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Treatment with selumetinib preserves cardiac function and improves survival in cardiomyopathy caused by mutation in the lamin A/C gene

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Aims Mutations in A-type nuclear lamins gene, *LMNA*, lead to a dilated cardiomyopathy. We have reported abnormal activation of the extracellular signal-regulated kinase1/2 (ERK1/2) signalling in hearts from *Lmna*$^{H222P/H222P}$ mice, which develop dilated cardiomyopathy. We therefore determined whether an inhibitor of ERK1/2 signalling that has been investigated in clinical trials for cancer has the potential to be translated to humans with *LMNA* cardiomyopathy.

Methods and results To evaluate the relevance of this finding in mice to patients, we analysed the ERK1/2 signalling in heart tissue from human subjects with *LMNA* cardiomyopathy and showed that it was abnormally activated. To determine whether pharmacological inhibitors of the ERK1/2 signalling pathway could potentially be used to treat *LMNA* cardiomyopathy, we administered selumetinib to male *Lmna*$^{H222P/H222P}$ mice starting at 16 weeks of age, after they show signs of cardiac deterioration, up to 20 weeks of age. Selumetinib is an inhibitor of ERK1/2 signalling and has been given safely to human subjects in clinical trials for cancer. Systemic treatment with selumetinib inhibited cardiac ERK1/2 phosphorylation and blocked increased expression of RNAs encoding natriuretic peptide precursors and proteins involved in sarcomere architecture that occurred in placebo-treated mice. Echocardiography and histological analysis demonstrated that treatment increases cardiac fractional shortening, prevents myocardial fibrosis, and prolongs survival. Selumetinib treatment did not induce biochemical abnormalities suggestive of renal or hepatic toxicity.

Conclusion Our results suggest that selumetinib or other related inhibitors that have been safely administered to humans in clinical trials could potentially be used to treat *LMNA* cardiomyopathy.

Keywords MAP kinase • ERK • Selumetinib • Dilated cardiomyopathy • A-type lamins • *LMNA*

1. Introduction

The lamin A/C gene, *LMNA*, encodes A-type nuclear lamins, intermediate filament proteins comprising the nuclear lamina of most differential mammalian somatic cells. Mutations in *LMNA* cause more than a dozen previously defined clinical entities primarily, often referred to as laminopathies, affecting either striated muscle, adipose tissue, peripheral nerve, or multiple systems with an accelerated ageing phenotype.[1] Most disease-causing *LMNA* mutations affect the heart, particularly as a dilated cardiomyopathy, which occurs with or without concurrent skeletal muscle involvement, usually Emery–Dreifuss muscular dystrophy or limb-girdle muscular dystrophy type 1B.[2–4]

Cardiomyopathy caused by *LMNA* mutation is characterized by chamber enlargement and systolic dysfunction of one or both ventricles. A key feature of *LMNA* cardiomyopathy is early atrioventricular conduction block and other conduction system defects.[2–4] *LMNA* mutations have been found in up to 7.5% of cases of dilated cardiomyopathy with a positive family history and in 3.6–11% of sporadic cases.[5,6] In one study of familial dilated cardiomyopathy with

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conduction block as prominent feature, 33% were found to have *LMNA* mutations.[7] Timely insertion of a pacemaker and/or intracardiac cardioverter defibrillator can decrease the risk of death from arrhythmias but most patients ultimately develop advanced heart failure. Approximately 55% of the patients with *LMNA* cardiomyopathy die of cardiovascular death or receive a heart transplant by 60 years of age.[5,8,9] While drugs such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, β-blockers, diuretics, and aldosterone antagonists may be of some benefit to patients with *LMNA* cardiomyopathy, there is no specific therapeutic intervention that improves cardiac function or prevents heart muscle deterioration.

Several genetically modified mouse lines have been created to help understand the pathogenic mechanisms of how alterations in A-type lamins cause disease.[10] *Lmna*$^{H222P/H222P}$ mice contain a mutation corresponding to one that causes Emery–Dreifuss muscular dystrophy in humans and develop cardiomyopathy with skeletal muscle involvement.[11] We have discovered abnormal activation of the Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK1/2) branches of the mitogen-activated protein kinase (MAPK) signalling cascade in hearts of these mice, which occurs prior to the onset of significant cardiomyopathy.[12] This finding has linked a cardiac disease-causing A-type lamin alteration to signal transduction pathways implicated in heart function and cardiomyopathy. Based on this discovery, we hypothesized that pharmacological inhibitors of these signalling pathways could improve heart function and prevent *LMNA* cardiomyopathy and have shown beneficial effects of treatment with such drugs in *Lmna*$^{H222P/H222P}$ mice.[13–15]

Our preclinical research showing abnormal activation of MAPK signalling pathways in hearts of *Lmna*$^{H222P/H222P}$ mice and that inhibitors of enzymes in these pathways have beneficial therapeutic effects has the potential to be translated to humans with *LMNA* cardiomyopathy. However, there are several limitations. First, it is not clear whether the same cell signalling abnormalities occur in hearts of human subjects with *LMNA* mutations. Secondly, JNK inhibitors have not advanced significantly in human clinical trials and the MAPK/ERK1/2 kinase (MEK1/2) inhibitor we previously used to block activation of ERK1/2, PD98059, is unsuitable for human use because of its poor pharmacokinetic profile and off-target toxicities. However, several biotechnology and pharmaceutical companies have MEK1/2 inhibitors in clinical development for cancer.[16] Among these MEK1/2 inhibitors, selumetinib (AZD6244/ARRY-142886)[17] has shown a high selectivity for its target by without activity against more than 40 other kinases.[18] Selumetinib has been studied in Phase I and Phase II clinical trials in patients with biliary cancers, colorectal cancer, myeloma, and hepatocellular carcinoma.[19–21] Thirdly, although often difficult to demonstrate in small animal studies, we have not yet reported a survival benefit of MAPK signalling inhibitors in *Lmna*$^{H222P/H222P}$ mice. Fourthly, a preliminary analysis of potential hepatic and renal toxicity in mice with cardiomyopathy has not been performed. We therefore carried out the current study to overcome these shortcomings.

2. Methods

2.1 Human heart tissue

Sections of explanted hearts from human subjects with *LMNA* mutations were obtained from Myobank-AFM de l'Institut de Myologie (Paris, France). Myobank-AFM is a non-profit service dealing with the collection, preparation, storage, and distribution of human tissue samples. The Myobank-AFM has received authorization to preserve and prepare tissues and cells of human origin for scientific purposes (French Ministry of Research—April 2008—authorization no. 2008-87), use computerized data files for sample tracking and banking activities (Commission Nationale Informatique et Libertés, CNIL—law no. 94-548), and import and export tissues and cells of human origin for scientific purposes in the context of international collaborations (French Ministry of Health, 2008—law no. 96-327). Myobank-AFM received the authorizations from the French Ministry of Health and from the Comity for Protection of Patient to share tissues and cells of human origin for scientific purposes, ensuring the donors the maintenance of anonymity, respect of their volition, and consent according to the legislation (http://www.institut-myologie.org/anglais/ewb_pages/r/recherche_banquetissus_activites2003.php). Control human heart samples were obtained from the National Disease Research Interchange (Philadelphia, PA, USA); information regarding donor confidentiality and consent can be found at http://www.ndriresource.org. Tissue samples received from either autopsy or transplanted consent donors were not obtained specifically for this study and provided by Myobank-AFM or the National Disease Research Interchange without patient identifiers; therefore, Institutional Review Board approval at Columbia University Medical Center was required. This study conforms to the Declaration of Helsinki for ethical principles for medical research involving research on identifiable human material and data.

2.2 Mice and treatment protocols

Lmna$^{H222P/H222P}$ mice were bred and genotyped as described previously.[11,13] Mice were fed chow and housed in a disease-free barrier facility with 12 h/12 h light/dark cycles. The study conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-53, revised 1996). The Institutional Animal Care and Use Committee at Columbia University Medical Center approved the use of animals and the study protocol. Selumetinib (Selleck Chemicals) was dissolved in dimethyl sulfoxide (DMSO; Sigma) at a concentration of 0.5 mg/mL. The placebo control consisted of the same volume of DMSO. Selumetinib was delivered at a dose of 1 mg/kg/day.[22] For biochemical analysis, echocardiographic analysis, and toxicity studies, selumetinib and DMSO were administered by ip injection using a 27 G 5/8 syringe starting when mice were 16 weeks of age and continuing until 20 weeks of age. For survival analysis, selumetinib and DMSO were diluted in the drinking water starting when mice were 16 weeks of age and continued until they suffered from significant distress or died. Specific signs of significant distress included (i) difficulty with normal ambulatory movement, (ii) failure to eat or drink, (iii) weight loss of more than 20%, (iv) depression, (v) rough or unkempt hair coat, and (vi) significant respiratory distress and were confirmed by consulting with veterinarians at the Institute of Comparative Medicine, Columbia University Medical Center. For most of these experiments, euthanasia of the animals was performed in a CO_2 chamber followed by cervical dislocation, according to the protocol of the Institute of Comparative Medicine. Euthanasia was confirmed by checking for lack of response to limb and tail pinch. Mouse hearts were then quickly excised by cutting the aorta.

2.3 Protein extraction and immunoblotting

Human or mouse heart tissue was homogenized in extraction buffer as described previously.[12,13] Extracted proteins were separated by SDS–polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and blotted with primary antibodies against total ERK1/2 (no. Sc-94, Santa-Cruz) and phosphorylated ERK1/2 (no. 9101, Cell Signaling). Secondary antibodies were horseradish peroxidase-conjugated (GE Healthcare). Recognized proteins were visualized by enhanced chemiluminescence (ECL, GE Healthcare). For quantification of phosphorylated ERK1/2 compared with total ERK1/2, immunoblots were scanned and analysed using ImageJ64 software.

2.4 Quantitative real-time RT–PCR analysis

Total RNA was extracted using the Rneasy isolation kit (Qiagen). Complementary DNA was synthesized using Superscript first-strand synthesis system according to the manufacturer's instructions (Invitrogen) on total RNA. For each replicate in each experiment, RNA from tissue samples of different animals was used. Primers were designed corresponding to mouse RNA sequences using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) for *Nppa* (forward 5′-gcttccaggccatattggag-3′, reverse 5′-ccctgcttcctcagtctgct-3′), *Mlc-1a* (forward 5′-cccaagcctgaagagatgag-3′, reverse 5′-agacaacagctgctccacct-3′) and *Mlc-2a* (forward 5′-tcaaggaagccttcagctgc-3′, reverse 5′-cggaacacttaccctcccg-3′). Real-time RT–PCRs contained HotStart-IT SYBR green qPCR Master Mix (Usb, Affymetrix), 200 nM of each primer, and 0.2 μL of template in a 25 μL reaction volume. Amplification was carried out using the ABI 7300 Real-Time PCR System (Applied Biosystems) with an initial denaturation at 95°C for 2 min followed by 50 cycles at 95°C for 30 s and 62°C for 30 s. Relative levels of mRNA expression were calculated using the $\Delta\Delta C_T$ method.[23] Individual expression values were normalized by comparison with *Gapdh* mRNA (forward 5′-tgcaccaccaactgcttag-3′, reverse 5′-ggatgcagggatgatgttc-3′) and *Hprt* mRNA (forward 5′-agttgagagatcatctccac-3′, reverse 5′-ttgctgacctgctggatttac-3′).

2.5 Natriuretic peptide A ELISA

Natriuretic peptide A in serum was detected using a commercially available kit (EIA-ANP-1, RayBiotech) as per the manufacturer's recommendations.

2.6 Transthoracic echocardiography

Lmna$^{H222P/H222P}$ mice were anaesthetized with 1.5% isoflurane inhalation and placed on a heating pad (37°C). Echocardiography was performed using a Visualsonics Vevo 770 ultrasound with a 30 MHz transducer applied to the chest wall. Cardiac ventricular dimensions were measured in two-dimensional mode and M-mode three times for the number of animals indicated. Fractional shortening (FS) was calculated using the following formula: FS (%) = [(LVEDD − LVESD)/LVEDD] × 100.

2.7 Histopathological analysis

Hearts from *Lmna*$^{H222P/H222P}$ mice were fixed in 4% formaldehyde for 48 h, embedded in paraffin, sectioned at 5 μM, and stained with haematoxylin and eosin and Gomori's trichrome. Representative stained sections were photographed using a Microphot SA (Nikon) light microscope attached to a Spot RT Slide camera (Diagnostic Instruments). Images were processed using Adobe Photoshop CS (Adobe Systems). To quantify myocardial fibrosis, micrographs of sections stained with Gomori's trichrome from each heart were processed (JMicroVision software) and blue-stained fibrotic tissue measured (ImageJ64 software). For quantification of nuclear length, cardiomyocyte nuclei were measured along the longitudinal length of a given cell (ImageJ64) in micrographs of sections of heart stained with haematoxylin and eosin.

2.8 Serum biochemical analysis

Serum was separated from blood drawn from mice and stored at −80°C until analysed. Routine clinical chemistry analysis was performed on an AutoAnalyzer at the Comparative Pathology Laboratory at Columbia University Medical Center.

2.9 Statistics

Values for scanned immunoblots, real-time RT–PCR, ELISA, fibrosis quantification, nuclear length, and blood chemistry were compared using an unpaired Student's *t*-test. Comparisons of the echocardiographic parameters between selumetinib- and DMSO-treated *Lmna*$^{H222P/H222P}$ were performed using a Welch's *t*-test; to validate these results, a non-parametric test (Mann–Whitney) was performed and concordance checked. Mouse survival was analysed using the Kaplan–Meier estimator followed by a log-rank test with $P < 0.05$ considered to be statistically significant.[24] Statistical analyses were performed using GraphPad Prism software.

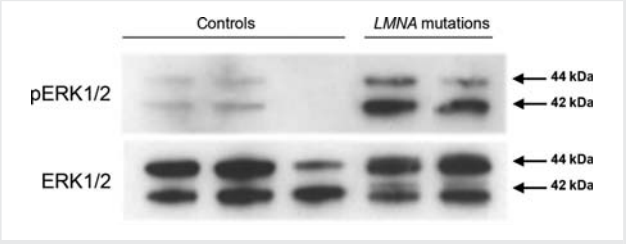

Figure 1 ERK1/2 activation in hearts from human subjects with *LMNA* cardiomyopathy. Immunoblots are shown using antibodies against phosphorylated ERK1/2 (pERK1/2) and total ERK1/2 (ERK1/2) to probe proteins extracted from human heart tissue from controls and individuals with *LMNA* cardiomyopathy.

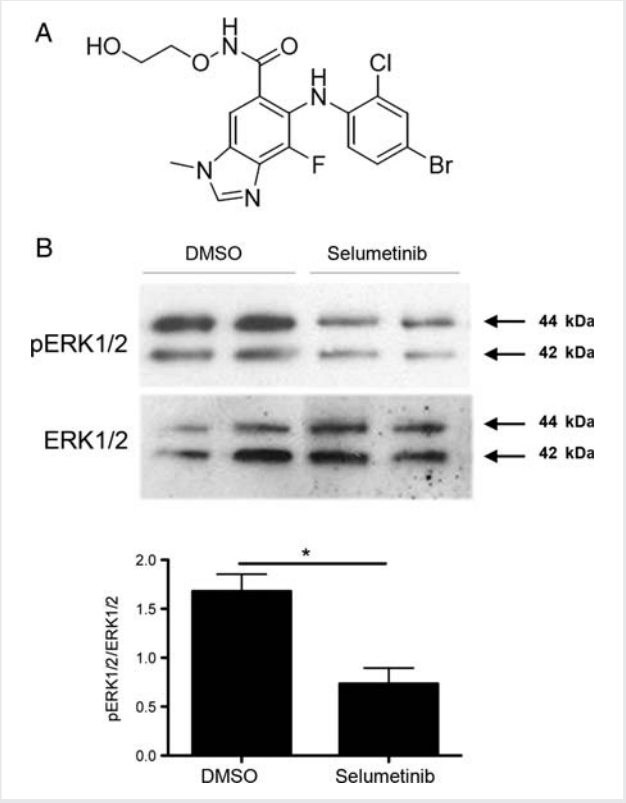

Figure 2 Selumetinib inhibits ERK1/2 signalling in hearts from male *Lmna*$^{H222P/H222P}$ mice. (*A*) Chemical structure of selumetinib. (*B*) Representative immunoblots using antibodies against phosphorylated ERK1/2 (pERK1/2) and total ERK1/2 (ERK1/2) to probe proteins extracted from hearts from *Lmna*$^{H222P/H222P}$ mice treated with selumetinib or DMSO. The bar graph shows means ± SEM signals pERK1/2/total ERK1/2 (relative expression from immunoblots of $n = 5$ mice treated with selumetinib and $n = 5$ mice treated with DMSO) of pERK1/2/total ERK1/2. *$P < 0.05$.

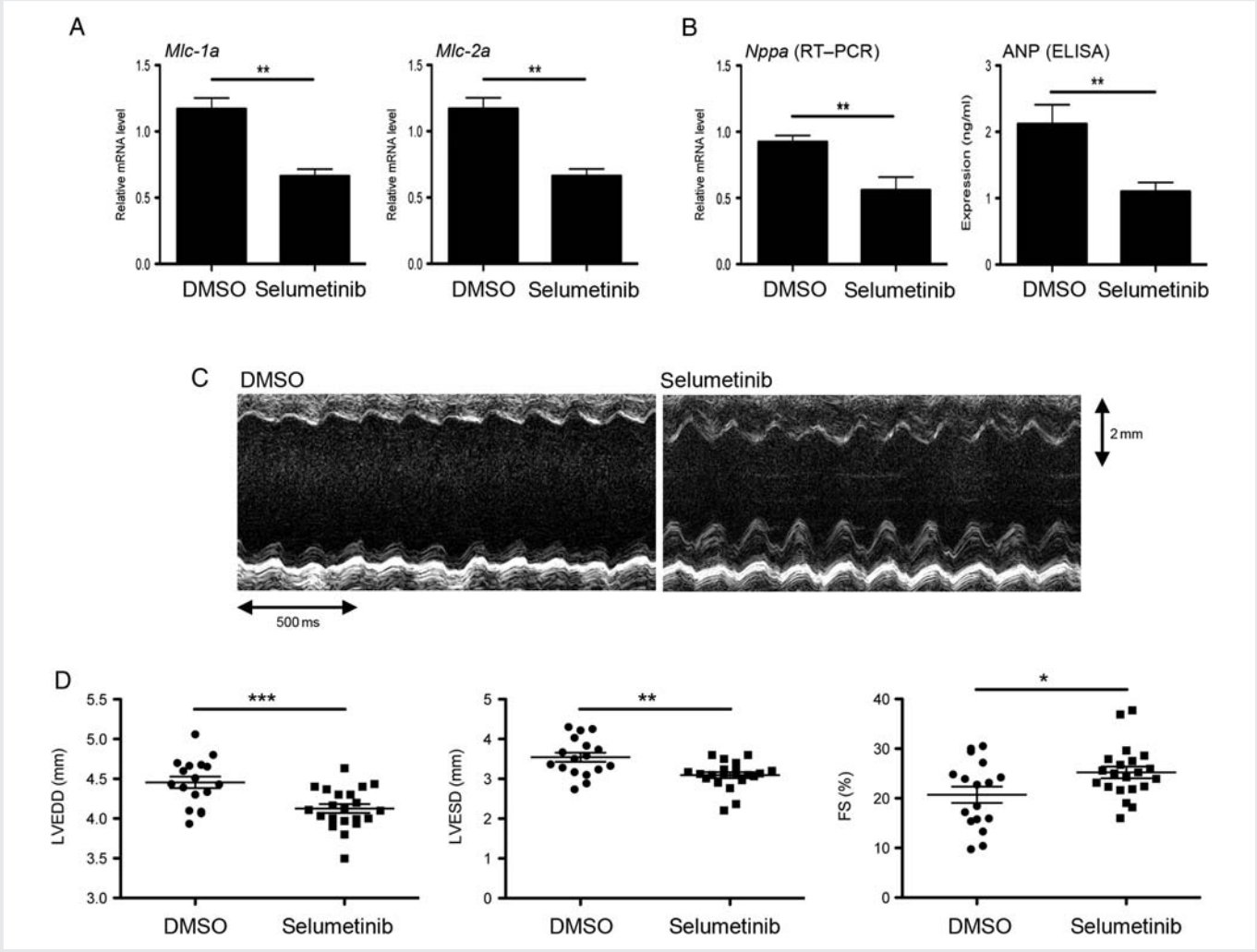

Figure 3 Selumetinib decreased expression of cardiac myosin light chains and natriuretic peptides and prevents left ventricular dilatation and deterioration of FS in *Lmna*$^{H222P/H222P}$ mice. Bar graphs indicate the expression levels of (*A*) *Mlc-1a* and *Mlc-2a* mRNAs encoding the cardiac isoforms of myosin light chains and (*B*) *Nppa* mRNA encoding the atrial natriuretic peptide A (ANP) and ANP expression in hearts *Lmna*$^{H222P/H222P}$ mice treated with selumetinib ($n = 7$) or DMSO ($n = 8$). Values for the real-time RT–PCR were obtained using the $\Delta\Delta C_T$ method using *Gapdh* as a housekeeping gene (similar values were obtained using *Hprt* as a housekeeping gene). Values for ELISA are the ratio of ANP expression at the end of the treatment/ANP expression before the treatment. Values for each individual mouse and means ± SEM are shown. $^{**}P < 0.005$. (*C*) Representative M-mode transthoracic echocardiographic tracings from 20-week only male *Lmna*$^{H222P/H222P}$ mice treated with DMSO or selumetinib. (*D*) Graphs showing mean left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), and FS in 20-week-old male *Lmna*$^{H222P/H222P}$ mice treated with selumetinib ($n = 21$) or DMSO ($n = 17$). Values for each individual mouse as well as means ± SEM are shown. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0005$.

3. Results

3.1 Abnormal activation of ERK1/2 signalling in hearts from human subjects with *LMNA* cardiomyopathy

We have previously shown abnormal activation of ERK1/2 signalling in hearts from *Lmna*$^{H222P/+}$ and *Lmna*$^{H222P/H222P}$ mice that develop cardiomyopathy.[12] Reduced expression of A-type lamins and expression of variants that cause cardiomyopathy in transfected cultured cells also activate ERK1/2 signalling.[12,16] However, no data on ERK1/2 signalling in hearts from human subjects with *LMNA* cardiomyopathy have been published previously. We therefore obtained samples of heart tissues from two human subjects with *LMNA* cardiomyopathy obtained after cardiac transplantation. One sample was from a 47-year-old woman with Emery–Dreifuss muscular dystrophy with an *LMNA* △K261 mutation and the other from a 62-year-old woman with limb-girdle muscular dystrophy type 1B carrying an *LMNA* IVS9 + 1g > a mutation. Control heart samples were obtained from a 57-year-old man who died from an intracranial bleed, a 15-year-old woman who died from a drug overdose, and a 46-year-old man who died from end-stage liver disease. Immunoblotting using antibodies against phosphorylated ERK1/2 and total ERK1/2 showed obvious increases in phosphorylated (activated) ERK1/2 in heart tissue of the patients with *LMNA* mutations compared with controls (*Figure 1*).

Table 1 Echocardiographic parameters in Lmna$^{H222P/H222P}$ mice treated with selumetinib or placebo (DMSO)

	Heart rate (b.p.m.)	LVEDD (mm)	LVESD (mm)	FS (%)	PW (mm)	Relative WT
DMSO ($n = 17$)	506.4 ± 4.7	4.4 ± 0.1	3.5 ± 0.1	20.7 ± 1.6	0.61 ± 0.01	0.26 ± 0.01
Selumetinib ($n = 21$)	511.6 ± 3.8	4.1 ± 0.1***	3.0 ± 0.1**	25.2 ± 1.2*	0.58 ± 0.01	0.28 ± 0.01

Values are means ± SEM. LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; FS, fractional shortening; PW, posterior wall thickness dimension; relative WT, relative wall thickness. *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$.

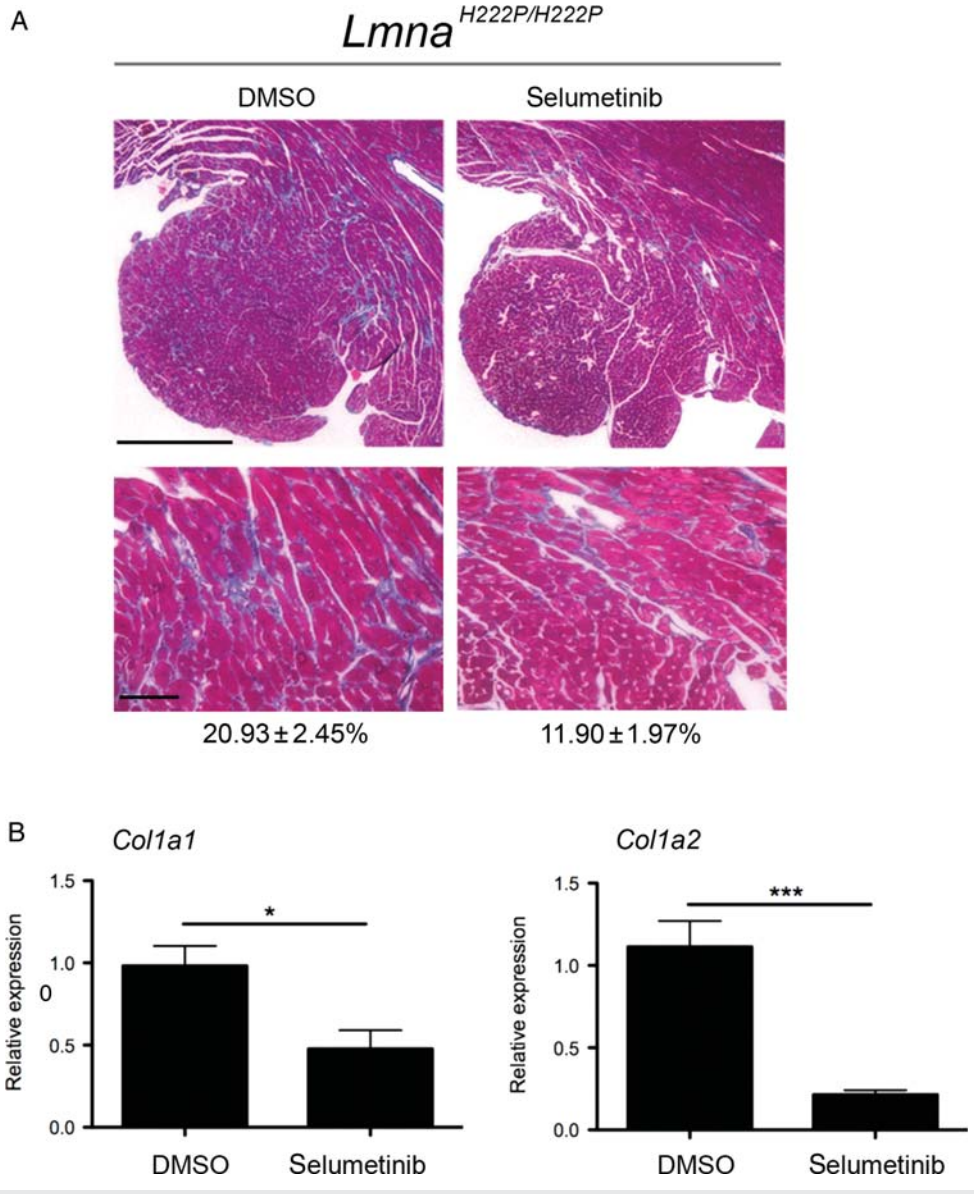

Figure 4 Selumetinib prevents cardiac fibrosis in *Lmna*$^{H222P/H222P}$ mice. (*A*) Representative heart tissue sections from male *Lmna*$^{H222P/H222P}$ mice treated with selumetinib or DMSO stained with Gomori's trichrome are shown. Fibrosis appears blue. Scale bar: 50 μm. Values (means ± SEM) reflect the myocardial fibrosis for each group (see Section 2). (*B*) Bar graphs showing expression levels of *Col1a1* and *Col1a2* mRNAs encoding collagens in hearts *Lmna*$^{H222P/H222P}$ mice treated with selumetinib ($n = 5$) or DMSO ($n = 5$). Values (means ± SEM) were obtained using the $\Delta\Delta C_T$ method using *Gapdh* as a housekeeping gene (similar values were obtained using *Hprt* as a housekeeping gene). *$P < 0.05$, ***$P < 0.005$.

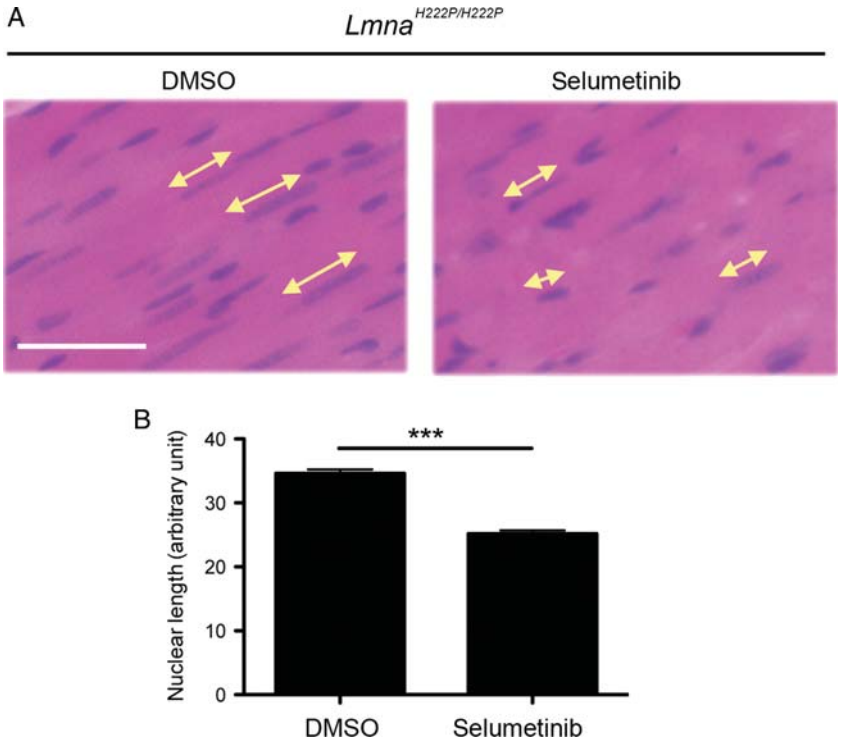

Figure 5 Selumetinib prevents abnormal elongation of cardiomyocyte nuclei in *Lmna*$^{H222P/H222P}$ mice. (*A*) Histological analysis of cross-sections of hearts from *Lmna*$^{H222P/H222P}$ mice treated with selumetinib or DMSO. Sections were stained with haematoxylin and eosin. Yellow lines with arrowheads demonstrate the measurement of nuclear length. Scale bar: 10 μm. (*B*) Quantification of nuclear elongation in cardiomyocytes from mice. Cardiomyocyte nuclei were measured along the yellow lines with arrowheads. Bars indicate the length of cardiomyocyte nuclei in the indicated hearts. Values are means ± SEM for $n = 200$ cardiomyocytes. ***$P < 0.0005$.

3.2 Selumetinib inhibits cardiac ERK1/2 activation, improves cardiac function and prevents fibrosis in *Lmna*$^{H222P/H222P}$ mice with cardiomyopathy

Selumetinib (*Figure 2A*) is a potent, highly specific allosteric MEK1/2 inhibitor, which does not bind to the ATP-binding site and compete with endogenous ATP.[18,19] We therefore assessed the efficacy of selumetinib in the treatment of cardiomyopathy in *Lmna*$^{H222P/H22P}$ mice. We administered selumetinib systemically (1 mg/kg by ip injection, daily) to male *Lmna*$^{H222P/H222P}$ mice starting at 16 weeks of age, when they have left ventricular dilatation and decreased cardiac FS,[11] and analysed them at 20 weeks of age. Systemic administration of selumetinib blocked the phosphorylation of ERK1/2 in hearts, decreasing it by ∼50% compared with mice treated with DMSO placebo (*Figure 2B*).

We previously reported an up-regulation of genes involved in sarcomere organization in the hearts of *Lmna*$^{H222P/H222P}$ mice.[12–15] We therefore assayed expression of *Mlc-1a* and *Mlc-2a* mRNAs, encoding cardiac isoforms of myosin light chains, in hearts from DMSO- and selumetinib-treated *Lmna*$^{H222P/H222P}$ mice. After treatment with selumetinib from 16 to 20 weeks of age, cardiac expression of both *Mlc-1a* and *Mlc-2a* mRNAs was significantly decreased compared with DMSO-treated *Lmna*$^{H222P/H222P}$ mice (*Figure 3A*). Cardiac expression of *Nppa* mRNA encoding natriuretic peptide A, the synthesis of which is increased in dilated hearts, was also significantly decreased in the hearts from selumetinib-treated *Lmna*$^{H222P/H222P}$ mice compared with hearts from those treated with DMSO (*Figure 3B*). The concentration of natriuretic peptide A in serum was also decreased by ∼50% in selumetinib-treated *Lmna*$^{H222P/H222P}$ mice compared with DMSO-treated *Lmna*$^{H222P/H222P}$ mice (*Figure 3B*). We further investigated the effects of selumetinib treatment on cardiac chamber diameters and contractility in *Lmna*$^{H222P/H222P}$ mice using M-mode transthoracic echocardiography (*Figure 3C*). Left ventricular end-diastolic diameter and left ventricular end-systolic diameter in *Lmna*$^{H222P/H222P}$ mice treated with selumetinib were significantly smaller than in mice treated with DMSO (*Figure 3D*). FS was significantly increased in *Lmna*$^{H222P/H222P}$ mice treated with selumetinib compared with the DMSO-treated mice (*Figure 3D*). Overall, these results showed that selumetinib had positive effects when administered after cardiac dysfunction occurred in *Lmna*$^{H222P/H222P}$ mice (*Table 1*). As treatment with selumetinib could induce changes in biological parameters at their baseline, we also treated *Lmna*$^{+/+}$ mice with selumetinib using the same dosing schedule and did not detect any differences in echocardiographic results compared with untreated mice (see Supplementary material online, *Table S1*).

Later stages cardiomyopathy caused by *LMNA* mutations are characterized by myocardial fibrosis. Gomori's trichrome staining of hearts from *Lmna*$^{H222P/H222P}$ mice at 20 weeks of age after treatment with selumetinib showed a significant reduction in fibrosis compared with hearts from DMSO-treated mice (*Figure 4A*). Hearts from DMSO-treated *Lmna*$^{H222P/H222P}$ mice had 20.93 ± 2.45% fibrotic tissue per total surface area examined compared with 11.90 ±

1.97% ($P < 0.05$) in mice treated with selumetinib. At the end of the treatment, hearts from selumetinib-treated *Lmna*$^{H222P/H222P}$ mice also had a two-fold reduction in expression of *Col1a1* and a four-fold decrease in *Co1a2* mRNAs that encode collagens compared with DMSO-treated mice (*Figure 4B*). These results demonstrated that selumetinib decreases progression of myocardial fibrosis in *Lmna*$^{H222P/H222P}$ mice, an end-stage irreversible pathology.

We have previously reported abnormal elongation of nuclei in cardiomyocytes of *Lmna*$^{H222P/H222P}$ mice.[13–15] Nuclei in cardiomyocytes of *Lmna*$^{H222P/H222P}$ mice treated with selumetinib *Lmna*$^{H222P/H222P}$ mice had an overall shape that was less elongated and more oval than those in cardiomyocytes of mice treated with DMSO (*Figure 5A*). The mean lengths of nuclei in cardiomyocytes in *Lmna*$^{H222P/H222P}$ mice treated with selumetinib were significantly shorter than those in hearts of mice in the DMSO-treated group ($P < 0.0005$) (*Figure 5B*). While other abnormalities in nuclear morphology have been observed in hearts of *Lmna*$^{H222P/H222P}$ mice when cardiac tissue was examined by electron microscopy,[11] we could not assess such ultrastructural alterations with the light microscopic methods we used.

3.3 Effect of selumetinib on survival of *Lmna*$^{H222P/H222P}$ mice with cardiomyopathy

Male *Lmna*$^{H222P/H222P}$ mice die between 4 and 9 months of age with a median survival of ~28 weeks.[11] Previous studies of MEK1/2 inhibitors or other interventions have not assessed a survival benefit. We therefore analysed the effect of oral administration of selumetinib, which is a more appropriate dosing for long-term study, on the survival of male *Lmna*$^{H222P/H222P}$ mice. Selumetinib or DMSO was dissolved in drinking water starting at 16 weeks of age. Cardiac ERK1/2 phosphorylation was inhibited in *Lmna*$^{H222P/H222P}$ mice drinking water containing selumetinib compared with water containing placebo, after 4 weeks treatment (*Figure 6A*). The inhibition of ERK1/2 phosphorylation was similar to that observed with ip dosing (*Figure 2B*). DMSO-treated male *Lmna*$^{H222P/H222P}$ mice had a maximum survival of 29 weeks and a median survival of 27 weeks. Selumetinib-treated *Lmna*$^{H222P/H222P}$ mice had a statistically significantly increased median survival of 30 weeks ($P < 0.0005$), with some mice living up to 32 weeks of age (*Figure 6B*). Hence, selumetinib treatment prolongs the lifespan of male *Lmna*$^{H222P/H222P}$ mice, consistent with its beneficial effects on cardiac function.

3.4 Preliminary analysis of potential renal, hepatic, and pancreatic toxicity of selumetinib in *Lmna*$^{H222P/H222P}$ mice with cardiomyopathy

Selumetinib has been studied in Phase I and Phase II human clinical trials.[20–22] However, its potential safety in subjects with cardiomyopathy is unknown. We therefore carried out a preliminary analysis of potential renal, hepatic, and pancreatic toxicity in the male *Lmna*$^{H222P/H222P}$ mice that were treated with selumetinib from 16 to 20 weeks of age. At the end of 4 weeks of treatment, we measured serum alkaline phosphatase activity, alanine aminotransferase activity, albumin concentration, globulin concentration, and bilirubin concentration to assess liver injury and function. We also measured serum creatinine and blood urea nitrogen concentrations as indicators of renal function and serum amylase activity as a marker of pancreatic injury. There were no statistically significant differences in any of these parameters between male *Lmna*$^{H222P/H222P}$ mice treated with selumetinib or placebo except for a decrease in serum alanine aminotransferase activity in the selumetinib-treated mice (*Table 2*). Significant abnormalities in serum chemistries have also not been reported in human clinical trials of selumetinib.[19–21]

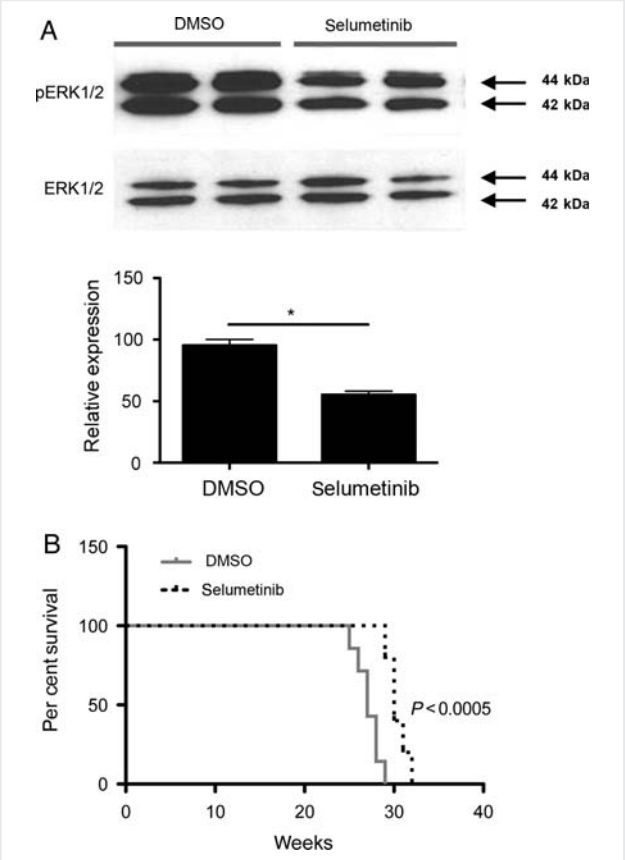

Figure 6 Oral selumetinib inhibits ERK1/2 activity in heart and prolongs survival of *Lmna*$^{H222P/H222P}$ mice. (*A*) Representative immunoblots using antibodies against phosphorylated ERK1/2 (pERK1/2) and total ERK1/2 (ERK1/2) to probe proteins extracted from hearts from *Lmna*$^{H222P/H222P}$ mice treated with selumetinib or DMSO in the drinking water. The bar graph shows means ± SEM signals pERK1/2/total ERK1/2 (relative expression from immunoblots of $n = 5$ mice treated with selumetinib and $n = 5$ mice treated with DMSO). *$P < 0.05$. (*B*) The Kaplan–Meier analysis of selumetinib-treated ($n = 10$) and DMSO-treated ($n = 7$) *Lmna*$^{H222P/H222P}$ mice.

4. Discussion

Dilated cardiomyopathy caused by *LMNA* mutation is a particularly aggressive inherited disease often leading to sudden death and heart failure. Despite its aggressive course, there is no specific therapeutic intervention that improves cardiac function or prevents heart muscle deterioration. We have previously reported aberrant activation of ERK1/2 signalling in hearts of *Lmna*$^{H222P/H222P}$ mice, a small animal model that phenocopies human cardiomyopathy caused by *LMNA* mutation.[12] This abnormal activation of cardiac ERK1/2 occurs prior to the development of significant cardiac muscle

Table 2 Selected serum chemistry values in male Lmna$^{H222P/H222P}$ mice 20 weeks of age treated for 4 weeks with selumetinib or placebo (DMSO)

Treatment	Alk Phos (U/L)	ALT (U/L)	Amylase (U/L)	BUN (mg/dL)	Albumin (g/dL)	Creatinine (mg/dL)	Globulin (g/dL)	Bilirubin (mg/dL)
DMSO ($n = 8$)	74.1 ± 8.0	106.0 ± 22.4	1,244.0 ± 244.7	34.1 ± 8.6	2.8 ± 0.5	0.3 ± 0.1	1.4 ± 0.5	<0.6
Selumetinib ($n = 15$)	76.1 ± 5.6	57.0 ± 10.5*	1,295.0 ± 153.8	25.7 ± 2.4	2.7 ± 0.2	0.3 ± 0.1	1.8 ± 0.2	<0.6

Values are means ± SEM. Alk Phos, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen. *$P < 0.05$.

damage or fibrosis, suggesting that it is a primary pathogenic process in *LMNA* cardiomyopathy. We have now shown similar abnormally activated ERK1/2 signalling in hearts of human subjects with *LMNA* cardiomyopathy. While it was not possible to obtain heart tissue from human subjects prior to the onset of clinical disease, this suggests that the same pathological process that occurs in model mice may occur in humans with the disease.

We have more recently showed that inhibiting ERK1/2 signalling using small molecule inhibitors of MEK1/2 can prevent the development of cardiomyopathy in *Lmna*$^{H222P/H222P}$ mice as well as improve cardiac function after there has been some deterioration in contractility.[13,15] We have now shown that an MEK1/2 inhibitor, selumetinib, which has been safely given to humans for other indications, has beneficial effects on cardiac pathology in *Lmna*$^{H222P/H222P}$ mice. Selumetinib treatment prevented further left ventricular dilatation and deterioration of cardiac contractility compared with placebo in male *Lmna*$^{H222P/H222P}$ mice when started at an age when these mice already have chamber dilatation and decreased FS. It also had other beneficial effects, including on the development of cardiac fibrosis, an end-stage and irreversible predominant feature of *LMNA* cardiomyopathy.

Our results also strongly suggested that selumetinib can prolong survival in male *Lmna*$^{H222P/H222P}$ mice. We showed that male *Lmna*$^{H222P/H222P}$ mice treated with DMSO starting at 16 weeks of age had a median survival of 27 weeks, similar to that previously reported,[11,25] while the selumetinib-treated mice had a median survival of 30 weeks. While the prolongation of survival induced by selumetinib was modest, it was highly statistically significant ($P < 0.0005$). In another study using SCH00013, a calcium-sensitizing agent, 50% survival time was significantly prolonged by 0.8 months in male *Lmna*$^{H222P/H222P}$ mice, although a Kaplan–Meier analysis of the overall mortality showed no statistical difference; in female *Lmna*$^{H222P/H222P}$ mice, there were significantly prolonged 50% survival time and reduced overall mortality.[25] In addition to cardiac disease, male *Lmna*$^{H222P/H222P}$ mice also develop myopathy of striated muscles other than the heart, including the diaphragm, by 24 weeks of age.[11] Hence, the early death of selumetinib-treated male *Lmna*$^{H222P/H222P}$ mice may very well be secondary to problems such as decreased locomotion or respiratory capacity rather than a complication of cardiomyopathy.

For selumetinib to be a useful drug for human subjects with *LMNA* cardiomyopathy, it would have to be safe for long-term use. In Phase II clinical trials of selumetinib given for 3 weeks for cancer, frequent but manageable adverse events were rash, nausea, and diarrhea.[20,21] These clinical trials used a dose of 1.5–2.0 mg/kg orally twice a day, which is three to four times the dose we used in the present mouse studies. In the male *Lmna*$^{H222P/H222P}$ mice with cardiomyopathy that received 1 mg/kg/day of selumetinib for 4 weeks, serum biochemical analysis did not reveal any evidence of renal, hepatic, or pancreatic toxicity. Renal and hepatic toxicities are common reasons for new drugs to fail in clinical trials and lack of renal toxicity may be of particular significance in subjects with reduced kidney perfusion secondary to heart failure and reduced kidney perfusion. While safety data on long-term administration of MEK1/2 inhibitors are not readily available, sorafenib, an inhibitor of Raf further upstream in the ERK1/2 signalling cascade, has been tolerated for longer than 2 years in human subjects.[26] While some studies have associated cardiac toxicity with the use of tyrosine kinase inhibitors, including sorafenib, the biological effects vary widely across the members of this family of drugs and left ventricular dysfunction does not seem to be common to most.[27]

Our promising preclinical results suggest that selumetinib could potentially be used to treat *LMNA* cardiomyopathy. The risks associated with this novel therapy would need to be low, particularly as the treatment may be needed for years. Such treatment may also be applicable to other cardiomyopathies in which there appears to be early abnormal activation of ERK1/2 signalling, such as Chagas disease, caveolin-3 gene mutations, and Noonan syndrome.[28–30] ERK1/2 hyperactivation also occurs in dilated, late-stage failing hearts resulting from several different aetiologies.[31] Ultimately, only prospective clinical trials in human subjects can determine the risks and benefits of selumetinib or other MEK1/2 inhibitors for patients with these cardiomyopathies.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Conflict of interest: H.J.W. and A.M. are inventors on a pending PCT patent application on methods for treating and/or preventing cardiomyopathies by ERK and JNK inhibition filed by the Trustees of Columbia University in the City of New York.

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