Immunologic Investigations into Transgene-Directed Immune-Mediated Myositis Following Delandistrogene Moxeparvovec Gene Therapy

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What does this study mean for the DMD community

- Peptides derived from micro-dystrophin exons 8 and/or 9 may induce a T-cell response leading to IMM in patients harboring deletions in this region. However, not all patients with genetic mutations involving exons 8 and 9 develop IMM following gene therapy administration.
- Knowledge of the patient's HLA presentation of micro-dystrophin peptides, in addition to the patient's specific type and location of genetic mutation within the *DMD* gene, may help with understanding the immune response to delandistrogene moxeparvovec micro-dystrophin.

Conclusions

- Results suggest that T-cell immune responses directed against micro-dystrophin peptides corresponding to *DMD* gene exons 8 and 9 led to the IMM events in these two patients.
  - Presence of T cells directed against three peptides, which are also included in delandistrogene moxeparvovec micro-dystrophin, that mapped to exons 8 and 9 of the *DMD* gene.
  - Greater probability for peptides derived from exons 8 and 9 to bind HLA-I molecules, increasing the potential for these specific peptide sequences to drive a cytotoxic immune response.
  - *DMD* gene deletion mutations involving exons 8 and 9, raising the potential for the immune system to recognize the corresponding protein sequences as foreign.
- The occurrences of IMM in ENDEAVOR are consistent with similar cases reported in other clinical trials evaluating gene therapies for DMD.[1–3]
- These data suggest the patients with deletions in the *DMD* gene that involve exons 8 or 9 may be at increased risk of IMM following micro-dystrophin gene therapy.

OBJECTIVE

- To determine the intricacies underlying the development of IMM in two patients with DMD who received delandistrogene moxeparvovec in ENDEAVOR (SRP-9001-103; NCT04626674).

BACKGROUND

- Delandistrogene moxeparvovec, a single-dose rAAVrh74 vector-based gene transfer therapy, was designed to address the absence of functional dystrophin in people with DMD by delivering a transgene encoding delandistrogene moxeparvovec micro-dystrophin, an engineered protein retaining the key functional domains of full-length dystrophin.[4–6]
- As of April 2024, delandistrogene moxeparvovec is approved in the USA, UAE, Qatar, Kuwait, Bahrain, and Oman for the treatment of ambulatory pediatric patients aged 4 through 5 years with DMD with a confirmed mutation in the *DMD* gene.[7–12]
  - Delandistrogene moxeparvovec is contraindicated in patients with any deletion in exon 8 and/or exon 9 of the *DMD* gene.
- Two serious adverse events of IMM were reported in ENDEAVOR, an open-label, multi-cohort Phase 1b study assessing delandistrogene moxeparvovec in patients with DMD.[13,14] The two patients experienced muscle weakness and received immunosuppressive treatment, including high-dose corticosteroids and tacrolimus.
- In these rare instances, IMM is believed to be caused by immune system reactions to micro-dystrophin in conjunction with the patient's specific genetic mutation.[1,15]
- Understanding the intricacies of the immune response underlying these cases of IMM is paramount in elucidating potential complications associated with gene therapy interventions for DMD.

METHODS

ELISpot assay

- The IFN-γ ELISpot assay was used to detect T cells directed at specific delandistrogene moxeparvovec micro-dystrophin peptides (**Supplementary Fig. 1**). A combination of peptides from regions of micro-dystrophin was selected to form a peptide pool – MDys pool 1, 2, or 3. The assay detected the specific peptide pools that elicited a T-cell response in the patients.

In silico HLA epitope mapping and scoring

- An *in silico* tool (NetMHCpan-4.1) was used to determine the propensity of each 9-mer peptide encoded by dystrophin exons 1–17 to bind each HLA-I molecule allele expressed by the patients. Based on the patients' HLA genotypes, individual EL rank values displayed by NetMHCpan-4.1 were used to calculate "epitope scores" for each dystrophin exon from 1–17.

Acknowledgments and disclosures

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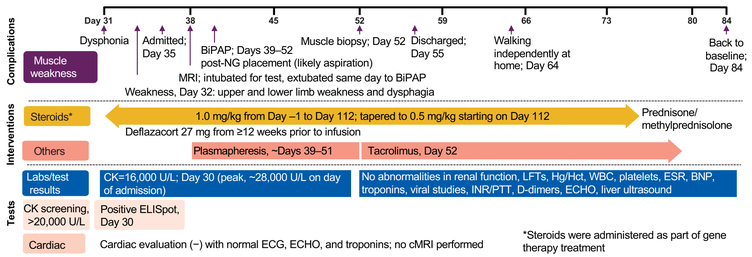
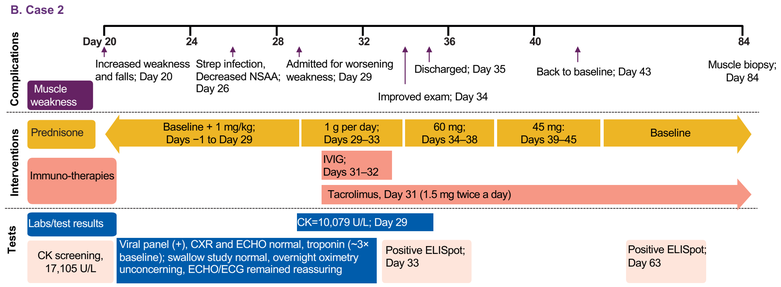
RESULTS

Figure 1. Outcome of the IMM cases

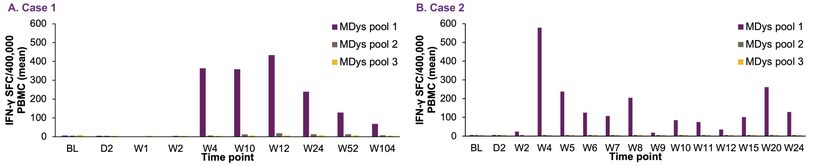


- **Case 1** occurred in a 9-year-old patient in Cohort 2 with a deletion of exons 3–43 of the *DMD* gene, 4–5 weeks post-infusion (**Fig. 1A**).
- The patient underwent six rounds of plasmapheresis and was started on tacrolimus before discharge. At discharge (Day 55), the patient did not need any respiratory support, and on Day 64 he regained the ability to walk independently. The patient recovered on Day 100 with sequalae (weakness). The patient was weaned off tacrolimus by Day ~980 post-infusion without reemergence of any symptoms of IMM.



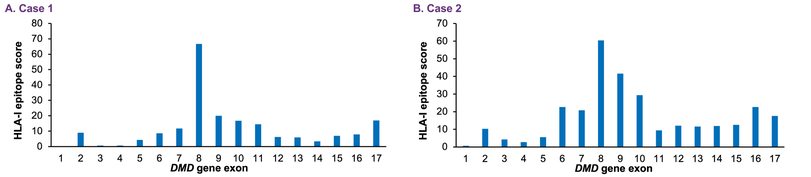
- **Case 2** occurred in a 7-year-old patient in Cohort 5a with a deletion of exons 8–9 of the *DMD* gene, 29 days post-dosing (**Fig. 1B**).
- The patient was started on tacrolimus and IVIG and remains on both as of ~220 days post-infusion. At discharge (Day 35), strength had improved significantly, with NSAA total score returning to baseline levels at Week 24 post gene therapy infusion.

Figure 2. Cellular immune response to micro-dystrophin



- ELISpot analysis revealed the patient samples contained T cells with elevated responses to peptides from MDys pool 1 **(Fig. 2).** Further analysis detected the specific peptides that were eliciting a T-cell response (Case 1, common peptides, 38 and 39; Case 2, common peptide, 32). These peptides map to exons 8 and 9 of the *DMD* gene (**Supplementary Fig. 2**).

Figure 3. *In silico* HLA epitope mapping based on HLA-I scores



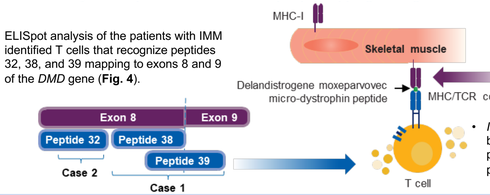
- *In silico* analysis indicated a greater probability for peptides derived from exons 8 and 9 to bind HLA-I, and consequently, a higher potential for these peptides to be presented at the cell surface and activate a response from specific T cells compared with other exons among 1–17 (**Fig. 3**).

Genotypes of patients in ENDEAVOR with mutations in exons 1–17 or 59–71

- 21 patients in ENDEAVOR had genetic mutations that overlapped the transgene region, including 17 patients with mutations in exons 1–17 and four patients with mutations in exons 59–71. Of these patients, only the two described in the present study experienced IMM. Notably, four patients with deletions involving exons 8 and/or 9 had no clinical evidence of IMM.

Figure 4. Summary of ELISpot and *in silico* HLA-epitope mapping findings

- ELISpot analysis of the patients with IMM identified T cells that recognize peptides 32, 38, and 39 mapping to exons 8 and 9 of the *DMD* gene (**Fig. 4**).



- *In silico* epitope mapping identified peptides encoded by exons 8 and 9 with high propensities to be presented by the patients' HLA-I molecules and the potential to drive an immune response (**Fig. 4**).

Abbreviations

ABD, actin-binding domain; BiPAP, Bilevel Positive Airway Pressure; BL, baseline; BNP, brain natriuretic peptide; CK, creatine kinase; cMRI, cardiac magnetic resonance imaging; cont; continued; CR, cysteine-rich domain; CT, C-terminal domain; CXR, chest X-ray; DMD, Duchenne muscular dystrophy; ECG, electrocardiogram; ECHO, echocardiogram; EL, eluted ligand; ELISpot, enzyme-linked immunosorbent spot; ESR, erythrocyte sedimentation rate; H, hinge domain; Hct, hematocrit; Hg, hemoglobin; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; INR, international normalized ratio; IMM, immune-mediated myositis; IVIG, intravenous immunoglobulin; LFT, liver function test; MDys, micro-dystrophin; MHC, major histocompatibility complex; MRI, magnetic resonance imaging; NG, nasogastric tube; NSAA, North Star Ambulatory Assessment; PBMC, peripheral blood mononuclear cell; PTT, partial thromboplastin time; R, spectrin-like repeat domain; rAAVrh74, recombinant adeno-associated virus rhesus isolate serotype 74; SD, standard deviation; SFC, spot-forming cells; TCR, T-cell receptor; WBC, white blood cell; W, week.

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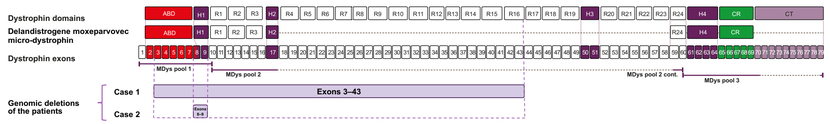
SUPPLEMENTARY METHODS

ELISpot assay

- To determine the region(s) of the micro-dystrophin most effective at stimulating T-cell responses, we synthesized 18-mer long peptides with 11 amino acid overlaps that covered the entirety of the protein expressed by the micro-dystrophin transgene.
- The individual peptides were divided into 3 pools (ie, MDys Pool 1, 2, and 3) based on the region of the protein from which they were derived.
- We exposed T cells to each pool of peptides and assessed their activation by measuring the amount of IFN-γ produced using an IFN-γ ELISpot assay.
- The assay was designed to detect the specific peptide pool(s) that elicited a T-cell response in the patients (**Supplementary Fig. 1**).
- An analysis was performed at the following time points:
  - Case 1 – baseline, day 2, and weeks 1, 2, 4, 10, 12, 24, 52, and 104;
  - Case 2 – baseline, day 2, and weeks 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20 and 24.
- To identify a specific T-cell epitope in pool 1, 51 individual peptides of pool 1 (exons 1–10) were distributed into 15 peptide pools. The matrix pooling strategy was adapted to ensure that each peptide was present in 2 separate pools (**Supplementary Fig. 2**).

Supplementary Figure 1. ELISpot assay pools



In silico HLA epitope mapping and scoring

- Based on the patients' HLA-I genotypes, individual ELrank values displayed by NetMHCpan-4.1 were used to calculate epitope scores for each dystrophin exon from 1–17.
- As low EL rank numbers correspond to higher affinities, EL ranks of each 9-mer peptide/HLA allele combination were transformed as follows: [transformed_score = –log2(EL_rank/zygosity^2)] and summed for each exon.
  - The zygosity of the allele, whether homozygous or heterozygous, was accounted for in this transformation.
- Any negative transformed scores were set to 0, establishing an upper EL rank threshold of 1.0 for heterozygous alleles and 4.0 for homozygous alleles.

SUPPLEMENTARY RESULTS

Supplementary Figure 2. ELISpot analysis of micro-dystrophin peptide pool MDys1 to identify potential T-cell targets

A. Case 1

Matrix peptides for epitope mapping

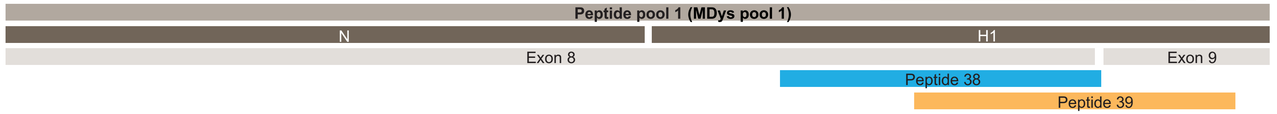
Pool ID	Peptide #										Pool #
S37 MDys pool 1.1–3*	.....										1–3
S37 MDys pool 1.4	32	33	34	35	36	37	38	39	40	41	4
S37 MDys pool 1.5*	.....										5
S37 MDys pool 1.6–11*	.....										6–11
S37 MDys pool 1.12	7	18	28	38	48						12
S37 MDys pool 1.13	8	19	29	39	49						13
S37 MDys pool 1.14–15*	.....										14–15

Common peptides in MDys pools with a positive T-cell response

Cytokine	IFN-γ	
Cells	400,000	
	Mean	SD
Medium	0.33	0.58
.....		
MDys pool 1.4	121.00	7.00
.....		
.....		
MDys pool 1.12	64.50	3.54
MDys pool 1.13	112.50	33.23
.....		

MDys pools with a positive T-cell response (pools 1.4, 1.12, and 1.13)

*MDys pool, peptide numbers, and IFN-γ response omitted for brevity



B. Case 2

Matrix peptides for epitope mapping

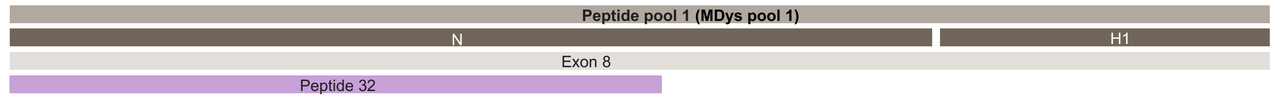
Pool ID	Peptide #										Pool #
S37 MDys pool 1.1–3*	.....										1–3
S37 MDys pool 1.4*	32	33	34	35	36	37	38	39	40	41	4
S37 MDys pool 1.5	.....										5
S37 MDys pool 1.6	1	11	22	32	42						6
S37 MDys pool 1.7–15*	.....										7–15

Common peptides in MDys pools with a positive T-cell response

Cytokine	IFN-γ	
Cells	400,000	
	Mean	SD
Medium	-	-
.....		
MDys pool 1.4	260.33	29.16
.....		
MDys pool 1.6	171.33	42.90
.....		

MDys pools with a positive T-cell response (pools 1.4 and 1.6)

*MDys pool, peptide numbers, and IFN-γ response omitted for brevity



Acknowledgments and disclosures

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Abbreviations

EL, eluted ligand; ELISpot, enzyme-linked immunosorbent spot; H, hinge domain; IFN-γ, interferon-gamma; MDys, micro-dystrophin; N, N-terminal.

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