Structure of STAR-0215 Bound to Active Plasma Kallikrein Reveals a Novel Mechanism of Enzyme Inhibition

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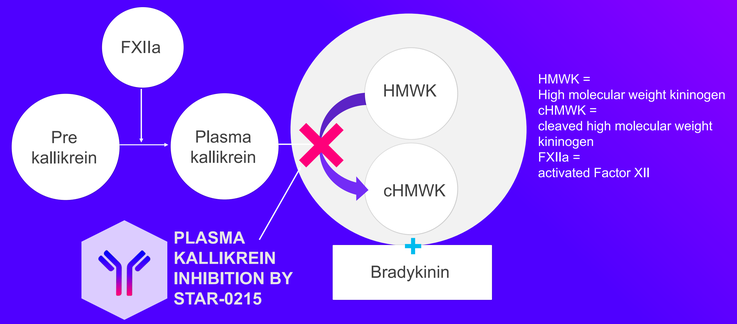
BACKGROUND:
Inhibition of plasma kallikrein activity is a validated mechanism for prevention of hereditary angioedema (HAE) attacks. STAR-0215 is a novel, potent and selective, long-acting monoclonal antibody plasma kallikrein inhibitor currently in clinical development.

We investigated STAR-0215 binding to plasma kallikrein and its inhibitory mechanism of action by utilizing Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) and Cryogenic Electron Microscopy (Cryo-EM).

Mechanism of HAE pathogenesis and the potential inhibitory role of monoclonal antibody inhibitor, STAR-0215



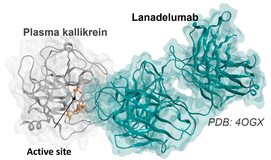
Methods

Plasma kallikrein alone and STAR-0215 Fab/plasma kallikrein complex were analyzed using Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS). Measurements were performed using an HDX sample handling robot in line with a Q-ToF Xevo G2-XS mass spectrometer. After deuterium exchange, the samples were quenched and frozen at 0°C before digestion and analysis using MS.

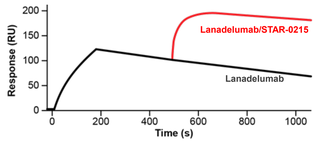
For structural determination of STAR-0215 Fab/plasma kallikrein complex using Cryo-EM, complexes were grid screened to evaluate the best conditions for imaging, followed by vitrification and imaging by a Krios G3i electron microscope. Image data were processed and analyzed to obtain a 2.62 Å high-resolution structure of the bound complex.

STAR-0215 Does Not Bind to Plasma Kallikrein Active Site

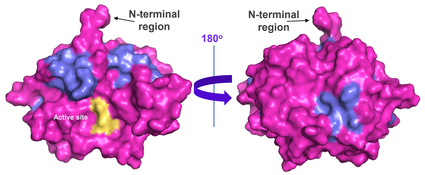
Lanadelumab binds directly to plasma kallikrein active site.



STAR-0215 binds plasma kallikrein in the presence of lanadelumab suggesting an allosteric binding mode.



HDX-MS confirms STAR-0215 allosteric binding



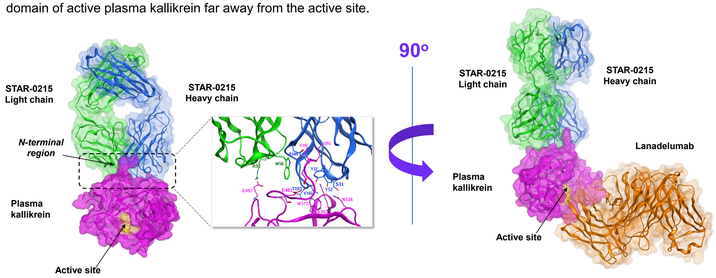
STAR-0215 in complex with full length human plasma kallikrein was analyzed by Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS).

The difference in mass of the free deuterated plasma kallikrein and deuterated STAR-0215/plasma kallikrein complex pinpoints areas affected during binding (colored blue).

Residues with the largest differences in deuterium uptake comparing the free plasma kallikrein to the STAR-0215/plasma kallikrein complex are highlighted in blue on the surface of the plasma kallikrein indicating a binding site distinct from the active site. The active site is highlighted in yellow.

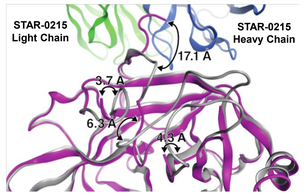
The Differentiated Plasma Kallikrein Binding of STAR-0215 is Consistent with its High Specificity

The 2.6 A high resolution structure of STAR-0215 in complex with plasma kallikrein shows STAR-0215 binding to the N-terminal domain of active plasma kallikrein far away from the active site.

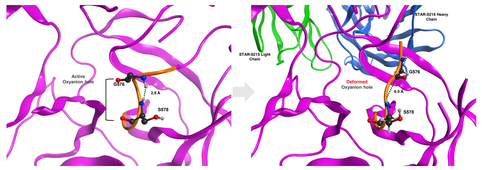


Structure of the STAR-0215 Fab/plasma kallikrein complex. The plasma kallikrein is depicted as a magenta transparent surface with the catalytic triad colored yellow. The heavy chain and the light chain of STAR-0215 are shown in blue and green surfaces, respectively. The inset showcases important interactions between the STAR-0215 Complementarity-determining regions (CDRs) and plasma kallikrein.

Binding of STAR-0215 to Plasma Kallikrein Induces Significant Conformational Changes Reverting it to a Zymogen-Like Inactive State



Structure overlay of the plasma kallikrein catalytic domain that is complexed with STAR-0215 Fab in magenta with the structure of active plasma kallikrein catalytic domain alone in gray (PDB 2ANW) demonstrates the conformational changes imposed by the STAR-0215 binding.



The oxyanion hole that is formed by the amides of G576 and S578 in plasma kallikrein is vital for enzymatic activity.

STAR-0215 binding to plasma kallikrein results in the distortion of the oxyanion hole and its subsequent inactivation.

CONCLUSIONS:

- The structure of STAR-0215 bound to plasma kallikrein confirms previous biophysical assays and reveals that allosteric binding of STAR-0215 inhibits plasma kallikrein activity by inducing conformational changes which render plasma kallikrein inactive by reconfiguring into a zymogen-like state.
- Highly selective binding of STAR-0215 to active plasma kallikrein (and against the prekallikrein precursor) as the N-terminal region is only generated upon activation by FXIIa cleavage of prekallikrein. This may avoid sink-effect and prevent potential target mediated drug disposition, thereby allowing more STAR-0215 to be available for plasma kallikrein inhibition.
- Allosteric binding site for STAR-0215 (instead of active site binding) may provide additional specificity against other S1 serine proteases with highly conserved active site regions.

These findings, together with recent Phase 1a clinical data that establish long half-life in healthy volunteers (Poster # 416), suggest that STAR-0215 is a potential best-in-class agent for prevention of HAE attacks.

The ALPHA-STAR Phase 1b/2 clinical trial of STAR-0215 in people with HAE is expected to initiate in Q1 2023.

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