

ASX Announcement

Race Discovers Multiple Novel FTO Inhibitor Candidates

- 39 unique FTO protein-binding molecules identified using state-of-the-art NMR fragment screening
- Identified compounds are confirmed FTO-binding chemical structures for the development of novel FTO targeting drugs
- Provides valuable new IP aiding the development of novel m⁶A RNA epigenetic pathway drugs

21 November 2024 – Race Oncology Limited (“Race”) is pleased to announce that it has successfully completed an FTO-targeted drug discovery program at Monash University’s Fragment Platform (MFP) (ASX announcement: 27 September 2022). This program used fragment-based screening by NMR to identify 39 molecular candidates (‘hits’) that specifically bind to the m⁶A RNA demethylase protein, FTO. These confirmed FTO-binding chemical scaffolds provide a platform for the development of novel and patentable molecules with the potential to become new drugs that specifically target the m⁶A RNA epigenetic pathway.

RNA epigenetics

Dysregulation (loss of control) of RNA epigenetics is a key driver of several metabolic diseases and many cancers.¹ One of the key proteins in this dynamic RNA regulatory system is the *Fatso* or *FaT and Obesity associated protein* (FTO), which acts as a global regulator of m⁶A RNA levels. There is significant scientific and pharmaceutical industry interest in developing FTO inhibitor drugs as potential new cancer treatments.^{2,3}

FTO inhibitors

Identification of selective FTO inhibitors is complicated by the high similarity between the enzymatic active sites of FTO and closely related members of the ALKBH protein family.⁴ NMR fragment screening offers an alternative to conventional high throughput screening and can identify drug-like molecules that bind outside the enzyme activity site with the potential to regulate protein activity.⁵

In 2022, Race commissioned the MFP to perform an NMR-based fragment screen to identify unique chemical structures that bind to the FTO or ALKBH5 proteins. This recently completed program identified 39 new FTO-binding chemical ‘hits’, with their binding confirmed by multiple NMR methods and surface plasmon resonance. This highly successful program is a major milestone towards Race developing potent and selective new FTO inhibitors.

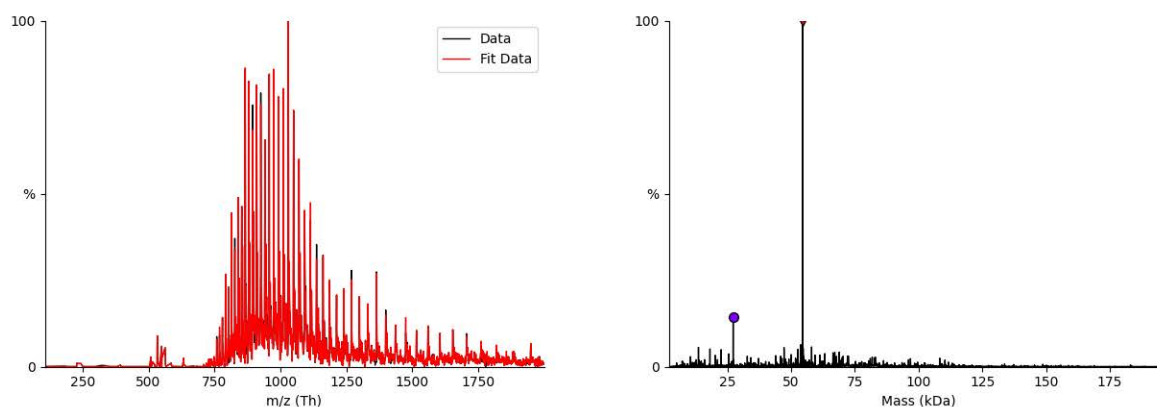
Race’s Vice President of Research, Prof. Mike Kelso, commented: *“Identification of chemical ‘hits’ that bind to a protein target of interest is a critical step in modern drug discovery. Our successful FTO program at Monash provides Race with valuable new IP in the RNA epigenetics space, an enormously exciting area at the cutting-edge of oncology research and drug development.”*

Study Highlights

FTO & ALKBH5 Protein Production

FTO and ALKBH5 (unlabelled and ^{15}N , ^2H -labelled) proteins required for fragment screening were expressed using the method of Khatiwada *et al.*⁶ The material produced was consistent with the desired FTO protein by gel electrophoresis, low-resolution mass spectrometry (MS) and ^{15}N TROSY NMR analysis (Figure 1). A yield of approximately 20 mg/L was obtained for both FTO and ALKBH5 proteins. Due to resource constraints, the fragment screening program focused on FTO.

(A)



(B)

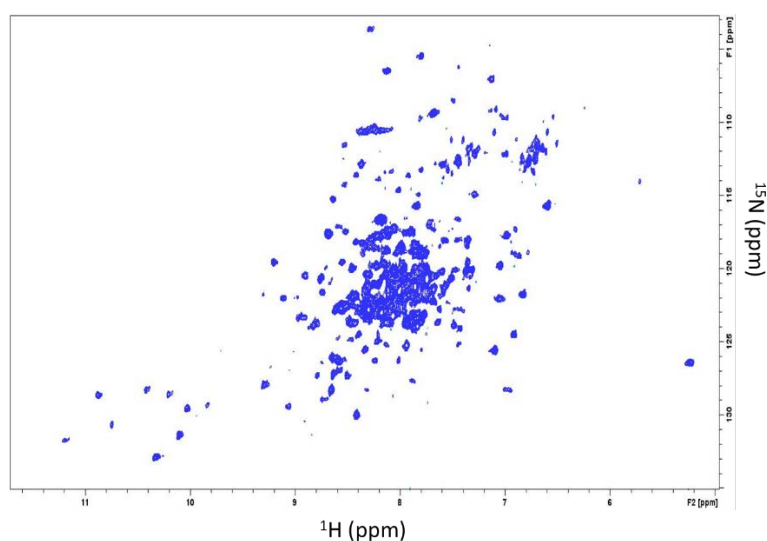


Figure 1. (A) Mass spectrum and (B) ^{15}N TROSY NMR spectrum of FTO protein.

NMR Screening of MFP Fragment Library

NMR and surface plasmon resonance (SPR) assays suitable for screening small molecule ‘fragments’ for binding to FTO were developed using the purified FTO protein. Reported FTO-binding compounds were used as positive controls. Primary screening with the purified FTO protein was performed using an efficient saturation transfer difference (STD) NMR protocol with the Monash Fragment Platform’s highly curated chemical fragment library. Flufenamic acid was used as a positive control for FTO-binding throughout the screen to confirm protein stability and assay consistency. The primary STD screen identified 96 FTO-

binding chemicals ('hits') from the extensive MFP library. Analysis of the screening data confirmed that FTO has the capacity to bind small molecules with high affinity.

FTO-binding of the 96 primary hits was further validated using three independent NMR-based detection methods (STD, CPMG and WaterLOGSY), along with surface plasmon resonance (SPR). This process identified 39 individual chemical compounds representing 9 chemical series' that showed FTO-binding in all four assays. A further 384 analogues (structurally related chemicals) of the validated hits were then screened for FTO-binding by SPR. Several analogue series showed improved affinity and clear structure-activity relationships, indicating their suitability for development into drug leads.

Next Steps

The next stage is to perform a 'hit-to-lead' medicinal chemistry campaign using the identified hits as starting scaffolds. This involves using synthetic chemistry to turn the hit fragments into lead drug candidates. These candidates will be screened for high FTO inhibitory potency and selectivity, efficacy in animal cancer models, low toxicity and acceptable metabolic properties. This is an expensive undertaking, and Race is currently evaluating options. No decision has been made at this stage to advance a hit-to-lead medicinal chemistry program.

Q&A.

What is NMR fragment screening and has it been used successfully to produce any marketed drugs?

Modern drug discovery requires the identification of 'hit' molecules that interact with a defined biomolecular target(s) responsible for a disease state. High throughput Screening (HTS) and Fragment Based Drug Design (FBDD) are two of the main approaches used by pharmaceutical companies for this critical step. In FBDD, a variety of techniques are used for the primary screening of a target (usually a protein) for 'hits', including fluorescence polarisation, X-ray crystallography and NMR spectroscopy. NMR fragment screening represents a particularly successful approach; for example, one study reported that 16 out of 20 clinical drug candidates found by FBDD had used NMR fragment screening for the initial 'hit' identification step.⁵ Venetoclax, a drug widely used to treat acute myeloid leukaemia and other haematological cancers, was developed using the NMR fragment screening approach.

What is the commercial significance of these discoveries?

The chemical structures identified by Race in the fragment screen at Monash have not been described in any previously reported FTO inhibitors. By identifying unique chemical species with validated binding to FTO, Race can now create FTO-inhibiting drugs that are structurally distinct from all previously described FTO inhibitors. This work ensures that: (1) the leads generated are patentable, providing valuable new IP for Race and (2) the leads could potentially overcome the potency and selectivity barriers that have hindered the clinical development of FTO inhibitors. Race has a clear opportunity to be first to the clinic with a purpose-built, targeted FTO inhibitor that might have utility beyond cancer.

References

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2. Li, Y., Su, R., Deng, X., Chen, Y., Chen, J. (2022) FTO in Cancer: Functions, Molecular Mechanisms, and Therapeutic Implications. *Trends in Cancer*, 8(7), 598 – 614.
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4. You, Y. et al. (2022) Recent Advances of m⁶A Demethylases Inhibitors and Their Biological Functions in Human Diseases. International Journal of Molecular Sciences 23, 5815.

5. Mureddu, L. G., Vuister, G. W. (2022) Fragment-Based Drug Discovery by NMR. Where Are the Successes and Where can It Be Improved? Frontiers in Molecular Biosciences 9:834453.

6. Khatiwada, B., Purslow, J. A., Underbakke, E. S., Venditti, V. (2020) N-terminal Fusion of the N-terminal Domain of Bacterial Enzyme I Facilitates Recombinant Expression and Purification of the Human RNA Demethylases FTO and Alkbh5. Protein Expression and Purification 167, 105540.

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About Race Oncology (ASX: RAC)

Race Oncology (ASX: RAC) is an ASX-listed clinical stage biopharmaceutical company with a dedicated mission to be at the heart of cancer care.

Race's lead asset, bisantrene, is a small molecule chemotherapeutic. Bisantrene has a rich and unique clinical history with demonstrated therapeutic benefits in both adult and paediatric patients, a well-characterised safety profile, and compelling clinical data demonstrating an anticancer effect and less cardiotoxicity over certain anthracyclines, such as doxorubicin.

Race is advancing a reformulated bisantrene (RC220) to address the high unmet needs of patients across multiple oncology indications, with a clinical focus on anthracycline combinations, where we hope to deliver cardioprotection and enhanced anticancer activity in solid tumours. Race is also exploring RC220 as a low-intensity treatment for acute myeloid leukaemia.

Race is investigating the effect of bisantrene on the m⁶A RNA pathway, following independent research published by the City of Hope identifying bisantrene as a potent inhibitor of FTO (Fat mass and obesity-associated protein). Dysregulation of the m⁶A RNA pathway has been described in numerous peer reviewed studies as a driver of a diverse range of cancers.

Race Oncology has collaborated with Astex, City of Hope, MD Anderson, Sheba City of Health, UNC School of Medicine, University of Wollongong and University of Newcastle, and is actively exploring partnerships, licence agreements or a commercial merger and acquisition to accelerate access to bisantrene for patients with cancer across the world.

Learn more at www.raceoncology.com.

If you have any questions on this announcement or any past Race Oncology announcements, please go to the Interactive Announcements page in our Investor Hub <https://announcements.raceoncology.com>

Race encourages all investors to go paperless by registering their details with the Company's share registry, Automic Registry Services, at www.automicgroup.com.au.

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