STAR-0215 Bound to Active Plasma Kallikrein Structure Uncovers a New Binding Mode

Nikolaos Bris, Pradeep Bista, Andrew Nichols
Astra Therapeutics Inc., Boston, MA

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).

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 Xavo G2-XS mass spectrometer. After deuterium exchange, the samples were quenched and frozen at -80°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.

Results
The 2.6 Å 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.

STAR-0215 does not bind to plasma kallikrein active site

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

The oxygen hole that is formed by the arginines of S576 and S578 in plasma kallikrein is vital for enzymatic activity.

Binding of STAR-0215 to Plasma Kallikrein induces significant conformational changes reverting it to a zymogen-like active site

The oxyanion hole is formed by the arginines of S576 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.
• STAR-0215 binds with high selectivity to active plasma kallikrein compared to prekallikrein as the N-terminal region is only generated upon activation by Factor XII 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 site binding by 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, suggest that STAR-0215 is a potential first choice agent for prevention of HAE attacks.

The ALPHA-STAR Phase 1b/2 clinical trial of STAR-0215 in people with HAE is enrolling and administering STAR-0215 to patients. Initial proof-of-concept results are anticipated by mid-2024.