Are malic enzymes a metabolic drug target in the war against cancer?

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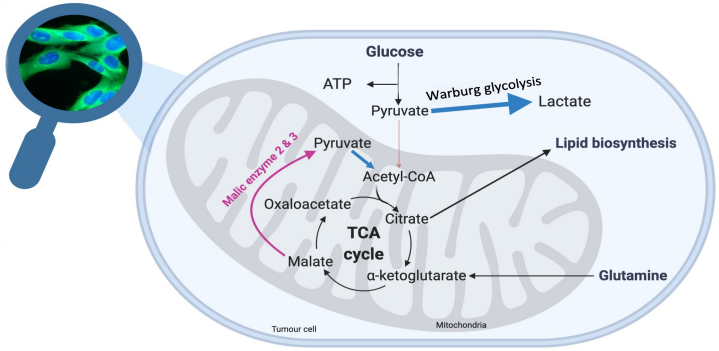
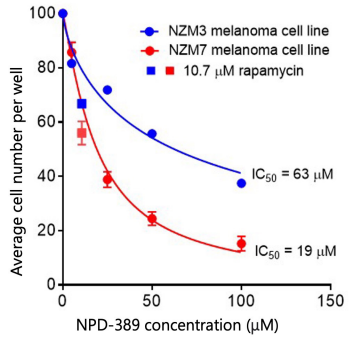
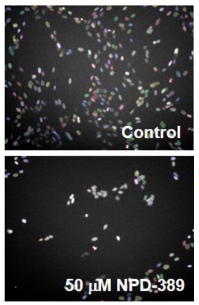
Introduction to Malic enzymes:



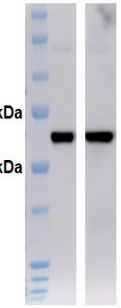
- **Cancer cells outcompete normal cells by altering their metabolism** to generate energy & building blocks by consuming more glucose & glutamine[1].
- Mitochondrial malic enzyme 2 (**ME2**) expression is increased in cancer cells[3]. Malic enzymes **convert mitochondrial malate into pyruvate** with the reduction of NAD(P)$^+$. Thus, enabling TCA cycle flux during lactate generation (Warburg glycolysis), promoting tumour cell growth and proliferation.

The small molecule, NPD-389, inhibits cellular proliferation in melanoma.



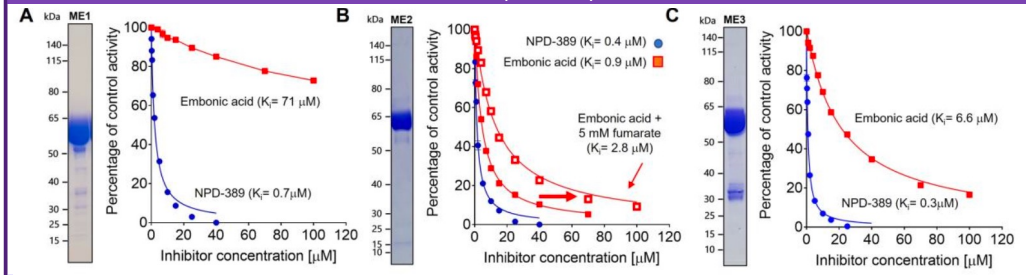


Cody Mullins, 2021



- NPD-389 profoundly inhibits cellular proliferation in human melanoma cancer cells, showing an almost 80% reduction in cell number at a 50 μM concentration.
- Western blot showing robust expression of ME2 in melanoma cell lines.

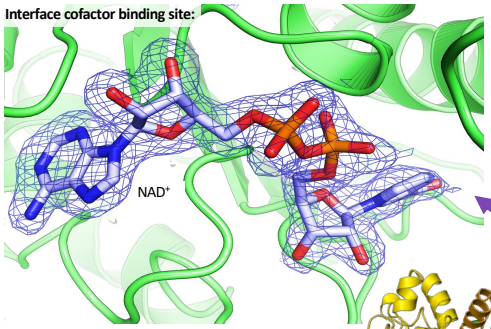
Differences in kinetics and inhibition across the malic enzyme family:

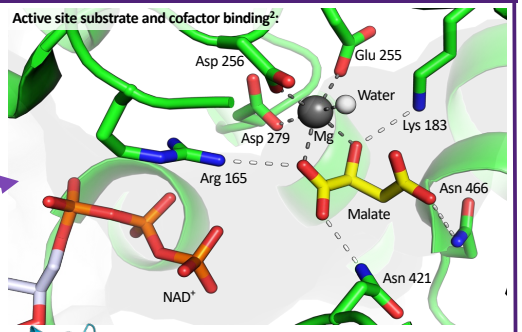


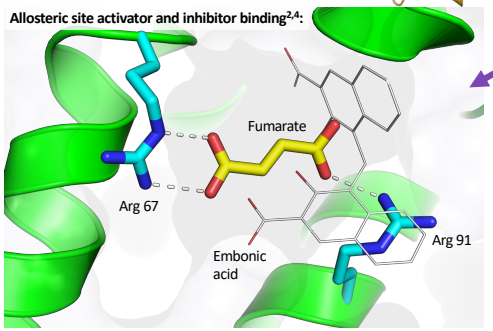
- Robust recombinant expression and purification of the three malic enzyme isoforms in LOBSTR *E.coli*.
- **NPD-389 potently inhibits all malic enzymes** at a low micromolar potency.
- **The unrelated small molecule, embonic acid and analogue MDSA, selectively inhibits ME2 (potently) and ME3** (10-fold decrease).
- This suggests they bind at different sites; **NPD-389**, near the **active site** and **EA/MDSA,** near the **fumarate binding site**.

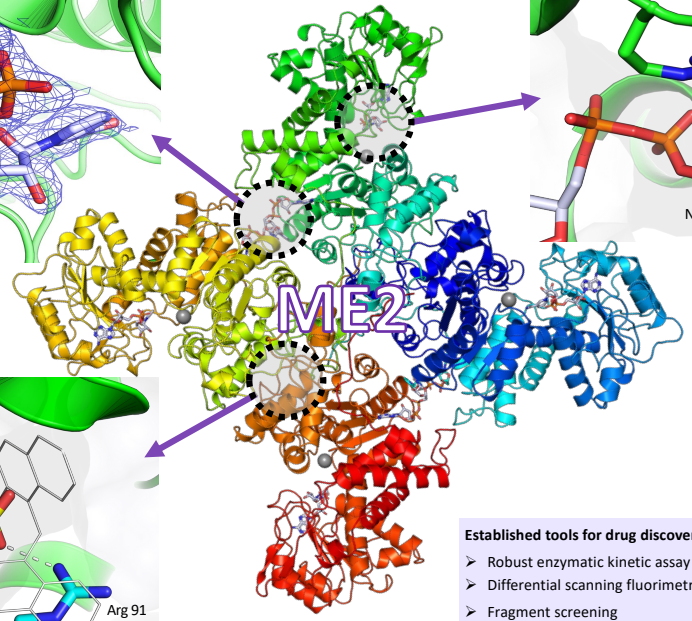
	ME1 c-NADP-ME	ME2 m-NAD(P)-ME	ME3 m-NADP-ME
L- Malate (K_M) mM	0.76 ± 0.03	5.3 ± 0.2	0.89 ± 0.03
NAD$^+$ (K_M) mM	N/A	0.66 ± 0.02	N/A
NADP$^+$ (K_M) mM	0.079 ± 0.003	1.2 ± 0.1	0.26 ± 0.01
Fumarate (K_M) mM	N/A	2.3 ± 0.1	N/A
Embonic acid (K_i) μM	71 ± 3	**0.89 ± 0.03**	**6.6 ± 0.2**
MDSA (K_i) μM	8.6 ± 0.4	**0.41 ± 0.07**	**0.23 ± 0.02**
Subcellular location	Cytosol	Mitochondria	Mitochondria

Molecular interactions to guide drug discovery in malic enzymes:



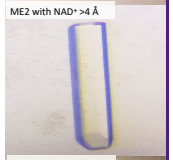


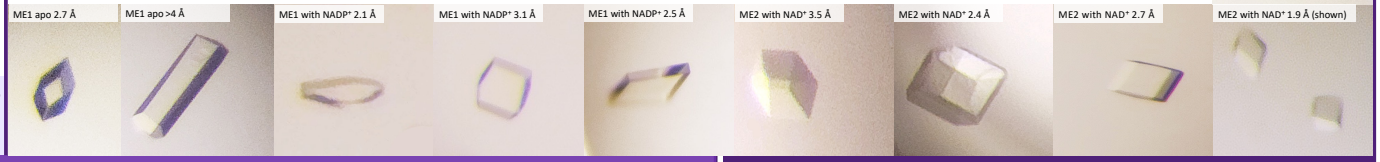




Established tools for drug discovery:

- Robust enzymatic kinetic assay (high-throughput)
- Differential scanning fluorimetry (high-throughput)
- Fragment screening
- Protein crystallography and structure determination using robotic microseed matrix seeding.





Expected findings and significance of my research:

- **Cancer is a leading cause of death worldwide.** Consequently, efficacious anticancer treatments are crucial to improving treatment outcomes and quality of life.
- Developing **small molecule anti-cancer drugs with high potency** minimises the toxic side effects associated with many other cancer treatments.
- The activation of ME2 is likely a feature of p53 inactivating mutations, making treatment with ME2-based therapeutics a **treatment option for a range of aggressive cancers.**
- ME2 is a promising cancer target because it is **upregulated in cancerous tissues.** It may be possible to selectively target ME2[3,4].
- We expect to determine the **structural binding interactions** between malic enzyme 2 and NPD-389 to inform future rational drug design.
- We will begin fragment screening to discover new, more potent ME inhibitors.

References:
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