Safety and Efficacy of DT-DEC01 Therapy in Non-Ambulatory Duchenne Muscular Dystrophy Patients up to 24 Months after Systemic Administration

Maria Siemionow*, MD, PhD; Grzegorz Biegański, MD; Jacek Wachowiak, MD, PhD; Jarosław Czarnota, MD; Krzysztof Siemionow, MD, PhD; Adam Niezgoda, MD, PhD; Anna Ziemiecka, MSc; Katarzyna Bożyk, MSc; Ahlke Heydemann, PhD

*corresponding author: siemiom@dystrogen.com

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. **Currently there is no cure for DMD.** To address this unmet need we introduced a novel DT-DEC01 therapy of **Dystrophin Expressing Chimeric (DEC) cells**, created by *ex vivo* fusion of human myoblasts derived from normal (allogeneic) and DMD-affected (autologous) donors.

We have previously reported safety and efficacy of DEC in the preclinical studies using *mdx* mouse models of DMD where DEC cells displayed phenotype and genotype of the parent cells, expressed dystrophin and maintained proliferative and myogenic differentiation potential. Moreover, the systemic effect of intraosseous DEC administration was confirmed by the **long-term amelioration of the cardiac, pulmonary and skeletal muscle function** with no evidence of adverse side effects and no need for immunosuppression [1-8]. Based on these encouraging data we introduced DEC cells into clinical applications [9-12]. This study presents **the long-term safety and efficacy of DT-DEC01, a novel DEC cell therapy assessed in DMD patients** after systemic-intraosseous administration.

Methods

This pilot open-label study was approved by the Bioethics Committee and was conducted in accordance with the GCP guidelines and the Declaration of Helsinki. All patients, their parents, and the donors provided informed consent to undergo muscle tissue biopsy followed by dosing of the DT-DEC01 therapy. Three DMD boys of age 11-16 years old received a single dose of $2x10^6$, $4x10^6$, $6x10^6$ cells/kg of the personalized DT-DEC01 therapy via systemic-intraosseous administration. No immunosuppression was used.

Safety was assessed up to 24 months by monitoring of the Adverse Events (AE), Serious Adverse Events (SAE), and Donor-Specific Antibodies (DSA) anti-HLA antibodies. Efficacy in non-ambulatory patients was assessed up to 24 months by: Performance of Upper Limb (PUL 2.0), grip strength, electromyography (EMG), echocardiography (ECHO), spirometry and daily activity by arm movements count measured by wristband activity tracker.

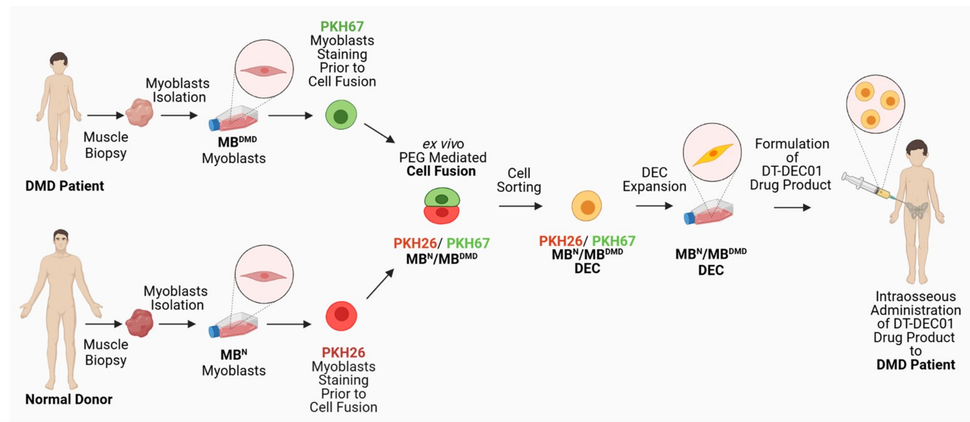

Figure 1. Manufacturing of DT-DEC01 Therapy. The protocol of myoblast cells isolation from muscle biopsies of DMD patient and normal donor, was followed by *ex vivo* cell fusion and DT-DEC01 manufacturing was employed as previously reported [9-11].

Results

Safety

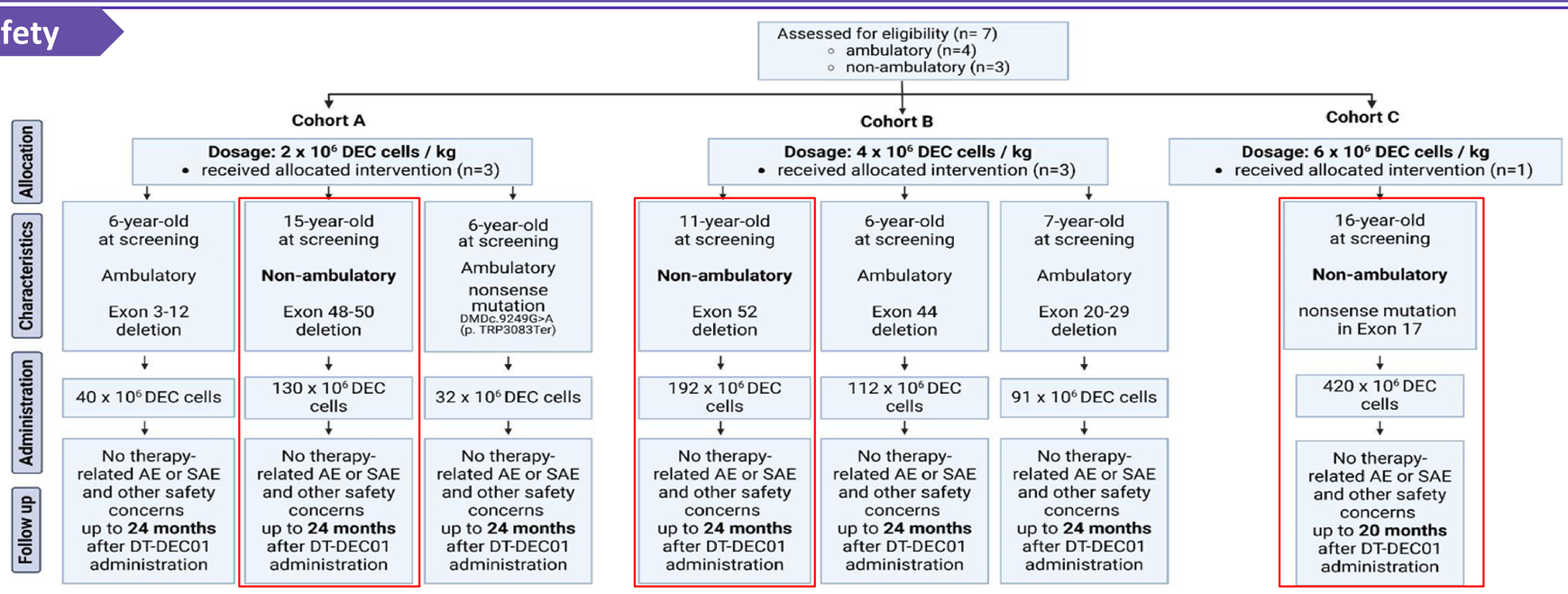

Figure 2. Consort Diagram. Baseline characteristics and safety outcomes assessed in seven DMD patients by monitoring of the Adverse Events (AE), Serious Adverse Events (SAE) and up to 24 months after systemic-intraosseous administration of DT-DEC01 therapy.

Seven DMD patients who met the inclusion criteria were enrolled to the study. Three non-ambulatory patients were allocated to three cohorts to receive the DT-DEC01 therapy at a single at dose of $2x10^6$, $4x10^6$ and $6x10^6$ cells per kg body weight respectively, via intraosseous systemic-administration. There were no therapy-related AE or SAE, DSA, or other safety concerns reported during 24-month follow-up period after DT-DEC01 administration.

Efficacy

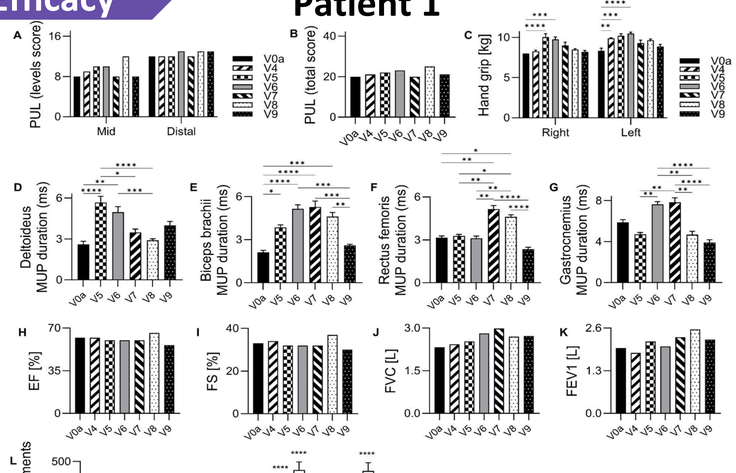

Fig. 3 The functional and EMG outcomes assessed in patient 1 up to 24 months after systemic-intraosseous DT-DEC01 administration. Compared to the baseline measurements (V0a), the results of the study showed several significant findings:

Assessment of upper limb performance (PUL 2.0) revealed: (**A**) mid-level activity was preserved, increase in distal-level activity (by 8%) at 24 months, (**B** increase in PUL total score by 5% up to 24 months. (**C**) Assessment of grip strength revealed 2.5% increase in the right and 6.4% increase in left hand at 24 months. EMG assessments of the average duration of Motor Unit Potential (MUP) showed increase: (**D**) in the deltoideus muscle by 53% at 24 months, (**E**) in the biceps brachii muscle by 23% at 24 months, (**F**) in the rectus femoris muscle by 46% at 18 months, (**G**) in the gastrocnemius muscle by 33% at 12 months. Echocardiography assessment revealed increase in (**H**) ejection fraction (EF) by 18% and (**I**) fractional shortening (FS) by 6% at 18 months. Spirometry assessment showed an increase in the respiratory parameters of (**J**) FVC by 17% and (**K**) FEV1 by 13%. (**L**) Average daily count of arm movements by 185% at 12 months, sustained at 9% at 18 months.

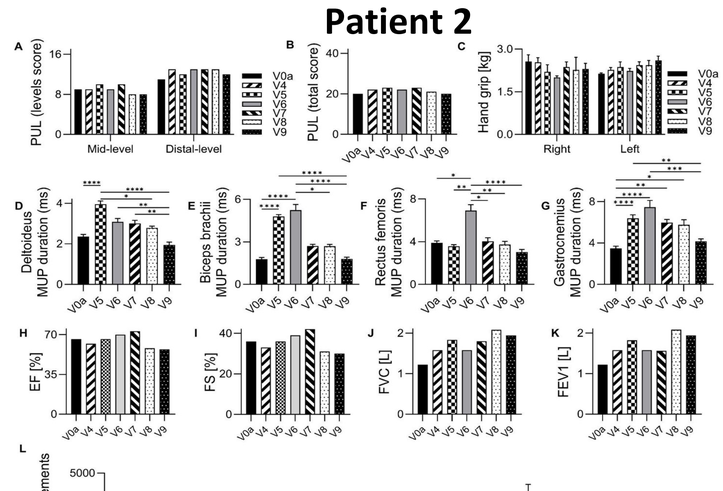

Fig. 4 The functional and EMG outcomes assessed in patient 2 up to 24 months after systemic-intraosseous DT-DEC01 administration. Compared to the baseline measurements (V0a), the results of the study showed several significant findings:

Assessment of upper limb performance (PUL 2.0) revealed: (**A**) increase in mid-level activity up to 12 months by 2 points, increase in distal-level activity by 1 point up to 24 months, (**B**) PUL total score increased by 15% up to 12 months and then was sustained at 5% at 18 months. (**C**) Assessment of grip strength revealed 22% increase in the left hand at 24 months. EMG assessments of the average duration of Motor Unit Potential (MUP) showed increase: (**D**) in the deltoideus muscle by 19% at 18 months, (**E**) in the biceps brachii muscle by 51% at 18 months, (**F**) in the rectus femoris muscle by 4% at 12 months, (**G**) in the gastrocnemius muscle by 20% at 24 months. Echocardiography assessment revealed increase in (**H**) ejection fraction (EF) by 11% and (**I**) fractional shortening (FS) by 17% up to 12 months. Spirometry assessment showed an increase in the respiratory parameters of (**J**) FVC by 59% and (**K**) FEV1 by 59% up to 24 months. (**L**) Average daily count of arm movements increased by 93% at 12 months, by 380% at 18 months and by 1150% at 24 months.

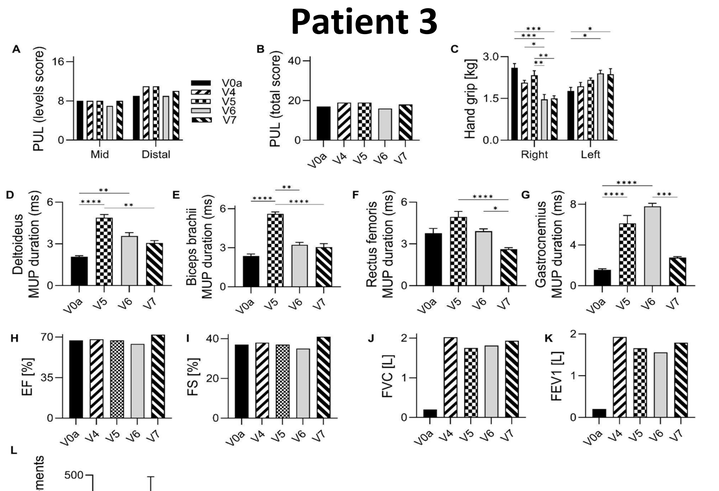

Fig. 5 The functional and EMG outcomes assessed in Patient 3 up to 12 months after systemic-intraosseous DT-DEC01 administration. Compared to the baseline measurements (V0a), the results of the study showed several significant findings: Assessment of upper limb performance (PUL 2.0) revealed: (**A**) maintenance in mid-level and improvement in distal-level activities by 22% at 3 months and by 11% up to 12 months. (**B**) PUL total score improved by 12% at 3 months and by 6% up to 12 months. (**C**) Grip strength improved in the left hand (by 34%) up to 12 months. The EMG assessments of average duration of the MUP revealed increase: (**D**) in the deltoideus by 73% at 6 months and by 49% at 12 months, (**E**) in the biceps brachii by 37% at 6 months and by 29% at 12 months, (**F**) in the rectus femoris by 31% at 3 months and (**G**) in the gastrocnemius increase by 78% up to 12 months after administration of DT-DEC01. (**H**) Echocardiography assessment revealed improvement in (**H**) EF by 7% and (**I**) FS by 11% up to 12 months. (**K**) FVC and (**L**) FEV1 values increased from 0.2L and were sustained at (J) 1.9L and (K) 1.8L at 12 months after DT-DEC01 administration. (**M**) Average daily count of arm movements increased by 24% up to 2 months.

Data expressed as mean ± SEM; statistical significance assessed by ANOVA (for grip strength) or Kruskal-Wallis test (for daily activity, EMG), * P≤0.05, ** P≤0.01, *** P≤0.001, **** P≤0.0001. **Abbreviations**: EF - Ejection Fraction, FEV1 - Forced Expiratory Volume in the first second, FS - Fractional Shortening, FVC - Forced Vital Capacity, PUL - Performance of Upper Limb, V0a – screening visit, V4 - Month 1, V5 - Month 3, V6 - Month 6, V7 - Month 12 after DT-DEC01 administration.

Conclusions

This study confirmed the **long-term safety of DT-DEC01, a novel Dystrophin Expressing Chimeric (DEC) cell therapy**, as evidenced by the **absence of treatment-related Adverse Events (AE) or Serious Adverse Events (SAE)**. Moreover, the **absence of anti-HLA Donor Specific Antibodies (DSA)** in all three patients up to 24 months after systemic-intraosseous administration further confirmed the lack of immune response and tolerability of DT-DEC01 therapy. Follow-up assessments demonstrated functional improvements **non-ambulatory patients** in PUL 2.0 test, grip strength and increased Motor Unit Potentials (MUP) duration by Electromyography (EMG). DT-DEC01 systemic benefits included enhanced **cardiac and respiratory function** assessed by echocardiography and spirometry**, as well as daily activity** recordings. Improvements in systemic functional tests are especially encouraging at this advanced stage of DMD, where disease progression typically leads to cardiac and pulmonary decline, rather than improvement. These findings introduce DT-DEC01 as a promising therapeutic option for all DMD patients, regardless of gene mutation or disease progression.

Acknowledgments

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