ISHLT 2025 Boston- Control Number: 5157

TX000045 (TX45), A Novel Fc-Relaxin-2 Fusion Protein: Bioengineering and Preclinical Development for the Treatment of Group 2 PH

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Abstract

Purpose
The therapeutic potential of Relaxin-2, a vasodilatory, antifibrotic, and anti-inflammatory peptide hormone, has been demonstrated in preclinical and clinical studies. However, its short in vivo half-life has limited its clinical utility. Native Relaxin has a high isoelectric point (pI), leading to non-specific binding in vivo and a poor pharmacokinetic (PK) profile. To address this, we engineered a potent Fc-Relaxin-2 fusion protein, TX45, with a superior pharmacokinetic (PK)/pharmacodynamic (PD) profile, renal arterial blood flow (RABF), and monocrotaline (MCT)-induced pulmonary hypertension (PH) model in rats.

Methods
TX45 is a single chain Fc-Relaxin-2 fusion molecule with an engineered Relaxin B-chain connected at its N-terminus to the Fc moiety and at its C-terminus to a linker and then an engineered Relaxin-2 A Chain. TX45 was optimized to reduce the pI by inclusion of multiple negatively charged amino acids in the linker and suitable substitutions in the Relaxin B-chain and A-chain sequences. Potency and specificity were tested using rat and human RXFP1 in vitro signaling assays. To characterize the pharmacology of TX45, we used RABF and the MCT-induced PH model in rats.

Results
High pI molecules bind to negatively charged heparin-proteoglycans and have poor PK properties characterized by rapid clearance in the first (alpha) phase of the concentration time profile. The pI of TX45 was reduced from >9 to ~7.8, with a corresponding loss in heparin binding and an improvement in PK. This improvement reduced the in vitro potency in our human and rat RXFP1 signaling assays. However, the loss of potency in vitro was more than compensated for in vivo, where TX45 increased RABF in rats in a dose-dependent manner, with an EC_{50} of 2.2 µg/mL (34 nM). TX45 is ~3-fold more potent against the human receptor compared to the rat receptor. Sub-chronic administration of TX45 in the MCT-PH model showed a significant decrease in right ventricular systolic pressure (RVSP), mean pulmonary arterial pressure (mPAP), and Fulton's Index compared to the control group. TX45 significantly reduced pulmonary vascular inflammation and improved the degree of pulmonary arterial muscularization.

Conclusion
The reduction in positive charge and heparin binding of TX45 translated into a greatly improved PK profile and superior PD effects in vivo, as measured by the dose-dependent increase in RABF. TX45 also significantly improved pulmonary hemodynamics in MCT-induced PH in rats. Collectively, our preclinical data support the clinical testing of TX45 in patients with Group 2 PH.

Background

- Human relaxin-2—a peptide hormone historically referred to as the "pregnancy hormone"—exhibits pronounced vasodilatory, anti-fibrotic, and anti-inflammatory properties, in addition to its vital role in pregnancy adaptations. Its receptor, RXFP1, is Gs coupled and is expressed in vascular smooth muscle, the endothelium, and cardiomyocytes. (1, 2).
- In clinical trials, intravenous infusion of serelaxin—a recombinant human relaxin—has shown promising effects. It enhanced renal blood flow in both healthy volunteers and patients with stable heart failure, simultaneously reducing filtration fraction and glomerular pressure, which may confer long-term renal protection. Invasive hemodynamic evaluations also revealed improvements in pulmonary capillary wedge pressure, mean pulmonary artery pressure, pulmonary vascular resistance, and systemic vascular resistance (3–5).
- Although the phase 3 RELAX-AHF-2 trial did not demonstrate a clinical benefit for relaxin in acute heart failure, a meta-analysis of six studies—including RELAX-AHF-2—suggested overall positive outcomes. Its short half-life necessitates dosing by continuous intravenous infusion, reinforcing the need for longer-acting relaxin therapies.

Objective

1) The primary challenge is that a native relaxin has a short half-life. Our objective was to fuse it with the Fc domain of human IgG1 to enhance the half-life.
2) In rat, Fc domain of human IgG1 may cause an anti-drug antibody response and reduce efficacy. Our objective was to remove B cells in rats and determine the efficacy of the molecule in a rat model of PH.

Methods

Optimization of TX45 molecule:
Both an initial Fc–Relaxin-2 fusion protein (TX-04) and the published Fc–Relaxin construct (RELAX0010), as described in the patent WO2021255127A1, showed an exaggerated alpha-phase decline in rats (6). We hypothesized that this may be due to their high isoelectric points (pI > 9) and corresponding high heparin binding. In response, we developed TX45 by strategically reducing its positive charge, lowering the pI to ~7.8, which decreased heparin binding and improved the pharmacokinetics in rats.

In vitro studies:

cAMP assay
We used an over-expression system to determine the cross-species in vitro potency of TX45 using HEK293. To determine selectivity of TX45, we used CHO-K1 Gs cell lines that stably expressed human RXFP1 or RXFP2.

In vivo Studies:

Animals
All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Avastus Preclinical Services (Cambridge, MA) and conducted in compliance with its guidelines.

Renal Arterial Blood Flow (RABF) measurements
Rats (320–380 g) were assigned to treatment groups receiving either vehicle (1X PBS, pH 7.4) or various doses of TX45 (0.03–10 mg/kg). Under urethane anesthesia, renal arterial blood flow (RABF) was measured using a perivascular flow probe and flowmeter. Following a 30–45-minute baseline recording, rats received a bolus injection of TX45 or vehicle, followed by a 90-minute observation. TX45 concentrations were quantified via a human IgG-Fc ELISA assay.

Monocrotaline model of pulmonary hypertension
The monocrotaline (MCT) rat model is a widely used system for studying pulmonary hypertension (PH) due to its ability to induce consistent pulmonary hypertension (PH) and right ventricular hypertrophy (7, 8). In this study, young male Sprague Dawley rats (200–240 g) were assigned to various treatment groups. On Study Day 1, rats in all groups except control group, received a single 60 mg/kg dose of MCT subcutaneously (1 mL/kg in DMSO). To deplete B-cell and reduce generation of anti-drug antibodies (ADAs), rats were also treated with an anti-mouse CD20 antibody (20 mg/kg, intraperitoneally) on Days 8, 9, 10, and 17. TX45 (10 mg/kg, intravenously) was given on Days 7, 10, 14, 17, 21, and 24. On Day 28, right ventricular pressure (RVP) and pulmonary arterial pressure (PAP) were measured. Hearts and lungs were excised and weighed, and the Fulton Index (ratio of right ventricular weight to left ventricle plus septum weight) was calculated to assess cardiac remodeling. Formalin-fixed lungs were analyzed for histopathological changes.

Statistical analysis:
Data are presented as mean ± SEM. Statistically significance determined using one-way ANOVA followed by Tuckey's multiple comparison tests.

Results

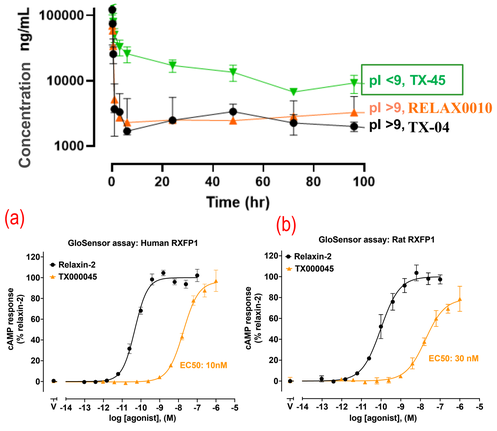

Figure 2: **High potency of TX45 at human and rat RXFP1. TX45 is potent at human** (a) **and** (b) **rat RXFP1.** The experiment was performed using HEK293 cells transiently expressing human and rat RXFP1. **TX45 is highly selective** at human RXFP1 but very weak at human RXFP2 (data not shown for brevity).

Figure 1: **TX45 showed better PK profile Vs earlier TX molecule and RELAX0010 in rats after lowering pI.** High-pI engineered molecules TX-04 and RELAX0010 made in-house exhibit rapid alpha-phase decline in rat PK studies, whereas TX45 with a lower pI shows enhanced pharmacokinetics.

Agonist	Human RXFP1 EC_{50} (nM) Mean ± SD (n)	Rat RXFP1 EC_{50} (nM) Mean ± SD (n)
Relaxin-2	0.05 ± 0.02 (6)	0.1 ± 0.02 (3)
TX-04	0.3 ± 0.06 (2)	1.2 ± 0.2 (3)
RELAX0010	**0.5 ± 0.1 (3)**	**0.9 ± 0.1 (2)**
TX45	**10 ± 4 (6)**	**30 ± 20 (3)**

Table 1: **TX45 at human and rat RXFP1 show loss in potency compared** to RELAX0010 in **in vitro assay.** Potencies were measured using a cAMP signaling assay with transiently expressed human or rat RXFP1 in HEK293 cells.

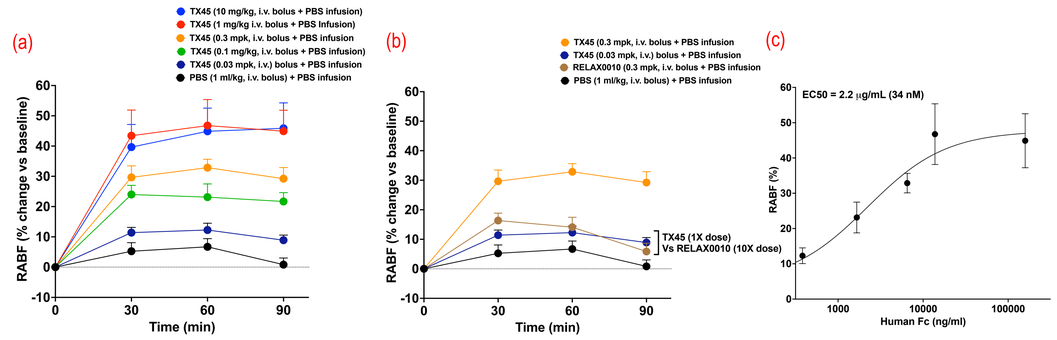

Figure 3: **Dose-dependent effect of TX45 on renal arterial blood flow (RABF) and Correlation with the exposure. TX45 significantly increased renal arterial blood flow (RABF) in a dose-dependent manner**. (a) Time-course analysis of RABF at baseline and up to 1.5 hours post-TX45 administration. Data points represent the mean baseline-corrected %RABF values for each treatment group at 30-, 60-, and 90-minutes post-dosing. (b) TX45 demonstrated superior efficacy in increasing RABF compared to RELAX0010 at a similar dose level. (c) Emax model fitting TX45 serum concentration vs %RABF at 1-hour post-administration.

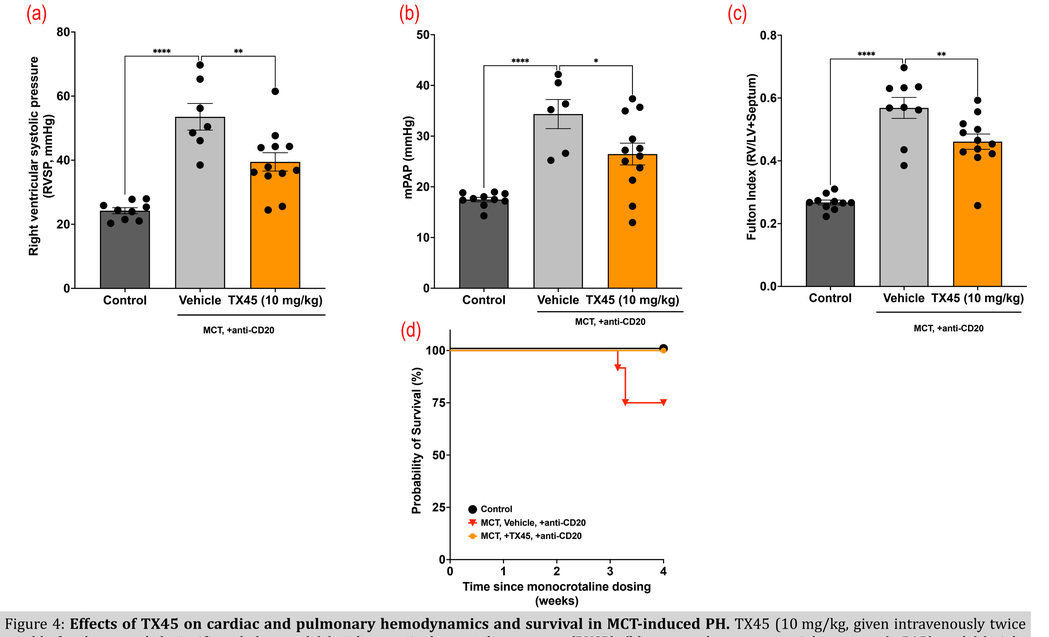

Figure 4: **Effects of TX45 on cardiac and pulmonary hemodynamics and survival in MCT-induced PH.** TX45 (10 mg/kg, given intravenously twice weekly for three weeks) significantly lowered (a) right ventricular systolic pressure (RVSP), (b) mean pulmonary arterial pressure (mPAP), and (c) right ventricular hypertrophy (Fulton Index, RV/[LV+S]). **Kaplan–Meier analysis (d) further revealed a 100% survival rate in TX45-treated rats**, compared with 75% in untreated controls. All data were collected at week 4. Asterisks (*) indicate statistically significant differences (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001) versus the vehicle group, determined using one-way ANOVA followed by Tuckey's multiple comparison test.

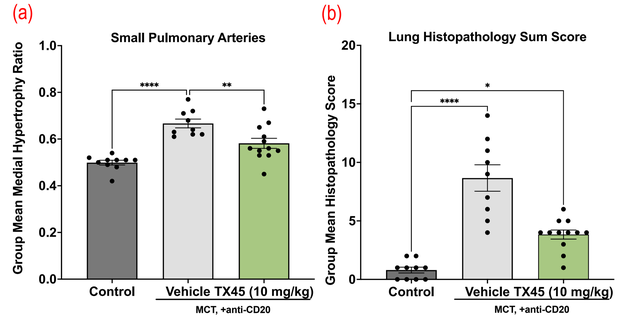

Figure 5: **Effects of TX45 on lung Inflammation and muscularization of small pulmonary arteries in a rat model of MCT-induced PH.** Group mean scores ± standard error of the mean are presented. Lung sum scores a) were significantly lowered by TX45 treatment in rats administered MCT and anti-CD20; reductions were driven by significantly lowered vasculitis, hemorrhage, and fibrin. Data analyzed by nonparametric 1-way analysis of variance with post hoc Dunn's multiple comparisons tests. * indicates p<0.05. ** indicates p<0.01. *** indicates p<0.001. **** indicates p<0.0001. b) TX45 significantly showed improvement of medial hypertrophy ratios of small arteries; ratio calculated as artery mural area / entire artery area (including lumen), with aSMA-Verhoeff-stained slides using image analysis quantification.

Summary

- TX45, a novel long-acting Fc-Relaxin fusion protein, exhibits significant therapeutic potential owing to its vasodilatory, anti-fibrotic, and anti-inflammatory properties.
- Protein engineering yielded a new molecule, TX45, that overcomes pharmacokinetic limitations associated with earlier relaxin molecular designs to achieve superior half-life and bioactivity.
- Notably, TX45 demonstrated an in vivo EC_{50} of 34 nM (2.2 µg/mL) in the rat RABF study aligning closely with our in vitro rat RXFP1 assay, , which yielded an EC_{50} of 30 nM.
- Preclinical studies also demonstrated dose-dependent efficacy to enhance renal blood flow and showcasing its ability and mitigate pulmonary hypertension pathologies, including elevated pulmonary pressures and right ventricular hypertrophy.
- Comprehensive animal toxicology studies established a strong safety profile for TX45, paving the way for Phase 1a clinical trials.
- Compared to alternative long-acting relaxin mimetics, TX45 demonstrates superior biophysical properties, enhanced efficacy, and improved pharmacokinetics, allowing for less frequent dosing in clinical settings.
- These optimized attributes position TX45 as a leading candidate for addressing chronic conditions, including Group 2 pulmonary hypertension secondary to chronic heart failure, and other forms of vascular diseases.

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Acknowledgments

We extend our sincere gratitude to the EC members of Tectonic Therapeutic, Inc., along with our investors and partners, whose generous financial support and invaluable guidance were instrumental in completing this preclinical work and advancing TX45 into clinical trials.