Potency Assay Development for CAP-1002: An Allogeneic Cell Therapy in Phase 3 Clinical Trial for DMD

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Abstract

Duchenne muscular dystrophy (DMD) is a severe, progressive, X-linked disease affecting both skeletal and cardiac muscle with severely reduced life expectancy. More than 80% of patients with DMD are non-ambulatory after the first decade. DMD triggers fibrosis, inflammation, and a maladaptive immune response. CAP-1002, the core technology product of Capricor consists of allogeneic cardiosphere-derived cells or CDCs, has been shown to be safe in multiple clinical trials and effective in reducing deterioration of upper limb function in patients with late stage DMD in a randomized, double-blind, placebo-controlled Phase 2 trial (HOPE-2). In accordance with the potential mechanism of action and clinical efficacy data for CAP-1002, the following assays were developed to better define CAP-1002 product identity and potency: 1) anti-fibrotic activity via fibroblast production of collagen using a co-culture system; 2) analysis of β-catenin in CAP-1002 cells and non-CAP-1002 cells, 3) analysis of CAP-1002 identity using a qRT-PCR panel generating from RNAseq data, 4) analysis of CAP-1002 clinical potency by RNA sequencing of CAP-1002 cells and non-CAP-1002 cells. Multiple CAP-1002 lots which were shown to be clinically potent were tested together with non-CAP-1002 cells as negative controls. Our results showed that the anti-fibrotic activity as demonstrated by the reduction in collagen expression induced by multiple CAP-1002 lots is consistent with clinical potency. At the same time, elevated β-catenin expression was detected by ELISA for all the clinical potent CAP-1002 lots. Finally, using RNAseq data from 20 potent CAP-1002 lots with known clinical efficacy and 16 non-CAP-1002 cell lines, a differentially expressed model was established, which could be used for CAP-1002 potency testing for all future CAP-1002 lots. This RNA profile identified a set of genes including HSPA6, IL6 and CXCL8, which gave the most different expression between CAP-1002 and non-CAP-1002 cells and could be used for CAP-1002 cell identity. Together these data support the use of these new assays for CAP-1002 product identity and potency.

Results

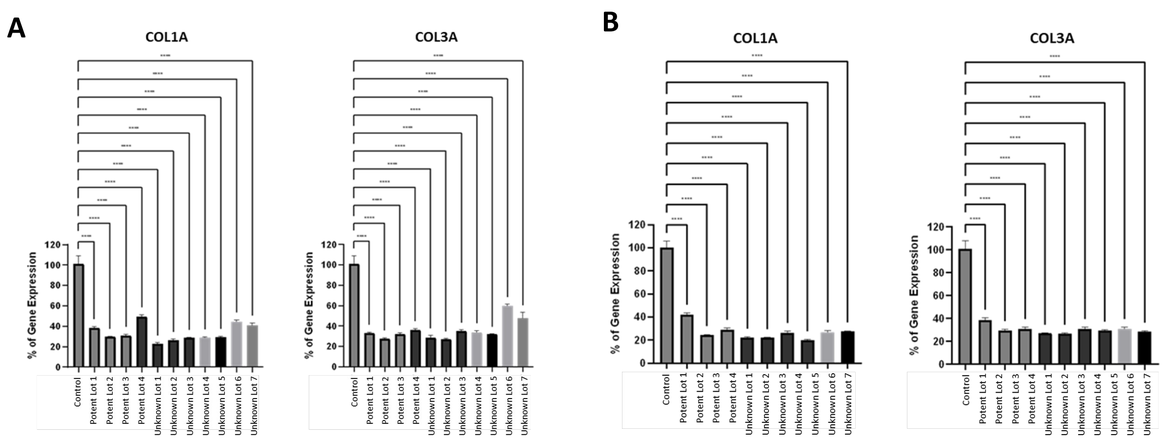

Figure 1*.CAP-1002 conditioned media (CM) from 11 lots (including 4 clinical lots and 1 repeat lot) significantly reduced the collagen gene expression (COL1A, COL3A) in fibroblasts after co-culture. Non-conditioned media (media control) was used as a control. COL1A and COL3A gene expression analysis was evaluated by qRT-PCR. Assay was performed in triplicate with data shown as average ± SD of assay triplicates. **** p<0.0001, *** p≤0.0005, ** p≤0.001.*

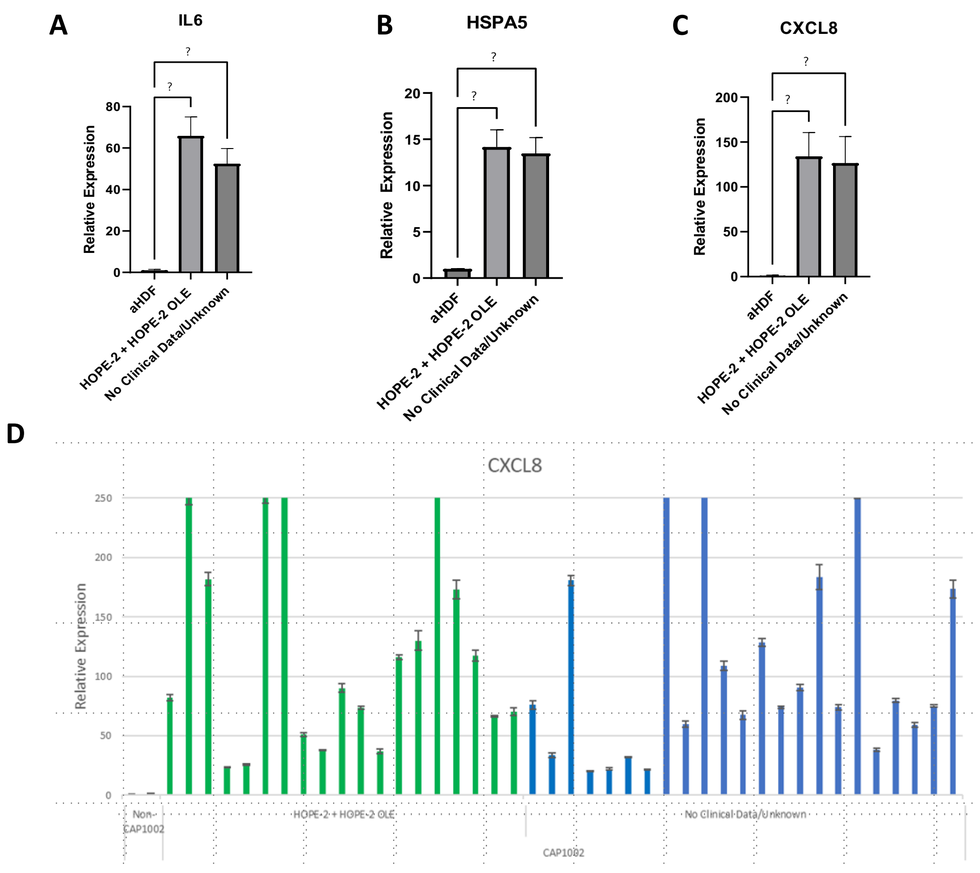

Figure 2. *IL6 (A),HSPA5 (B), and CXCL8 (C) gene expression is statistically significantly upregulated in CAP-1002 cells, including 19 CAP-1002 lots used in HOPE-2 or HOPE-2 OLE (middle bars) and CAP-1002 lots with unknown clinical efficacy (right bars), when compared to adult human dermal fibroblasts (aHDF, left bars). (D) CXCL8 gene expression is highly upregulated in 43 lots of CAP-1002, including 19 CAP-1002 lots used in HOPE-2 or HOPE-2 OLE (green bars), when compared to adult human dermal fibroblasts (aHDF, grey bars). CAP-1002 cells with unknown clinical efficacy are shown in blue bars.*

A

Sample Name	Correlation p<0.00001	Sample Classification
Potent Lot 1	0.9944	Potent
Potent Lot 2	0.9942	Potent
Potent Lot 3	0.9928	Potent
Potent Lot 4	0.9919	Potent
Potent Lot 5	0.99	Potent
Potent Lot 6	0.9876	Potent
Potent Lot 7	0.9869	Potent
Potent Lot 8	0.986	Potent
Potent Lot 9	0.9843	Potent
Potent Lot 10	0.977	Potent
Potent Lot 11	0.9735	Potent
Potent Lot 12	0.9719	Potent
Potent Lot 13	0.9675	Potent
Potent Lot 14	0.9654	Potent
Potent Lot 15	0.9653	Potent
Potent Lot 16	0.9626	Potent
Potent Lot 17	0.9608	Potent
Potent Lot 18	0.9366	Potent
Potent Lot 19	0.9244	Potent
Potent Lot 20	0.9119	Potent
Cardiac Endothelial Cells Lot 1	0.8965	Non-Potent
aHDF Lot 1	0.8929	Non-Potent
Cardiac Fibroblast Lot 1	0.8907	Non-Potent
Cardiac Smooth Muscle	0.8891	Non-Potent
Cardiac Endothelial Cells Lot 2	0.887	Non-Potent
MSC Lot 1	0.8836	Non-Potent
aHDF Lot 2	0.8834	Non-Potent
MSC Lot 2	0.8605	Non-Potent
Cardiac Fibroblast Lot 2	0.7396	Non-Potent
Human aortic smooth muscle cells (HASMC-1)	0.649	Non-Potent
Human atrial cardiac fibroblasts (HACF-1)	0.6283	Non-Potent
HEK293	0.5889	Non-Potent
MSC Lot 3	0.5572	Non-Potent
Human aortic endothelial cells (HAEC-1)	0.5106	Non-Potent
aHDF Lot 3	0.4037	Non-Potent
THP-1	0.3594	Non-Potent

CAP-1002

Non-CAP-1002

B

Sample Name	Correlation p<0.00001	Sample Classification
Potent Lot 1	0.9944	Potent
Potent Lot 2	0.9942	Potent
Test Lot 1	0.9932	Test The Model
Potent Lot 3	0.9928	Potent
Potent Lot 4	0.9919	Potent
Test Lot 2	0.9913	Test The Model
Test Lot 3	0.9907	Test The Model
Potent Lot 5	0.9900	Potent
Test Lot 4	0.9897	Test The Model
Test Lot 5	0.9893	Test The Model
Potent Lot 6	0.9876	Potent
Potent Lot 7	0.9869	Potent
Potent Lot 8	0.9860	Potent
Potent Lot 9	0.9843	Potent
Test Lot 6	0.9789	Test The Model
Potent Lot 10	0.9770	Potent
Potent Lot 11	0.9735	Potent
Potent Lot 12	0.9719	Potent
Potent Lot 13	0.9675	Potent
Potent Lot 14	0.9654	Potent
Potent Lot 15	0.9653	Potent
Potent Lot 16	0.9626	Potent
Potent Lot 17	0.9608	Potent
Potent Lot 18	0.9366	Potent
Test Lot 7	0.9350	Test The Model
Potent Lot 19	0.9244	Potent
Potent Lot 20	0.9119	Potent
Cardiac Endothelial Cells Lot 1	0.8965	Non-Potent
aHDF Lot 1	0.8929	Non-Potent
Cardiac Fibroblast Lot 1	0.8907	Non-Potent
Cardiac Smooth Muscle	0.8891	Non-Potent
Cardiac Endothelial Cells Lot 2	0.8870	Non-Potent
MSC Lot 1	0.8836	Non-Potent
aHDF Lot 2	0.8834	Non-Potent
Test Lot 8	0.8831	Test The Model*
MSC Lot 2	0.8605	Non-Potent
Test Lot 9	0.8507	Test The Model*
Test Lot 10	0.8304	Test The Model*
Cardiac Fibroblast Lot 2	0.7396	Non-Potent
Human aortic smooth muscle cells (HASMC-1)	0.6490	Non-Potent
Human atrial cardiac fibroblasts (HACF-1)	0.6283	Non-Potent
HEK293	0.5889	Non-Potent

Figure 3. *(A) Gene expression profile data of clinically potent CAP-1002 lots (green) and non-CAP-1002 cells (labeled as non-potent, red). CAP-1002 lots. Using the most stringent, conservative model with a P value of <0.00001, 100% of known clinically potent CAP-1002 lots (highlighted green) rank higher than 100% of "assumed non-potent", non-CAP-1002 cells (highlighted red). (B) Gene expression profile data of clinically potent CAP-1002 lots (green) and non-CAP-1002 cells (labeled as non-potent, red). Test the model samples are duplicate RNA samples that were independently sequenced a second time and are shown in white. Test the model samples with poor quality RNA are labeled as "test the model*.*

Conclusions

- Conditional media (CM) collected from all eleven lots of CAP-1002 induced a statistically significant reduction in both COL1A and COL3A expression when compared to the non-conditioned media control, indicating that CAP-1002 may play a role in ameliorating fibrosis, which is a clear pathological feature observed in DMD patients.
- Expression of three genes, CXCL8, IL6 and HSPA5, is significantly upregulated in 41 lots of tested CAP-1002 cells compared with Non-CAP-1002 cells, suggesting that these genes could be potentially used for CAP-1002 cell identity.
- Differentially expressed gene (DE) model was built based on the RNAseq data from potent CAP-1002 cells (Used in HOPE 2 and HOPE 2 OLE) and non-CAP-1002 cells, which could be used to predict CAP-1002 potency.

Disclosure

- CAP-1002 is proprietary of Capricor Therapeutics, Inc (NASDAQ:CAPR)