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Full Reference: Targeting Mitochondrial Metabolism and Tumour Hypoxia – A Promising Strategy to Improve the Radiosensitivity of Diffuse Midline Gliomas. Faiqa Mudassar[1,2], Cecilia Chang[3], Prunella Ing[1,2], Kristina M Cook[2,4], Geraldine O'Neill[5,6], *Han Shen[1,2] and *Eric Hau[1,2,7,8]
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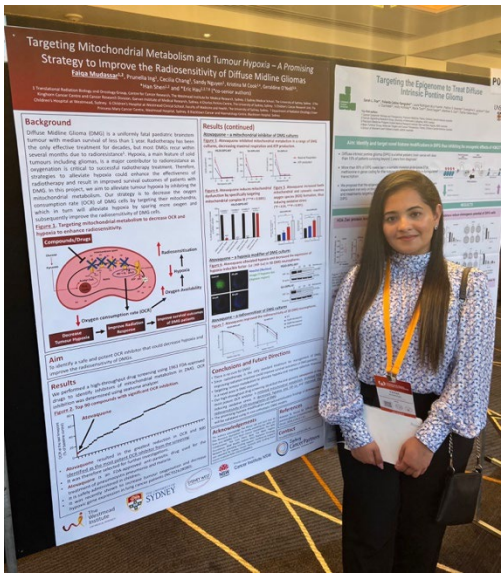
Conference/Meeting Name: Children's Brain Cancer Conference 2023
Location: Brisbane, QLD, Australia
Dates: 23-24th March 2023
Presentation Type: Poster Presentation



"CBCC2023 Day 2 with my supervisor – Dr Han Shen"

"CBCC2023 was an amazing opportunity. Got to share my research and hear from and meet some great researchers in my field. I am very grateful for the travel grant from Sydney Cancer Partners and Cancer Institute NSW that supported me to attend this wonderful conference."

The Children's Brain Cancer Conference was held on 23-24th March 2023 at Sofitel Hotel Brisbane Central. This conference brought together some of the brightest minds working on children's brain

cancer research. It was attended by 120 delegates with 14 invited speakers with several oral and poster presentations. The two-day conference covered a range of themes including gliomagenesis and preclinical tumour models, immunotherapy for treating gliomas, clinical translation, clinical trial design, survivorship and emerging breakthroughs in children's brain cancer research. The key invited speakers included Dr Pratiti (Mimi) Bandopadhayay, A/Prof Raelene Endersby, A/Prof Misty Jenkins, Prof Di Yu, Dr Carolyn Shembrey, Prof David Eisenstat, Prof Jorden Hansford, Prof Darren Hargrave, Dr Kim Carter, Dr Donald Mabbott, Dr Erin Pitt, Dr Hana Starobova and Prof Chris Jones, A/Prof Andrew Ellisdon.

It felt amazing to meet some great researchers in my field, all with a common goal to contribute towards finding a cure for the deadly and incurable paediatric brain tumours. All the presentations showcased exciting and novel research happening in this area. It was great to see everyone actively engaging in the question/answer sessions following each presentation.

I travelled to the conference with my PhD co-supervisor Dr Han Shen who introduced me to a lot of his previous colleagues working in the DMG area. We learnt and discussed a range of ideas to apply to our ongoing work at WIMR. Both of us met A/Prof Raelene and discussed how her lab performs serial *in vivo* passage of medulloblastoma cells using orthotopic xenograft models as we intended to perform similar experiment using our DMG models. This helped us troubleshoot the issues we have faced when isolating DMG tumour cells from the mouse brain. We also met Prof Geraldine and our other collaborators including Dr Ryan Dutchatel and discussed our ongoing animal work and shared ideas.

Overall, CBCC2023 was an amazing learning experience. It provided me with a wealth of new knowledge and experimental ideas which I could apply to my PhD project. It was great to see a lot of the work focusing on drug repurposing for DMG treatment as it is a faster approach towards clinical translation of compounds from bench to bedside. The learning outcomes of CBCC2023 are relevant to the wider Sydney Cancer Partners membership as all the presentations had a clinical translation element with an overall aim to improve survival outcomes of paediatric cancer patients.

A nice conference dinner was arranged for the attendees at the end of conference day 1. The conference concluded with a networking function at the end of day 2. I am very thankful to Sydney Cancer Partners and Cancer Institute NSW for the travel grant that supported me attend this fantastic conference.

Targeting Mitochondrial Metabolism and Tumour Hypoxia – A Promising Strategy to Improve the Radiosensitivity of Diffuse Midline Gliomas

Faiqa Mudassar[1,2], Prunella Ing[1], Cecilia Chang[3], Sandy Nguyen[1], Kristina M Cook[2,4], Geraldine O'Neill[5,6], *Han Shen[1,2] and *Eric Hau[1,2,7,8] (*co-senior authors)

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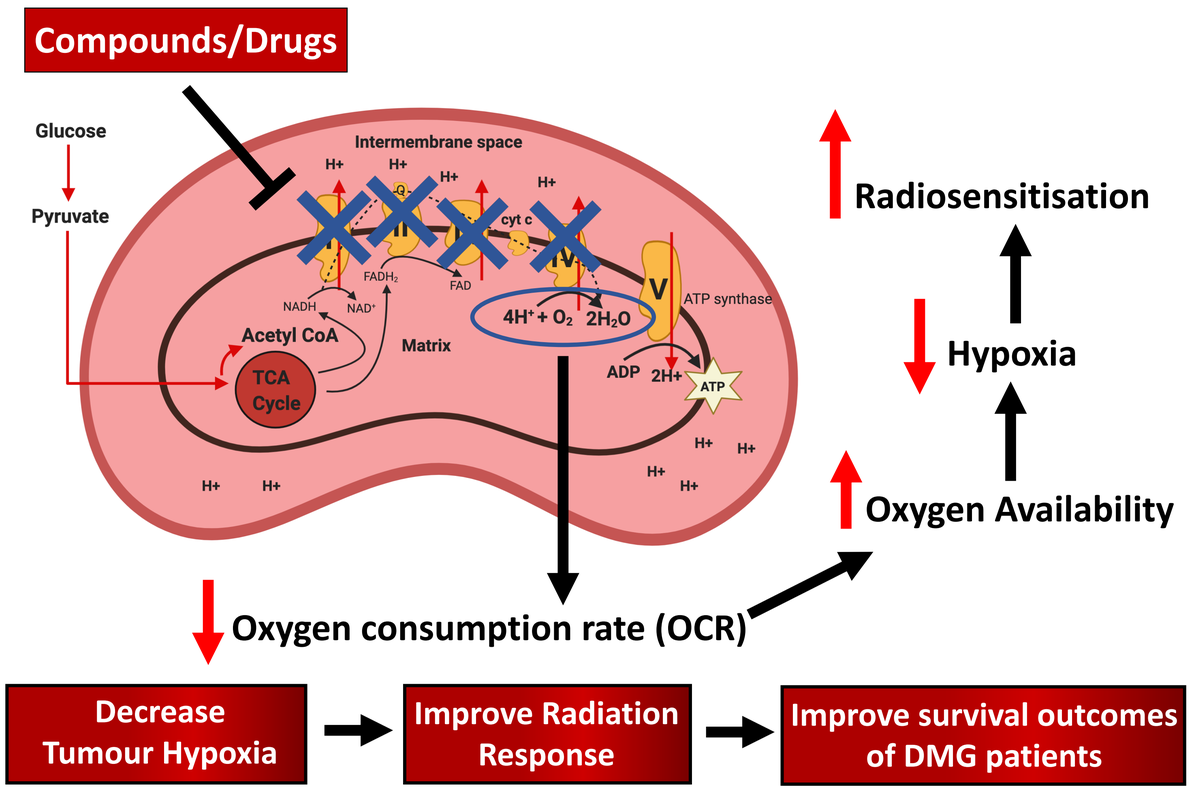
Background

Diffuse Midline Glioma (DMG) is a uniformly fatal paediatric brainstem tumour with median survival of less than 1 year. Radiotherapy has been the only effective treatment for decades, but most DMGs recur within several months due to radioresistance[1]. Hypoxia, a main feature of solid tumours including gliomas, is a major contributor to radioresistance as oxygenation is critical to successful radiotherapy treatment. Therefore, strategies to alleviate hypoxia could enhance the effectiveness of radiotherapy and result in improved survival outcomes of patients with DMG. In this project, we aim to alleviate tumour hypoxia by inhibiting the mitochondrial metabolism. Our strategy is to decrease the oxygen consumption rate (OCR) of DMG cells by targeting their mitochondria, which in turn will alleviate hypoxia by sparing more oxygen and subsequently improve the radiosensitivity of DMG cells.

Figure 1. Targeting mitochondrial metabolism to decrease OCR and hypoxia to enhance radiosensitivity.



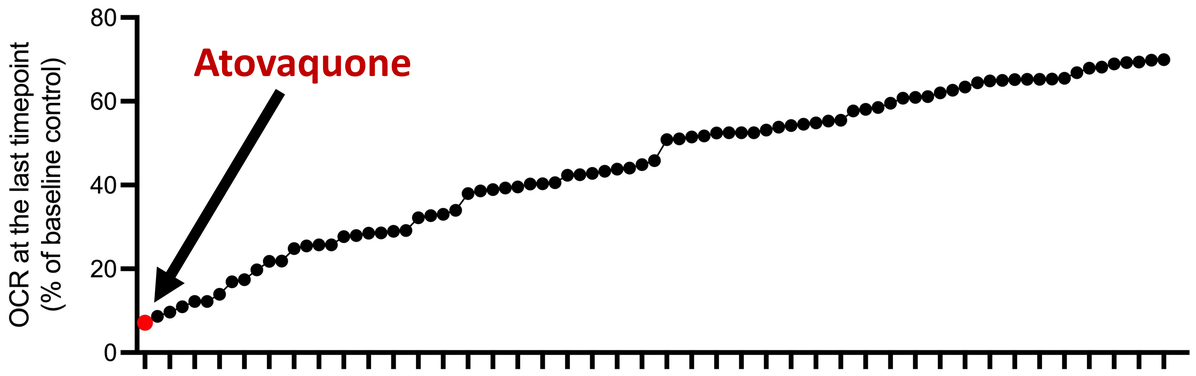
Aim

To identify a safe and potent OCR inhibitor that could decrease hypoxia and improve the radiosensitivity of DMGs.

Results

We performed a high-throughput drug screening using 1963 FDA-approved drugs to identify inhibitors of mitochondrial metabolism in DMG. OCR inhibition was determined using seahorse analyzer.

Figure 2. Top 90 compounds with significant OCR inhibition.



- **Atovaquone** resulted in the greatest reduction in OCR and was identified as the most potent OCR inhibitor from the screening.
- It was therefore selected for further investigations.
- **Atovaquone** is an FDA-approved anti-parasitic drug used for the treatment of pneumocystis pneumonia and malaria.
- It is safely administered in children.
- It was recently shown to increase tumour oxygenation and decrease hypoxic gene expression in lung cancer patients (NCT02628080).

Results (continued)

Atovaquone – a mitochondrial inhibitor of DMG cultures

Figure 3. Atovaquone inhibited mitochondrial metabolism in a range of DMG cultures, decreasing maximal respiration and ATP production.

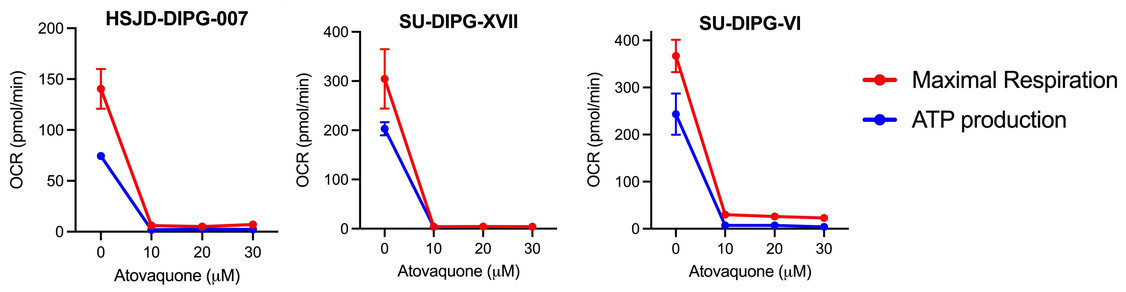


Figure 4. Atovaquone induces mitochondrial dysfunction by specifically targeting mitochondrial complex III (*P < 0.0001).**

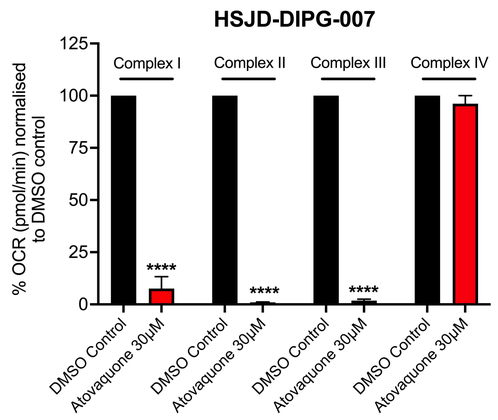
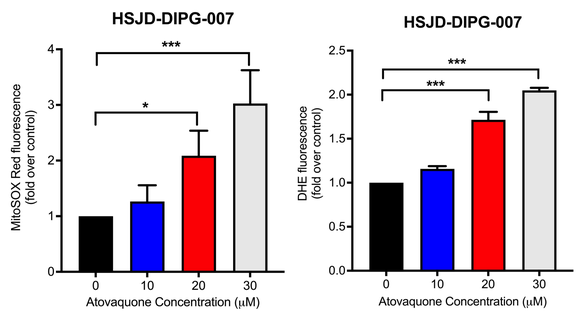
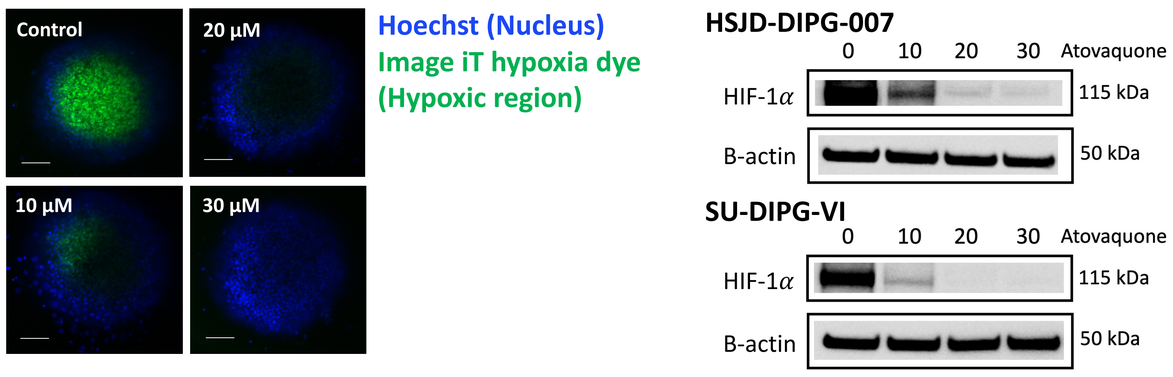


Figure 5. Atovaquone increased both mitochondrial and cytosolic reactive oxygen species (ROS) formation, thus inducing oxidative stress (*P < 0.05, ***P < 0.001).



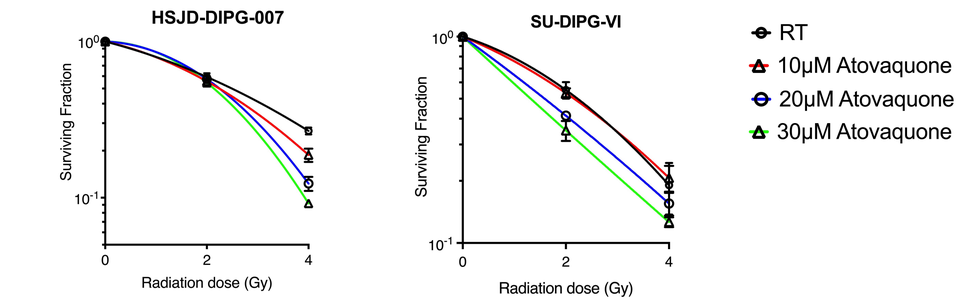
Atovaquone – a hypoxia modifier of DMG cultures

Figure 6. Atovaquone alleviated hypoxia and decreased the expression of hypoxia-inducible factor -1α (HIF-1α) in 3D DMG neurospheres.



Atovaquone – a radiosensitizer of DMG cultures

Figure 7. Atovaquone improved the radiosensitivity of 3D DMG neurospheres.



Conclusions and Future Directions

- There is no cure for DMG!
- Since radiotherapy is the only standard treatment for management of DMG, improving radiation response could improve survival outcomes of DMG patients.
- Targeting tumour metabolism to alleviate hypoxia and improve radiation response is a novel area in DMG research.
- Our high-throughput drug screening identified the anti-parasitic **atovaquone** as the most potent OCR inhibitor. It inhibited mitochondrial metabolism, increased ROS inducing oxidative stress. It decreased hypoxia and HIF-1α and improved the radiosensitisivity of 3D neurospheres of DMG.
- The promising hypoxia modification and radiosensitisation findings of **atovaquone** will be validated using *in vivo* orthotopic DMG models.

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