Oral ARV-027 Induces PolyQ-AR Degradation and Improves Muscle Strength and Endurance in a Murine Model of SBMA

James Gregory[1], Valerie Guss[1], Michele Matchett[1], Debra Nickischer[1], Huensuk Kim[1], Dustin Revell[1], Catherine Braine[1], Wendy Wu[1], Rashaun Wilson[1], John Corradi[1], Yeontae Jeong[1], Yosif Ganat[1], Antonella Falconieri[2], Maria Pennuto[2], Sethu Sankaranarayanan[1], Adetola Ojo[3], Gabrielle Davis[3], Heather Montie[3], Angela M Cacace[1]

[1]Arvinas Operations, Inc., New Haven, CT, USA; [2]University of Padova, Padova, Italy; [3]Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA

Objective

- To evaluate the preclinical activity of ARV-027, an orally bioavailable PROteolysis TArgeting Chimera (PROTAC) targeting polyglutamine-expanded (polyQ) androgen receptor (AR), the root cause of disease in spinal and bulbar muscular atrophy (SBMA), including:
  - Measuring ARV-027-mediated degradation of polyQ-AR in a mouse model of rapidly progressing SBMA (AR100Q)
  - Testing whether peripheral polyQ-AR degradation improves muscle function in an SBMA model
  - Defining muscle tissue effects of ARV-027 using proteomics and RNA sequencing (RNA-seq)

Key Findings

- **Potent degradation:** ARV-027 induced robust degradation of polyQ-AR in human myotubes derived from SBMA patient induced pluripotent stem cells (iPSCs)
- **Oral pharmacodynamics (PD) in mice:** Single oral doses induced dose-dependent degradation of AR in muscle that was sustained for ≥24 hours
- **Chronic exposure:** In-diet dosing of ARV-027 (~15 mg/kg/day) induced marked degradation of polyQ-AR in muscle in male AR100Q mice
- **Efficacy:** 60–70% reductions in muscle polyQ-AR levels fully rescued treadmill endurance, improved muscle strength, and extended survival by 59% in a rapidly progressing mouse model of SBMA
- **Mechanism insight:** Muscle RNA-seq indicates upregulation of oxidative-metabolism and other proteins/genes may underlie improved motor function

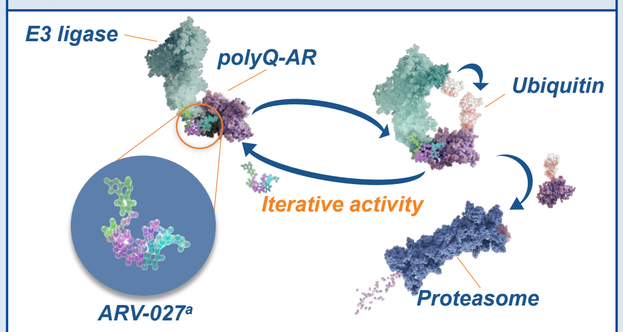
Conclusions

- ARV-027 shows robust pharmacokinetic (PK)/PD–driven degradation of muscle polyQ-AR with clear functional improvement and extended survival in a rapidly progressing polyQ-AR overexpression model
- These findings support future clinical investigation of ARV-027 as a disease-modifying therapy in patients with SBMA by reducing toxic polyQ-AR in muscle; Investigational New Drug-enabling studies are ongoing

Background

- SBMA is an X-linked disease caused by a CAG expansion in the AR gene that produces a polyQ-AR (>~38 CAG repeats)
- polyQ-AR aggregates, sequesters key cellular proteins, and causes an androgen-dependent toxic gain-of-function—disrupted proteostasis and transcription, impairment of the ubiquitin-proteasome system, mitochondrial stress, and progressive degeneration[1]
- In skeletal muscle, a primary disease target, polyQ-AR impairs contractility and oxidative metabolism and drives atrophy, leading to weakness and loss of endurance, making polyQ-AR in muscle a key therapeutic target[2,3]
- ARV-027 is a peripherally-restricted PROTAC that drives ubiquitin–proteasome degradation of polyQ-AR in skeletal muscle (**Figure 1**)

Figure 1: Mechanism of action of ARV-027



[a]General PROTAC protein degrader is shown.
polyQ-AR=polyglutamine-expanded androgen receptor; PROTAC=PROteolysis TArgeting Chimera.

Methods

In vitro polyQ-AR degradation

- Myotubes derived from iPSCs from a patient with SBMA (NINDS ND50091, AR53Q) were treated with ARV-027 for 24 hours; polyQ-AR protein levels were quantified by capillary immunoassay and normalized by total protein levels

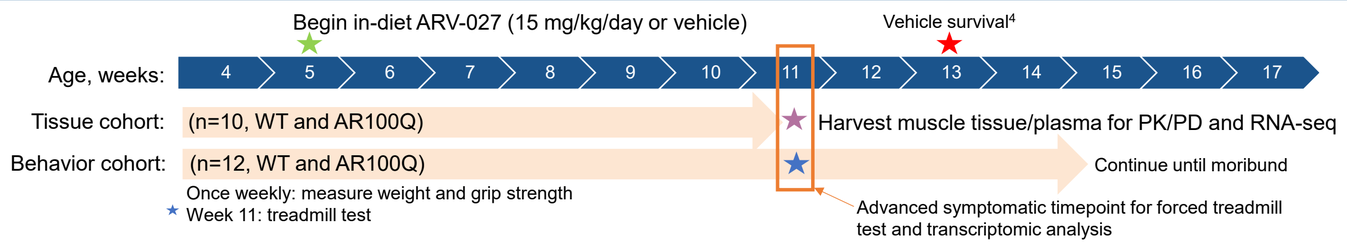
PK and PD of oral ARV-027 in WT mice

- Male C57BL/6 mice (6–8 weeks of age) received ARV-027 (0.3–60 mg/kg) or vehicle once daily for 3 days; 24 hours after the last dose, plasma and levator ani/bulbocavernosus (LABC) muscle were collected to measure ARV-027 exposure and AR degradation (quantified by enzyme-linked immunosorbent assay [ELISA]), respectively

Chronic in-diet dosing of ARV-027 in a transgenic mouse model of SBMA (AR100Q)[4]

- Male AR100Q and wild-type (WT) littermate mice received ARV-027 15 mg/kg/day or vehicle in chow starting at 5 weeks of age (**Figure 2**)
  - **Tissue cohort:** Mice were sacrificed at the age of 11 weeks for plasma and muscle collection. Levels of AR, polyQ-AR, and high-molecular-weight (HMW) polyQ-AR in muscle were quantified by capillary immunoassay (ProteinSimple, Jess) and normalized to total protein levels
  - **Behavioral cohort:** Bodyweight and 4-limb grip strength (Ugo Basile; 6 trials/mouse/timepoint, blinded) were measured once weekly; forced treadmill test (Panlab; graded acceleration) was conducted at week 11 and distance to exhaustion was recorded; mice were euthanized when moribund
- **Transcriptomics:** Tibialis anterior muscle was analyzed by RNA-seq (Roche RNA HyperPrep with RiboErase; NovaSeq PE100; ~50MM reads/sample); differential gene expression was analyzed using DESeq2 and limma for pathway score differentiation

Figure 2: Study of ARV-027 in a mouse model of SBMA expressing human polyQ-AR (AR100Q)[4]

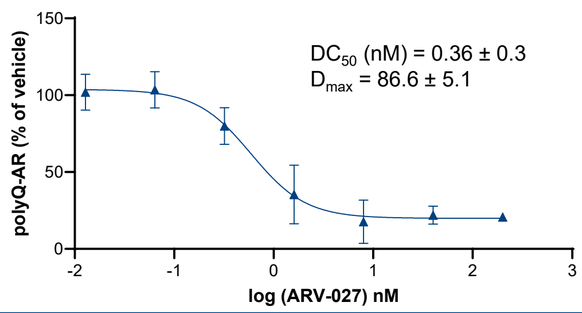


AR100Q transgenic mice express full-length human AR with a 100 glutamine-residue pathogenic polyQ tract under the control of the cytomegalovirus immediate-early enhancer and the chicken beta-actin (pCAGGS) promoter.[4]
PD=pharmacodynamic; PK=pharmacokinetic; polyQ-AR=polyglutamine-expanded androgen receptor; SBMA=spinal and bulbar muscular atrophy; WT=wild-type.

Results

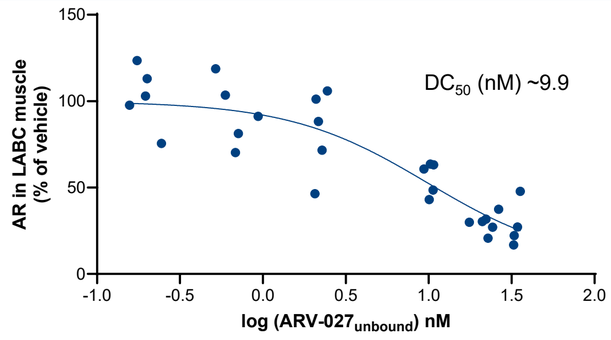
- ARV-027 induced potent degradation of polyQ-AR in human myotubes differentiated from iPSCs from a patient with SBMA (**Figure 3**)
- ARV-027 induced AR degradation in muscle with free half-maximal degradation concentration (DC_{50}) ~10 nM and mean maximum level of degradation (D_{max}) of 71% (60 mg/kg/day) in WT mice (**Figure 4**)
- In male AR100Q SBMA mice, chronic ARV-027 exposure reduced levels of monomeric AR and polyQ-AR in LABC and quadriceps muscle; reductions in HMW polyQ-AR were smaller and significant only in the quadriceps muscle (**Figure 5**)
- In-diet dosing with ARV-027 rescued weight loss, improved grip strength, fully restored treadmill endurance, and increased lifespan by 59% vs vehicle in male AR100Q mice (**Figure 6**)
- Muscle RNA-seq analyses supported ARV-027-mediated rescue of gene expression (**Figure 7A**), including upregulation of oxidative-metabolism genes (**Figure 7B**), consistent with observed improvements in motor function

Figure 3: ARV-027 dose response curve of polyQ-AR degradation in human myotubes differentiated from SBMA iPSCs



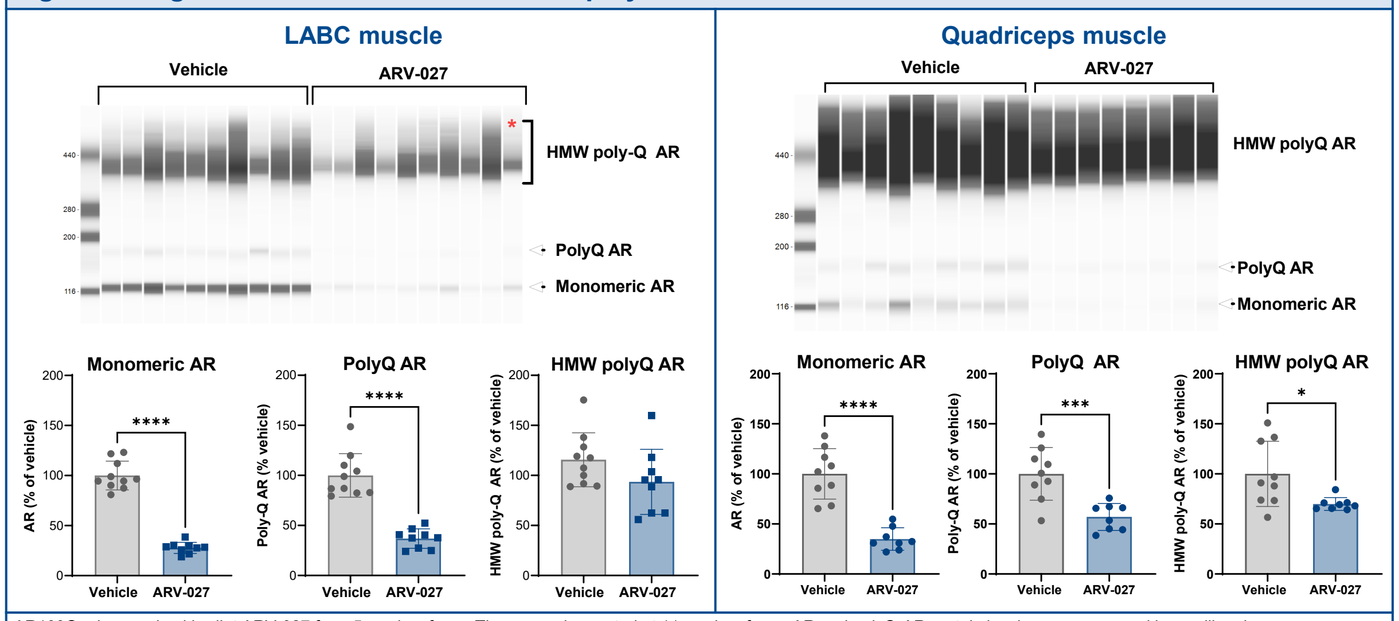
Representative curve from a single experiment; each concentration was measured in triplicate. DC_{50}/D_{max} are mean (SD) values from 5 independent experiments.
DC_{50}=half-maximal degradation concentration; D_{max}=maximum level of degradation; iPSC=induced pluripotent stem cells; polyQ-AR=polyglutamine-expanded androgen receptor; SBMA=spinal and bulbar muscular atrophy.

Figure 4: WT AR degradation in LABC muscle in WT mice treated with ARV-027



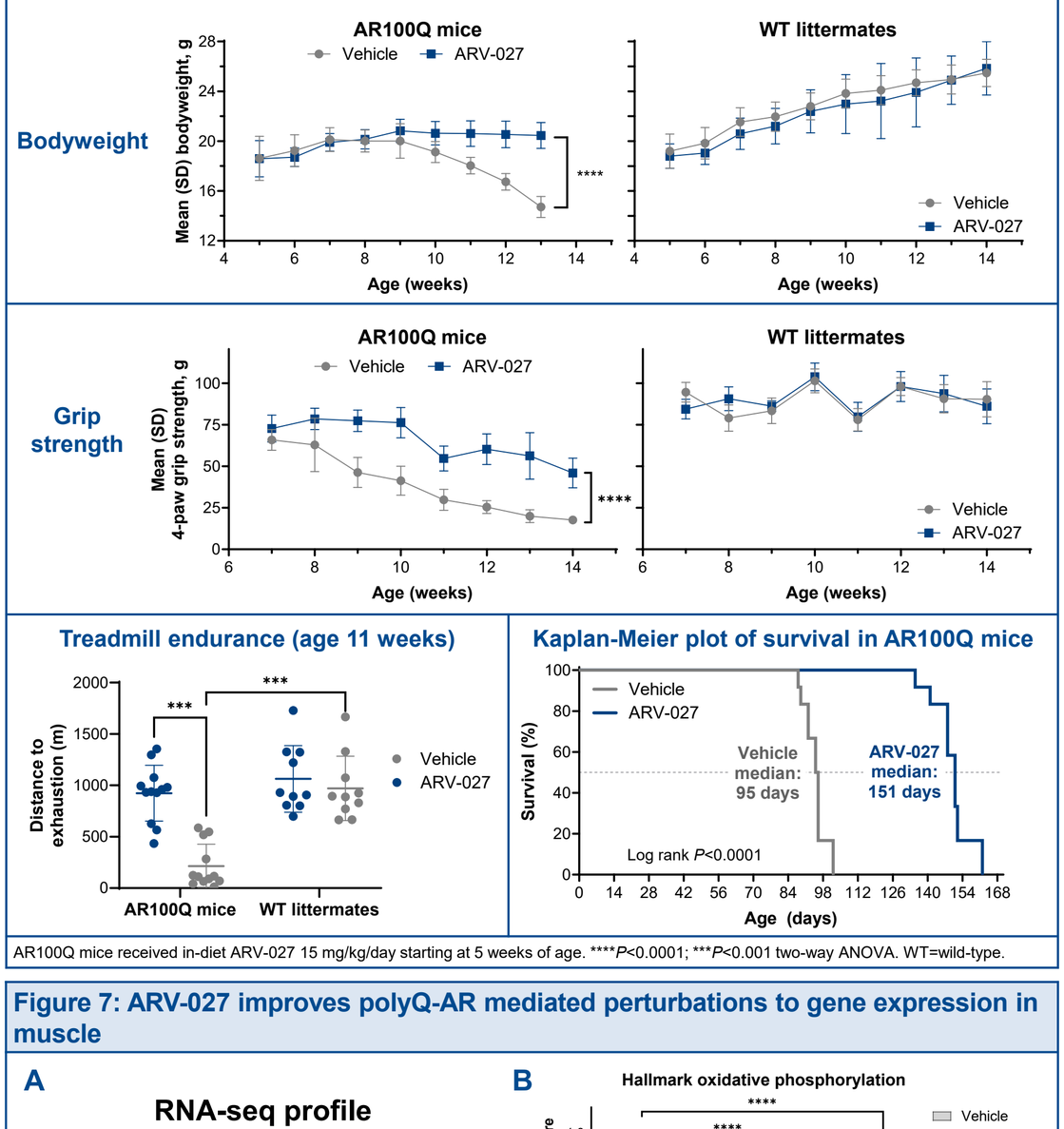
C57BL/6 WT mice were treated with ARV-027 QD for 3 days (0.3–60 mg/kg/day). AR protein (% of vehicle) levels are plotted vs log_{10}-transformed unbound ARV-027 plasma concentrations with a 4-parameter nonlinear fit (top fixed at 100; bottom > 0).
AR=androgen receptor; DC_{50}=half-maximal degradation concentration; LABC=levator ani/bulbocavernosus; QD=once daily; WT=wild-type.

Figure 5: Degradation of monomeric AR and polyQ-AR in muscle from AR100Q mice treated with ARV-027



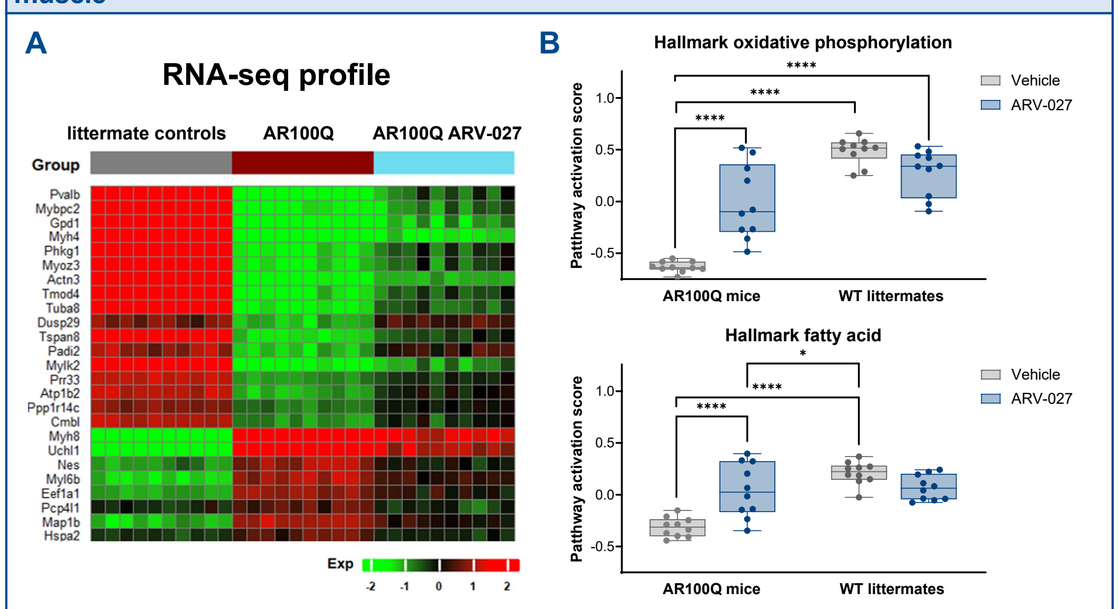
AR100Q mice received in-diet ARV-027 from 5 weeks of age. Tissue was harvested at 11 weeks of age. AR and polyQ-AR protein levels were measured by capillary immunoassay. The red asterisk denotes lane excluded from analysis due to failed protein normalization. ****P<0.0001; ***P<0.001; *P<0.05 unpaired t-test.
AR=androgen receptor; LABC=levator ani/bulbocavernosus; HMW=high-molecular-weight; polyQ=polyglutamine-expanded.

Figure 6: ARV-027 improves bodyweight, muscle strength, endurance and survival in AR100Q mice



AR100Q mice received in-diet ARV-027 15 mg/kg/day starting at 5 weeks of age. ****P<0.0001; ***P<0.001 two-way ANOVA. WT=wild-type.

Figure 7: ARV-027 improves polyQ-AR mediated perturbations to gene expression in muscle



****P<0.0001; *P<0.05 limma two-group comparison (modified t-test) corrected for multiple hypotheses. Tibialis anterior muscle was harvested at 11 weeks of age and analyzed by RNA-seq.
polyQ-AR=polyglutamine-expanded androgen receptor; RNA-seq=RNA sequencing; WT=wild-type.

References

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Acknowledgments

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Contact

Jamie Gregory: James.gregory@arvinas.com