T-cell response to micro-dystrophin in a patient treated with delandistrogene moxeparvovec gene therapy: A case of immune-mediated myositis

S Khan,[1] H Haegel,[2] A Hollenstein,[2] C Wandel,[2] D Asher,[1] DA Griffin,[1] RA Potter,[1]* I Moeller,[1] T Singh,[1] LR Rodino-Klapac[1]

[1]Sarepta Therapeutics, Inc., Cambridge, MA, USA; [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland

*Presenting on behalf of the author group (email address: medinfo@sarepta.com)

What does this study mean for the DMD community?

- Peptides derived from micro-dystrophin exons 8 and 9 may induce a T-cell response leading to immune-mediated myositis (IMM) in patients with deletion of these exons.
- Knowledge of the patient's human leukocyte antigen (HLA) alleles, in addition to the patient's deletion in the *DMD* gene, may help with assessing the risk of immune response to delandistrogene moxeparvovec micro-dystrophin.

Conclusions

- The immunological investigation of IMM in a patient with DMD treated with delandistrogene moxeparvovec gene therapy indicated that exons 8 and/or 9 appear to be highly immunogenic.
- These results are consistent with clinical trials of other investigational DMD gene therapies suggesting that patients with deletions in regions of the *DMD* gene overlapping those expressed in a given micro-dystrophin may be at an increased risk of an IMM event following gene therapy.
- We hypothesize that a combination of the following factors poses the highest risk for IMM and led to IMM in this patient: Deletion of exon 8 or 9; presence of T cells recognizing exons 8 or 9 as non-self; HLA type with strong HLA presentation of peptides mapping to exons 8 or 9.
- Work is currently underway to better understand these risk factors and to find ways to safely administer delandistrogene moxeparvovec to patients with potentially higher-risk *DMD* mutations.

Objective

- To better understand the patient and antigenic features mediating IMM in a patient with DMD treated with delandistrogene moxeparvovec.

Background

- Delandistrogene moxeparvovec is an rAAV vector-based gene therapy, designed to compensate for the absence of functional dystrophin in DMD by delivering a transgene encoding delandistrogene moxeparvovec micro-dystrophin, an engineered protein that retains key functional domains of the wild-type protein.[1–3]
- Delandistrogene moxeparvovec is approved in the USA and UAE for the treatment of ambulatory pediatric patients aged 4 through 5 years with DMD with a confirmed mutation in the *DMD* gene.[4,5,*,†]
- ENDEAVOR (NCT04626674) is an open-label, multi-cohort Phase 1b study assessing delandistrogene moxeparvovec in patients with DMD.[6,7] A case of IMM occurred in ENDEAVOR Cohort 2 in a 9-year-old patient with a deletion of exons 3–43 of the *DMD* gene, 35 days post-dosing.
- In individuals who have a portion of the *DMD* gene sequence deleted, there is a risk of the transgene being recognized as foreign and, in turn, eliciting an immune response. One requisite of being detected by the immune system is the presentation of peptide fragments of the transgene by HLA-I or HLA-II.
- Here, we present the results of the investigation of this case.

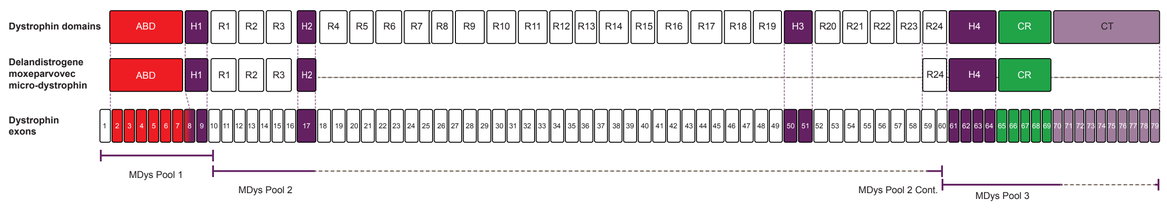
*Delandistrogene moxeparvovec is contraindicated in patients with any deletion in exon 8 and/or exon 9 in the *DMD* gene. †As of August 2023.

Methods

ELISpot assay

- The IFN-γ ELISpot assay was used to detect T cells directed at specific delandistrogene moxeparvovec micro-dystrophin peptides (**Figure 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 pool that elicited a T-cell response in the patient. An analysis was performed at the following time points: baseline, Day 2, and Weeks 1, 2, 4, 10, 12, 24, 52, and 104.

Figure 1. ELISpot assay pools

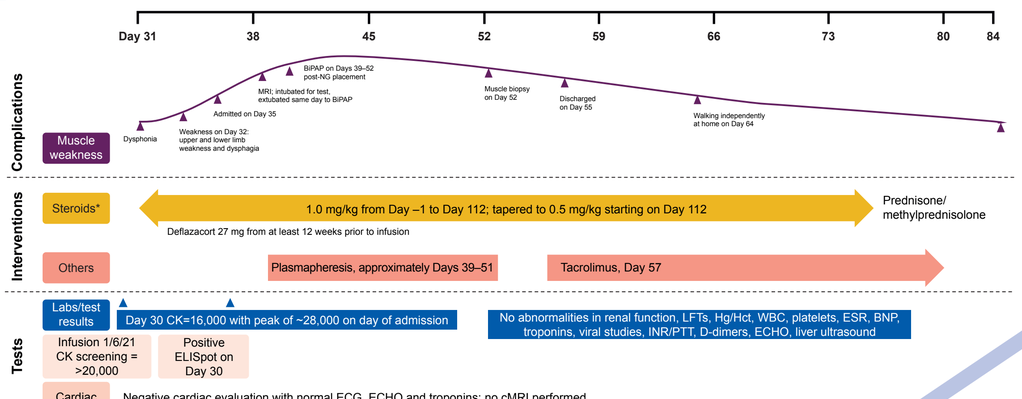


In silico HLA epitope mapping

- An *in silico* tool (NetMHCpan) was used to determine MDys peptide binding with either HLA-I (using 9-mer peptides) or HLA-II (using 15-mer peptides) molecules that correspond to the patient's specific HLA allele combinations.
- HLA-I and HLA-II scores for exons 1–17 were determined by multiplying the number of predicted strong binder peptides by the number of alleles binding these peptides.

Results

Figure 2. Outcome of the IMM clinical event



*Steroids were administered as part of gene therapy treatment.

- The patient underwent a series of six rounds of plasmapheresis and was started on tacrolimus before discharge and is still on tacrolimus as of June 2023 (**Figure 2**).
- At discharge (Day 55), the patient did not need any respiratory support, and on Day 67 he regained the ability to walk independently.
- The patient recovered on Day 100 with sequalae (weakness).

Figure 3. Generation of MDys peptide pools to determine relative antigenic strength

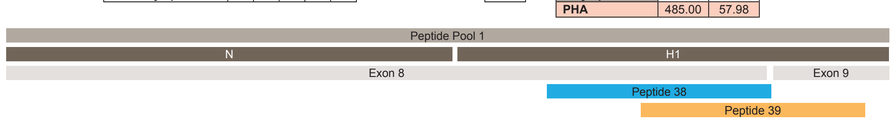
Matrix peptides for epitope mapping

Pool ID	Peptide #										Pool #
S37 MDys pool 1.1	1	2	3	4	5	6	7	8	9	10	1
S37 MDys pool 1.2	11	12	14	15	16	17	18	19	20	21	2
S37 MDys pool 1.3	22	23	24	25	26	27	28	29	30	31	3
S37 MDys pool 1.4	32	33	34	35	36	37	38	39	40	41	4
S37 MDys pool 1.5	42	43	44	45	46	47	48	49	50	51	5
S37 MDys pool 1.6	1	11	22	32	42						6
S37 MDys pool 1.7	2	12	23	33	43						7
S37 MDys pool 1.8	3	14	24	34	44						8
S37 MDys pool 1.9	4	15	25	35	45						9
S37 MDys pool 1.10	5	16	26	36	46						10
S37 MDys pool 1.11	6	17	27	37	47						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	9	20	30	40	50						14
S37 MDys pool 1.15	10	21	31	41	51						15

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

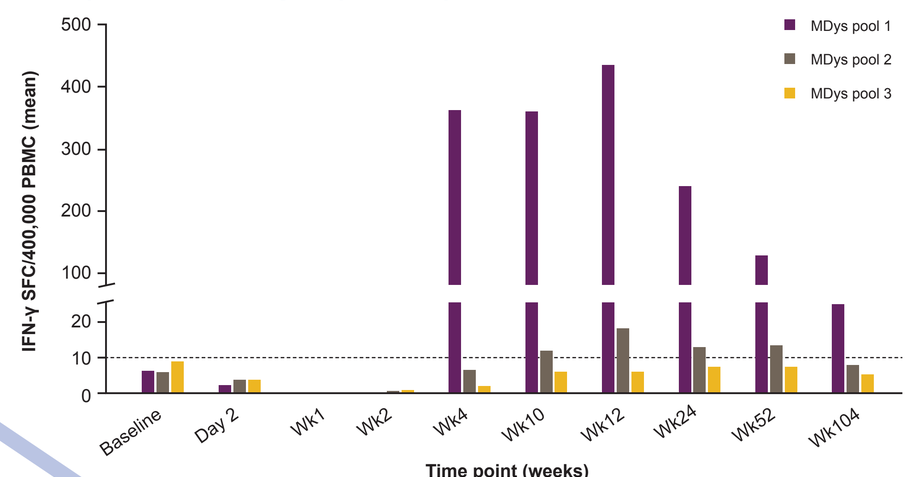
Cytokine	IFN-γ	
Cells	400000	
	Mean	SD
Medium	0.33	0.58
MDys pool 1.1	1.00	1.00
MDys pool 1.2	1.67	1.53
MDys pool 1.3	5.00	3.46
MDys pool 1.4	121.00	7.00
MDys pool 1.5	2.33	2.08
MDys pool 1.6	2.00	1.73
MDys pool 1.7	2.00	0.00
MDys pool 1.8	2.00	1.41
MDys pool 1.9	0.50	0.71
MDys pool 1.10	1.00	1.41
MDys pool 1.11	2.00	0.00
MDys pool 1.12	64.50	3.54
MDys pool 1.13	112.50	33.23
MDys pool 1.14	1.50	0.71
MDys pool 1.15	2.00	2.83
PHA	485.00	57.98

MDys pools with a positive T-cell response (Pools 4, 12, and 13)



- To determine the regions of the micro-dystrophin most effective at stimulating T-cell responses, we cleaved micro-dystrophin into 51 small peptides, which we distributed into 15 pools that were comprised of varying regions of the micro-dystrophin. These pools of peptides were then presented to T cells in the context of HLA, and we measured activation by IFN-γ production using ELISpot.
- Upon further analysis, the 51 peptides in MDys Pool 1 were grouped into 15 different pools to detect the specific peptides that were eliciting a T-cell response in the patient. ELISpot analysis suggested three peptide pools that mounted a T-cell response (in green), of which the common peptides were 38 and 39. Peptides 38 and 39 were identified to induce T-cell responses in ELISpot (IFN-γ secretion) and map to exons 8 and 9 of the *DMD* gene (**Figure 3**).

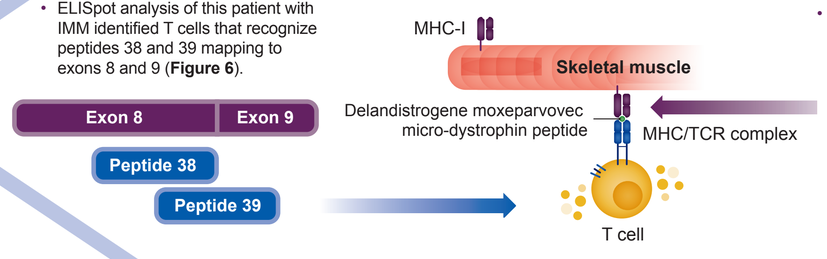
Figure 4. Cellular immune response to micro-dystrophin in a patient with IMM



- ELISpot analysis suggested that the IMM resulted from T cell-mediated responses directed against specific delandistrogene moxeparvovec micro-dystrophin peptides with elevated responses to peptides from MDys Pool 1 (**Figure 4**).

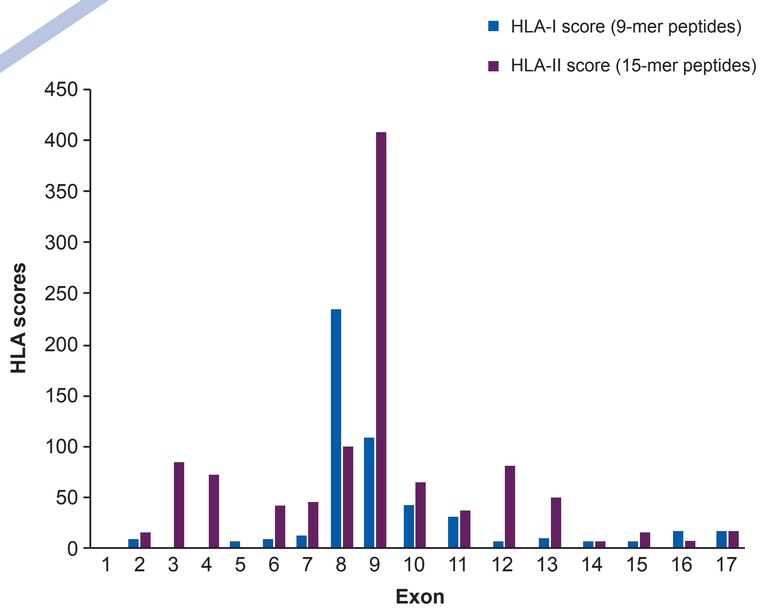
Figure 6. Summary of ELISpot and *in silico* HLA-epitope mapping findings

- ELISpot analysis of this patient with IMM identified T cells that recognize peptides 38 and 39 mapping to exons 8 and 9 (**Figure 6**).



- *In silico* epitope mapping identified peptides encoded by exons 8 and 9 with high propensities to be presented by the patient's HLA-I and HLA-II molecules and drive an immune response (**Figure 6**).

Figure 5. *In silico* HLA epitope mapping based on HLA scores in a patient with IMM



- *In silico* analysis of HLA presentation in this patient suggested a high risk of immunogenicity for exons 8 and 9 of the *DMD* gene (**Figure 5**).

Abbreviations

ABD, actin-binding domain; BiPAP, Bilevel Positive Airway Pressure; BNP, brain natriuretic peptide; CK, creatine kinase; cMRI, cardiac magnetic resonance imaging; Cont; continued; CR, cysteine-rich domain; CT, C-terminal domain; DMD, Duchenne muscular dystrophy; ECG, electrocardiogram; ECHO, echocardiogram; 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; LFT, liver function test; MDys, micro-dystrophin; MHC, major histocompatibility complex; MRI, magnetic resonance imaging; NG, nasogastric tube; PBMC, peripheral blood mononuclear cell; PTT, partial thromboplastin time; R, spectrin-like repeat domain; rAAV, recombinant adeno-associated virus; SD, standard deviation; SFC, spot-forming cells; TCR, T-cell receptor; UAE, United Arab Emirates; WBC, white blood cell; Wk, week.

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