Abstract 4871: Dysregulation of MYC-Family Proteins Sensitises Cancers to NMT Inhibition: Identification of NMTi Sensitivity and Mechanism

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1 Introduction and Background

N-Myristoylation

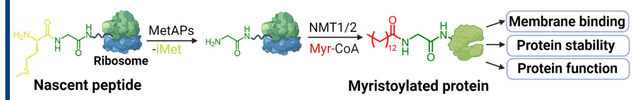


Fig 1. *N*-myristoylation, an essential modification. *N*-myristoylation is catalysed by *N*-myristoyltransferases 1/2 (NMT) and is predominantly co-translational[1]. After initiator methionine removal to reveal a N-terminal glycine, NMTs transfer a myristate group from myristoyl-CoA to the nascent peptide of selected proteins.

NMT as a cancer target

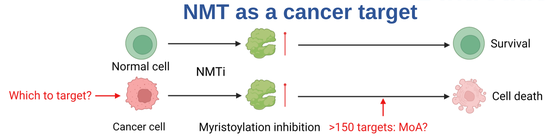
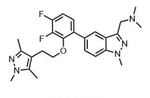
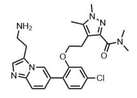


Fig 2. NMT as a cancer target. NMT has been suggested as a cancer target, but a mechanistic rationale to identify patients for targeted therapy is lacking[2]. The mode of action (MoA) of NMT inhibitors (NMTi) is also difficult to dissect given its pleiotropic MoA[3].

NMT Inhibitors





Compound	K_D (nM)	IC50 (nM)
IMP1088	0.1 ± 0.01	1.8
MYX1715	0.09 ± 0.01	<1

Fig 3. NMT inhibitors used. The structures and inhibitory potency against human NMT1.

This work

- Screening of NMTi across 245 cell lines, followed by ssGSEA, identified an **NMTi sensitivity signature** (NSS) and indicated that sensitivity is predicted by **MYC family proteins status** as well as a **membrane protein signature.**
- MYC family proteins are known to increase oncogenic load and translation stress, and we hypothesize that this increases sensitivity to NMT inhibitors.
- We have used 'omics analyses to identify pathways involved in NMTi MoA, including **induction of the unfolded protein response (UPR) and loss of Complex I activity**, which was functionally validated in DLBCL models.
- We have also shown that NMTi are efficacious *in vivo* in multiple cancer types.

2 Predicting Sensitivity to NMTi

Strategy to Identify a Sensitivity Signature

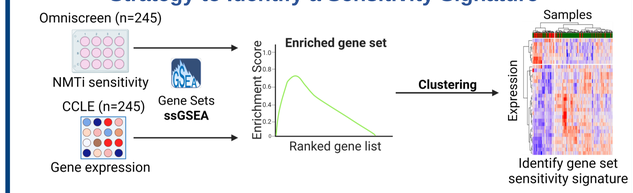


Fig 4. Identification of NMTi Sensitivity Signature (NSS). Sensitivity to MYX1715 was determined on a panel of 245 cancer lines. ssGSEA was performed on their transcriptome and the gene sets whose expression best define NMTi sensitivity were identified.

Low membrane protein expression, low protein secretion and high MYC target expression predicts NMTi sensitivity

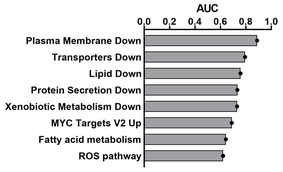


Fig 5. Gene sets which best discriminate between NMTi-sensitive and insensitive lines. Gene sets are consistent with higher oncogenic load in cells.

LINCS L1000 data shows that the UPR imitates NMTi KO.

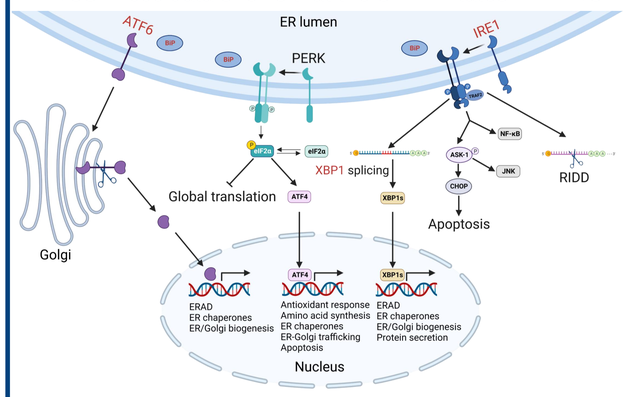


Fig 6. Components of the unfolded protein response (UPR) phenocopy NMT KO. The LINCS L1000[1] dataset on knockdown of UPR proteins was analysed. Knockdown of genes in red phenocopy NMTi in most of the cell lines in the LINCS L1000 dataset.

3 Diffuse Large B-Cell Lymphoma

NMTi Sensitivity Correlates with MYC in a Tet-Off System

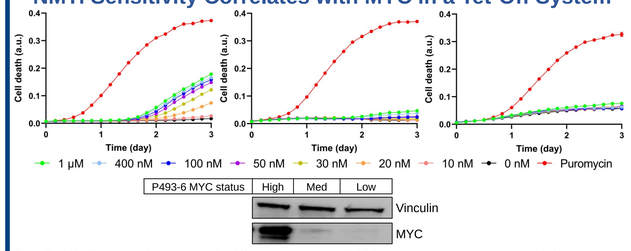


Fig 7. NMTi toxicity in the MYC-regulatable P493-6 cells depends on MYC status. Effect of IMP1088 in in the high (top left), medium (top middle) and low (top right) MYC states. Western blot showing MYC levels in P493 cells in each state (bottom).

Proteomics Identifies a Strong Impact on Mitochondria

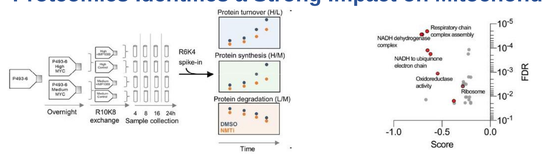


Fig 8. Effect of NMTi on proteome dynamics. Experimental set up (left). 1D enrichment on differential synthesis of proteins in high MYC cells (right).

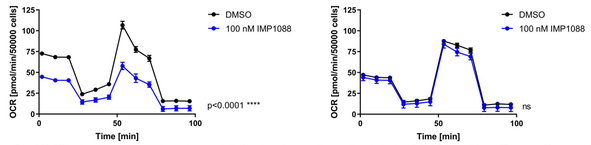


Fig 9. NMTi induces mitochondrial dysfunction in high MYC cells. The effect of IMP1088 on mitochondria in high (left) and medium (right) MYC P493-6 cells.

MYC status	Med		High	
IMP1088	-	+	-	+

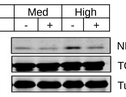


Fig 10. The NMT substrate and complex I assembly factor NDUFAF4 is lost upon NMT inhibition. Loss of NDUFAF4 myristoylation by IMP1088 treatment leads to its degradation, which is associated with Complex I defects[4].

4 Neuroblastoma

NMTi Sensitivity Correlates with MYCN Status *in vitro*

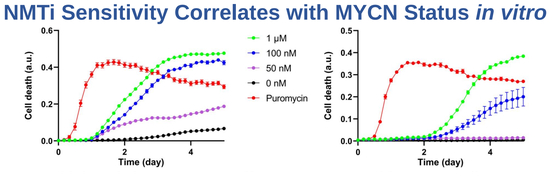


Fig 11. Sensitivity of the MYCN tet-off SHEP21N neuroblastoma cells to IMP1088. Response of SHEP21N cells to IMP1088 in the high MYCN (left) and low MYCN (right).

Cell line	IMR5	BE(2)C	Kelly	SHEP21N (high MYCN)	SHEP21N (low MYCN)	SHEP	SKNSH	SKNAS
MYCN	Y	Y	Y	Y	N	N	N	N
MYC	N	N	N	N	N	N	Low	Y
IC50 / nM	95	45	35	65	300	N/A	N/A	N/A
Max AUC (% puro)	0.89	0.78	0.94	0.80	0.54	0.28	0.33	0.22

Table 1. Summary of IMP1088 sensitivity of neuroblastoma lines.

4 Neuroblastoma cont.

IMP1088 Does Not Directly Impact MYC Family Proteins

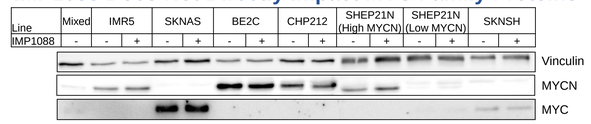


Fig 12. MYC proteins are not targets of NMT inhibitors.

5 NMT substrates are directly associated with impacted pathways.

Many NMT substrates are involved in ER/Golgi function

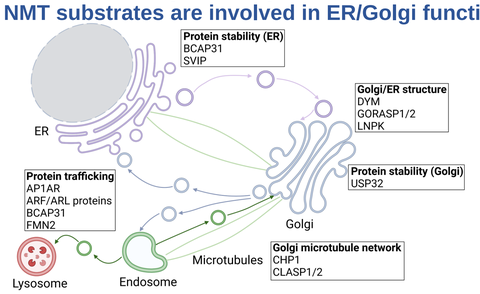
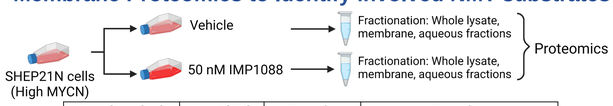


Fig 13. Schematic showing which NMT substrates are involved in ER/Golgi function.

Membrane Proteomics to Identify involved NMT substrates



-Log(p-value)	Log2(FC)	Protein	Function
3.41	-0.94	ARF1; ARF3	Golgi trafficking
3.04	-0.71	ARF4	Golgi trafficking
3.37	-0.73	ARF5	Golgi trafficking
3.39	-0.96	ARF6	Golgi trafficking
4.88	-0.90	ARL1	Golgi trafficking
3.55	-0.65	ARL5B	Golgi trafficking
2.90	-1.12	NDUFAF4	Complex I assembly
2.79	-1.07	TMEM261	Complex I assembly
2.66	-0.94	LAMTOR1	mTOR signalling

Fig 14. Proteomic strategy to identify NMTi MoA in a MYCN-dependent context. A selection of significantly and highly affected NMT substrates are shown.

Mechanisms of NMTi under high translation stress

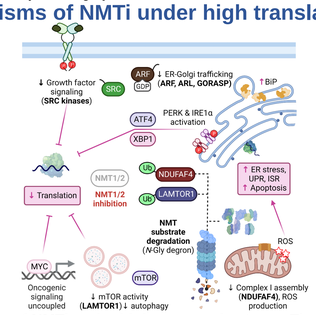


Fig 15. The effects of NMTi under high oncogenic stress. The interaction between the ER/Golgi, lysosomes and mitochondria, which are highly coupled, may be important.

6 NMTi is efficacious in cancer models *in vivo*

Diffuse Large B-Cell Lymphoma

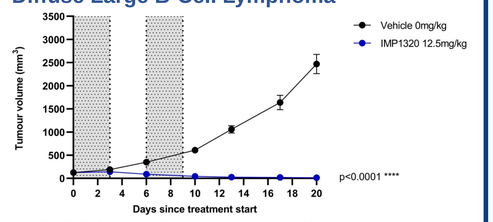


Fig 16. Efficacy of MYX1715 in a DLBCL xenograft model. DOHH2 cells were implanted into IL2R-NSG mice.

Neuroblastoma

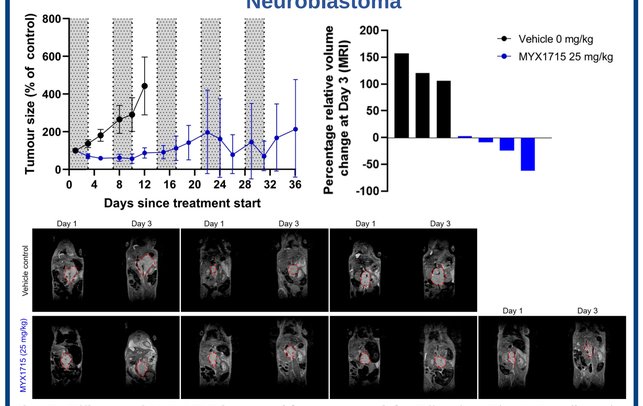


Fig 17. Efficacy of MYX1715 in neuroblastoma models. Effect in a Th-*MYCN* allograft model, in which Th-*MYCN* tumour cells were implanted in 129SvJ mice (top left). MYX1715 prevents tumour growth in a Th-*MYCN* GEMM model (top right, bottom).

Gastric Cancer

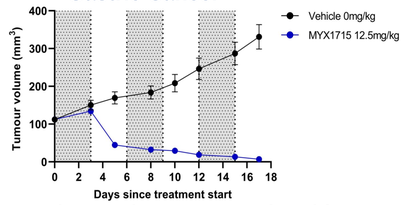


Fig 18. Efficacy of MYX1715 in a gastric cancer xenograft model. SNU-620 cells were implanted into NCG mice.

7 Conclusions

- Sensitivity to NMT inhibitors can be predicted by low expression of membrane associated proteins, low protein secretion and high MYC-family protein activity.
- Knockdown of key UPR pathway proteins phenocopies the NSS.
- High MYC family protein activity sensitises cells to NMTi, likely through increasing the basal oncogenic load and translational stress in these cells.
- The MoA of NMTi likely involves activation of the UPR, mitochondrial dysfunction and loss of mTOR activity.
- NMT inhibitors are highly effective *in vivo* in multiple cancer types.

References

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5. Figures created with BioRender.com