Docket #: S17-473

High-affinity, Small-molecule, CLC-2 Chloride Ion Channel Probes

Chloride channels, which control the flux of Cl– ions across cell membranes, are notoriously difficult to drug. Most small molecule chloride channel inhibitors act on many anion channels with little to no selectivity among the nine members of the CLC family and other chloride channels (e.g., VRAC, CFTR, TMEM16A). Researchers at Stanford have developed AK-42, a first-in-class molecule that selectively targets the CLC-2 chloride channel (17 nM IC50) with unprecedented selectivity (>1000-fold) over CLC-1, the most closely related CLC family member. CLC-2 plays a role in certain forms of epilepsy/neuronal hyperexcitability and white matter degeneration in the brain but is poorly understood in these contexts.

Discovery and validation of AK-42:

- **Screening and optimization**: Meclofenamate, an NSAID, was identified as a lead from a high-throughput patch-clamp electrophysiology screen of FDA-approved drugs. SAR studies from this lead using CHO cells overexpressing CLC-2 enabled the optimization of AK-42 to low-nanomolar potency. Analogous studies with CLC-1 demonstrated >1000-fold selectively for CLC-2.
- **Off-target evaluation**: AK-42 shows no off-target engagement against a panel of 61 common ion channels, receptors, and transporters in the CNS, including other chloride channels (VRAC, CFTR, TMEM16A). Functional screening was performed by the NIH Psychoactive Drug Screening Program.
- **Knockout validation**: AK-42 specificity was validated with homozygous CLC-2 knockout mice, using electrophysiology recordings of endogenous CLC-2 currents in mouse brain slices. AK-42 attenuated steady-state CLC-2 currents in neurons from wild-type mice but had no effect on those from Clcn2–/– animals.
- **Binding site validation**: Independent structural studies from Stanford and a competing lab disclosed cryo-EM structures of AK-42 bound to CLC-2, providing further validation that the compound directly acts on the channel by binding

near the ion conduction pore, giving insight into the molecular basis of potency/selectivity.

Despite the relatively untapped potential of chloride channels as drug targets, recent successes in the biotech sphere have illustrated the relevance of such proteins for disease treatment—In 2024, the FDA approved TRIKAFTA® from Vertex Pharmaceuticals, targeting the CFTR chloride channel as treatment for cystic fibrosis. Within the CLC family, NMD Pharma has shown promising results with a small molecule targeting CLC-1 for the treatment of neuromuscular disorders and has now been granted orphan drug designation by the FDA for treatment of myasthenia gravis.

It is likely that many pharmaceutically relevant indications for CLC chloride channels have not been discovered yet because of the lack of tools to acutely, reversibly, and selectively modulate CLC function. AK-42 fills this void for CLC-2 and has spurred requests for the compound from other scientists around the world. Commercializing AK-42 as a research tool would provide greatly expanded access to the compound for establishing mechanistic evidence for the role of CLC-2 in different physiological contexts, including in epilepsy, leukoencephalopathies, and glioma cancers. It has been difficult for Stanford to keep up with the requests to share compound, which currently is synthesized in-house in the lab where it was invented. AK-42 has already proven useful for parsing the role of CLC-2 in Cl– secretion within the intestinal epithelium, where various channels and transporters are critical for maintaining gut health. Outside the CNS, CLC-2 malfunction has also been shown to play a role in primary aldosteronism, one of the most prevalent causes of secondary hypertension.

Stage of Development: *Ex Vivo* Proof of Concept

Research is ongoing to develop imaging probe molecules. Derivative AK-42 imaging probe structures that retain potency against CLC-2 are anticipated to be published in the coming year. Stanford has also begun to collaborate with other researchers to study CLC-2 in primary cells and in vivo physiological contexts using AK-42. The recent availability of high resolution cryo-EM structures of CLC-2 in both the apo-form and with AK-42 bound, along with analogous structures of CLC-1, is guiding computational modeling and SAR studies to advance new inhibitors of both channels and to develop selective imaging probes. A derivative of AK-42 has been validated as a selective fluorescent probe for recombinant CLC-2 expressed heterologously. Experiments are in progress to validate the utility of this tool in primary cells and tissue.

Applications

- **Research tools**:
  - Pharmacological tools
  - Imaging probes
- **Drug discovery and development** for aldosteronism, and central nervous system diseases like epilepsy, leukoencephalopathy, and glioma treatments
  - Therapeutic leads

Advantages

- First-in-class, small-molecule (non-peptide) modulators
- High affinity, selectivity, and specificity

Publications

- Xu, M., Neelands, T., Powers, A.S., Liu, Y., Miller, S.D., Pintilie, G.D., Du Bois, J., Dror, R.O., Chiu, W., Maduke, M. (2024). CryoEM structures of the human CLC-2 voltage-gated chloride channel reveal a ball-and-chain gating mechanism. *eLife* . 12:RP90648.
- Ma, T., Wang, L., Chai, A. et al. Cryo-EM structures of ClC-2 chloride channel reveal the blocking mechanism of its specific inhibitor AK-42. *Nat Commun* 14, 3424 (2023).
- Oak AA, Chu T, Yottasan P, Chhetri PD, Zhu J, Du Bois J, Cil O. Lubiprostone is non-selective activator of cAMP-gated ion channels and Clc-2 has a minor role in its prosecretory effect in intestinal epithelial cells. *Mol Pharmacol*. 2022 Jun 9;102(2):106–15.
- Koster, A. K., Reese, A. L., Kuryshev, Y., Wen, X., McKiernan, K. A., Gray, E. E., Wu, C., Beenhakker, M. P., Huguenard, J. R., Maduke, M., & Du Bois, J. (2020). Development and validation of a potent and specific inhibitor for the CLC-2 chloride channel. *Proceedings of the National Academy of Sciences, 117.* (51), 32711-32721.
- Koster, A. K. (2019). Expanding the Small-Molecule Toolbox for Studying CLC Chloride Channel Physiology and Biophysics. Stanford University.

Patents

- Published Application: 20200016103
- Issued: 11,173,137 (USA)

Innovators

- Anna Koster
- Merritt Maduke
- Justin Du Bois

Licensing Contact

Mona Wan

Senior Licensing Manager for Special Projects

Email