Electromyography as an Efficacy Biomarker with Improvement in Motor Unit Potentials after DT-DEC01 Therapy in Duchenne Muscular Dystrophy

DYSTROGEN THERAPEUTICS

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked lethal disease, caused by mutations in the dystrophin gene leading to muscle degeneration, wasting and weakness.

Electromyography (EMG) is an objective electrophysiological biomarker of muscle fiber function and has been used extensively to study muscular dystrophies.

A novel **DT-DEC01 therapy of Dystrophin Expressing Chimeric (DEC) cells** was created by fusion of human myoblasts derived from normal (allogeneic) and DMD-affected (autologous) donors.

This study aimed to determine the preliminary efficacy of DT-DEC01 therapy by standard needle EMG assessed up to 12-months after systemic-intraosseous administration of DT-DEC01 therapy.

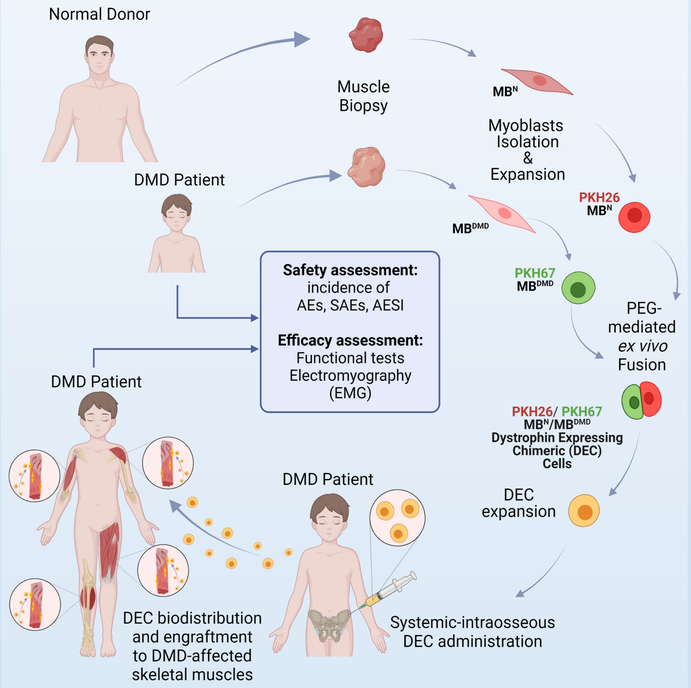

Fig. 1 Outline of the first-in-human Pilot study assessing safety and efficacy of systemic-intraosseous administration of DT-DEC01 therapy in DMD patients. Manufacturing of DEC begins with muscle biopsies from DMD patient and normal donor, followed by myoblasts isolation and expansion, PKH staining and PEG fusion creating DEC cells, further DEC sorting, expansion, product formulation and DEC administration to DMD patient.

Methods

First- in- human study was performed in 5-18 years old boys (n = 3) with genetically confirmed DMD (Bioethics Committee approval no. 46/2019).

Standard electromyography assessed duration of motor unit potentials (MUP) and amplitudes in the selected muscles of: deltoideus, biceps brachii, rectus femoris and gastrocnemius, at screening visit (V0a) and at 3, 6 and 12 months (visits V5, V6 and V7) after systemic-intraosseous administration of a single dose of DT-DEC01 therapy (2x10^6 cells / kg).

No immunosuppression was used.

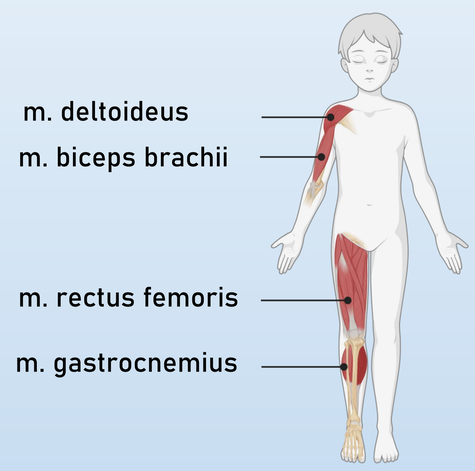

Fig. 2 EMG assessment of duration of MUP and amplitudes in the selected muscles (deltoideus, biceps brachii, rectus femoris and gastrocnemius) of DMD Patients.

Acknowledgments

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Figures 1 and 2 were created with BioRender.com.

Results

Patient 1

DMD: exon 3-12 deletion
Age at enrollment: 6 years
Ambulatory Glucocorticoids: 1.5 years
Weight: 21 kg
DT-DEC01 dose: 2 x 10^6 cells/kg

No AE or SAE recorded up to 15-months after intraosseous administration of DT-DEC01 therapy

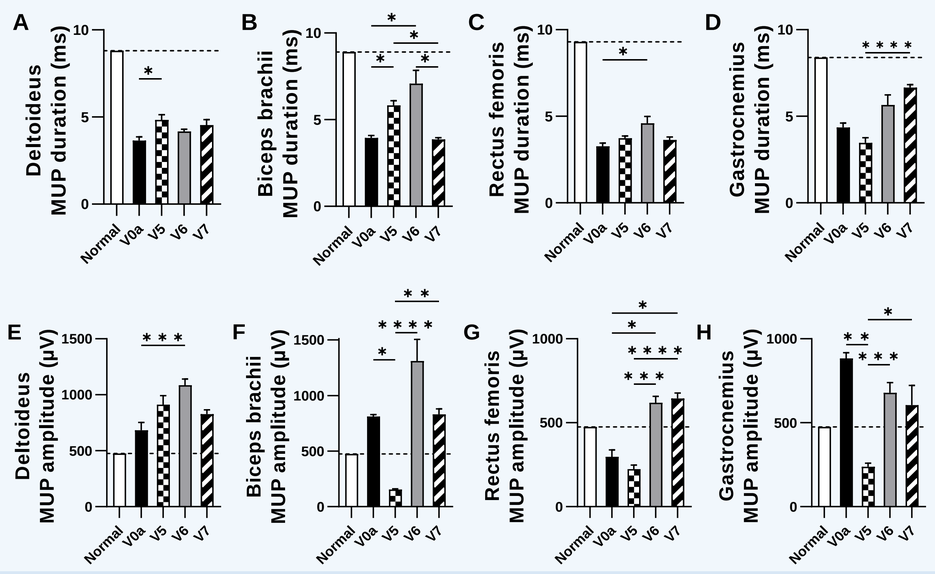

Fig. 3 EMG outcomes assessed in Patient 1 up to 12 months after systemic-intraosseous DT-DEC01 administration.
EMG assessment of average MUP duration revealed: A) increase in deltoideus by 24.5% ± 8.3%, **B)** preservation of duration in biceps brachii (97.5% ± 2.8% of baseline duration), **C)** increase in rectus femoris by 11.0% ± 5.4% and **D)** significant increase in gastrocnemius by 52.7% ± 4.0%. **EMG assessment of average MUP amplitudes revealed: E)** increase in deltoideus by 20.8% ± 5.8%, **F)** amplitude preservation in biceps brachii (102.5% ± 5.9% of baseline duration), **G)** significant increase in rectus femoris by 116.5% ± 10.9% and **H)** amplitude decrease in gastrocnemius by 31.5% ± 13.3%.
Normal values of MUP duration and amplitude in non-DMD affected muscles are indicated in white bars.
Data are expressed as mean ± SEM, the average of 10 MUP measurements is shown.
Statistical significance assessed by Kruskal-Wallis test, * P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001.

Patient 2

DMD: exon 48-50 deletion
Age at enrollment: 15 years
Non-ambulatory Glucocorticoids: 11 years
Weight: 65 kg
DT-DEC01 dose: 2 x 10^6 cells/kg

No AE or SAE recorded up to 13-months after intraosseous administration of DT-DEC01 therapy

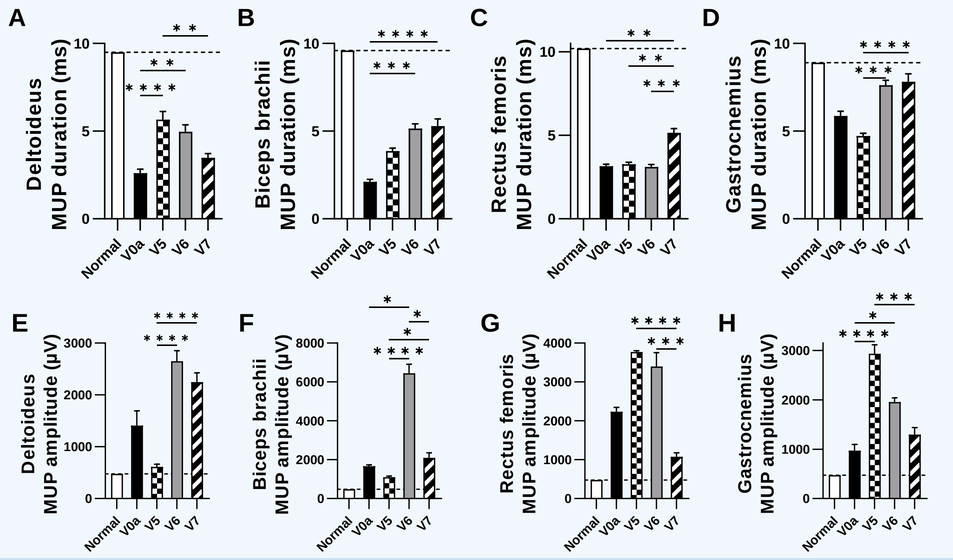

Fig. 4 EMG outcomes assessed in Patient 2 up to 12 months after systemic-intraosseous DT-DEC01 administration.
EMG assessment of average MUP duration revealed significant increase: A) in deltoideus by 33.6% ± 9.3%, **B)** in biceps brachii 149.6% ± 19.3%, **C)** in rectus femoris by 63.5% ± 7.7% and **D)** in gastrocnemius by 33.2% ± 7.6%. **EMG assessment of average MUP amplitude revealed: E)** increase in deltoideus by 59.8% ± 12.5%, **F**) increase in biceps brachii by 26.1% ± 15.4%, **G**) amplitude decrease in rectus femoris by 51.7% ± 4.4% and **H**) amplitude increase in gastrocnemius by 33.7% ± 14.6%.
Normal values of MUP duration and amplitude in non-DMD affected muscles are indicated in white bars.
Data are expressed as mean ± SEM, the average of 10 MUP measurements is shown.
Statistical significance assessed by Kruskal-Wallis test, * P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001.

Patient 3

DMD: Nonsense mutation
Age at enrollment: 6 years
Ambulatory Glucocorticoids: 2 years
Weight: 16 kg
DT-DEC01 dose: 2 x 10^6 cells/kg

No AE or SAE recorded up to 11-months after intraosseous administration of DT-DEC01 therapy

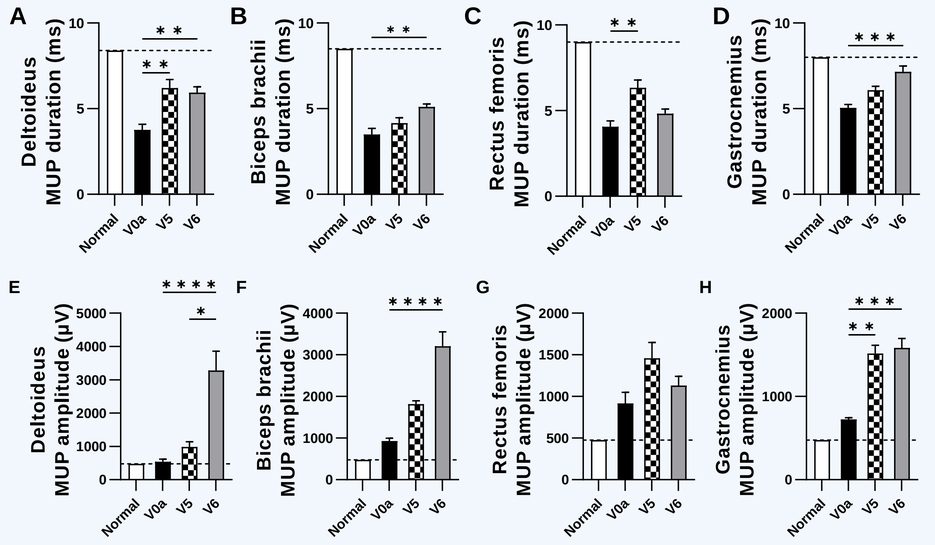

Fig. 5 EMG outcomes assessed in Patient 3 up to 6 months after systemic-intraosseous DT-DEC01 administration.
EMG assessment of average MUP duration revealed: A) significant increase in deltoideus by 58.0% ± 9.1%, **B)** significant increase in biceps brachii by 45.9% ± 5.1%, **C)** increase in rectus femoris by 18.9% ± 6.6% and **D)** significant increase in gastrocnemius by 45.3% ± 6.9%. **EMG assessment of average MUP amplitudes revealed: E)** significant increase in deltoideus by 506.8% ± 107.6%, **F)** significant increase in biceps brachii by 245.5% ± 37.2%, **G)** increase in rectus femoris by 23.7% ± 12.0% and **H)** significant increase in gastrocnemius by 119.0% ± 15.4%.
Normal values of MUP duration and amplitude in non-DMD affected muscles are indicated in white bars.
Data are expressed as mean ± SEM, the average of 10 MUP measurements is shown.
Statistical significance assessed by Kruskal-Wallis test, * P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001.

Summary of MUP outcomes in Patients 1-3

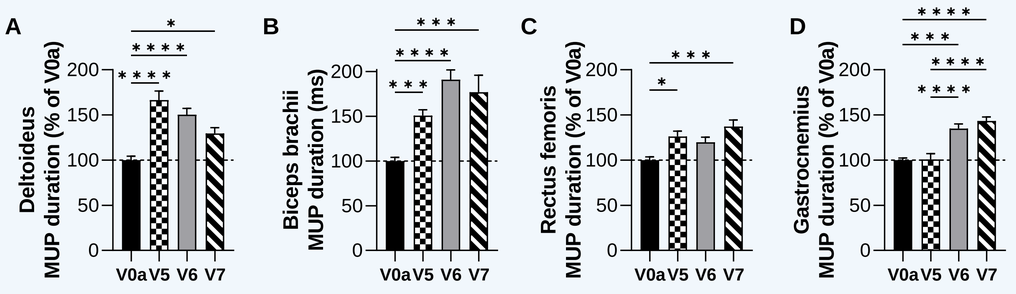

Fig. 6 Summary of the MUP outcomes assessed by EMG in three DMD Patients up to 12 months after systemic-intraosseous DT-DEC01 therapy administration.
Assessment of average MUP duration in three patients revealed significant increase in all tested muscles: A) in deltoideus by 29.6% ± 6.3%, **B)** in biceps brachii by 76.9% ± 18.9%, **C**) in rectus femoris by 37.3% ± 7.2% and **D**) in gastrocnemius by 43.3% ± 4.5%.
Data expressed as mean ± SEM, the average of 30 MUP measurements is shown.
Statistical significance assessed by Kruskal-Wallis test, *P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001.

Summary of Polyphasic MUP outcomes in Patients 1-3

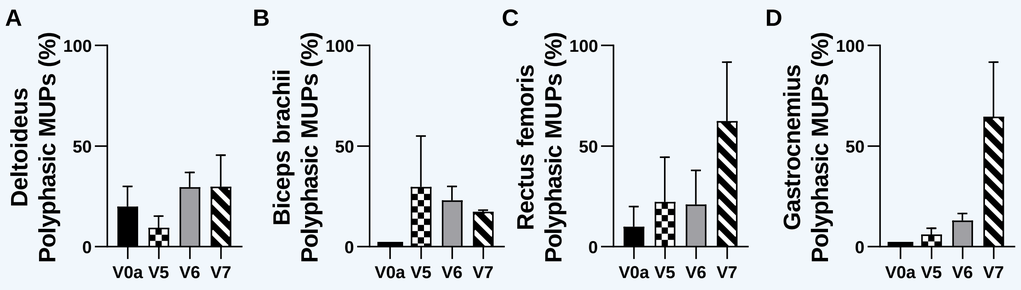

Fig. 7 Summary of polyphasic MUP outcomes assessed by EMG in three DMD Patients up to 12 months after systemic-intraosseous administration of DT-DEC01 therapy.
EMG assessment of the polyphasic MUPs in three patients revealed increased percentage of the polyphasic MUPs when compared to baseline level in all tested muscles: A) in deltoideus from 20.0% up to 29.9%, **B**) in biceps brachii from 0.0% up to 17.5%, **C**) in rectus femoris from 10.0% up to 62.5% and **D**) in gastrocnemius from 0.0% up to 64.6%.

Conclusions

This study confirmed significant increase in MUP and amplitudes of the assessed muscles of both, ambulatory and non-ambulatory DMD patients up to 12-month after intraosseous administration of DT-DEC01 therapy. The recorded EMG parameters may reflect an active process occurring in the muscles of DMD patients after DT-DEC01 administration, indicating increase in muscle fiber volume resulting in longer duration and higher amplitudes of MUP.

In addition, percentage of the polyphasic MUP increased in the assessed muscles over 12-months follow-up. This may be an electrophysiological sign of a muscle response to DT-DEC01 therapy by the regrowth and remodeling of the shape and volume of the regenerating muscle fibers, thus giving the rise to the polyphasic MUPs in the DMD-affected muscles.

Since EMG is an objective and minimally invasive method of assessment of muscle health and disease, therefore, we propose EMG as the reliable and sensitive electrophysiological biomarker of restoration of dystrophic muscle activity and function in DMD patients.