# 2. STUDY SYNOPSIS

| **Name of Company:** | **Name of Finished Product:** | **Name of Active Ingredient:** |
|---|---|---|
| aTYR Pharma, Inc. | ATYR1940 | ATYR1940 |

**Title of Study:**

A Placebo-controlled, Randomized, Multiple Ascending Dose Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Biological Activity of ATYR1940 in Adult Patients with Molecularly Defined Genetic Muscular Dystrophies

**Investigators and/or Study Centers:**

The study was performed in 4 study centers in 4 countries (United States, France, Netherlands, and Italy).

**Publication (reference):**

A poster presentation based on preliminary results of this study was presented at the 21st International congress of the World Muscle Society in Grenada, Spain; October 2016. An abstract of this presentation is published as cited:

Gershman A, Chiang K, Do M, et al. A randomized, double-blinded, placebo-controlled, multiple ascending dose study to evaluate the safety, tolerability, pharmacokinetics, immunogenicity, and biological activity of ATYR1940 in adult patients with facioscapulohumeral muscular dystrophy (FSHD). Neuromuscul Disord. 2016; 6(Suppl 2): S167.

| **Studied Period:** | **Phase of development:** |
|---|---|
| 04 Sep 2014 to 14 Dec 2015 | Phase 1b/2a |

**Objectives:**

The primary objective of this study was:

- To evaluate the safety, tolerability, pharmacokinetics (PK), and immunogenicity of multiple doses of IV ATYR1940 in adults 18 to 65 years of age, inclusive, with facioscapulohumeral muscular dystrophy (FSHD).

The secondary objectives of this study were:

- To explore pharmacodynamics (PD) changes in the following parameters:
  - FSHD-related inflammatory immune responses in muscle, as assessed by quantitative magnetic resonance imaging (MRI)
  - FSHD-related inflammatory immune state in peripheral blood, as assessed by:
    - Circulating immune proteins, such as cytokines
    - Ex vivo inflammatory protein (including cytokines) release from peripheral blood mononuclear cells (PBMCs)
    - Immunophenotyping (general and FSHD-related) of circulating PBMCs

| **Name of Company:** | **Name of Finished Product:** | **Name of Active Ingredient:** |
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- Explore PD changes in the following clinical parameters:
  - Manual muscle testing (MMT), as determined by the Investigator
  - Individualized Neuromuscular Quality of Life (INQoL) instrument, as determined by the patient

**Methodology:**

Study ATYR1940-C-002 was a multi-national, multi-center, double-blind, randomized, placebo-controlled, ascending dose study designed to evaluate the safety, tolerability, PK, immunogenicity, and PD effects of ATYR1940 in patients with FSHD.

Patients were screened for study eligibility during the Screening period within 3 weeks before Baseline (i.e., Day 1, the first day of study drug administration). Eligible patients, as determined based on Screening assessments, were randomly assigned to treatment with ATYR1940 or placebo.

After randomization, patients entered the 1-week single-blind placebo period during which all patients received a single 30-minute IV infusion of placebo. Thereafter, patients entered the double-blind treatment period and received 30-minute IV infusions of study drug (ATYR1940 or placebo) according to their random treatment assignment. The number of patients enrolled in each cohort, the ratio at which patients were assigned to ATYR1940 or placebo, and the duration of study drug treatment were dependent on the cohort; the planned dose cohorts are summarized in **Table S1**.

**Table S1 Planned Dose Cohorts**

| Cohort | Dose Regimen | N | Duration of Blinded Study Drug Treatment | Ratio ATYR1940: Placebo |
|---|---|---|---|---|
| 1 | 0.3 mg/kg Once weekly | 4 | 4 weeks | 3:1 |
| 2 | 1 mg/kg Once weekly | 8 | 4 weeks | 3:1 |
| 3 | 3 mg/kg Once weekly | 8 | 12 weeks | 3:1 |

After completion of a given cohort through at least Week (W) 6 in the study, enrollment in the next scheduled cohort(s) commenced after review by the Data Monitoring Board (DMB).

A DMB consisting of at least 3 independent physicians with expertise in clinical research, biologic drugs and the clinical management of muscular dystrophies, as well as, an independent statistician, reviewed interim safety data from all patients within each cohort through at least W6. The DMB made a recommendation regarding commencement of enrollment in the next cohort(s), as well as the dose regimen to be employed in the next cohort(s).

During treatment, patients attended the study center weekly. Patients in Cohorts 1 and 2 also attended the study center mid-week after the fourth study drug dose. After completion of the 1 week single-blind

| **Name of Company:** | **Name of Finished Product:** | **Name of Active Ingredient:** |
|---|---|---|
| aTYR Pharma, Inc. | ATYR1940 | ATYR1940 |

placebo period and the double-blind treatment period, all patients were to attend a follow-up visit 1 week after the last study drug dose. After completion of the 1-week follow-up visit, patients were to attend follow-up visits 1 month and 3 months after the last Study drug dose. The 3-month Follow-up visit was considered the End of Study (EOS) visit.

During the study, safety was assessed by physical examination, including neurologic evaluation, documentation of adverse events (AEs), including serious adverse events (SAEs), safety laboratory tests, electrocardiograms (ECGs), vital signs, pulmonary function tests, and pulse oximetry following IV dosing. Blood samples for measurement of anti-drug antibodies (ADA) and Jo-1 antibodies were also collected. In addition, serial blood samples for PK assessments were collected, and PD assessments for biological activity were performed.

**Number of Patients (Planned and Analyzed):**

No formal sample size calculation was performed. Up to 44 patients aged 18 to 65 years, inclusive, were planned to be enrolled in this study at multiple study centers in the United States (US) and Europe (EU).

A total of 20 patients were enrolled and randomized to receive placebo (n=5) or ATYR1940 (n=15): Cohort 1 (0.3 mg/kg/week), n=3; Cohort 2 (1 mg/kg/week), n=6; or Cohort 3 (3 mg/kg/week), n=6.

**Diagnosis and Main Criteria for Inclusion:**

Study patients included males and females, aged 18 to 65 years, inclusive with an established, genetically-confirmed, diagnosis of FSHD with clinical findings meeting existing criteria. Patients must have been, in the Investigator's opinion, willing and able to complete all study procedures, and provide written informed consent after the nature of the study had been explained and prior to the performance of any research-related procedures. Patients randomized to ≥Cohort 2 must have had imaging findings meeting defined criteria for muscle inflammation in at least 1 skeletal muscle.

Patients were excluded from the study if he/she had received an immunomodulatory agent or had a history of such treatment, corticosteroids, or high-dose non-steroid anti-inflammatory agents (NSAIDS) within the pre-specified wash-out periods. Patients could not receive curcumin or albuterol, have evidence of alternative diagnosis other than FSHD, have severe retinopathy, or have a history or evidence of obstructive or restrictive lung disease. Patients who had positive results for anti-synthetase syndrome/Jo-1 antibody (Ab), Epstein-Barr virus (EBV), cytomegalovirus, any chronic infection or tuberculosis were excluded. Patients were excluded if he/she had evidence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematological, metabolic, dermatological, or gastrointestinal disease, or had a condition that required immediate surgical intervention. Any patient with anemia, elevated gamma-glutamyl transferase (GGT; >2xupper limit of normal), or serum creatinine levels, or any abnormal baseline findings/medical condition(s) that in the Investigator's opinion, might have jeopardized the patient's safety were excluded. Vaccination(s) within 8 weeks of Baseline or planned during the study were prohibited. Patients who underwent a muscle biopsy with 30 days of Baseline or

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|---|---|---|
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who had initiated or had a significant adjustment to his/her statin treatment were excluded. Patients could not have received a product that putatively enhances muscle growth or activity on a chronic basis, have previously received ATYR1940, or used any investigational product or device with 30 days prior to Baseline. Patients of child-bearing potential must have agreed to use contraception.

**Test Product, Dose and Mode of Administration, Batch Number(s):**

ATYR1940 is a 505 amino acid protein identical to the wild-type amino acids 2-506 of human HARS. ATYR1940 was formulated at a target concentration of 25 mg/mL as a sterile, non-pyrogenic solution in a formulation buffer containing histidine, sodium chloride, and polysorbate 20 at pH 7.3 and filled into type 1 borosilicate glass vials (5 mL) with butyl rubber stoppers and aluminum seals. The fill volume of the clinical trial material was 4 mL. The product was non-preserved and was suitable for a single-dose use by IV administration.

Within each cohort, patients were randomly assigned to receive ATYR1940 or placebo, as presented in **Table S1** above.

Packing Lot Number (and Catalent Item Codes) for ATYR1940, 25 mg/mL used in this study were: CMC13002 (CLB-F-739-120711-B2) and CMC13002 (CLB-F-739-120711-D1).

All study drug (ATYR1940) was administered via IV infusion over 30 minutes.

**Reference Therapy, Dose and Mode of Administration, Batch Number(s):**

Placebo was supplied as the formulation buffer.

Packing Lot Number (and Catalent Item Codes) for placebo used in this study were: CMD13001 / (CLB-F-739-120711-C) and CMD13001 (CLB-F-739-120711-A2).

All study drug (placebo) was administered via IV infusion over 30 minutes.

**Duration of Treatment:**

Patients received an initial study drug infusion of placebo, followed by once weekly IV study drug administration (ATYR1940 or placebo) for 4 or 12 weeks, depending on the cohort in which the patient was enrolled (**Table S1**).

**Criteria for Evaluation:**

**Pharmacodynamics:**

The PD effects of ATYR1940 were evaluated by the following:

- Changes in FSHD-related inflammatory immune responses in skeletal muscle, as assessed by quantitative MRI.
- Changes in FSHD-related inflammatory immune state in peripheral blood, as assessed by:

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|---|---|---|
| aTYR Pharma, Inc. | ATYR1940 | ATYR1940 |

- Circulating immune proteins such as cytokines.
- *Ex vivo* inflammatory immune protein (including cytokines) release from PBMCs.
- Immunophenotyping (general and FSHD-specific) of circulating PBMCs.

- Changes in the following clinical parameters:
  - MMT, as determined by the Investigator.
  - INQoL instrument, as determined by the patient.

**Pharmacokinetics:**

To the extent the systemic drug concentration data allowed, the following PK parameters were determined or calculated from the drug concentration time data as follows using WinNonlin:

- $C_{max}$: Observed maximum serum concentration
- $t_{max}$: Time to attain maximum serum concentration
- $k_{el}$: Elimination rate constant
- $t_{1/2}$: Elimination half-life
- $AUC_{0-t}$: Area under the serum concentration-time curve up to time t, where t is the last point with concentrations above the lower limit of quantitation (LLOQ)
- $AUC_{0-inf}$: Total AUC up to the last measurable concentration plus the AUC extrapolated from the last measurable concentration (Clast at tlast) to infinity: AUC0-t +Clast/λz
- $\%AUC_{extrap}$: Percentage of estimated part for the calculation of AUC0-inf:
- ([AUC0-inf- AUC0-t]/AUC0-inf)*100%
- Dose Normalized $AUC_{0-inf}$: $AUC_{0-inf}$ divided by dose to assess dose proportionality.
- CL: Clearance
- $V_z$: Volume of distribution
- $V_{ss}$: Volume of distribution at steady state

**Safety:**

The safety and tolerability endpoints of this study are:

- Change from Baseline of physical examination, including neurologic examination
- Incidence of AEs, including serious and severe AEs
- Change from Baseline in safety laboratory test results
- Change from Baseline in ECG findings
- Change from Baseline in vital sign measurements and pulmonary evaluations (pulmonary function tests and pulse oximetry)
- ADA titers and Jo-1 Ab test results
- Incidence of infusion reactions and infusion site examination findings

| **Name of Company:** | **Name of Finished Product:** | **Name of Active Ingredient:** |
|---|---|---|
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**Statistical Methods:**

Statistical analyses of safety, PK, and PD data were primarily descriptive in nature. Continuous variables were summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). Categorical variables were summarized showing the number and percentage (n, %) of patients within each classification.

All AEs (serious and non-serious) that occurred after study drug administration in the single blind placebo period and before the end of study, regardless of relationship to study drug, were classified based on SOC and PT using MedDRA. Number and percentage of patients who experienced the following were summarized by treatment group: treatment-emergent adverse events (TEAEs), TEAEs by greatest intensity, TEAEs by strongest relationship, serious TEAEs, TEAEs leading to study withdrawal.

**Summary and Conclusions:**

**Patient Characteristics at Study Entry:**

A total of 20 patients were enrolled and randomized to receive placebo (n=5) or ATYR1940: Cohort 1 (0.3 mg/kg/week), n=3; Cohort 2 (1 mg/kg/week), n=6; or Cohort 3 (3 mg/kg/week), n=6. Of the randomized patients, all received the single-blind placebo infusion and at least one dose of study drug in the double-blind treatment period; therefore, all were included in the Intent-to-treat (ITT) population. No patient was excluded from the Per Protocol (PP) population. Sixteen out of 20 patients completed the study, including follow-up visits. Four patients (placebo, n=1; ATYR1940, 3.0 mg/kg, n=3) enrolled into an extension study after completing the treatment period. One patient discontinued ATYR1940 treatment before completing the treatment period due to generalized infusion-related reactions.

Of the randomized patients, 12 (60%) were male and 8 (40%) female; all patients were white and of non-Hispanic or non-Latino descent. The mean age for the pooled placebo group was slightly older (54.0 years) than the mean ages of the ATYR1940 treated cohorts (range: 44.3 to 45.3 years). The average weights in the pooled placebo group (87.5 kg) and ATYR1940, 0.3 mg/kg (89.3 kg) were heavier compared to ATYR1940, 1.0 mg/kg (74.4 kg) and ATYR1940, 3.0 mg/kg 3 (72.0 kg), and therefore, had higher mean BMIs. Given the small sample size there was a minor imbalance in baseline parameters between groups.

All patients reported having FSHD1, the dominant form of FSHD, and most patients had at least 4 D4Z4 repeat units. Although variable, patients in the pooled placebo group and ATYR1940, 0.3 mg/kg group had a shorter mean duration of FSHD from the time of their first symptoms to first dose of study drug (15.8 years and 10.3 years, respectively) compared to patients in ATYR1940, 1.0 mg/kg (24.3 years) and ATYR1940, 3.0 mg/kg (26.6 years) groups. Mean FSHD CSS ranged from 2.5 (0.3 mg/kg ATYR1940) to 3.67 (1.0 mg/kg ATYR1940).

| **Name of Company:** | **Name of Finished Product:** | **Name of Active Ingredient:** |
|---|---|---|
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**Summary of Pharmacodynamics:**

Using targeted MRI, the change from baseline in muscle inflammation (i.e., muscle water [ms]) and fatty infiltration (fat fraction percentage by Dixon scan) showed minimal mean and median changes during the study with high variability. The change from baseline in Dixon-Total contractile muscle volume showed small mean and median increases for the placebo group and small mean and median decreases for the ATYR1940 group(s) at W6 and W14 (Cohort 3 only). Of those patients with a follow-up Targeted MRI, only 1 patient (Patient 05-002, who received ATYR 3.0 mg/kg) had a shift in Mercuri score, showing increased severity at W6 and a further increase at W14.

A trend toward improvement in muscle strength was shown from results of the MMT, particularly for the upper limb muscles. At 6 weeks, the mean percent change from baseline in MMT total scores was 2.8% and 3.1% for ATYR1940 0.3 mg/kg and 1.0 mg/kg groups, respectively, compared to 1.34% for placebo, indicating a slight improvement in muscle strength over the 6-week period. This improvement was more pronounced in the lower limb score 0.06, 3.07 and 3.50 % in placebo, 0.3 mg/kg and 1.0 mg/kg respectively. At 14 weeks, the mean percent change from baseline for MMT for patients in Cohort 3 indicated stabilization (0.70; range: -5.9 to 9.2) when compared to placebo (-1.4; range: -1.5 and -1.3). Similar changes were observed when upper and lower limb scores were compared.

Mean change from baseline in overall INQoL score at Week 6 indicated improvement in quality of life for the ATYR1940-treated patients in Cohort 2 (-2.98) and Cohort 3 (-3.78); whereas, the mean changes from baseline for the placebo group and ATYR1940-treated patients in Cohort 1 were 4.12 and 2.77, respectively. For the ATYR1940-treated patients in Cohort 3, the INQoL score improved further from -3.78 at Week 6 to -9.90 at Week 14. Additionally, a post-hoc analysis of the change from baseline score using an ANOVA model showed a statistical significant improvement in INQoL for patients who received 12 weekly doses of ATYR1940, 3.0 mg/kg compared to those who received placebo (p=0.030). It is not known at present time what degree of change in the overall INQoL score represents a clinically meaningful change.

Due to the small number of patients and minimal changes, no conclusions could be drawn regarding sleep status and functional assessment of lower limbs following study treatment.

There were no notable changes in PD biomarkers or immunophenotyping data following study drug administration within a treatment group, between patients who received ATYR1940 compared to placebo, or across ATYR1940 doses.

**Summary of Pharmacokinetics:**

The PK population consisted of 15 patients who had Baseline and sufficient post-Baseline samples to permit analysis of PK parameters: 3 patients who received ATYR1940, 0.3 mg/kg and 6 patients each who received ATYR1940 1.0 mg/kg and 3.0 mg/kg.

For the 3 doses of ATYR1940 (03, 1.0, and 3.0 mg/kg), exposure increased with increasing dose in a

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|---|---|---|
| aTYR Pharma, Inc. | ATYR1940 | ATYR1940 |

dose-proportional manner. Peak serum concentrations generally occurred at or shortly before the end of the 30 minute infusion, with mean $T_{max}$ values of approximately 0.5 hours, followed by a mono-phasic decline. Arithmetic mean $C_{max}$ ranged from 1360 to 21,900 ng/mL at W2, and mean $AUC_{0\text{-}inf}$ ranged from 7650 to 136,000 ng*h/mL at W2. $C_{max}$ and $AUC_{0\text{-}inf}$ values did not notably increase with administration of multiple doses.

The mean total systemic clearance (CL) was low (ranging from approximately 0.384 to 0.681 mL/min/kg) and apparent volume of distribution ($V_{ss}$) was small (approximately 0.2 L/kg), which resulted in a terminal half-life of approximately 3 to 5 hours across all dose levels and treatment weeks. There was no notable accumulation of ATYR1940 over multiple dosing weeks at all dose levels.

**Summary of Safety:**

Nearly all patients (19/20) received all study drug infusions over the 4 week (Cohorts 1 and 2) or 12 week (Cohort 3) treatment period. Safety was evaluated over the entire observation (study) period which comprises the exposure period (interval between first and last dose) plus the follow up period. Adverse event rates were not adjusted for patient years of exposure and instead presented as a crude incidence rate.

All patients (100%) had at least one TEAE during the study, and all TEAEs were mild (Grade 1) or moderate (Grade 2) in intensity. The TEAE experienced by more than 2 ATYR1940 treated patients included headache, cough, arthralgia, and back pain. These events were consistent with a baseline medical condition (seasonal allergy, FSHD) or also occurred with placebo (headache).

One TEAE of infusion-related reaction assessed by the Investigator as non-serious was later upgraded by the Sponsor as serious adverse event. The patient had 3 events of infusion-related reaction during successive ATYR1940 infusions (eight, ninth, and tenth). These events had an overlapping symptom complex including flushing, chills, headache, non-cardiac chest pain, diaphoresis and dyspnea. The symptoms were mild or moderate and resolved the same day with treatment. The reactions were found to be associated with complement activation. There was no evidence for mast cell degranulation (serum tryptase levels were normal). There was trace hematuria with the last infusion (infusion 10, Week 11) and at the last assessment at Week 25. While this may be suggestive of deposits of circulating immune complexes, there was however no proteinuria or casts. Urinalysis will continue to be monitored for all patients experiencing an infusion related reaction. There was clear and substantial complement activation for the 1 patient who experienced 3 generalized infusion-related reactions with consecutive doses of 3.0 mg/kg ATYR1940; this was not observed for any other patient with samples analyzed. No evidence of mast cell activation was observed.

The pre-infusion Jo-1 Ab levels during the 3 successive infusion-related reactions were low (1.9, 2.8 and 4.7 U/mL) when compared to levels considered diagnostic (>10 U/mL) of anti-synthetase syndromes. A role for Jo-1 anti-bodies in infusion related reactions could not necessarily be established as there was

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|---|---|---|
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another patient who had Jo-1 values of 2.7 without an infusion reaction. No patient in this study met the criterion of Jo-1 Ab positivity (i.e., >10.0 U/mL, a level considered diagnostic of anti-synthetase syndrome) or fell into a range considered equivocal (i.e., 7.0 to 10.0 U/mL).

The patient with the infusion-related reactions did not have confirmed ADA during the first 2 infusion related reactions and had confirmed ADA after the ninth infusion of ATYR1940. All 20 patients were evaluated for ADA, with 6 of 15 ATYR1940-treated patients confirmed to be positive, with titers ranging from 1:41 to 1:1312. There was no compelling evidence that increased dose was associated with confirmed ADA. In patients with a confirmed ADA, there was no consistent pattern in the ADA titers (increase, decrease or plateau) with continued ATYR1940 dosing. Patients who developed ADA continued to be dosed with ATYR1940 without clinical events. ADA didn't appear to predict infusion related reactions. ADA trended downwards in all patients after the treatment period. Finally, the development of ADA did not affect the PK of ATYR1940 and was not associated temporally with adverse symptoms or safety laboratory abnormalities.

Review of the shifts and summary statistic for hematology, serum chemistry, vitals, ECG, and pulmonary function tests from baseline to end of treatment did not reveal any trends within or across treatment groups. There was also no evidence of ATYR1940-mediated immunosuppression; specifically, no study drug-related infections, leukopenia, or neutropenia.

Urine analysis did not reveal any apparent trend either or across treatment groups.

**Conclusions:**

ATYR1940 is generally safety in adult patients with FSHD and was well tolerated over a range of doses of 0.3 to 3.0 mg/kg. Over the dose range studied, exposure of ATYR1940 increased with increasing dose in a dose-proportional manner with no notable accumulation of ATYR1940 over multiple dosing weeks at all dose levels. In this study, ATYR1940 was administered over a relatively short period of 4 to 12 weeks. Improvement or stabilization in muscle strength and function was observed over the assessment period. A longer duration of ATYR1940 treatment is likely required to appropriately assess the effects of ATYR1940 on FSHD.

**Date of the Report:** Final: 12 June 2017