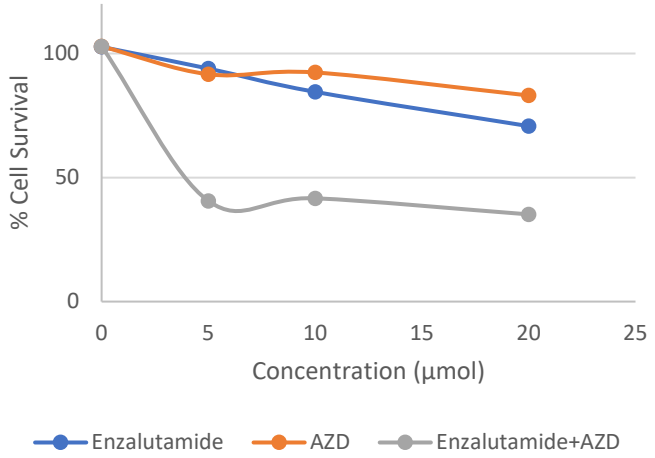

Fig. 5B

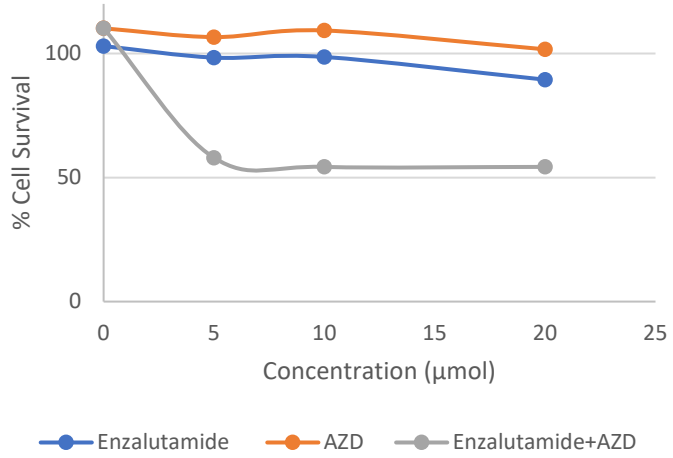

Fig. 6A

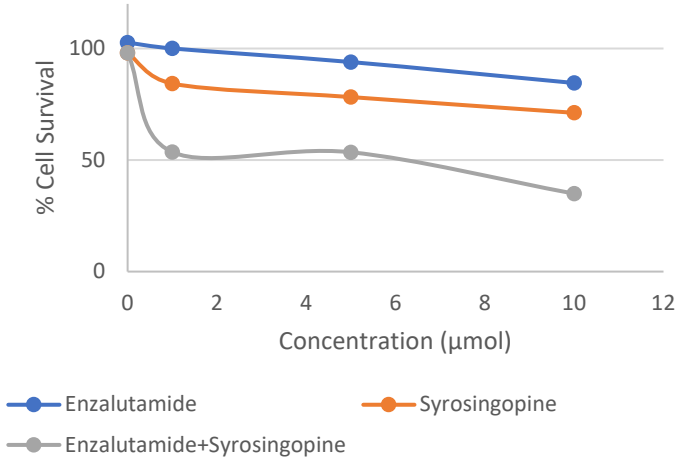

Fig. 6B

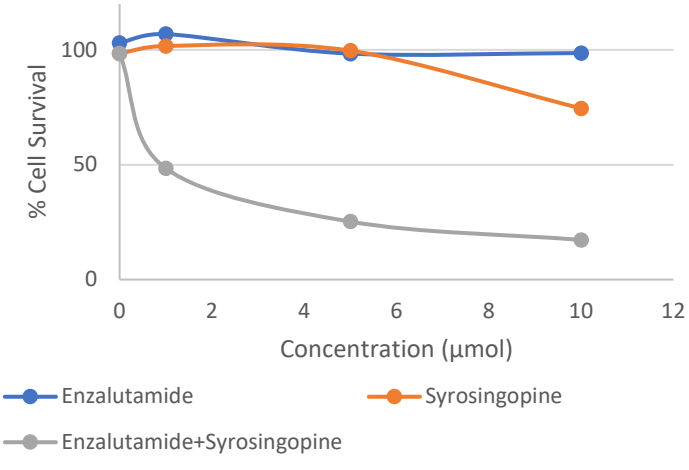

Fig. 7A

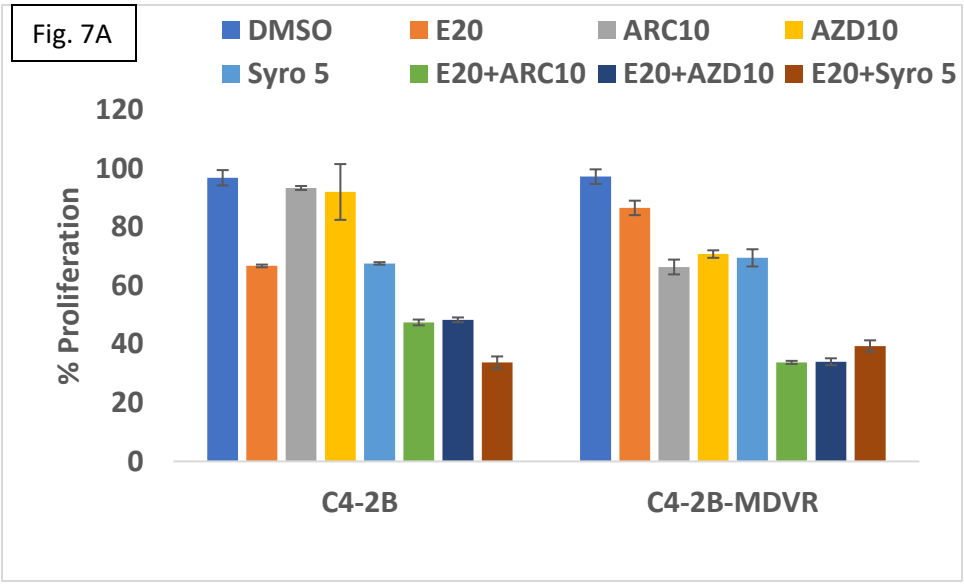

Fig. 7B

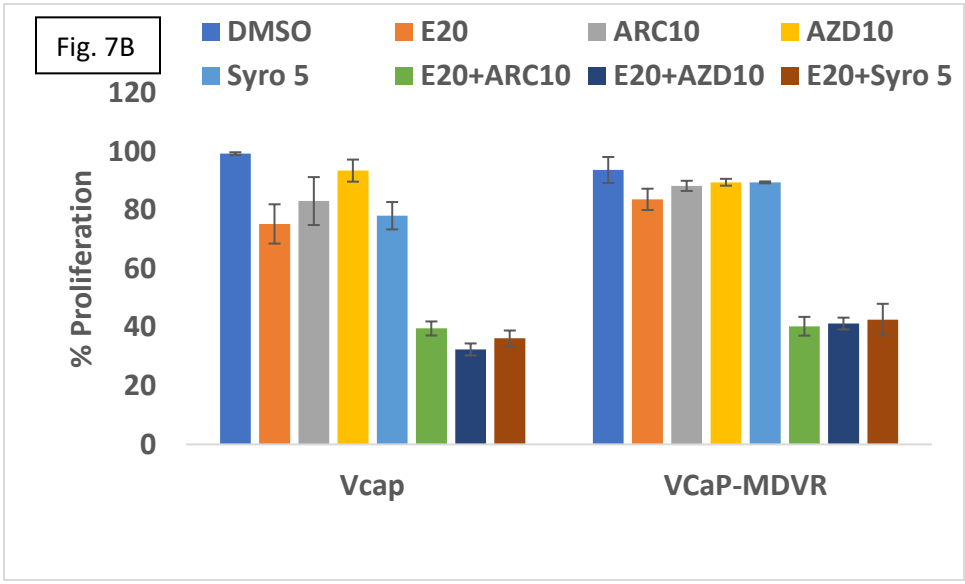

Fig. 8A

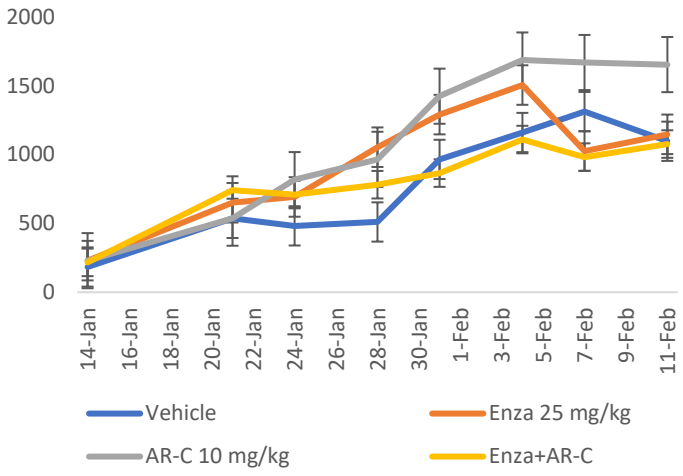

Fig. 8B

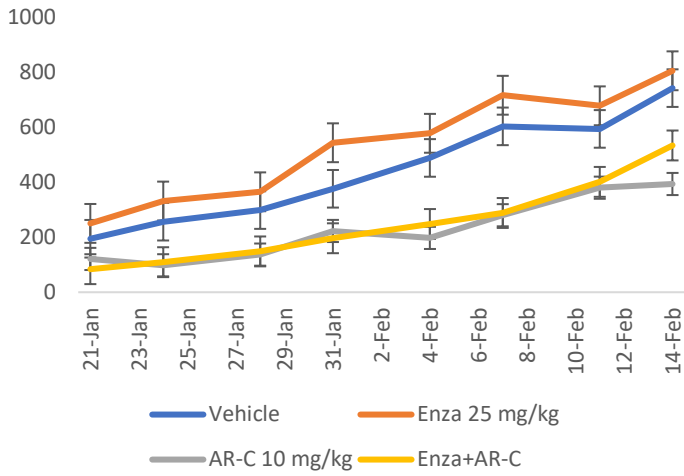

Fig. 9A

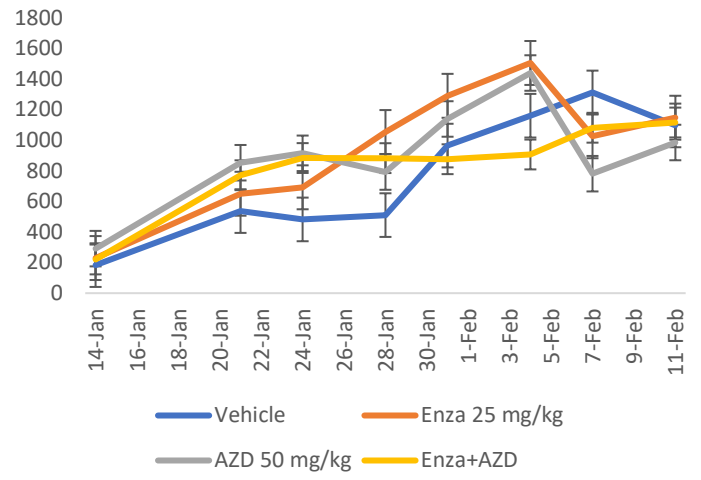

Fig. 9B

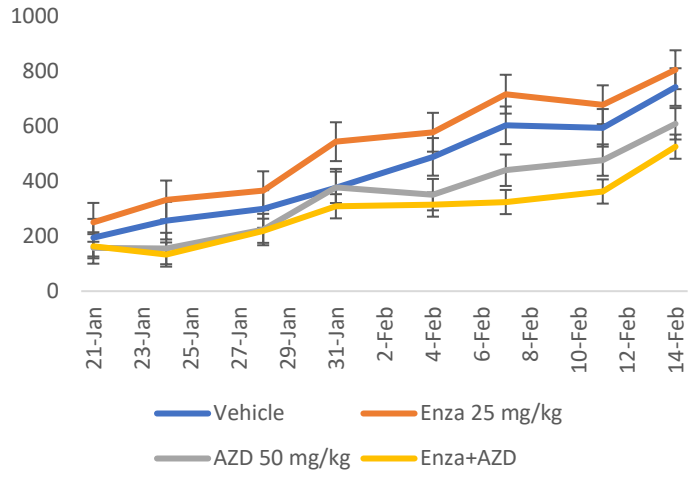

Fig. 10A

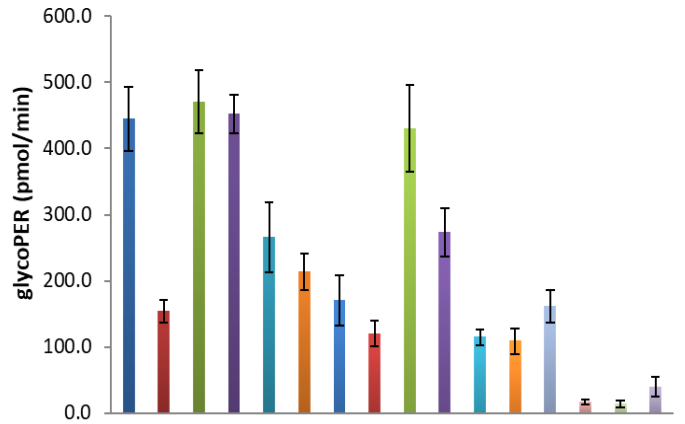

Fig. 10B

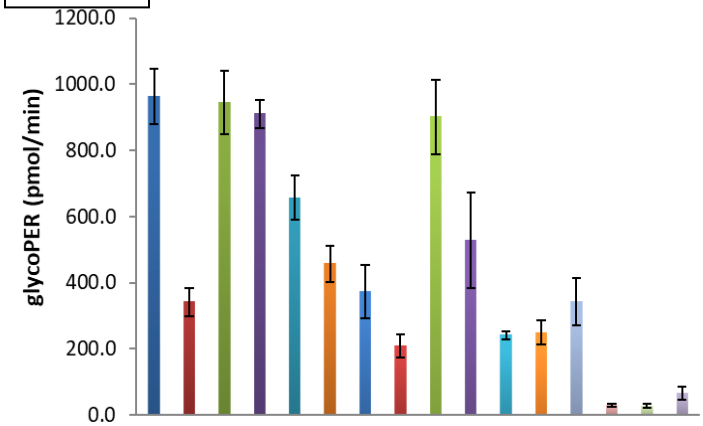

Fig. 10C

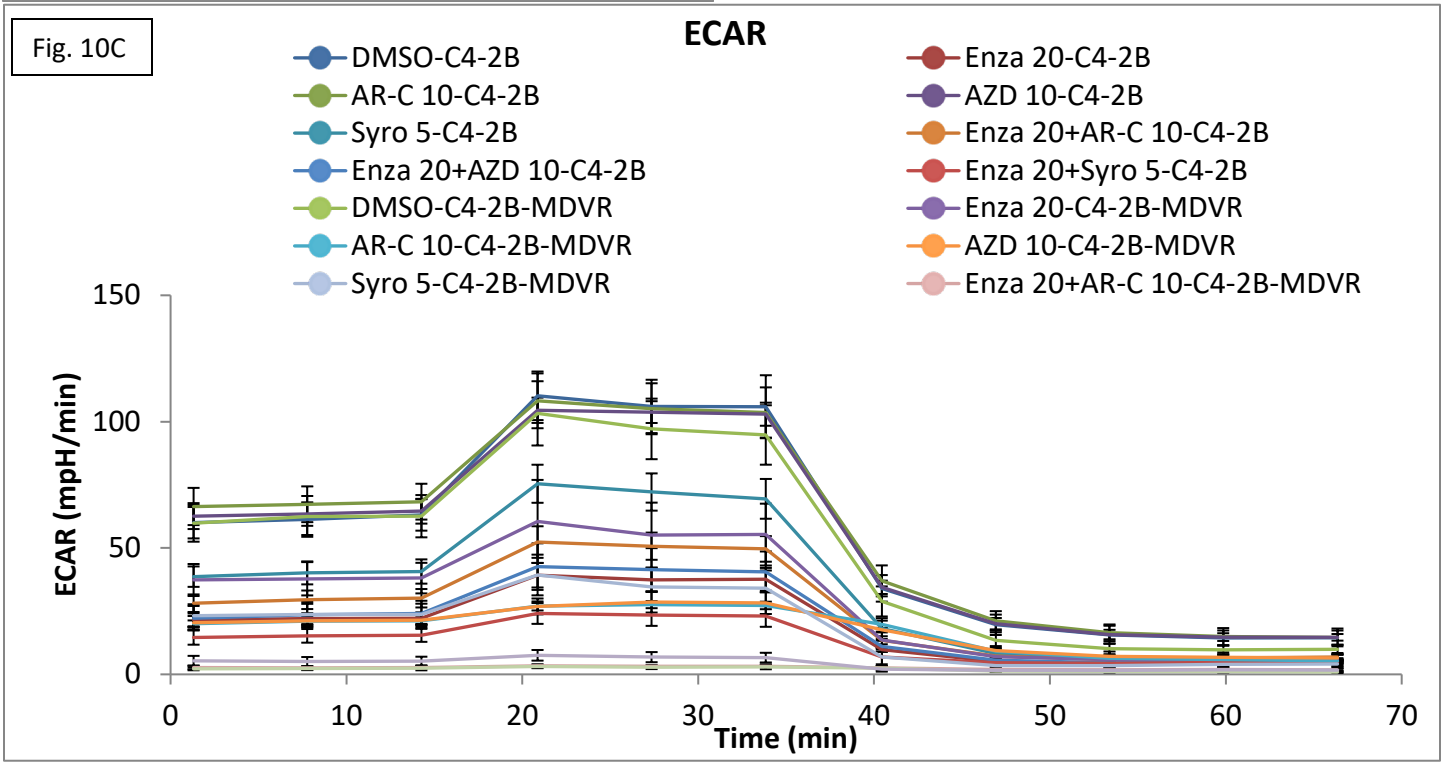

Fig. 11A

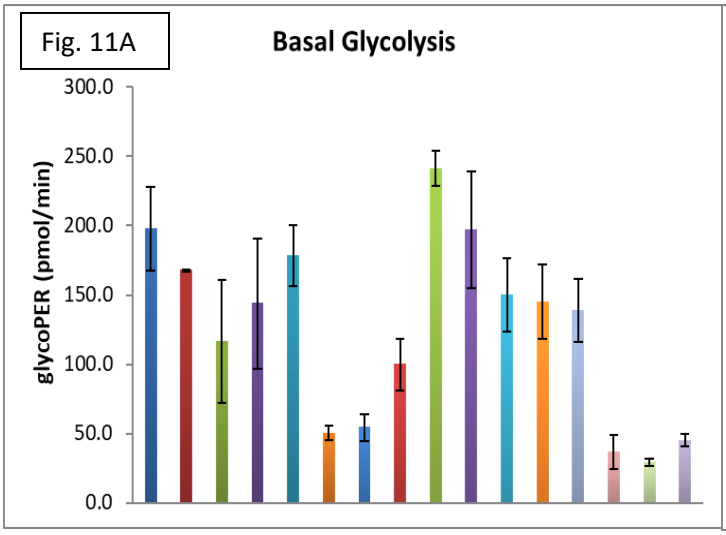

Fig. 11B

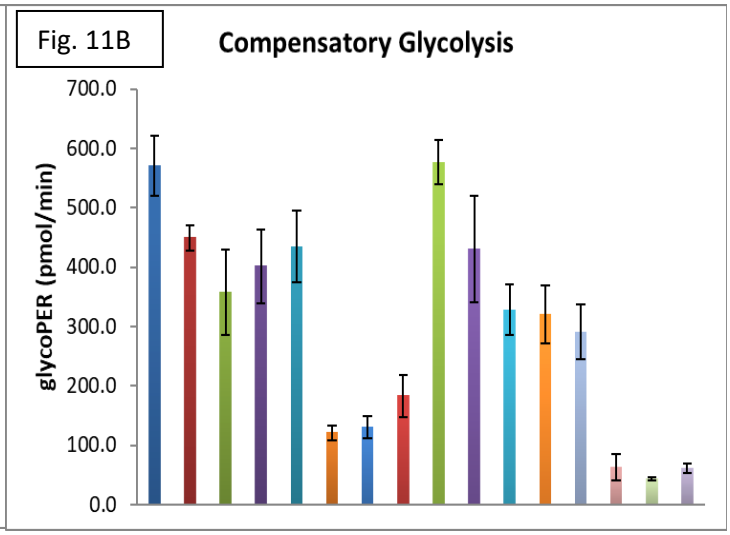

Fig. 11C