The Clinical Candidate Xanomeline Displays a Binate Orthosteric and Allosteric Binding and Pharmacological Profile at the M_4 mAChR

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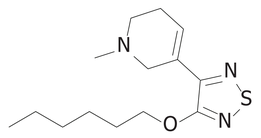
Background

- The M_4 muscarinic acetylcholine receptor (mAChR) has emerged as a drug target of high therapeutic interest due to its expression in brain regions involved in the regulation of psychosis, cognition, and addiction
- The investigational M_1/M_4 preferring mAChR agonist xanomeline demonstrated clinical efficacy and was generally well tolerated in the 5-week, randomized, double-blind, placebo-controlled, phase 2 EMERGENT-1 (NCT03697252)[1] and phase 3 EMERGENT-2 (NCT04659161)[2] studies
- Initially, xanomeline had been considered to bind only to the orthosteric acetylcholine binding site; however, recent studies have shown much greater complexity as to the precise nature of ligand-receptor binding interactions, including efficacy-driven selectivity, subtype-dependent wash-resistant binding, and an atypical interaction with positive allosteric modulators (PAMs)
- Understanding how xanomeline binds to both the orthosteric and allosteric binding pockets of the M_4 mAChR and determining the molecular mechanisms behind its unique pharmacological profile are key for the design of novel antipsychotics that target the M_4 mAChR

Xanomeline

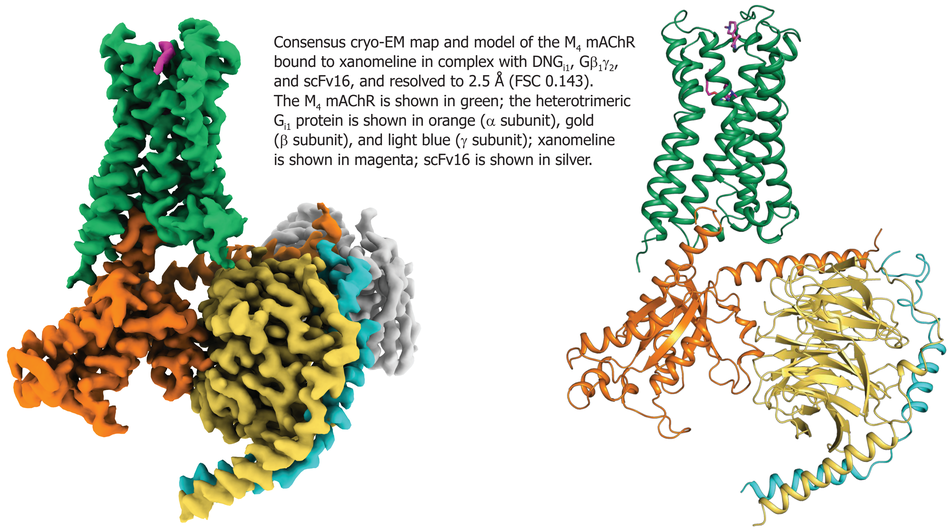


Methods

- We determined a cryogenic electron microscopy (cryo-EM) structure of xanomeline bound to the human M_4 mAChR in complex with the heterotrimeric G_{i1} transducer protein
- Molecular dynamics (MD) simulations were used to corroborate the additional allosteric binding mode that was identified in the cryo-EM structure
- The allosteric binding mode of xanomeline was further validated using site-directed mutagenesis

Results

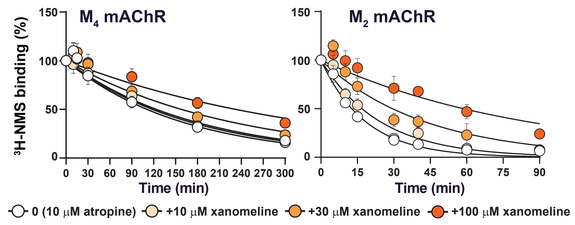
Consensus Cryo-EM Map and Model of the Xanomeline-Bound M_4 mAChR–G_{i1} Complex



Consensus cryo-EM map and model of the M_4 mAChR bound to xanomeline in complex with DNG_{i1}, $G\beta_1\gamma_2$, and scFv16, and resolved to 2.5 Å (FSC 0.143). The M_4 mAChR is shown in green; the heterotrimeric G_{i1} protein is shown in orange (α subunit), gold (β subunit), and light blue (γ subunit); xanomeline is shown in magenta; scFv16 is shown in silver.

cryo-EM, cryogenic electron microscopy; DNG_{i1}, dominant negative form of G_{i1}; FSC, Fourier shell correlation; $G\beta_1\gamma_2$, G-protein beta-1 and gamma-2 subunit complex; mAChR, muscarinic acetylcholine receptor; scFv16, single-chain variable Fab fragment.

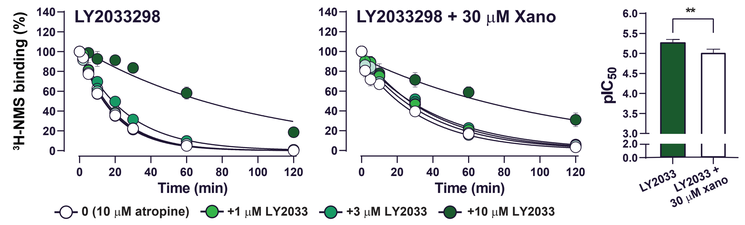
Verifying Allosteric Mode Through Radioligand Binding



^{3}H-NMS, [^{3}H]-N-methylscopolamine; mAChR, muscarinic acetylcholine receptor.

- Xanomeline alters orthosteric antagonist ligand dissociation, a hallmark of allosteric modulators[4]
- Largest allosteric effect observed at the M_2 mAChR subtype

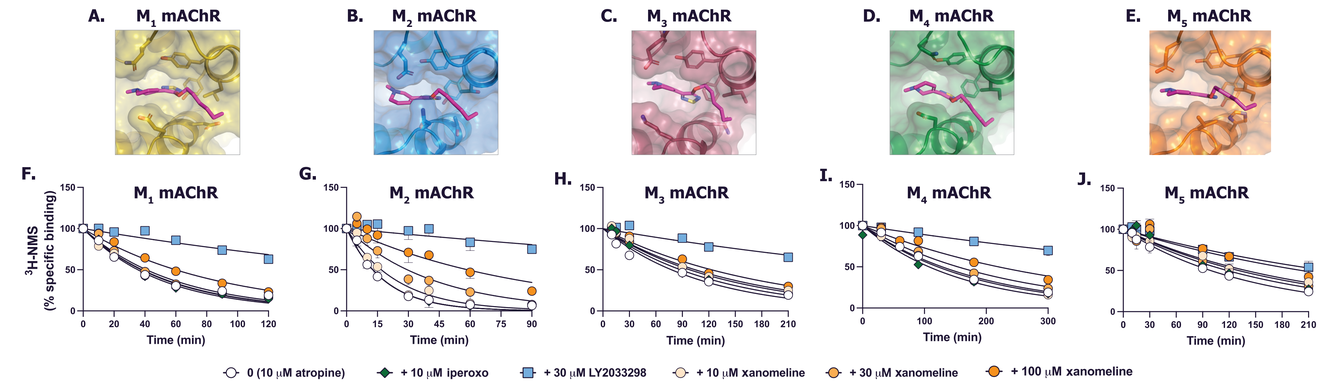
Xanomeline Competes With "Traditional" Positive Allosteric Modulators at the M_2 mAChR



**$P \leq 0.01$ via 2-tailed Student's t test.
^{3}H-NMS, [^{3}H]-N-methylscopolamine; LY2033, LY2033298; mAChR, muscarinic acetylcholine receptor; pIC, predicted toxicity value; xano, xanomeline.

- LY2033298: $M_4 > M_2$ mAChR selective positive allosteric modulator
- Previous study: xanomeline dose-response curve collapses in the presence of LY2033298 at the M_2 mAChR[5]
- Data suggest xanomeline competes with LY2033298, perhaps explaining the collapse in dose-response curve
- Xanomeline is a dual orthosteric/allosteric ligand, in part explaining its unique pharmacology

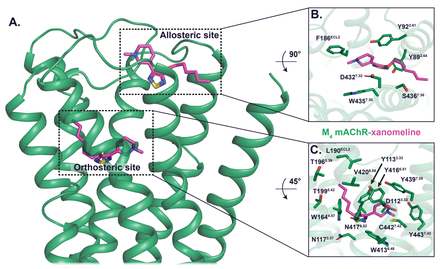
Xanomeline Binds to the Allosteric Binding Site at All mAChR Subtypes



A-E. Xanomeline was placed in the allosteric site of either an active structure (M_1 and M_2 mAChR) or active state homology model (M_3 and M_5 mAChR) followed by an energy minimization and compared with the M_4 mAChR cryo-EM structure. For all subtypes, xanomeline and the surrounding residues display a similar pose. **F-J.** Effect of increasing concentrations of xanomeline, LY2033298 30 μM, or iperoxo 10 μM in the presence of atropine 10 μM on the dissociation of ^{3}H-NMS at the M_1 mAChR, M_2 mAChR, M_3 mAChR, M_4 mAChR, and M_5 mAChR. At all subtypes, xanomeline is able to slow the dissociation of ^{3}H-NMS, indicating an allosteric character.

^{3}H-NMS, [^{3}H]-N-methylscopolamine; cryo-EM, cryogenic electron microscopy; mAChR, muscarinic acetylcholine receptor.

Analysis of the M_4 mAChR Orthosteric and Allosteric Binding Sites of Xanomeline

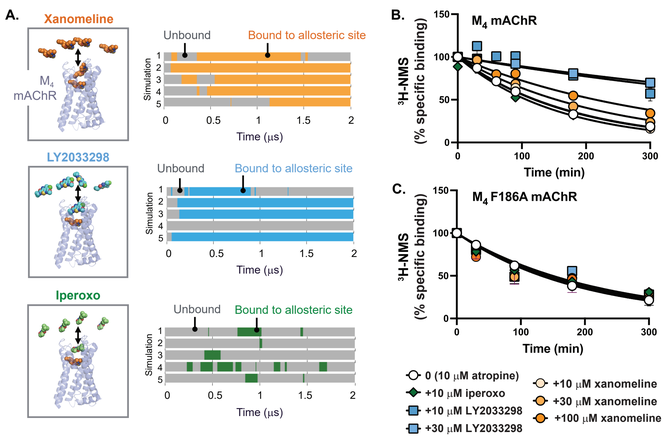


A. Xanomeline is bound in both the orthosteric and allosteric binding sites of the M_4 mAChR. **B.** Xanomeline in the common extracellular vestibule mAChR allosteric binding site with allosteric site residues (shown as green sticks). **C.** Xanomeline is bound in the canonical orthosteric binding site of the mAChRs positioned under a closed tyrosine lid composed of residues Y113, Y416, and Y439. The hexyloxy aliphatic tail of xanomeline sticks up toward the extracellular region of the M_4 mAChR.

Superscript numbers represent Ballesteros–Weinstein nomenclature[3] for residue structural position within G-protein coupled receptor transmembrane helices.

ECL2, extracellular loop 2; mAChR, muscarinic acetylcholine receptor.

Computational and Pharmacological Validation of Xanomeline Binding in the M_4 mAChR Allosteric Site



A. In molecular dynamics simulations, xanomeline spontaneously binds to the M_4 mAChR allosteric site for a similar fraction of time as the PAM LY2033298 and for a greater fraction of time than the orthosteric agonist iperoxo. Simulations were initiated with free ligands in solution (xanomeline, LY2033298, or iperoxo at the same concentration) and a xanomeline molecule bound to the orthosteric site. Each horizontal bar represents an independent simulation and shows when the allosteric binding site has a ligand bound. **B.** Pharmacological validation for the allosteric character of xanomeline at the M_4 mAChR. Increasing concentrations of xanomeline slow down the dissociation of ^{3}H-NMS similar to the PAM LY2033298, whereas iperoxo has no influence on ^{3}H-NMS dissociation. **C.** Mutation of a key residue in the allosteric binding site (F186A) removes the ability of xanomeline and LY2033298 to have an allosteric influence on ^{3}H-NMS dissociation. For **B** and **C**, data represent the mean ± SEM of 3-9 individual experiments performed in duplicate.

^{3}H-NMS, [^{3}H]-N-methylscopolamine; mAChR, muscarinic acetylcholine receptor; PAM, positive allosteric modulator; SEM, standard error of the mean.

Summary and Conclusions

- Xanomeline unexpectedly displayed dual orthosteric and allosteric binding at the M_4 mAChR
- In MD simulations, xanomeline spontaneously binds to the M_4 mAChR allosteric site as tightly as the M_4 mAChR PAM LY2033298 and much more tightly than the orthosteric agonist iperoxo
- Xanomeline displayed allosteric modulation at all 5 mAChRs through delayed ^{3}H-NMS off rates and was competitive with the allosteric modulator LY2033298 at the M_2 mAChR
- The significance of the xanomeline dual orthosteric and allosteric binding is the subject of ongoing studies

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Disclosures

CCF and SMP are employees of and hold equity in Karuna Therapeutics. All other authors were employees of Monash University, Stanford University, University of Glasgow, or the University of Tokyo at the time of the study. SMP has or had business relationships with Alnylam Pharmaceuticals, Sage Therapeutics, and Voyager Therapeutics. DW, PMS, and AC have an ongoing business relationship with Septerna.

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