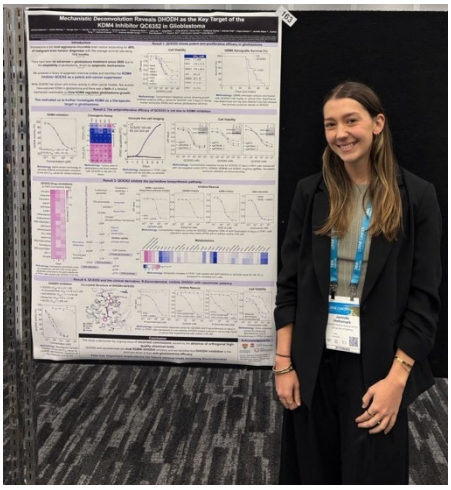
Name: Jacinda Rose Holtsmark
Position & Affiliation: PhD Candidate, The University of Sydney
Full Reference: Holtsmark J, Sterling J, Joun G, Du T, Kuchibhotla M, Johns T, De Weck A, Loo L, Neely G, Shard C, Tran K, Gomez G, Patil A, Saxton A, Baker J, Feng Y, Kraemer A, Mueller-Knapp S, Workman P, Shi DD, Losman J, Munoz L. *Mechanistic Deconvolution Reveals DHODH as the Key Target of the KDM4 Inhibitor QC6352 in Glioblastoma.*
Conference/Meeting Name: Lorne Cancer 2026
Location Lorne, Melbourne
Dates: 12 – 14th February, 2026
Presentation Type: Poster



The Lorne Cancer Conference was an exceptionally valuable experience and represents a significant milestone in my professional development to date. I had the privilege of engaging with a diverse cohort of accomplished and dedicated researchers who have made substantial contributions to the field of cancer research. As an early-career researcher, the opportunity to learn from leading experts across Australia—including my supervisor, Lenka Munoz—as well as distinguished international speakers from the United States, the United Kingdom, and Singapore, was both intellectually stimulating and professionally enriching.

In particular, the presentations delivered by Masha Poyurovsky, whose work focuses on therapeutic drug discovery, provided valuable insight into the pharmaceutical dimensions of drug development. Her research highlighted the rigorous validation processes required to ensure that candidate targets are biologically relevant and therapeutically actionable. This perspective strongly resonated with my own research, which addresses the persistent issue of erroneous conclusions arising from the use of inadequate or non-orthogonal chemical tools to interrogate proteins of interest. Such methodological limitations contribute to irreproducibility within the scientific literature and may ultimately impede the successful development of anticancer therapies, particularly when drugs are designed to target proteins that do not represent true cancer vulnerabilities. Gaining insight into the stringent validation frameworks employed within the pharmaceutical industry reinforced the critical importance of methodological robustness and target verification.

Additionally, the work presented by Nicholas Hunting, which elucidates the regulatory networks governing natural killer (NK) cell immunity and informs drug discovery initiatives aimed at enhancing NK cell–based cancer immunotherapies, offered a complementary perspective on translational research. While distinct from my primary focus, this approach further underscored the diverse strategies being pursued to improve therapeutic outcomes in oncology.

Overall, the conference reinforced the essential role of rigorous, high-quality research in translating fundamental scientific discoveries into meaningful therapeutic innovation and improved patient outcomes.

Mechanistic Deconvolution Reveals DHODH as the Key Target of the KDM4 Inhibitor QC6352 in Glioblastoma

Jacinda Holtsmark [1 2], Jayden Sterling [1 2], George Joun [1 2], Tian Du [2 3], Mani Kuchibhotla [4 5], Terrance Johns [1 5], Antoine De Weck [6 7], Lipin Loo [2 3], Greg Neely [2 3], Chloe Shard 8, Karen Tran [1 2], Guillermo Gomez [8], Ashwini Patil [9], Angus Saxton [10], Jennifer Baker [11], Yuchen Feng [1 2], Andreas Kraemer [12 13 14], Susanne Mueller-knapp [12 13 14 15], Paul Workman [15 16], Diana D. Shi[17], Julie-Aurore Losman[18] Lenka Munoz [1 2]

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Introduction

Glioblastoma is the **most aggressive incurable** brain tumour accounting for **49% of malignant brain tumour diagnoses** with the average survival rate being **14.6 months**.

Stupp et al., 2005

There have been **no advances** in **glioblastoma treatment since 2005** due to the **adaptability** of glioblastoma, driven by **epigenetic mechanisms**.

Yao et al., 2024

We screened a library of epigenetic chemical probes and identified the **KDM4 inhibitor QC6352 as a potent anti-cancer suppressor**

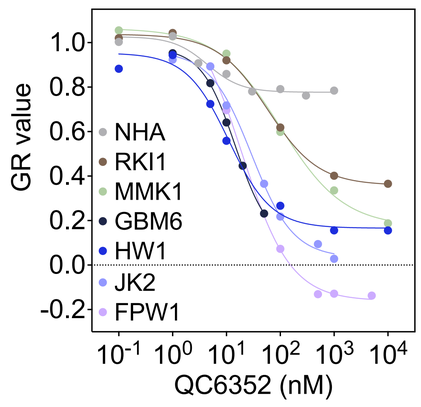
While QC6352 has shown anti-tumour activity in other cancer models, few studies have explored KDM4 in glioblastoma and there was a **lack** of a detailed mechanistic examination on **how KDM4 regulates glioblastoma growth**.

Li, M. et al., 2020; Wang, Z. et al., 2023; Lee, D.H. et al., 2021; Mallm, J.-P. et al., 2020.

This motivated us to further investigate KDM4 as a therapeutic target in glioblastoma

Result 1. QC6352 shows potent anti-proliferative efficacy in glioblastoma

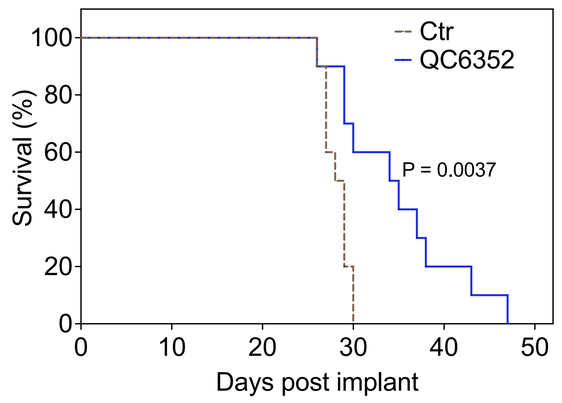
Cell Viability



	GR_{50} (nM)
NHA	> 1,000
RKI1	133 ± 41.9
MMK1	119 ± 29.6
JK2	28 ± 6
GBM6	20 ± 16
FPW1	21 ± 4.1
HW1	3 ± 3.0

Methodology: Concentration-response curves showing growth inhibition potency (GR_{50}) values for QC6352 over 5 days in normal human astrocytes (NHA) and various glioblastoma cell lines.

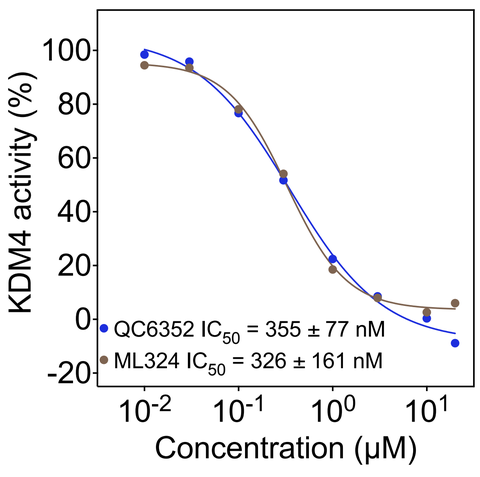
GBM6 Xenografts Survival (%)



Methodology: GBM6 orthotopic tumours and treated with QC6352 (100 mg/kg) or vehicle (Ctr). Significance was determined with log rank (Mantel-Cox) test between the survival curves for vehicle vs QC6352

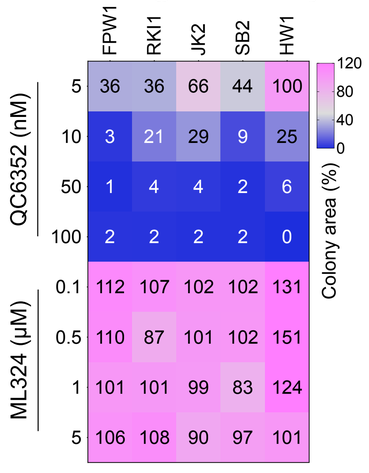
Result 2. The antiproliferative efficacy of QC6353 is not due to KDM4 inhibition

KDM4 inhibition



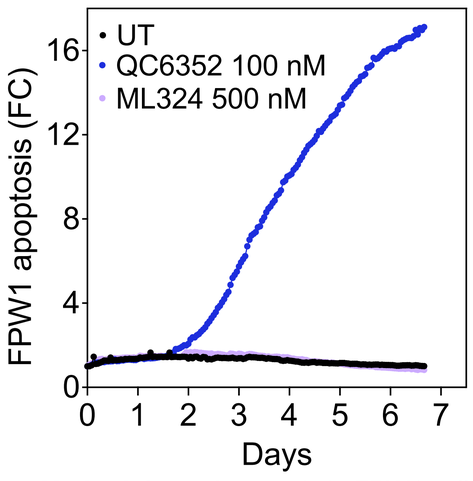
Methodology: Alpha screen technology to generate KDM4A concentration-inhibition curves and IC_{50} values for tested inhibitors.

Clonogenic Assay



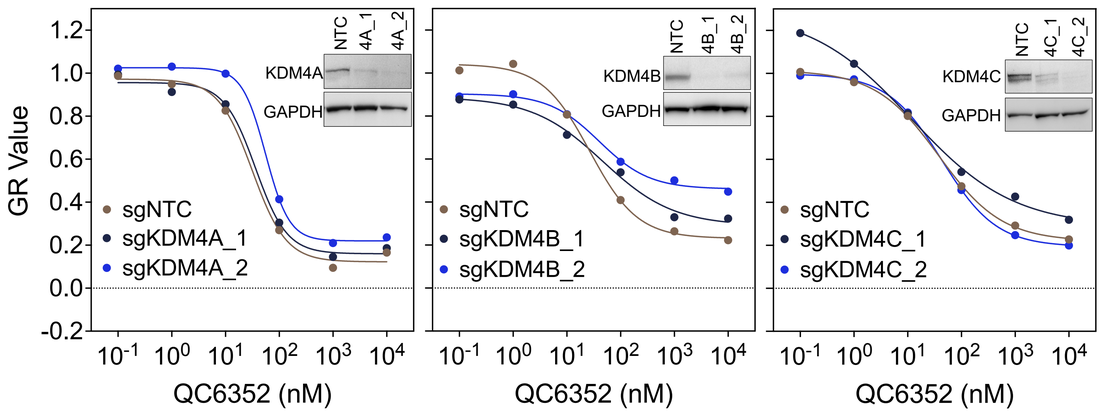
Methodology: Colony area of glioblastoma cell lines treated with QC6352 or ML324 (10 days).

Incucyte live cell imaging



Methodology: Apoptosis in FPW1 cells treated with ML324 (ML) or QC6352 (QC).

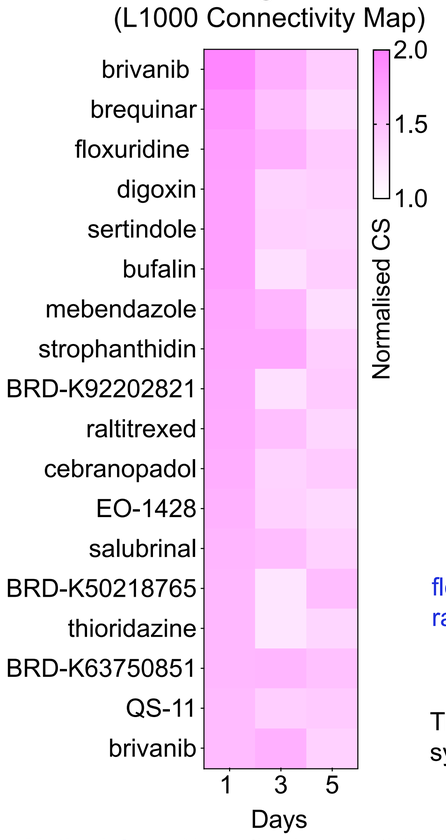
Cell Viability



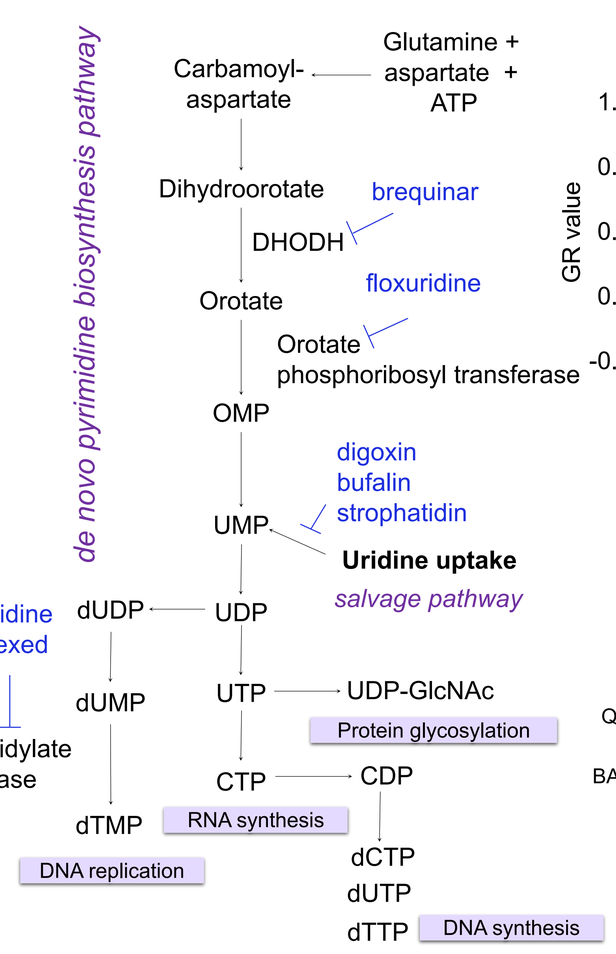
Methodology: Concentration-response curves for QC6352 (5 days) in RKI1 cells transduced with non-targeted control (NTC), KDM4A, KDM4B and KDM4C targeting sgRNAs. Successful knockout validated via immunoblotting.

Result 3. QC6352 inhibits the pyrimidine biosynthesis pathway

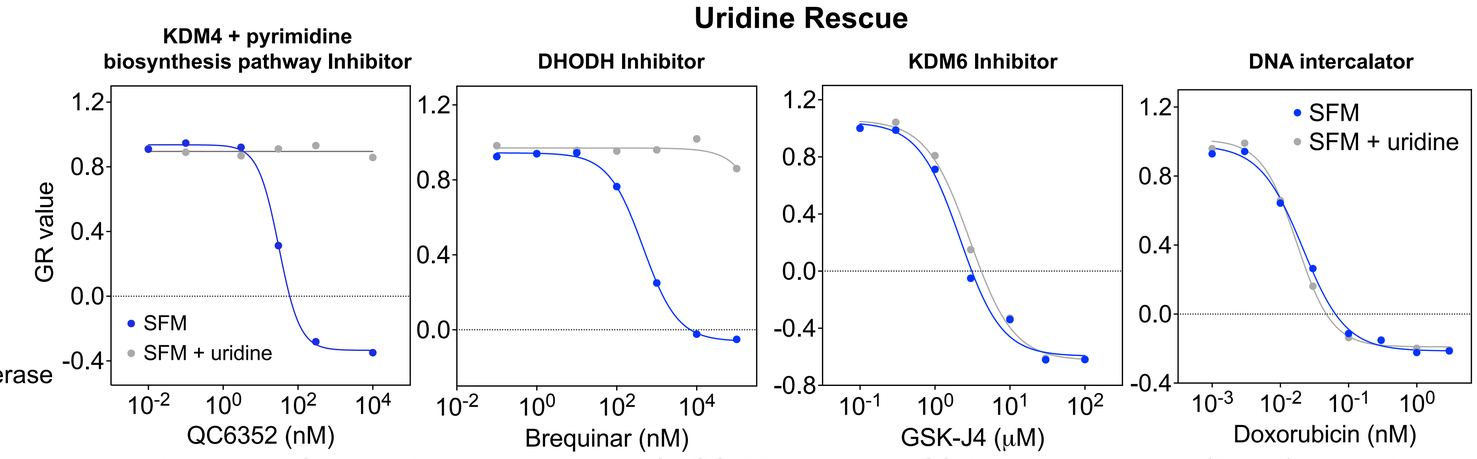
QC6352-Drug correlations (L1000 Connectivity Map)



Methodology: Normalised connectivity score for compounds with the most similar gene signatures compared to the gene signatures of FPW1 cells treated with QC6352 (100 nM).

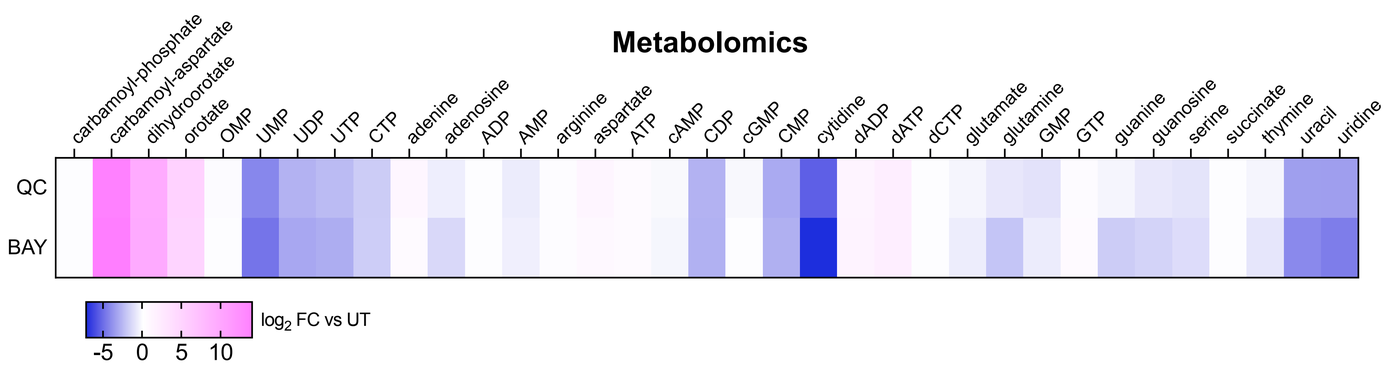


Uridine Rescue



Methodology: Concentration-response curves for QC6352, brequinar, GSK-J4 and Doxorubicin (5 days) in FPW1 cells cultured in serum-free media (SFM) with or without uridine (100 μM)

Metabolomics

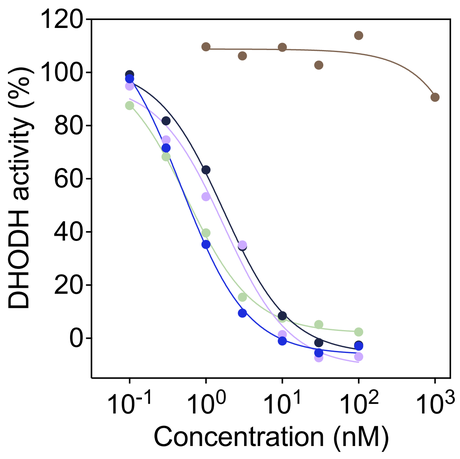


Methodology: Metabolite changes in FPW1 cells treated with BAY2402234 or QC6352 (both 50 nM, 24 h) compared to untreated (UT) cells.

Result 4. QC6352 and the clinical derivative, R-Zavondemstat, inhibits DHODH with nanomolar potency

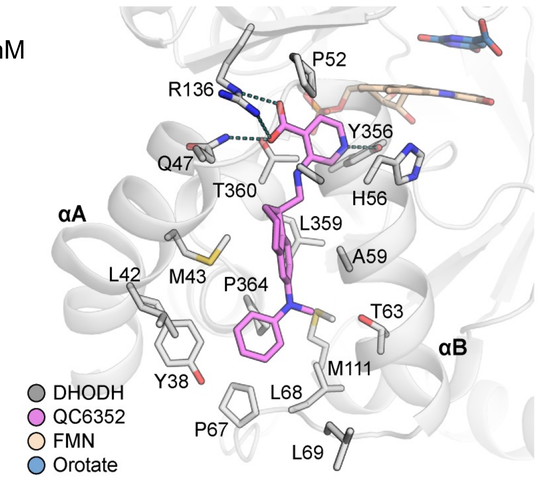
DHODH Inhibition

- QC6352: IC_{50}= 0.3 ± 0.1 nM
- R-Zavondemstat: IC_{50} = 0.78 ± 0.3 nM
- BAY2402234: IC_{50} = 1.1 ± 0.8 nM
- Brequinar: IC_{50}= 2.8 ± 1.0 nM
- ML324: IC_{50} > 1,000 nM

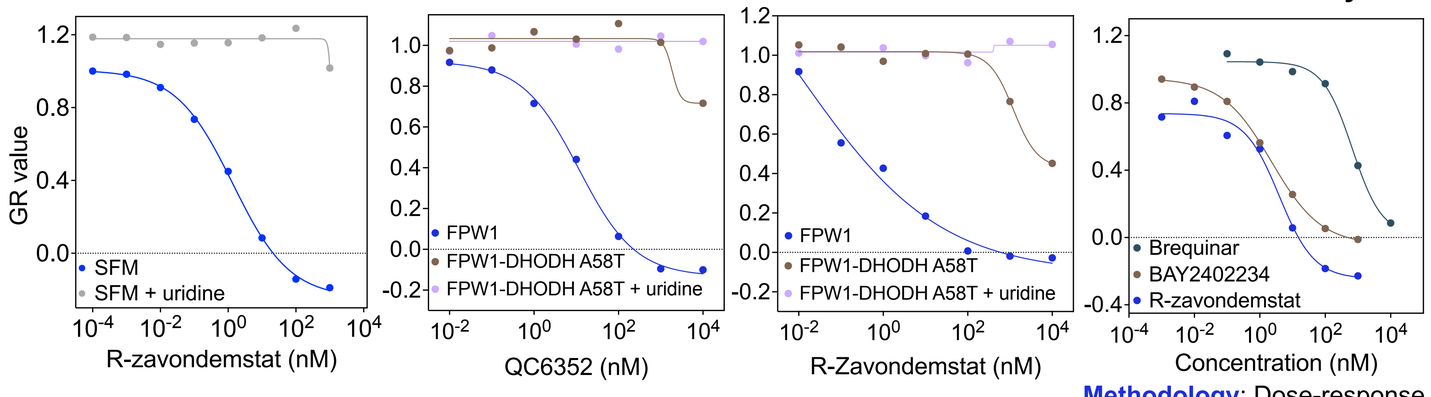


Methodology: Recombinant DHODH inhibition curves and IC_{50} values for QC6352, BAY2402234, brequinar, R-Zavondemstat and ML324

Co-crystal Structure of DHODH-QC6352



Uridine Rescue



Methodology: Concentration-response curves for QC6352 and R-zavondemstat (5 days) in FPW1 or FPW1 cells transduced to over-express DHODH mutant cultured in serum-free media or serum-free supplemented with uridine (100 μM)

Cell Viability

Methodology: Dose-response curves for brequinar, BAY2402234 and R-Zavondemstat in FPW1 (5 days)

Conclusion

This study underscores the ongoing issue of **incorrect conclusions** caused by the **absence of orthogonal high-quality chemical tools**.

QC6352 and zavondemstat are **dual KDM4–DHODH** inhibitors and we identified that **DHODH inhibition** is the dominant driver of their **anti-glioblastoma efficacy.**

This has important implications for future clinical trials involving Zavondemstat

Acknowledgements

Jacinda Holtsmark received conference funding from Sydney Cancer Partners via a grant from Cancer Institute NSW (2021/CBG0002).