Hit finding and assay enablement for MGAT1, a novel glycosyltransferase involved in cancer cell immune evasion

Katarzyna B. Handing, Mu-Sen Liu, Douglas A. Whittington, Sining Sun, Rebecca Salerno, William D. Mallender, Jon Come, Scott Throner, Andrew Maynard, Patrick McCarren, John P. Maxwell, Serge Gueroussov, Kiera Vassallo, Yingnan Chen, Jannik N. Andersen, Wenhai Zhang

Abstract # C104

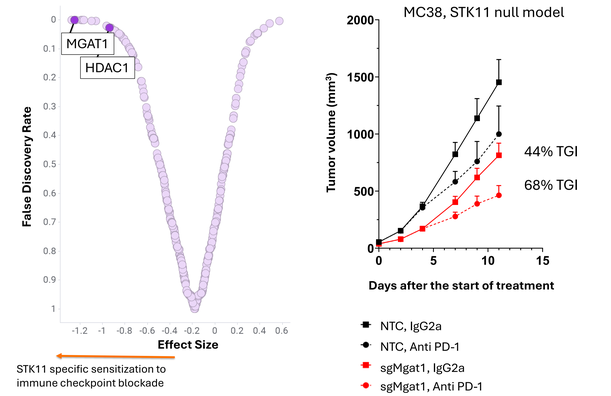
INTRODUCTION

MGAT1 is an N-glycosyltransferase required for N-glycan synthesis. Cell-surface glycans can function as immune checkpoints[1]. MGAT1 knockout in STK11-mutant cancers enhances immune recognition and T-cell–mediated killing, with enzymatic activity being necessary for this phenotype. Another gene identified in the same screen, the deacetylase enzyme of the CoREST complex, is the target of our TNG260 inhibitor currently in Phase 1 clinical trials[2].

We discovered novel MGAT1 binders and inhibitors with sub-micromolar potency through two independent approaches: a UDP-Glo™ high-throughput screen measuring enzymatic inhibition, and a DNA-encoded library screen identifying reproducible binders. High-resolution crystal structures revealed detailed interactions at a binding site distinct from the catalytic pocket, and SPR competition assays confirmed noncompetitive binding with respect to UDP.

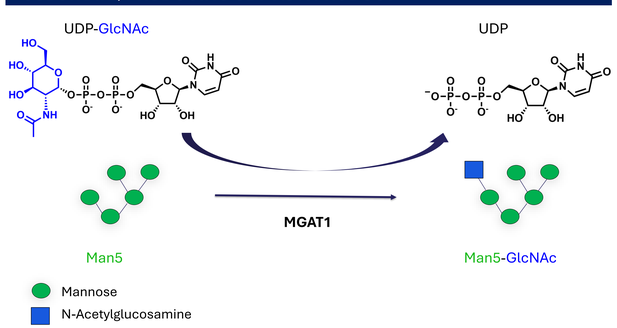
Together, these findings validate allosteric inhibition of MGAT1 as a novel and tractable strategy to block its activity, laying the foundation for future structure-based optimization of MGAT1 inhibitors with potential applications in cancer immunotherapy.

1. MGAT1 KNOCKOUT RE-SENSITIZES STK11-MUTANT MOUSE TUMORS TO IMMUNE CHECKPOINT BLOCKADE



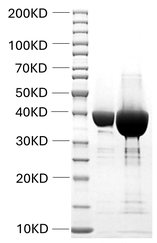
MGAT1 is a top sensitizer identified in *in vivo* STK11 null MC38 screens (left)
MGAT1 knockout is synergistic with anti-PD1-1 treatment in STK11 null MC38 model *in vivo* (right).

2. MGAT1 TRANSFERS N-ACETYLGLUCOSAMINE TO ITS MAN5 SUBSTRATE, PRODUCING UDP AS A PRODUCT

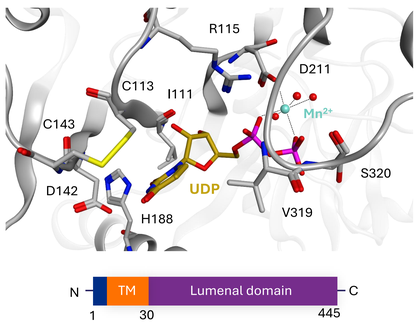


Schematic illustration of the MGAT1-catalyzed transfer of a GlcNAc moiety onto a growing N-glycan.

3. MGAT1 CAN BE ROBUSTLY PRODUCED AND CHARACTERIZED IN BIOCHEMICAL AND BIOPHYSICAL ASSAYS

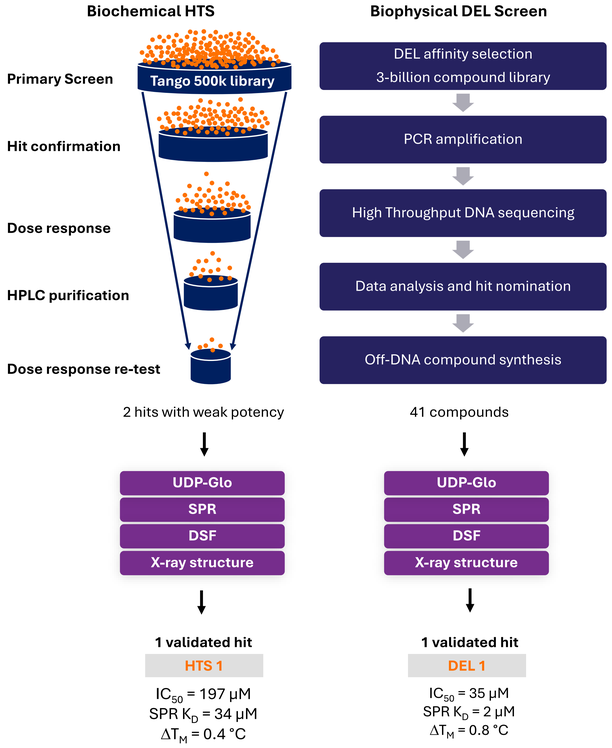


M = Marker
Lane 1 = 2 µg
Lane 2 = 10 µg



High quality protein produced (>95% pure by SDS-Page) as a monomer (Analytical SEC), without post-translational modifications (LC-MS). First X-ray structure determined of human MGAT1 lumenal domain in complex with UDP at 1.6 Å resolution.

4. NOVEL SMALL-MOLECULE COMPOUNDS TARGETING MGAT1 CAN BE IDENTIFIED IN ACTIVITY AND BINDING SCREENS

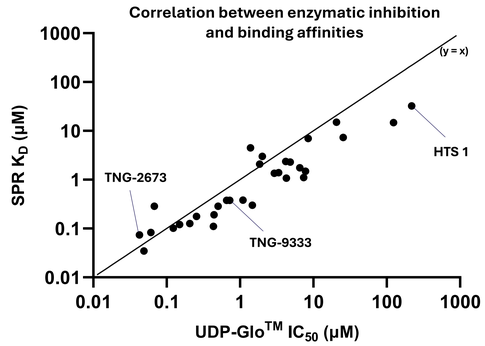


Summary of UDP-Glo biochemical HTS. One validated hit was identified (left).
Summary of DEL binding screen. One validated hit was identified (right).

5. SAR DEVELOPMENT OF MGAT1 HTS COMPOUND SERIES RESULTS IN NANOMOLAR INHIBITORS

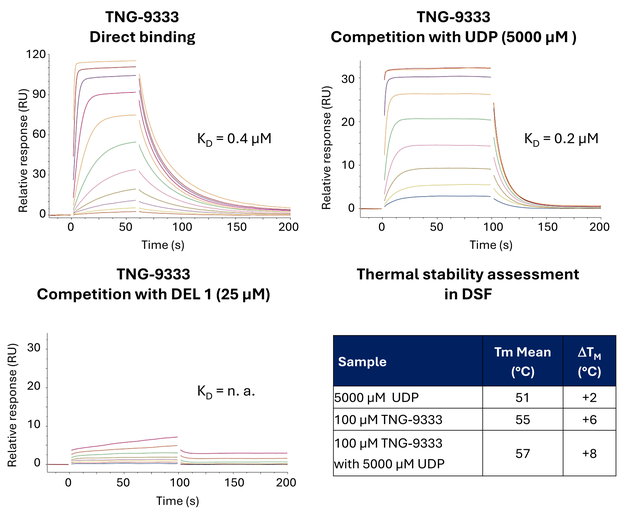
	HTS 1	TNG-9333	TNG-2673
MW, LogD, TPSA	335, 2.6, 82	419, 2.2, 110	478, 2.6, 108
MGAT1 UDP-Glo™ IC_{50} (µM)	197	0.814	0.043
MGAT1 SPR			
Steady-state K_D (µM)	32.2	0.38	0.07
Kinetic K_D (µM)	-	0.38	0.03
Kinetic solubility (µM)	11.8	178	96.4
Human hepatocyte clearance (mL/min/1x10⁶ cells)	54.4	*3.2*	<1.35

MGAT1 inhibitors and their associated physical property and potency data. HTS 1 was identified via an MGAT1 UDP-Glo™ biochemical HTS of 500K compounds.



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

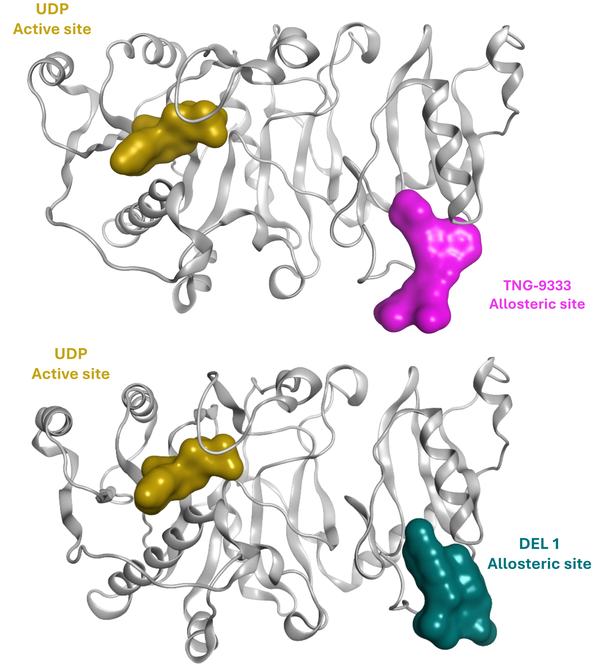
6. MGAT1 COMPOUNDS IDENTIFIED IN BIOCHEMICAL HTS AND DEL BINDING SCREENS BIND IN ALLOSTERIC POCKET



Sample	Tm Mean (°C)	ΔT_M (°C)
5000 µM UDP	51	+2
100 µM TNG-9333	55	+6
100 µM TNG-9333 with 5000 µM UDP	57	+8

SPR data showing TNG-9333 binding affinity was unaffected by saturating UDP, indicating independent binding, a conclusion further supported by the additive stabilization observed in DSF. In contrast, TNG-9333 binding was reduced in the presence of saturating DEL 1, suggesting the two share a common site.

7. ALLOSTERIC BINDING OF HITS IDENTIFIED IN BIOCHEMICAL HTS AND DEL SCREEN CONFIRMED BY CRYSTALLOGRAPHY



Ternary X-ray structures of MGAT1 with UDP and TNG-9333 (2.0 Å resolution), or UDP and DEL 1 (1.3 Å resolution), confirm SPR and DSF results, showing that UDP and TNG-9333 bind distinct sites, while DEL 1 shares the site with TNG-9333.

SUMMARY

- **MGAT1 is identified as a novel, druggable, and tractable immune evasion target in STK11-mutant cancers.**
- **Established robust protein production, biophysical/biochemical assays, and solved the first human MGAT1 crystal structure to enable drug discovery.**
- **Applied two orthogonal approaches to discover novel chemical matter: a UDP-Glo™ HTS for enzymatic inhibition and a DEL screen for direct binders.**
- **Hit expansion and medicinal chemistry optimization improved potency by >1,000-fold, yielding nanomolar inhibitors.**
- **Mechanistic studies (SPR, DSF) showed that HTS- and DEL-derived compounds bind a shared allosteric pocket while co-binding with UDP, a conclusion further supported by X-ray crystallography.**
- **MGAT1 program is available for partnering.**

Acknowledgements

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

References

1. Pereira M. S. et al. Glycans as Key Checkpoints of T Cell Activity and Function. Front Immunol. (2018) doi: 10.3389/fimmu.2018.02754.
2. 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.