Inhibition of MGAT1 overcomes STK11-driven immune evasion in non-small cell lung cancer

Kiera M. Vassallo, Yingnan Chen, Katarzyna B. Handing, Alvin Lu, Samuel Meier, Shangtao Liu, Binzhang Shen, Teng Teng, Yi Yu, Lei Ji, Alan Huang, Chengyin Min, Brian Haines, William D. Mallender, Sining Sun, Mu-Sen Liu, John P. Maxwell, Andrew Maynard, Douglas A. Whittington, Jon H. Come, Scott Throner, Jannik N. Andersen, Wenhai Zhang, Serge Gueroussov

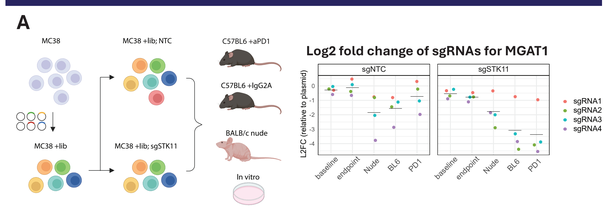
Tango Therapeutics, Boston, MA USA

Abstract #1225

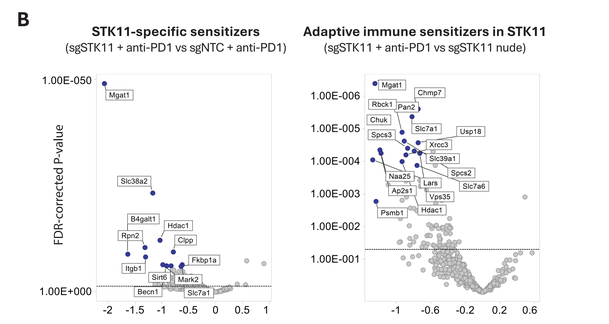
ABSTRACT

Background: Checkpoint inhibitors have emerged as a standard of care therapy for non-small cell lung cancer (NSCLC). Mutations in STK11 occur in 20% of NSCLC and are associated with poor responsiveness to anti-PD-1 agents, creating an increased need to identify novel therapeutic targets for this tumor subtype. **Methods:** Functional genomic screening utilizing CRISPR-Cas9 is a powerful tool to identify new therapeutic targets in cancer. We applied this technology specifically to STK11-mutant syngeneic tumor models in mice to identify targets which restore sensitivity to anti-PD-1 therapy. **Results:** Knockout of MGAT1, an enzyme critical for maturation of high-mannose N-glycans into hybrid and complex glycan types, was able to reverse STK11-mutant-driven resistance to anti-PD-1 treatment. Parallel in vitro screens using co-culture systems showed that disruption of N-glycosylation was also a powerful mechanism in sensitizing tumor cells to direct killing by antigen-matched CD8 T cells. Our investigation suggests that global disruption of N-glycans likely impacts the ability of T cells to recognize tumor cells. The role of MGAT1 in immune evasion was dependent on its enzymatic activity, making it a promising target for therapeutic small molecule inhibitor development. **Conclusions:** Our work implicates N-glycosylation as a key mediator of immune evasion in STK11 mutant NSCLC and nominates MGAT1 a novel target for therapeutic development to overcome this mechanism.

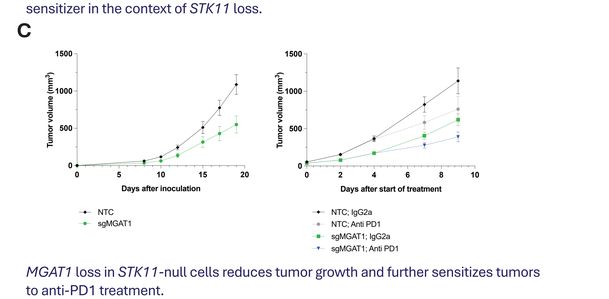
1. LOSS OF *MGAT1* CAN OVERCOME STK11-DRIVEN IMMUNE EVASION IN MC38 CELLS



In vivo screen setup and corresponding dropout of individual *MGAT1* guides with increasing immune pressure.

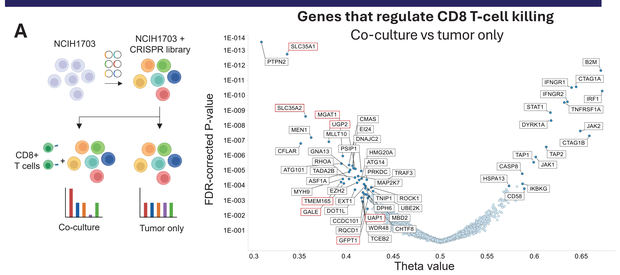


MGAT1 is a top hit as both an *STK11*-specific sensitizer and as an adaptive immune sensitizer in the context of *STK11* loss.

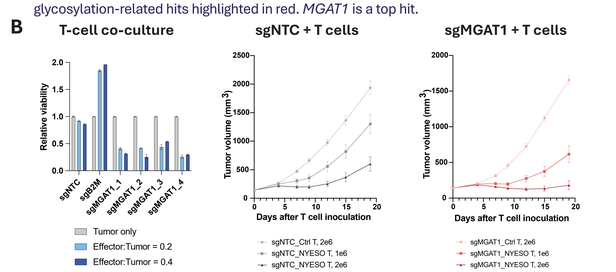


MGAT1 loss in *STK11*-null cells reduces tumor growth and further sensitizes tumors to anti-PD1 treatment.

2. *MGAT1* LOSS SENSITIZES HUMAN TUMOR CELLS TO ANTIGEN-SPECIFIC T-CELL-MEDIATED KILLING IN VITRO

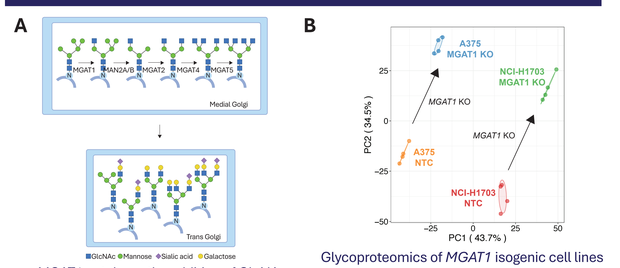


In vitro co-culture screen setup and corresponding dropout analysis with glycosylation-related hits highlighted in red. *MGAT1* is a top hit.



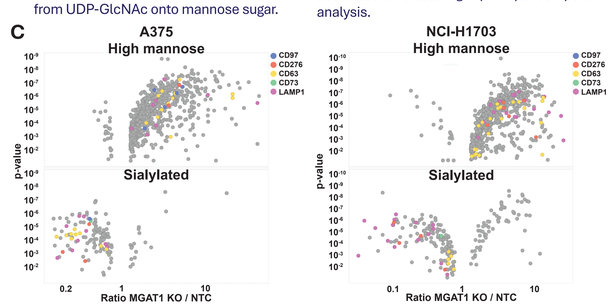
MGAT1 knockout reduces cancer cell viability in T cell co-culture assay and reduces tumor growth in T cell-dependent manner.

3. *MGAT1* LOSS RESULTS IN GLOBAL GLYCOPROTEOME REMODELING

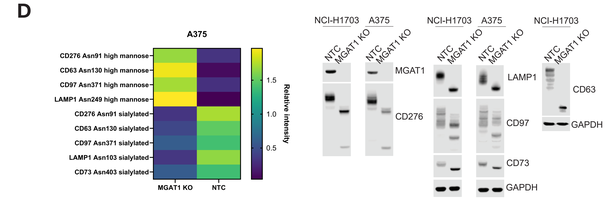


MGAT1 catalyzes the addition of GlcNAc from UDP-GlcNAc onto mannose sugar.

Glycoproteomics of *MGAT1* isogenic cell lines show distinct clustering in principal component analysis.

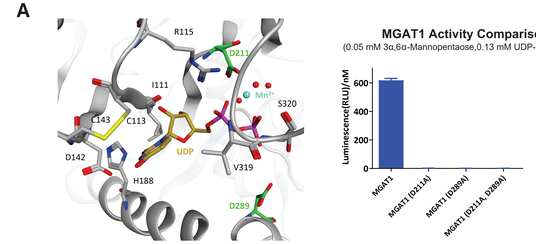


MGAT1 knockout increases prevalence of high mannose N-glycans and decreases prevalence of sialylated N-glycans in two isogenic cell line pairs.

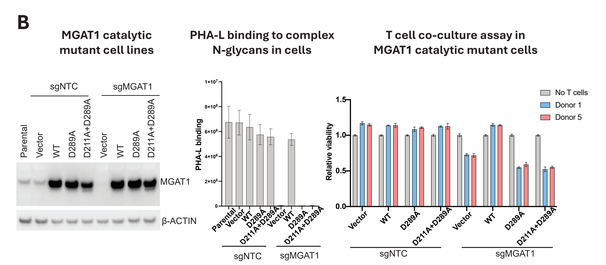


Immune-regulatory glycoproteins show shift toward high mannose modification and away from sialylation marks upon *MGAT1* knockout, and corresponding molecular weight shift is consistent with reduced glycosylation.

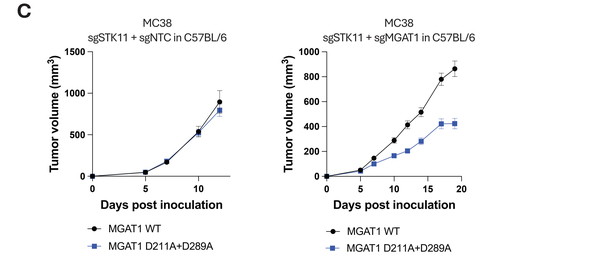
4. CATALYTIC ACTIVITY OF MGAT1 IS REQUIRED FOR IMMUNE EVASION



D211 and D289 are identified as catalytic residues in first human MGAT1 apo structure. Mutations in these residues abrogate MGAT1 catalytic activity in UDP-Glo biochemical assay.



Cell lines generated with *MGAT1* catalytic residue mutations (left) are insufficient to rescue reduced L-PHA binding to high mannose glycans in MGAT1 knockout cells (middle). Catalytic residues are also necessary to rescue cancer cell viability in T cell co-culture assay (right).

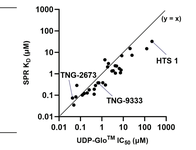


MGAT1 catalytic mutants are insufficient to rescue tumor growth in sgSTK11 + sgMGAT1 syngeneic models.

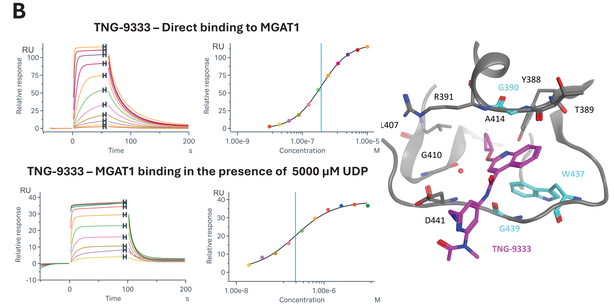
5. MGAT1 ACTIVITY CAN BE INHIBITED BY SMALL MOLECULE INHIBITORS

A

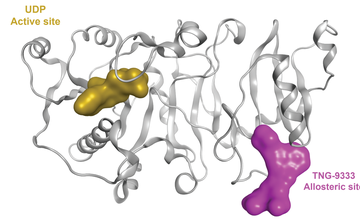
	HTS 1	TNG-9333	TNG-2673
MW, LogD, TPSA	335, 2.6, 82	419, 2.2, 110	478, 2.6, 108
MGAT1 UDP-Glo™ IC_{50} (μM)	197	0.814	0.043
MGAT1 SPR Steady-state K_D (μM) Kinetic K_D (μM)	32.2 -	0.38 0.38	0.07 0.03
Kinetic solubility (μM)	11.8	178	96.4
Human hepatocyte clearance (μL/min/1x10^6 cells)	54.4	3.2	<1.35



>1000-fold improvement in biochemical potency achieved from initial HTS hit with strong correlation between binding and biochemical UDP-Glo potency.



TNG-9333 binds MGAT1 to form a binary complex. TNG-9333 binds MGAT1 in the presence of saturating UDP to form a ternary complex, suggesting an allosteric mechanism.



Ternary structure of MGAT1 in complex with substrate and inhibitor shows TNG-9333 binding in allosteric site.

SUMMARY

- **MGAT1 is identified as a novel, druggable, and tractable immune evasion target in *STK11*-mutant cancers.**
- **Tumor sensitivity to MGAT1 loss is driven, at least in part, by T-cell-mediated killing.**
- **Glycoproteomic data reveals global changes in protein glycosylation upon *MGAT1* knockout, including key immune regulatory proteins.**
- **MGAT1 catalytic activity is required for T-cell-mediated killing in vitro and in vivo.**
- **First crystal structures of human MGAT1 in complex with small molecule inhibitors have been solved.**
- **Medicinal chemistry efforts have achieved >1000-fold improvement in potency from the initial HTS hit with good correlation between enzymatic activity and binding affinity.**
- **MGAT1 program is enabled for drug discovery and available for licensing.**

Acknowledgements

The authors gratefully acknowledge the generous contributions from the scientific teams at WuXi AppTec, PTM Bio, BioMetas, Biortus, and Evotec.

References

Ahronian, L. G. et al. TNG260 is a Small-Molecule CoREST Inhibitor that Sensitizes STK11-Mutant Tumors to Anti-PD-1 Immunotherapy. Cancer Res. (2025) doi:10.1158/0008-5472.can-25-0998.