INTRODUCTION

Pompe disease (PD) is an autosomal recessive, lysosomal storage disease caused by deficiency of acid α-glucosidase (GAA). Glycogen accumulation is seen in the affected organ such as skeletal muscle, heart, and liver. Hypertrophic cardiomyopathy is frequently seen in the infantile onset Pompe disease. On the other hand, cardiovascular complication of the late-onset Pompe disease is considered as less frequent and severe than that of infantile onset. There are few investigations which show cardiovascular complication of late onset Pompe disease due to the shortage of appropriate disease model. We have generated late-onset Pompe disease-specific induced pluripotent stem cell (iPSC) and differentiated them into cardiomyocytes. Differentiated cardiomyocyte shows glycogen accumulation and lysosomal enlargement. Lentiviral GAA rescue improves GAA enzyme activity and glycogen accumulation in iPSC. The efficacy of gene therapy is maintained following the cardiomyocyte differentiation. Lentiviral GAA transfer ameliorates the disease-specific change in cardiomyocyte. It is suggested that Pompe disease iPSC-derived cardiomyocyte is replicating disease-specific changes in the context of disease modeling, drug screening, and cell therapy.

ARTICLE

Disease modeling and lentiviral gene transfer in patient-specific induced pluripotent stem cells from late-onset Pompe disease patient

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Pompe disease (PD) is an autosomal recessive inherited metabolic disease caused by deficiency of acid α-glucosidase (GAA). Glycogen accumulation is seen in the affected organ such as skeletal muscle, heart, and liver. Hypertrophic cardiomyopathy is frequently seen in the infantile onset Pompe disease. On the other hand, cardiovascular complication of the late-onset Pompe disease is considered as less frequent and severe than that of infantile onset. There are few investigations which show cardiovascular complication of late onset Pompe disease due to the shortage of appropriate disease model. We have generated late-onset Pompe disease-specific induced pluripotent stem cell (iPSC) and differentiated them into cardiomyocytes. Differentiated cardiomyocyte shows glycogen accumulation and lysosomal enlargement. Lentiviral GAA rescue improves GAA enzyme activity and glycogen accumulation in iPSC. The efficacy of gene therapy is maintained following the cardiomyocyte differentiation. Lentiviral GAA transfer ameliorates the disease-specific change in cardiomyocyte. It is suggested that Pompe disease iPSC-derived cardiomyocyte is replicating disease-specific changes in the context of disease modeling, drug screening, and cell therapy.

Molecular Therapy — Methods & Clinical Development (2015) 2, 15023; doi:10.1038/mtm.2015.23; published online 8 July 2015

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Received 17 March 2015; accepted 21 May 2015
RESULTS

Generation of Pompe disease pluripotent stem cell and its characterization

Three iPSC clones from one late-onset PD patient and one clone from one normal control were analyzed. Pluripotency markers, Oct3/4, Sox2, Klf4, Myc, Nanog, Gdf3, Rex1, DPPA2, and DPPA4, were analyzed by reverse transcription polymerase chain reaction (RT-PCR). All of the pluripotency markers were expressed in both PD and control iPSC almost equally except relatively low expression of GDF3 in control iPSC (Figure 1a).

Pluripotency markers such as SSEA-4, Tra-1–60, and Tra-1–81 were also analyzed by immunofluorescence and all of the markers were positively stained equally both control and PD iPSC without difference between both cell lines (Figure 1b).

Next we have conducted embryoid body formation and checked pluripotency. Ectoderm (PAX6 and MAP2), mesoderm (Brachury and MSX1), and endoderm (FOXA2 and AFP) markers were analyzed by RT-PCR. In all cell lines, at least one of ectoderm, mesoderm, and endoderm markers expression was confirmed (Figure 1c). Pluripotency was also evaluated by directed differentiation to three germ layers confirmed by immunofluorescence. The expression of Otx2 (Ectoderm), Brachury (Mesoderm), and Sox17 (Endoderm) were observed in all patient and control cell lines (Figure 1d).

These results showed that both control and Pompe disease iPSCs have similar characteristics as pluripotent stem cell.

Pompe disease pluripotent stem cells have disease-specific phenotypes

Gene analysis showed compound heterozygote mutation, c.796C>T and c.1316T>A, which is corresponding to late onset Pompe disease (Figure 2a).

Figure 1 Characterization of iPSC cell lines. (a) Reverse transcription polymerase chain reaction of iPSC cell lines (Control, Pompe1, Pompe2, and Pompe3). Oct3/4, Sox2, Klf4, Myc, Nanog, Gdf3, Rex1, DPPA2, DPPA4, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression were analyzed. (b) Immunofluorescence of iPSC cell lines (Pompe1, Pompe2, Pompe3, and control). ALP stain, SSEA-4 (Alexa488), Tra-1–60 (Alexa488), and Tra-1–81 (Alexa488) were analyzed. Scale bar, 100 µm. (c) RT-PCR of embryoid body from iPSC cell lines (Control, Pompe1, Pompe2, and Pompe3). PAX6, MAP2, Brachury, MSX1, FOXA2, AFP, and GAPDH were analyzed. (d) Immunofluorescence of directed differentiated three germ layers. Otx2 (Alexa488), Brachury (Alexa488), and Sox17 (Alexa488) were analyzed. Scale bar, 100 µm.
Electron microscopy showed lysosomal accumulation of glycogen in Pompe disease iPSCs compared to control (Figure 2b). Patient-specific iPSC lines show the weak expression of GAA compared to the healthy control iPSC (Figure 2c). Pompe disease iPSCs have increased glycogen content compared to the healthy control iPSC (Figure 2d). Pompe disease iPSCs have disease-specific characteristics both pathologically and biochemically.

Pathological hallmarks of cardiomyocyte derived from Pompe disease iPSC
We have differentiated iPSCs into cardiomyocyte according to the differentiation protocol shown in Figure 3a. Robust differentiation was also possible in healthy control and Pompe disease iPSCs (Figure 3b). Beating cardiomyocyte was observed around 10 days after the differentiation in healthy control and patient specific iPSCs (Supplementary Movies S1 and S2).

RT-PCR showed the expression of cardiomyocyte markers including MLC2A, MLC2V, NKX2.5, MYH6, MYH7, and cardiac troponin I (cTnI) in control and Pompe disease iPSC-derived cardiomyocytes (Figure 3c).
Cardiac troponin T (cTnT) positive, mature cardiomyocyte derived from control and Pompe disease iPSCs were observed by immunofluorescence (Figure 3d). From these observations, we successfully differentiated cardiomyocyte form PD and control iPSCs and characteristics of both cardiomyocyte were similar.
Electron microscopy showed control and Pompe disease iPSC-derived cardiomyocyte has sarcomeric cardiac fibers with gap junction. Massive accumulation of glycogen in the lysosome of the cardiomyocyte derived from Pompe disease iPSCs, not from control, were also observed (Figure 3e). Interestingly, there is no remarkable change in the structure of the cardiomyocyte fiber, such as disarray and hypertrophy.

Cellular pathology of late-onset Pompe disease is reflected in patient-specific iPSC-derived cardiomyocyte.
GAA rescue ameliorates the disease phenotype of Pompe disease iPSCs
Third-generation lentiviral vector which express GAA has been generated (Figure 4a). Then we have infected lentiviral vector to Pompe disease iPSCs at the multiplicity of infection of 0, 10, 50, and 100. GAA enzyme activity was increased in dose-dependent manners (Figure 4b). Glycogen contents were significantly decreased by GAA transduction by lentiviral vector only in the highest multiplicity of infection ($P < 0.01$). Glycogen contents were not normalized in treated iPSCs within 48 hours (Figure 4c).

Immunofluorescence using anti-GAA antibody reveals that GAA expression of patent specific iPSC was increased by lentiviral gene therapy according to the strength of the transfection (Figure 4d). Immunofluorescence using anti-GAA antibody and anti-LAMP-2 antibody also reveals that rescued GAA in Pompe disease iPSC was co-localized with lysosomal protein, LAMP-2, which is indicating expressed GAA was localized in lysosome (Figure 4e).
Electron microscopy showed amelioration of disease hallmarks in iPSC morphology such as glycogen accumulation and lysosomal enlargement (Figure 4f).
Lentiviral GAA transfer ameliorates pathological and biochemical abnormality seen in patient-specific iPSC.

The efficacy of GAA rescue remains after cardiomyocyte differentiation
The efficacy of lentiviral gene transfer after the cardiomyocyte differentiation has been assessed. Dose-dependent expression of GAA was observed in enzyme assay in every iPSC-derived cardiomyocyte (Figure 5a). Immunofluorescence shows GAA positive-cTnT positive

![Figure 2](image-url)  
**Figure 2**  
**Disease-specific change of Pompe disease iPSCs.**  
**a** Mutation analysis shows compound heterozygous mutation (c.796 C>A and c.1316 T>A).  
**b** Electron microscopy of iPSC cell lines (Pompe-1, Pompe2, Pompe3, and control). Arrow is demonstrating accumulated glycogen. Upper scale bar, 5 µm; lower scale bar, 1 µm.  
**c** GAA enzyme assay of iPSC cell lines (Control, Pompe1, Pompe2, and Pompe3). Data were expressed as means ± SEM.  
**d** Glycogen assay of iPSC cell lines (Control, Pompe1, Pompe2, and Pompe3). Data were expressed as means ± SEM.
cardiomyocyte in each Pompe disease derived cardiomyocyte after gene transfer (Figure 5b). Electron microscopy showed the amelioration of cellular pathology, such as glycogen accumulation and lysosomal enlargement after the GAA gene transfer (Figure 5c).

The efficacy of lentiviral gene transfer is maintained after the cardiac differentiation and cellular pathology is improved by GAA rescue in dose-dependent manner.

**DISCUSSION**

Pompe disease is classified into the infantile type and the late-onset type according to the clinical phenotype. One of the remarkable differences between infantile and late-onset type is the disease onset and clinical phenotype is not identical. Cardiovascular complication is another important phenotype and major clinical finding particularly in the infantile onset disease. However, the cardiovascular complication is now seeking attention even in the LOPD.

Some report showed that cardiovascular complication such as cardiac hypertrophy and arrhythmia are sometime seen in the LOPD, usually less frequent and severe than infantile onset. In recent, Lee et al. showed five LOPD patients complicated with hypertrophic cardiomyopathy. It is difficult to investigate the disease mechanism of cardiac complication of LOPD because it is difficult to obtain human sample and there is no appropriate disease model which simulates cardiac complication of LOPD.

To solve this problem, we have generated iPSC from LOPD patient and attempted to conduct disease modeling. Our results are compatible with the previous clinical observations because pathological change in the cardiomyocyte is reproduced in vitro. Our model is useful for the investigation of pathological mechanism occurred in the cardiomyocyte of LOPD. Since the discovery of human pluripotent stem cell, a lot of research about patient-specific iPSC has been carried out. Disease modeling, drug screening, and cell therapy are main topics of the stem cell research. Our investigation shows that disease modeling of LOPD and gene correction with lentiviral gene transfer.

Most of the Pompe disease patients are currently receiving enzyme replacement therapy (ERT). In infantile form, the drastic improvement of survival is achieved according to the ERT. Similarly, in late-onset form, clinical improvement is reported. However, the refractory mechanism to enzyme replacement therapy (ERT)
such as neutralizing antibody formation and autophagic buildup is considered.\textsuperscript{15,16} Lentiviral gene therapy is one of the alternative treatment modality to PD patient.\textsuperscript{17} Stem cell therapy such as differentiated pluripotent stem cell replacement is another strategy to treat monogenic disorder and can be combined with the viral gene therapy and homologous recombination.

In our model, gene dosage effect in pluripotent stem cell was evaluated in the context of lentiviral gene therapy to monogenic disease. Our data show that high-intensity gene therapy could rescue the disease phenotype of PD. Disease biochemical phenotype is recovered in terms of enzyme activity, glycogen accumulation, and colocalization of enzyme. Glycogen reduction is statically significant compared to nontreated iPSC but the total content of glycogen was not normalized in 48 hours after transduction. There are two possible explanations why glycogen level was not normalized even if enzyme activity increased over wild-type iPSC. One of the explanations is that very few cells were transduced and glycogen was not reduced in untransduced cells. The other explanation is that 48 hours is not enough to reduce glycogen profoundly. Using same vector construct expressing fluorescent protein, almost 60% wild type were transducted (data not shown). Thus, the former possibility is unlikely. It is speculated that it takes more time to normalize

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**Figure 4** Gene transfer to Pompe disease iPSC. (a) Vector construct. GAA was cloned into downstream of EF-1-α promoter. (b) GAA enzyme assay of transfected iPSC cell lines (Control, Pompe1, Pompe2, and Pompe3). Transfection is conducted at the multiplicity of infection of 0, 10, 50, and 100. Data were expressed as means ± SEM. (c) Glycogen assay was conducted in iPSCs (Control, Pompe1, Pompe2, and Pompe3). Data were expressed as means ± SEM. (d) Immunofluorescence of transfected iPSCs (Pompe1, Pompe2, and Pompe3). GAA (Alexa568) were stained with DAPI. Scale bar, 100 μm. (e) Immunofluorescence of transfected iPSCs (Pompe1) GAA (Alexa568) and LAMP-2 (Alexa488) were stained to confirm colocalization. Scale bar, 100 μm. (f) Electron microscopy of iPSCs after lentiviral GAA transfer. Upper scale bar, 5 μm, lower scale bar, 1 μm.
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Figure 5 Cardiac differentiation after gene transfers. (a) Acid α-glucosidase (GAA) enzyme assay of differentiated cardiomyocyte (Control, Pompe1, Pompe2, and Pompe3). Data were expressed as means ± SEM. (b) Immunofluorescence of differentiated cardiomyocyte (Pompe1). Cardiac troponin T (Alexa488) and GAA (Alexa568) were stained with DAPI. Scale bar, 100 µm. (c) Electron microscopy of cardiomyocyte (Pompe1) after gene transfer. Black arrow is indicating sarcomeric cardiac fiber and white arrow is indicating enlarged lysosome. Upper scale bar, 5 µm, lower scale bar, 1 µm.

glycogen content in cells even if very high level of enzyme is exist in cells.

Cell therapy based on iPSC technology is a promising therapeutic modality in the context of the treatment of genetic diseases. In fact, clinical trial of iPSC-based therapy is ongoing in the retinal disease. Gene addition such as lentiviral gene transfer will be combined with stem cell differentiation and differentiated stem cell might be transplanted. In terms of safety, homologous recombination is much more promising because we should concern tumorigenicity if we use integrating viral vector. On the other hand, higher expression will be achieved in lentiviral gene transfer and the amount of transplanted cell to be required to therapeutic effect can be reduced compared to homologous recombination. Thus lysosomal storage disorder is the ideal target of gene transfer based iPSC cell therapy because the cross correction of therapeutic protein might improve transduced cells.

Tedesco et al.18 successfully treated limb-girdle muscular dystrophy model mouse by human patient derived pluripotent stem cell. In this study, they differentiated patient-derived pluripotent stem cell into meso-angioblast and then infected lentiviral vector targeting for α-sarcoglycan. Transplanted immunodeficient model mice lacking α-sarcoglycan showed phenotypical correction and it is proof of principle study for differentiated pluripotent stem cell replacement combined with lentiviral gene transfer.

Other strategy is differentiated pluripotent stem cell combined with gene correction without chromosomal integration such as sleeping beauty transposase system. Filareto et al.19 showed that they have generated pluripotent stem cell from tip tail fibroblast of mice lacking both dystrophin and utrophin and replaced therapeutic gene by sleeping beauty transposase system targeting for micro-utrophin. They transplanted differentiated gene corrected pluripotent stem cell and disease phenotype of model mouse is recovered. Our study also suggests that it is possible to investigate differentiated pluripotent stem cell replacement therapy also to the late-onset Pompe disease.

Limitation of our study is the number of cell lines of iPSC. We could not obtain iPSC from the infantile onset and did not compare the phenotypes. Three different clones from one patient were examined in this study. Previous reports have used infantile-onset PD iPSC, so our model is unique in the context of the phenotypic variation of monogenic disease. Our PD iPSCs have residual enzyme activity which might cause more modest disease-specific change and treatment efficacy might be easy to be obtained. Comparison between infantile- and late-onset Pompe disease might be important because underlying mechanism could not be identical and the difference among patients could be evaluated by pluripotent stem cell research. Inclusion of infantile/early-onset PD cardiomyocyte would have also added valuable information to our study. Further study including multiple patient cell lines should be conducted to investigate disease mechanism and variation of infantile and late onset Pompe disease. It is suggested that our investigation could bring the possibility of expansion of the pluripotent stem cell research in the field of Pompe disease research.

MATERIALS AND METHODS

iPSC generation
Patient-specific iPSC (HSP0175, 0176, 0177; Pompe1, 2, 3) were kindly provided from RIKEN BRC. Healthy control iPSC (HPS0223; Control) was also provided from RIKEN BRC20. Both iPSC cell lines were generated by introducing four reprogramming factors, Klf-4, Oct3/4, Sox-2, and c-Myc, by Sendai virus.21

iPSC culture
iPSC cell lines were cultured by Dulbecco's Modified Eagle's Medium/F12 (Sigma Aldrich, St Louis, MO), 20%KSR (Life Tech, Carlsbad, CA), 1%glutamine (Life Tech), 1%Penicillin-streptomycin (Life Tech), 0.01% L-melcaptoethanol (Life Tech), 5 ng/ml bFGF (Wako Pure Chemical, Japan). Radiation inactivated SNL cells were used as feeder cells and collagen I coated dish (AGC TECHNO GLASS, Japan) was used. In feeder-free culture, Corning Synthemax (Corning, Tewksbury, MA) was used as extracellular matrix and mTeSR1 (StemCell Technologies, Vancouver, Canada) was used as culture medium.
In vitro differentiation
To form embryoid body, 1*10^11 feeder depleted iPSC was transferred to petri dish and maintained by floating culture for 28 days. IPSC medium containing bFGF was changed every 2 days. Directed differentiation was conducted by Human iPSC characterization kit (R&D systems, Minneapolis, MN).

Cardiac differentiation
Confluent iPSC was transferred to petri dish (BD Biosciences, San Jose, CA) for aggregation, after 8–24 hours cells were allowed to attach Laminin coated six-well plate (BD) at the density of 3*10^10 cells in each well. Cardiomyocyte was induced by the cardiac differentiation protocol using Wnt inhibitor (KY02111) as described before. To briefly describe, CHIR99021 and BIO were added to the medium until 72 hours after the differentiation and then KY02111 and XAV933 were added to the medium until 10 days after the differentiation.

Mutation analysis
Genomic DNA was extracted from iPSCs. For gene analysis, intron-franking primer was designed to detect genetic mutation (Supplementary Table S1).

RT-PCR
Total RNA was collected and RT-PCR was done by Primescript One-step RT-PCR kit (TAKARA BIO, Japan). RT-PCR primers are shown in (Supplementary Table S2).

Immunofluorescence
iPSC was reacted with primary antibody, SSEA-4, Tra-1–60 and Tra-1–81 using E5 cell characterization kit (Miltenyi Biotec, Germany). Differentiated three germ layers were stained with anti-Otx2, Brachury and Sox17 antibodies (R&D) and nuclei were stained with 4,6-diamidino-2-phenylindole dihydrochloride (Dojindo, Japan).

Cardiomyocyte was stained by cardiac troponin T antibody (Thermo Fisher Scientific, Waltham, MA), anti-GAA antibody and nuclei were stained with DAPI (Dojindo).

Electron microscopy
Electron microscopy analysis was done after 2% glutaraldehyde fixation and followed by 1% osmium tetroxide fixation. Ethanol dehydration followed by epon embedding was done.

Lentivirus generation and infection
Third-generation lentivirus vector plasmid (CS2-EF1a-MCS) was kindly provided form Dr. Miyoshi (RIKEN BRC). GAA was cut out using EcoR1 site and followed by 1% osmium tetroxide fixation. Ethanol dehydration followed by epon embedding was done.

Enzyme assay
Collected cells were homogenized into sterile water and protein concentration was measured by BCA protein assay kit (Thermo Scientific). GAA enzyme assay was done by 4-MU-a-glucopyronside (Sigma) assay as previously described.

Glycogen content analysis
Collected cells were homogenized into sterile water. Glycogen was measured by the Glycogen Colorimetric/Fluorometric assay kit (BioVision, Milpitas, CA).

CONFLICT OF INTEREST
T.Q., H.J., and Y.E. have active research support from Genzyme Japan Co., Ltd. and Shire Japan Co., Ltd. These activities have been fully disclosed and are managed under a Memorandum of Understanding with the Conflict of Interest Resolution Board of The Jikei University School of Medicine.

ACKNOWLEDGMENTS
We thank Division of Molecular Cell Biology, Core Research Facilities for Basic Science for providing fluorescence microscopy, cell analyzer and electron microscopy. Y.S. is supported by Ishidzu Shun Memorial Scholarship. H.K. is supported by Kawanou Memorial Foundation.

REFERENCES
1. Hers, HG (1963). Alpha-Glucosidase deficiency in generalized glycogenstorage disease (Pompe's disease). Biochem J 86: 11–16.
2. Kishnani, PS and Howell, RR (2004). Pompe disease in infants and children. J Pediatr 144(5 Suppl): S35–S43.
3. Kishnani, PS, Corzo, D, Nicolino, M, Byrne, B, Mandel, H, Hwu, WL et al. (2007). Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. Neurology 68: 99–109.
4. van der Ploeg, AT, Clemens, PR, Corzo, D, Escolar, DM, Florence, J, Goeневeld, GJ et al. (2010). A randomized study of aligusidase alfa in late-onset Pompe's disease. N Engl J Med 362: 1396–1406.
5. Aluti, A, Bisaccio, L, Saramazzu, S, Ferrua, F, Cicalese, MP, Baricordi, C et al. (2013). Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science 341: 233151.
6. Biffi, A, Montini, E, Liorioli, L, Cesani, M, Fumagalli, F, Plati, T et al. (2013). Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science 341: 233169.
7. Khanna, R, Powe, AC Jr, Lur, Y, Soska, R, Feng, J, Dhulipala, R et al. (2014). The pharmacological chaperone AT2220 increases the specific activity and lysosomal delivery of mutant acid alpha-glucosidase, and promotes glycosin reduction in a transientic mouse model of Pompe disease. PLoS One 9: e102092.
8. Huang, HP, Chen, PH, Hwu, WL, Chuang, CY, Chien, YH, Stone, L et al. (2011). Human Pompe disease-induced plupotent stem cells for pathogenesis modeling, drug testing and disease marker identification. Hum Mol Genet 20: 4851–4864.
9. Forsha, D, Li, JS, Smith, PB, van der Ploeg, AT, Kishnani, P and Pasquali, SK; Late-Onset Treatment Study Investigators (2011). Cardiovascular abnormalities in late-onset Pompe disease and response to enzyme replacement therapy. Genet Med 13: 625–631.
10. Hobson-Webb, LD, Proia, AD, Thurberg, BL, Banugadia, S, Prater, SN and Kishnani, PS (2012). Autosopy findings in late-onset Pompe disease: a case report and systematic review of the literature. Mol Genet Metab 106: 462–469.
11. Fox, UJ, Daley, QG, Goldman, SA, Haard, J, Kamp, TJ and Trucco, M (2014). Stem cell therapy. Use of differentiated pluripotent stem cells as replacement therapy for treating disease. Science 345: 1247391.
12. Lee, DH, Qiu, WJ, Lee, J, Chien, YH and Hwu, WL (2014). Hypertrophic cardiomyopathy in pompe disease is not limited to the classic infantile-onset phenotype. JIMD Rep 17: 71–75.
13. Priori, SG, Napolitano, C, Di Pasquale, E and Condorelli, G (2013). Induced pluripotent stem cell-derived cardiomyocytes in studies of inherited arrhythmias. J Clin Invest 123: 84–91.
14. Garate, Z, Davis, BR, Quintana-Bustamante, O and Segovia, JC (2013). New front in regenerative medicine: site-specific gene correction in patient-specific induced pluripotent stem cells. Hum Gene Ther 24: 571–583.
15. Râben, N, Schreiner, C, Baum, R, Takkita, S, Xu, S, Xie, T et al. (2010). Suppression of autophagy permits successful enzyme replacement therapy in a lysosomal storage disorder–mimic Pompe disease. Autophagy 6: 1078–1089.
16. Kishnani, PS, Goldenberg, PC, DeArmy, SL, Heller, J, Benjamin, D, Young, S et al. (2010). Cross-reactive immunologic material status affects treatment outcomes in Pompe disease infants. Mol Genet Metab 99: 26–33.
17. Kyozen, SO, Iizuka, S, Kobayashi, H, Kimura, T, Fukuda, T, Shen, J et al. (2010). Neonatal gene therapy using lentiviral vector for murine Pompe disease: long-term expression and glycogen reduction. Gene Ther 17: 521–530.
18. Tedesco, FS, Gerli, MF, Perani, L, Benedetti, S, Ungaro, F, Cassano, M et al. (2012). Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy. Sci Trans Med 4: 140a89.
19. Filareto, A, Parker, S, Darabi, R, Bories, L, Lascovin, M, Schaal, T et al. (2013). An ex vivo gene therapy approach to treat muscular dystrophy using inducible pluripotent stem cells. Nat Commun 4: 1549.
20. Ono, M., Hamada, Y., Horiuchi, Y., Matsuo-Takasaki, M., Imoto, Y., Satomi, K. et al. (2012). Generation of induced pluripotent stem cells from human nasal epithelial cells using a Sendai virus vector. PloS One 7: e42855.

21. Hamasaki, M., Hashizume, Y., Yamada, Y., Katayama, T., Hohjoh, H., Fusaki, N. et al. (2012). Pathogenic mutation of ALK2 inhibits induced pluripotent stem cell reprogramming and maintenance: mechanisms of reprogramming and strategy for drug identification. Stem Cells 30: 2437–2449.

22. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861–872.

23. Minami, I., Yamada, K., Otsuij, T.G., Yamamoto, T., Shen, Y., Otsuka, S. et al. (2012). A small molecule that promotes cardiac differentiation of human pluripotent stem cells under defined, cytokine- and xeno-free conditions. Cell Rep 2: 1448–1460.