2022

# Sustainable healthcare: A future for all

Princess Alexandra Hospital Health Symposium 23–26 August 2022

# Establishing a real time metabolic assay for bladder cancer models to assess metabolic vulnerabilities and identify potential targets for combination therapy

**Alivia Calabrese**[1,2,3,4], Jennifer Gunter [1,3], Patrick B. Thomas [1,2,3,4], Elizabeth D. Williams [1,2,3,4], Ian Vela [1,2,3,4,5]

1. Queensland University of Technology (QUT), Faculty of Health, School of Biomedical Sciences at Translational Research Institute, Woolloongabba, Queensland 4102, Australia.
2. Queensland Bladder Cancer Initiative (QBCI), Brisbane, Queensland 4102, Australia.
3. Australian Prostate Cancer Research Centre – Queensland (APCRC-Q), Brisbane, Queensland 4102, Australia
4. Centre for Personalised Analysis of Cancers (CPAC), Brisbane, Queensland 4102, Australia.
5. Department of Urology, Princess Alexandra Hospital, Woolloongabba, Queensland 4102, Australia.

## Summary

Bladder cancer presents as a highly heterogenous disease that frequently recurs post-therapy with limited chemotherapy options for patients if ineligible, or if chemotherapy treatment has failed. Altered cellular metabolism promotes tumourigenesis and resistance to standard-of-care (SOC) therapies. Understanding the intricate bioenergetics of bladder cancer, *in situ,* in the tissue microenvironment may identify novel therapeutic targets to improve patient outcomes. Current models are limited to 2D tumour cell lines which fail to recapitulate the TME contribution. Hence, there is a critical need for more representative ex vivo models to capture tumour-specific metabolic alterations of BC and identify potentially targetable pathways leading to disease progression.


## Methodology

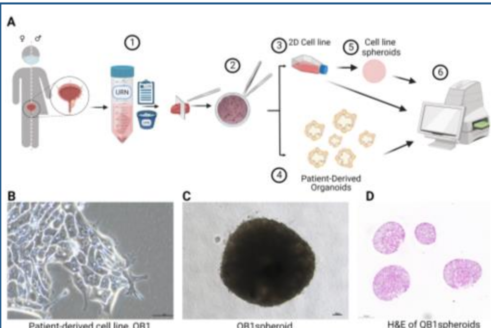



**Figure 1. A)** Pipeline from patient tissue to bioenergetic studies. 1) Patient tissue arrives from surgery and is immediately finely dissected in the lab. 2) Tissue is filtered for cell lines and organoids. 3) Largest filtration is seeded to create 2D cell lines and validated (via genomics). 4) Organoids develop over time a period of 7-14 days. 5) Cell lines grown in 2D and 3D spheroids. 6) Metabolic analysis using Seahorse XFe96 analyser. **B)** 2D culture of primary cell lines. **C)** Spheroid in culture. **D)** Histology of spheroids with necrotic core.

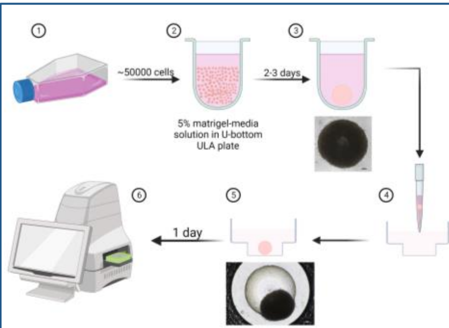



**Figure 2. Generation of cell line spheroids to use in the Seahorse XFe Analyser**. 1) Grow cells in T75 flask and passage. 2) Seed cells at ~50 000 cells in 5% Matrigel/media solution in U-bottom Ultra-low attachment plate. 3) Allow spheroids to grow for 2-3 days. 4) Seed spheroid into Seahorse plate. 5) Allow spheroid settle in well overnight 6) Proceed with Seahorse experiment.

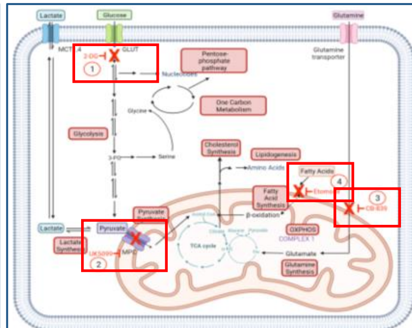

**Figure 3. Metabolic pathways of cancer cell.** Metabolic inhibitors act on major fuel entry points to determine fuel dependency. These inhibitors are used during Seahorse XFe96 analysis to identify dependency and flexibility (red crosses) in metabolic pathways to record measurements of oxygen consumption rate and extracellular respiration rate as an indicator of metabolic activity.

## Results

- Our results show that bladder cancer cell lines derived from primary patient tissue are highly dependent on glucose, shown by a loss of OXPHOS when this fuel source is blocked with UK5099 (72% loss in spare respiratory capacity). In the established bladder cancer cell lines (5637 and TSU-Pr1) there is a 60% and 35% loss in spare respiratory capacity respectively. Hence, glucose is the predominant fuel source for bladder cancer in patient-derived cells.
- Preliminary 3D bioenergetic studies show more fuel flexibility, with the greatest sensitivity when glutamine is blocked from entering the TCA.
- Addition of metformin increases sensitivity when in combination with cisplatin and gemcitabine (SOC treatments for bladder cancer).

### 2D Results

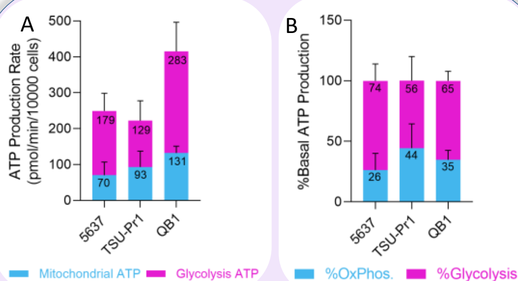



**Figure 4. Determining the source of ATP generation using the Seahorse XFe.** A)Total ATP produced by bladder cancer cell lines via either glycolysis or the mitochondria. B) Percentage of basal ATP generated through the use of OXPHOS or glycolysis.

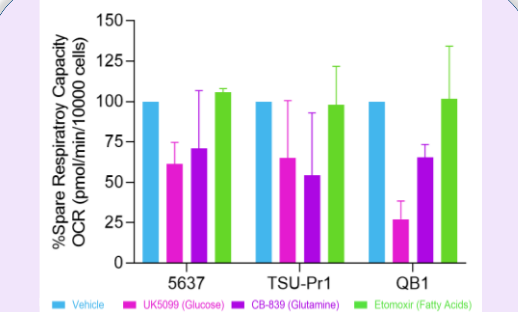



**Figure 5. Spare Respiratory Capacity (SRC) of 2D cell lines using the Seahorse XFe.** SRC of cell lines in stress conditions to determine fuel source dependencies using UK5099 (blocks pyruvate into the mitochondria), CB-839 (blocks glutamine from entering the mitochondria) and Etomoxir (blocks fatty acids entry into the mitochondria)

### 3D Results

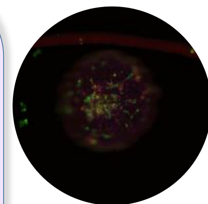

**Figure 6. QB1 spheroid stained with Hoechst, MitoTracker Green and MitoTracker Orange.** Z-Stacked images of 50k cells QB1 spheroid

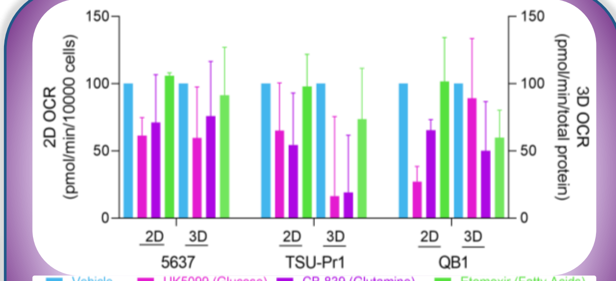



**Figure 7. Comparing the Spare Respiratory Capacity (SRC) of bladder cancer cell lines in 2D and 3D models.** Using the numerous inhibitors (UK5099, CB-839, and Etomoxir) to identify similarities or differences fuel dependencies between models.

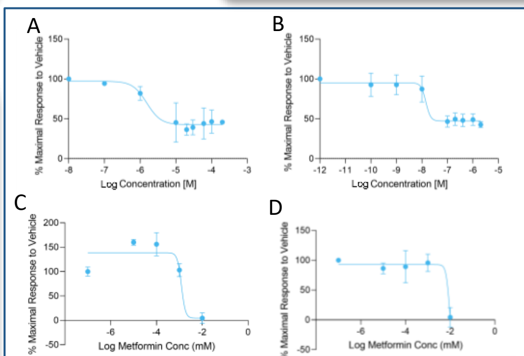



**Figure 8. QB1 cell line in 2D treated with SOC chemotherapy treatments and in combination with Metformin.** A-B) QB1 cell line treated with Cisplatin (A) or Gemcitabine (B) only across 9-point dose curve. C-D) QB1 cell line treated with Cisplatin at 1uM (C) and Gemcitabine at 10nM (B) with metformin at increasing concentrations (10, 1, 0.1, 0.01mM) increases sensitivity to treatment.

## Conclusions

- By understanding the major metabolic pathways that are critical to bladder cancer tumourigenesis and resistance to SOC therapies will provide additional context to novel potential combination SOC therapies and personalised medicine approaches to improve patient outcomes.
- Our data indicates that glycolysis is the main metabolic pathway used by bladder cancer to generate cellular energy and contributes to cell survival and can be exploited *in vivo* to monitor effects.

## References & Acknowledgements


1. Australian Institute of Health and Welfare, 2019; Available at: https://canceraustralia.gov.au/affected-cancer/cancer-types/bladder-cancer/bladder-cancer-statistics.
2. Afonso J et al. *Nature Reviews Urology. 2020;17(2):77-106.*
3. Conde VR et al. *Experimental Cell Research. 2015;335(1):91-8.*
4. Massari F et al. *Cancer Treatment Reviews. 2016;45:46-57.*



This research was supported by funding from a Princess Alexandra Research Foundation award, and the Medical Research Future Fund (MRFF) Rapid Applied Research Translation Program CPAC. The Translational Research Institute receives support from the Australian Government.



Alivia.calabrese@hdr.qut.edu.au
https://orcid.org/0000-0003-4837-379X
linkedin.com/in/alivia-calabrese-3377451b7
@QBCI_
@qbci_aus


PA Research Foundation · QBCI · cpac · QUT · TRI · APCRC-Q Australian Prostate Cancer Research Centre Queensland · QUT Centre for Genomics and Personalised Health