**Introduction**: Omega-3 eicosyeycicosanoids are bioactive lipid mediators generated by cytochrome P450 epoxygenases from omega-3 fatty acids, such as EPA. Omega-3 eicosyeycicosanoids exert cardioprotective effects by improving Ca2+ handling and mitochondrial function and limiting pro-inflammatory and pro-fibrotic responses. We concluded the preclinical development of a metabolically robust small molecule (OMT-28) that mimics the structure and function of the EPA-derived omega-3 eicosyeycicosanoid 17,18-epoxyeicosatetraenoic acid (17,18-EEQ). Here we validate the ability of OMT-28 to target key pathomechanisms that govern the occurrence and persistence of atrial fibrillation (AF).

**Methods**: Preclinical mechanistic studies were performed in neonatal rat cardiomyocytes (NRCMs), embryonic chicken atrial cardiomyocytes (CAMs), and atrial cardiomyocytes (HAMs) from patients undergoing open-heart surgery. AF vulnerability was analyzed by programmed electrical stimulation in mice subjected to 2 weeks chronic β-adrenergic stress. A first-in man safety and pharmacokinetics study was conducted in 75 healthy volunteers (NCT03078738).

**Result**: In vitro, OMT-28 reduced spontaneous beating of NRCMs with an EC50 of 1.6 nM, being thus about 10- and 1000-fold more potent than 17,18-EEQ and EPA. OMT-28 reduced Ca2+-transient amplitudes by 40-60% both in spontaneously beating CAMs and voltage-clamped HAMs. Cell shortening was unaffected by OMT-28 (100 nM) in HAMs, whereas time-to-peak and relaxation times were accelerated by 51±5% and 65±4%, respectively. In NRCMs, OMT-28 (1 µM) increased Akt-Ser437 (+143±56%) and phospholamban-Ser16 (+61±18%) phosphorylation. Based on multiple pharmacological interventions, we discovered that OMT-28 likely acts via an unknown Gαi protein-coupled receptor that elicits PI3K/Akt/eNOS/PKG signaling. Beyond rapid non-genomic effects, long-term treatment with OMT-28 protected NRCMs against LPS-induced inflammatory injury as well as loss of mitochondrial function in response to hypoxia/reoxygenation, consistent with OMT-28-mediated activation of sirtuin-1 and PPARα. OMT-28 (0.625 mg/kg) decreased the vulnerability of mice to electrically inducible AF from 73% to 18%; AF burden and episode duration were also significantly reduced. In healthy volunteers, OMT-28 (4-60 mg) showed high oral bioavailability and favorable pharmacokinetics (plasma half-life about 57 h) with no adverse side effects. No QT prolongation was detected using high-resolution ECGs.

**Conclusion**: OMT-28 might constitute a novel approach for AF treatment by targeting Ca2+ handling.
mitochondrial function, and inflammation in atria. This strategy is currently clinically tested in a Phase II study on OMT-28 in the maintenance of sinus rhythm after electrical cardioversion in patients with persistent AF (NCT03906799).