Development of a Novel Fusion Protein, JUV-161, that Enhances Muscle Regeneration for Treatment of Myotonic Dystrophy Type 1

Hee-Ju Kim[1], Zhihua Li[1], Rohit R. Jadhav[1], Thach Mai[1], Ashil Koranne[1], Han Song[1], Trang Vuong[1], Mohammad A. Tabrizi[1], Jeremy D. O'Connell[1], Hanadie Yousef[1]

[1]Juvena Therepeutics, Redwood City, CA. juvenatherapeutics.com

Introduction

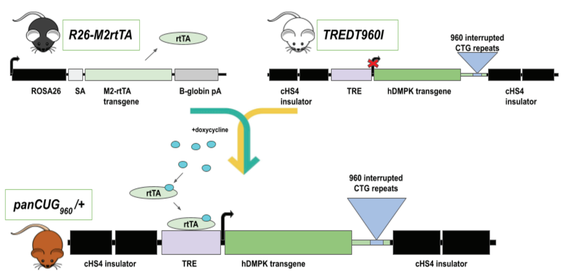
Stem cell secreted proteins are increasingly appreciated to drive regenerative effects across a multitude of tissues and cell types. An important application of these effects are leveraging them to both identify and develop biologics that elicit rejuvenation in specific biological processes and systems that undergo degeneration in aging, disease, or developmental conditions.

Utilizing this approach, Juvena Therapeutics developed JUV-161 as a recombinant fusion protein to agonistically target MAPK/ERK and PI3K/AKT regenerative cascades. These pathways are the major signaling mediators in skeletal muscle to enhance myogenesis, muscle survival, metabolism, and strength.

To advance the preclinical development of JUV-161, Juvena Therapeutics developed a pan- inducible, TREDT960I transgenic mouse model containing a human genomic segment containing exons 11-15 of DMPK gene with 960 interrupted CTG repeats (CUG960) under direction of the tetO (tet-responsive element) promoter. This panCUG960/+ murine model encompasses the key aspects of DM1 muscle deterioration, as shown using both functional and histological testing to confirm distal muscle wasting and RNA foci accumulation in impacted tissue.

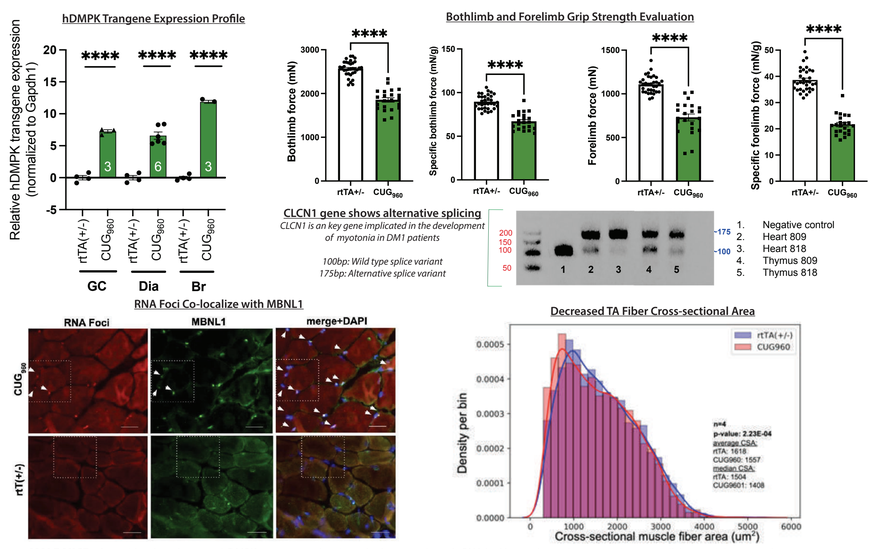
Administration of JUV-161 in this DM1 mouse model resulted in significant improvements in grip strength, coinciding with significantly increased tibialis anterior cross-sectional area in both male and female mice. Binding assays coupled with mouse PK profiling reflected potential clinical translatability of the preclinical results obtained in the DM1 mouse model. Receptor binding along with evaluation of receptor sequence homology (>94% across all species evaluated; not shown) allowed selection of the pharmacologically relevant species (rat and dog) for the conduct of PK/PD and nonclinical safety studies. Based on the results from these studies and the promising activity of JUV-161 in preclinical and nonclinical studies, Juvena therapeutics is planning to evaluate the potential therapeutic benefit of JUV-161 in adult-onset DM1 patients in 2024. JUV-161 treatment could improve muscle strength, endurance, mass, and glucose regulation, leading to reduced atrophy together with faster walk speeds and reduced fall rates, in adult-onset DM1 patients.

Mouse Model Development



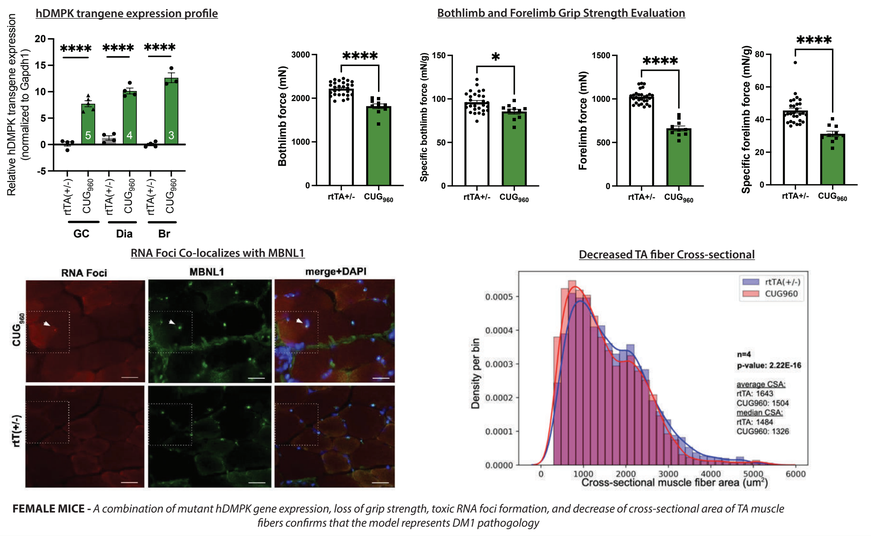
Animals were fed 2g/kg doxycycline for 9-10 weeks beginning at PN1. Mice were then evaluated for grip strength and euthanized to assess transgene expression, foci development with MBNL1 co-localization, aberrant splicing and TA fiber cross-sectional area size

MALES



MALE MICE - *A combination of mutant hDMPK gene expression, loss of grip strength, toxic RNA foci formation, subsequent aberrant splicing, and decrease of cross-sectional area of TA muscle fibers confirms that the model represents DM1 pathogology*

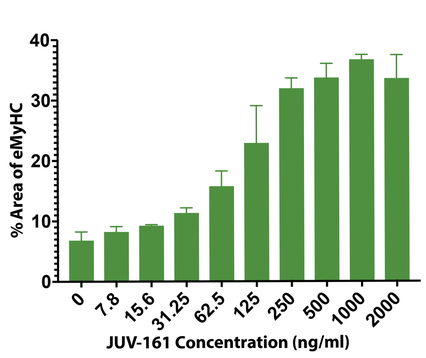
FEMALES

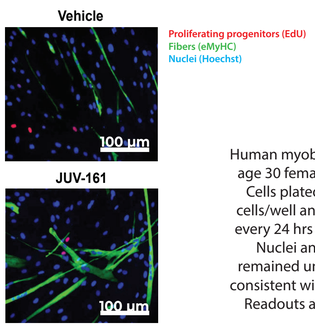


FEMALE MICE - *A combination of mutant hDMPK gene expression, loss of grip strength, toxic RNA foci formation, and decrease of cross-sectional area of TA muscle fibers confirms that the model represents DM1 pathogology*

Results

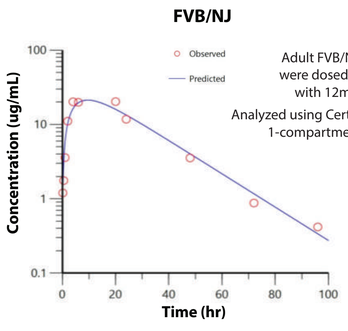
JUV-161 treatment in vitro leads to enhanced myogenesis via concentration-dependent human myoblast differentiation





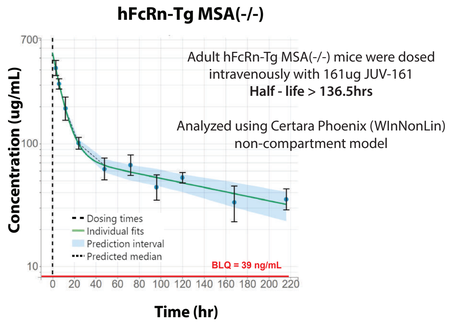
Human myoblasts from age 30 female donor. Cells plated at 20K cells/well and treated every 24 hrs for 72hrs. Nuclei and EdU remained unaffected consistent with control. Readouts assed via

Administration of JUV-161 in mice shows predictable PK profile



Adult FVB/NJ animals (n=3) were dosed subcutaneously with 12mg/kg JUV-161

Analyzed using Certara Phoenix (WInNonLin) 1-compartment 1st order model



Adult hFcRn-Tg MSA(-/-) mice were dosed intravenously with 161ug JUV-161

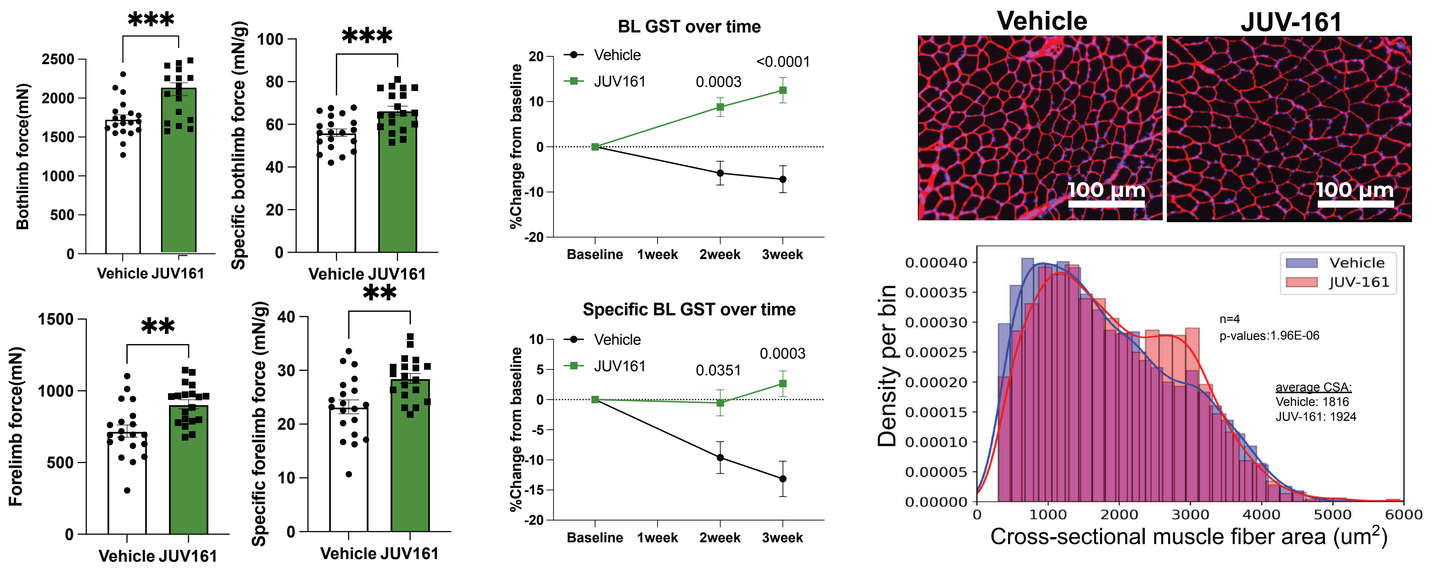
Half - life > 136.5hrs

Analyzed using Certara Phoenix (WInNonLin) non-compartment model

Results - in vivo Efficacy of JUV-161

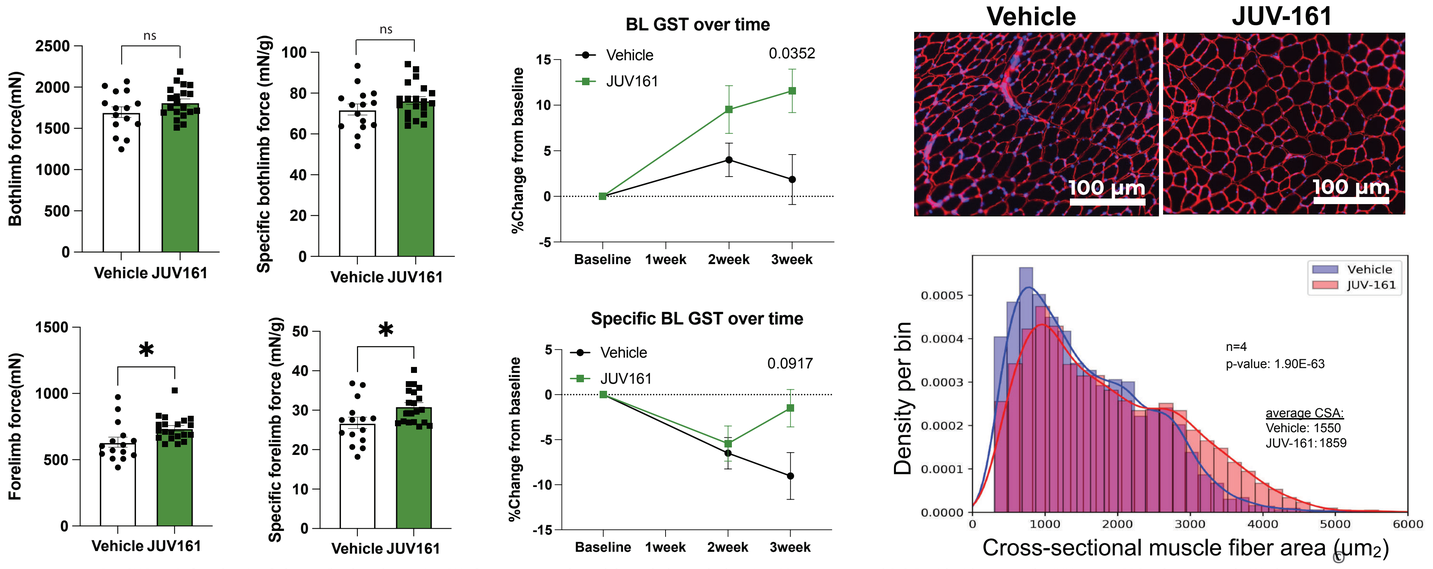
Male and female panCUG960 mice treated with JUV-161 Q.O.D for 3 weeks showed greatly improved grip strength and TA cross-sectional area

MALES



PanCUG960/+ male mice fed on 2g/kg doxy beginning PN1 for 9-9.5 weeks and through the study duration. Mice were treated Q.O.D with either 12mg/kg JUV-161 or PBS subcutaneously. Week 3 grip strength shown (left)

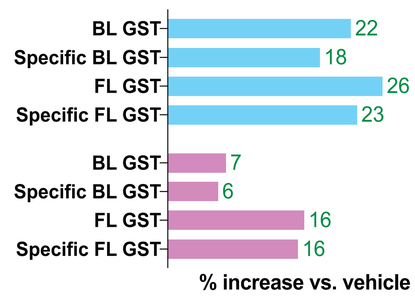
FEMALES



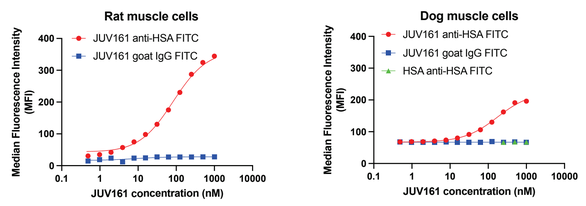
PanCUG960/+ female mice fed on 2g/kg doxy beginning PN1 for 10-10.5 weeks and through the study duration. Mice were treated Q.O.D with either 12mg/kg JUV-161 or PBS subcutaneously. Week 3 grip strength shown (left)

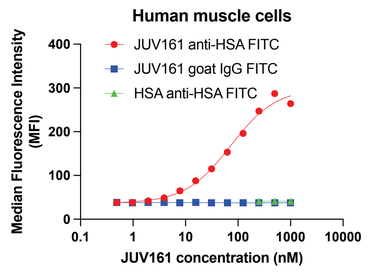
In vivo Efficacy of JUV-161: Graphic Summary

	Male		Female	
	JUV-161	Vehicle	JUV-161	Vehicle
BL GST (mN)	2117	1734	1816	1697
Specific BL GST (mN/g)	66.44	56.14	76.35	71.99
FL GST (mN)	905.7	719.3	736.5	632.4
Specific FL GST (mN/g)	28.51	23.21	30.99	26.78

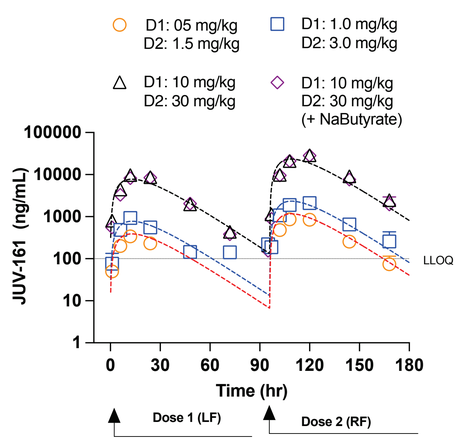


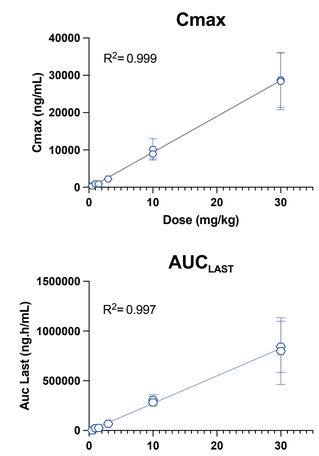
JUV-161 binds to rat, dog and human primary muscle cells in a concentration-dependent manner





Rat PK profile following subcutaneous dosing





A 1-compartment model closely fits the data following the first dose and allowes PK profile predictions following the second dose

Conclusions

- The pan-CUG960/+ DM1 mouse model recapitulated myopathic and functional manifestations of DM1, consistent with organ degeneration in patients with DM1, enabling assessment of reversal and/or amelioration of these changes following administration of JUV-161 to these mice
- Treatment with JUV-161 across multiple concentrations in vitro resulted in dose-proportional increases in percentage of Myosin Heavy Chain muscle fibers
- Subcutaneous administration of JUV-161 in FVB/NJ mice results in a linear PK profile
- In the pan-CUG960/+ DM1 mouse model, administration of JUV-161 was associated with reversal of skeletal muscle mass losses (*not shown), increase in skeletal muscle fiber cross-sectional areas, and ultimately significant improvements in functional assessments including forelimb and both-limb grip strength
- JUV-161 shows specific and concentration-dependent binding to rat, dog and human primary muscle cells, supporting rat and dog as relevant species for the conduct of pharmacology/toxicology studies
- Subcutaneous administration of JUV-161 in rats resulted in dose-proportional increases in the exposure and the linear PK profiles. Additionally, the PK profile following administration of the second dose was both linear and stationary (time-independent). These results indicated no complexities in JUV-161 absorption or elimination following SC dosing in rats

funded by CIRM CALIFORNIA'S STEM CELL AGENCY NIH National Institutes of Health