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**Introduction:** An oral testosterone (T) replacement therapy (TRT) would be the preferred choice for many hypogonadal men. Until recently, the only oral TRT approved in the US was methyl-T which has been associated with hepatotoxicity. The safety of a novel oral T undecanoate (TU) formulation was evaluated in hypogonadal men for up to 2 years. **Subjects and Methods**: Two open-label, multicenter, dose-titration trials were conducted in hypogonadal men (serum T ≤ 300 ng/dL) age 18-75 years. Trial I was a randomized, active-controlled, 2-arm, 12-month study. Trial 2 was a long-term extension of those who completed Trial 1. Statistical analyses were only conducted with the subjects who completed Trial 1 and continued treatment in Trial 2, thus providing up to 2 full years of data. Safety was assessed by physical exam, AE reporting, and routine clinical laboratory measurements. **Results**: Overall, up to 81 subjects were available for evaluation. T concentration increased from 208.3 ± 102.4 ng/dL (Mean ± SD) at baseline (BL) to 470.1 ± 396.5 ng/dL after 24 Mo of therapy with oral TU, and 84% of men achieved T in eugonadal range (300-1000 ng/dL) after 90 days of therapy. Mean T concentrations remained in the eugonadal range throughout Trial 2. There were no clinically significant changes in liver function tests - ALT (28.0 ± 12.3 to 26.6 ± 12.8 U/L), AST (21.8 ± 6.8 to 22.0 ± 8.2 U/L), and bilirubin (0.58 ± 0.22 to 0.52 ± 0.19 mg/dL) throughout the two studies. At Day 270, one subject had an ALT level of 227 U/L, which was > 4x the ULN (ULN for ALT = 45 U/L). Despite continued use of oral TU, ALT was measured again on Day 290, and the level dropped to 87 U/L, < 2x ULN. This was the only instance of an LFT elevation. There was a modest initial increase in prostate-related growth endpoints (i.e. PSA and prostate volume) that stabilized over time. There were not any significant changes in IPSS total score (-0.06 ± 3.9 vs BL). There were significant, yet modest, increases in mean HCT (+2.52 ± 3.7% vs BL, $p < 0.001$) and cuff systolic BP (+5.6 ± 15.0 mmHg vs BL, $p = 0.006$). The change in prostate-related growth variables and CV endpoints changed initially and stabilized throughout the 2 trials. For example, systolic BP consistently showed a mean increase from BL between 3 - 6 mmHg. **Conclusion:** This oral TU formulation is an effective long-term therapy for hypogonadal men and has a safety profile consistent with other approved T products. Notably, no evidence of liver toxicity was observed. The long-term efficacy and safety profile of oral TU may provide a treatment option that avoids issues associated with other TRTs, such as injection site pain or transference to partners and children.

## Steroid Hormones and Receptors

### STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

### *Single-Cell RNA Sequencing Reveals Novel Populations of Fibroblasts and Transcriptomic Changes Within Abdominal Skeletal Muscle in a Mouse Model of Aromatase-Induced Inguinal Hernia*

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**Introduction:** Inguinal hernia is a highly prevalent condition in men, of which the only currently available treatment is invasive surgical repair. An inguinal hernia often results from a protrusion of the intra-abdominal contents through a weakened region of the lower abdominal wall, but the etiology is unknown. One potential cause is aging-related steroid hormonal changes, which coincide with an increased incidence of hernia in aged men. Our group previously developed the first mouse model of inguinal hernia ($Arom^{Hum}$) that is generated via the humanized expression of the enzyme aromatase, which converts androgens to estrogens. In the lower abdominal muscle (LAM), an aromatase-mediated increase in tissue estrogen causes fibroblast proliferation, fibrosis, and myocyte atrophy, resulting in hernias. However, the molecular mechanism of this phenotype remains unclear. In this study, we aimed to find genome-wide transcriptomic differences in $Arom^{Hum}$ compared to WT mice at a single-cell resolution. We hypothesized that in relation to WT mice, $Arom^{Hum}$ mice would have distinct fibroblast signatures that arise from the increased estrogen exposure to LAM tissue. **Methods:** LAM was harvested from 9-10-week-old male WT and $Arom^{Hum}$ mice (n=3 each) and digested into a single-cell suspension. Cells were processed via the 10X Genomics Chromium platform for single-cell RNA sequencing. The 6 samples combined yielded a total of ~63,000 cells. Data was analyzed using Cell Ranger v3, Seurat v3, Slingshot, and PROGENy R packages. **Results:** UMAP visualization of WT and $Arom^{Hum}$ LAM tissue revealed 22 cell clusters, which we grouped into 10 broad cell types through known marker gene expression. $Arom^{Hum}$ LAM contained a significantly higher proportion of fibroblasts than WT (44% vs. 27% of total analyzed cells), and $Arom^{Hum}$ fibroblasts expressed more pro-fibrotic genes, such as *Timp1*, *Spon2,* and *Postn*. In $Arom^{Hum}$ and WT combined, we found 6 clusters of fibroblast-like cells. Two of these clusters (clusters 2 and 3) were heavily represented by cells derived from $Arom^{Hum}$ mice (85-90% of cells in each cluster), which we termed "hernia-associated fibroblasts" (HAFs). Cluster 3 HAFs expressed high levels of *Esr1* (gene encoding ERα), as well as estrogen-responsive genes such as *Pgr* and *Greb1,* and was enriched for estrogen, hypoxia, and TGFβ signaling pathways. Cluster 2 HAFs expressed genes associated with a pathological state, such as *Lbp, Cthrc1, Mmp3,* and *Il33*, and was enriched for the NF-κB and TNF-α signaling pathways. **Conclusions:** We found that LAM fibrosis in $Arom^{Hum}$ may result from the expansion of two distinct populations of HAFs - one is estrogen-responsive, and another is pathologic. Further in vitro / in vivo experiments are required to determine the relative contributions of these sub-populations of HAFs to fibrosis and inguinal hernias, leading to developing novel intervention strategies.

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