Aging Pathobiology and Therapeutics 2025; 7(3): 218-225 ***218***
DOI: 10.31491/APT.2025.09.185

RESEARCH

Pharmacologic activation of Δ133p53α reduces cellular senescence in progeria patients-derived cells

Sebastien M. Joruiz[a, #], Delphine Lissa[a, #], Natalia Von Muhlinen[a], Patricia K. Dranchak[b], James Inglese[b, c], Izumi Horikawa[a, *], Curtis C. Harris[a]

[a] *Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.*
[b] *Division of Preclinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD 20852, USA.*
[c] *Metabolic Medicine Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20814, USA.*

Abstract

Background: Patients with Hutchinson-Gilford progeria syndrome (HGPS) show accelerated aging phenotypes and have shortened lifespan, with implications in physiological aging processes as well. While therapeutic approaches targeting the disease-causing abnormal protein, progerin, have been developed, further efforts to explore mechanistically distinct and complementary strategies are still critical to better treatment regimens. We previously showed that lentiviral vector-driven expression of Δ133p53α, a natural inhibitory isoform of p53, rescued HGPS patients-derived fibroblasts from early entry into cellular senescence, which is a downstream event of progerin-induced DNA damage. We also performed a quantitative high-throughput screen (qHTS) of approved drug and investigational agent libraries, leading to the identification of celastrol and AZD1981 as compounds that upregulate Δ133p53α protein levels.

Materials and Methods: To investigate whether celastrol and ADZ1981 upregulate endogenous Δ133p53α in HGPS-derived fibroblasts and reduce their senescence-associated phenotypes, we performed western blot assays (Δ133p53α, progerin, and $p21^{WAF1}$, which mediates p53-induced senescence and is inhibited by Δ133p53α), senescence-associated β-galactosidase (SA-β-gal) staining, enzyme-linked immunosorbent assay (IL-6, which is a proinflammatory cytokine secreted from senescent cells), and qRT-PCR assays ($p21^{WAF1}$ and IL-6).

Results: Treatment with celastrol (0.1 μM for 24 h) or AZD1981 (10 μM for 24 h) reproducibly increased Δ133p53α expression and decreased $p21^{WAF1}$ expression in two strains of fibroblasts derived from HGPS patients. These compounds reduced the percentage of SA-β-gal-positive senescent cells and the secretion of IL-6 into culture medium in both of these fibroblast strains, irrespective of their different basal levels of senescence and IL-6 secretion. These compounds had no effect on the level of progerin.

Conclusion: Celastrol and ADZ1981 upregulate endogenous Δ133p53α and, reproducing the effects of its vector-driven expression, inhibit cellular senescence and IL-6 secretion in HGPS-derived fibroblasts. Their progerin-independent action suggests that they may synergize with currently available progerin-targeting therapies. This study also warrants further investigation of these compounds for potential applications in other diseases and conditions in which Δ133p53α-regulated senescence plays a role.

Keywords: p53 isoform, cellular senescence, IL-6, fibroblasts, aging, Hutchinson-Gilford progeria syndrome, progerin, pharmacologic activation

# S.M.J. and D.L. contributed equally to this work.
* Corresponding author: Izumi Horikawa
Mailing address: Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Building 37, Room 3054, Bethesda, Maryland 20892, USA.
Email: horikawi@mail.nih.gov
Received: 30 July 2025 / Revised: 08 August 2025
Accepted: 26 August 2025 / Published: 30 September 2025

Introduction

The human *TP53* gene encodes not only the full-length protein consisting of 393 amino acids (simply referred to as "p53" in general and in this manuscript as well) but also at least a dozen of naturally occurring isoforms due to alternative RNA splicing and/or alternative usage of transcription and translation initiation sites [1].

Among these *TP53*-encoded isoforms, Δ133p53α is an N-terminally truncated isoform that is generated via an alternative transcription initiation from intron 4 and an alternative translation from the methionine codon 133 [1, 2]. In various types of normal human cells, the expression level of Δ133p53α is primarily regulated via protein turnover involving chaperone-assisted selective autophagy [3-5], which leads to diminished expression of endogenous Δ133p53α upon cellular senescence [2, 3, 5-9]. The retroviral or lentiviral vector-driven expression of Δ133p53α prevents or delays normal human cells from entering cellular senescence [2, 3, 5, 6, 8, 9], supporting the direct role of Δ133p53α in regulating human cell senescence. Of particular importance, Δ133p53α-expressing cells, not entering senescence, exhibit their inherent normal functions, such as neuroprotective activity of Δ133p53α-expressing astrocytes [5, 8-10] and anti-tumor activity of Δ133p53α-expressing T lymphocytes [11]. Although inhibition of p53 activities could lead to genome instability and increased oncogenesis [12, 13], Δ133p53α preferentially inhibits p53-mediated senescence in a dominant-negative manner, while maintaining or even enhancing p53-mediated DNA repair [7-9, 11, 14, 15]. This selective inhibitory nature of Δ133p53α suggests a potential therapeutic value of Δ133p53α in senescence-associated diseases and conditions with minimum risk of genome instability or oncogenesis. In an attempt to pharmacologically activate Δ133p53α for future therapeutic applications, we performed a cell-based quantitative high-throughput screen (qHTS) on approved drugs, investigational agents and chemical probe libraries using fluorescently labeled Δ133p53α protein, which identified two compounds (celastrol and AZD1981) that upregulate Δ133p53α at the protein level in normal cells [16]. Celastrol, a pentacyclic quinone methide, is a natural product used in traditional Chinese medicine. The molecule has multiple molecular mechanisms, likely due to its thiol reactivity [17-19]. These activities include acting as an Hsp70 inducer and proteasome inhibitor [20, 21]. AZD1981 is a prostaglandin DP2 receptor antagonist developed for asthma [22].

Hutchinson-Gilford progeria syndrome (HGPS) is a genetic disease caused by a mutation in the *LMNA* gene, which produces an abnormal nuclear envelope protein called progerin [23]. Patients with HGPS exhibit premature aging phenotypes in childhood and have a short lifespan, with an average life expectancy of 13-15 years [24]. Although the occurrence of HGPS is very rare, this disease also has significant implications for natural aging processes at both the organismal and cellular levels, as well as for interventions against aging in healthy individuals [25, 26]. Similar to the premature aging phenotypes observed in patients with HGPS, their derived cells in culture (often fibroblasts) display a premature onset of cellular senescence accompanied by proinflammatory cytokine production, which is attributed to progerin-induced accumulation of DNA damage and hyperactivation of p53 [27-29]. Our previous study showed that lentiviral expression of Δ133p53α delayed the onset of cellular senescence and reduced the production of the proinflammatory cytokine IL-6 in fibroblasts derived from HGPS patients, concurrent with mitigated DNA damage and repressed expression of $p21^{WAF1}$, a p53-inducible gene that mediates cellular senescence [7]. In this study, we investigate whether treatment with celastrol or AZD1981 can reproduce the effects of lentiviral expression of Δ133p53α in HGPS-derived fibroblasts, exploring the potential therapeutic value of these compounds in HGPS.

Materials and Methods

Cells and cell culture

A fibroblast strain AG11513, from an 8-year-old female HGPS patient, was obtained from Coriell Institute for Medical Research (https://catalog.coriell.org/). Another fibroblast strain HGADFN271, from a 1-year-3-month-old male patient, was obtained from Progeria Research Foundation (https://www.progeriaresearch.org/). These cells were grown in DMEM medium (Corning #15-013-CV) supplemented with 10% fetal bovine serum (Sigma-

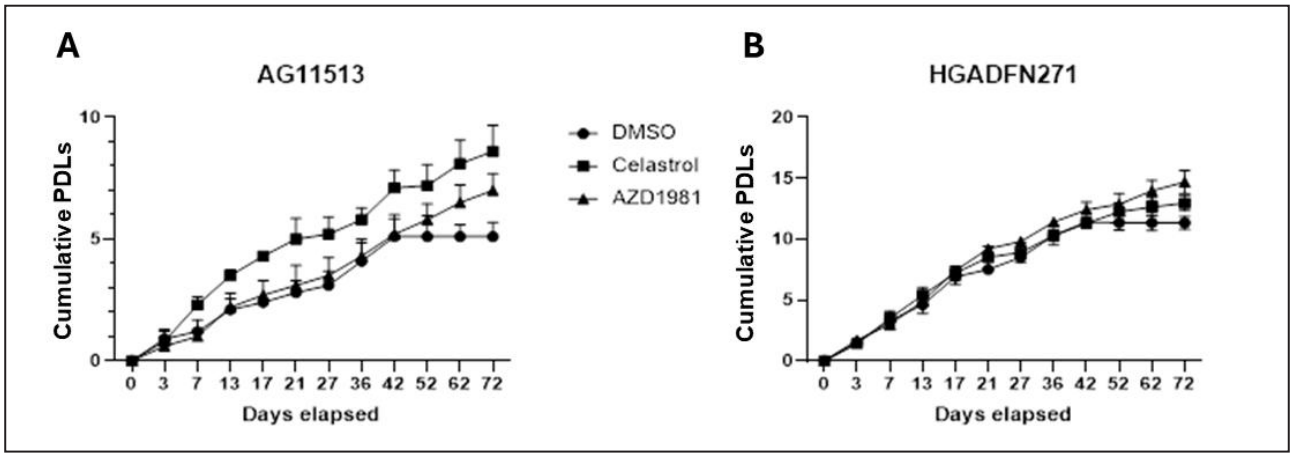

Figure 1. Cell proliferation in HGPS patients-derived fibroblasts AG11513 (A) and HGADFN271 (B) untreated and treated with celastrol or AZD1981. Both fibroblast strains were treated with 0.1 μM celastrol, 10 μM AZD1981, or DMSO alone (untreated control), which was supplemented freshly every 3 or 4 days. Cell proliferation was monitored by periodical cell counting for 72 days and the cumulative population doubling levels (PDLs) were calculated. Note the differences in cumulative PDLs between the two fibroblast strains.

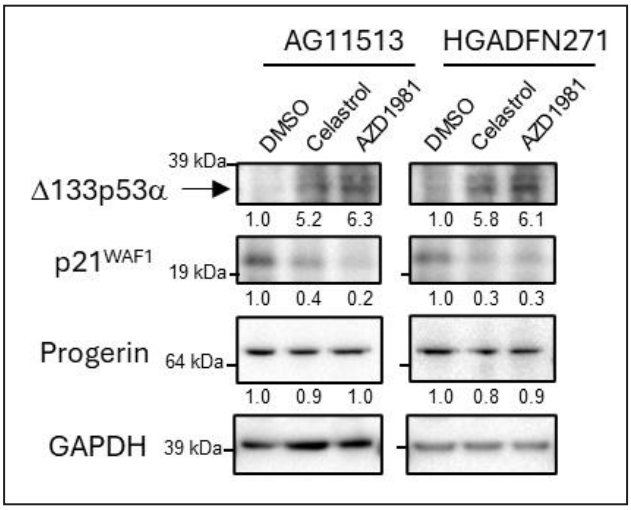

Figure 2. Western blot analysis of HGPS patients-derived fibroblasts AG11513 and HGADFN271 treated with celastrol or AZD1981. The cells were treated with 0.1 μM celastrol, 10 μM AZD1981, or DMSO alone for 24 h, maintained in cell culture for 4 days in the absence of compound, and then harvested. In this figure, Δ133p53α was detected using a rabbit polyclonal antibody MAP4 [2, 30]. The original image obtained with MAP4 before cropping the bands, along with an image obtained using a sheep polyclonal anti-p53 antibody SAPU, which also detects Δ133p53α [1, 30], are shown in Supplementary Figure 1. The quantitative expression levels of Δ133p53α, $p21^{WAF1}$ and progerin, normalized to GAPDH and calculated relative to DMSO alone, are shown below the images.

Aldrich #F0926), 1% L-glutamine (Thermo Fisher #25030081) and 1% penicillin/streptomycin (Thermo Fisher #10378016) at 37 °C under a humidified atmosphere of 5% CO_2. Cells were passaged at a split ratio of 1:3 (AG11513) or 1:4 (HGADFN271). To visualize nuclear morphology, cells on a Lab-Tek II CC2 Chamber Slide (Thermo Fisher #154739) were fixed with 4% paraformaldehyde for 15 min and mounted with Mounting Medium containing 4',6-diamidino-2-phenylindole (DAPI) (Vectashield, H-1200).

Compounds and treatment

Celastrol was purchased from Cayman Chemical (#70950) and dissolved in DMSO as 100 μM stock solution. AZD1981 was also purchased from Cayman Chemical (#20763) and dissolved in DMSO as 10 mM stock solution. In our previous qHTS study using EGFP-labeled Δ133p53α proteins [16], when well-fitted and two-asymptote concentration-response curves were obtained, celastrol and AZD1981 showed AC_{50} (activity concentration 50%) values of 3.43–4.85 μM and 1.22–7.69 μM, respectively. While 10 μM AZD1981 was non-cytotoxic during 24-h treatment of normal human fibroblasts and astrocytes, celastrol was only tolerated at 0.1 μM, but not at 1 or 10 μM, under the same conditions. We therefore defined 10 μM AZD1981 and 0.1 μM celastrol as the standard treatment concentrations in the previous study [16] and applied these concentrations to the current study as well. Thus, in this study, cells were treated with celastrol at a final concentration of 0.1 μM, AZD1981 at a final concentration of 10 μM, or DMSO vehicle control for 24 h in Figures 2, 3 and 4, as previously performed [16]. Following 4 days of cell culture, cells were harvested for assays mentioned below. In Figure 1, celastrol (0.1 μM), AZD1981 (10 μM) or DMSO was supplemented freshly every 3 or 4 days over 72 days, and the cumulative population doubling levels (PDLs) were calculated as previously described [7].

Western blot analysis

Preparation of protein lysates, gel electrophoresis, transfer to PVDF membranes, antibody incubation, and signal detection were performed as previously described [16]. Primary antibodies used were: rabbit polyclonal anti-Δ133p53α antibody MAP4 (diluted at 1:5,000) [2, 30], sheep polyclonal anti-p53 antibody SAPU (diluted at 1:5,000) [1, 30], anti-progerin antibody (Abcam ab153757, diluted at 1:500), anti-$p21^{WAF1}$ antibody (Cell Signaling #2947, diluted at 1:1,000), and anti-GAPDH antibody (EMD Millipore, MAB374, diluted at 1:1,000). The quantitative image analysis was performed using the ChemiDoc Imaging System (Bio-Rad) and the Image Lab software (Bio-Rad, ver. 6.1).

Senescence-associated β-galactosidase (SA-β-gal) staining

SA-β-gal activity was stained using SA-β-Galactosidase Staining Kit (Cell Signaling #9860). Data of SA-β-gal-positive cells (%) were presented as mean ± SD from 3 biological replicates in 3 independent experiments. At least 100 cells were observed in each replicate.

Enzyme-linked immunosorbent assay (ELISA)

Quantification of IL-6 in culture media was carried out using Human IL-6 ELISA Kit (Sigma-Aldrich, RAB0306). Standard curves were drawn from recombinant IL-6 provided in the kit. Data were normalized to cell numbers and presented as mean ± SD from biological triplicates.

RNA isolation and quantitative RT-PCR (qRT-PCR)

Total RNA samples were prepared using RNeasy Plus Micro Kit (QIAGEN #74034). Reverse transcription was carried out using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher #4368814). qRT-PCR assays were performed using Taqman Gene Expression Master Mix (Thermo Fisher #4369016). The primer/probe sets used were (Thermo Fisher): $p21^{WAF1}$ (Hs00355782_m1), IL-6 (Hs00174131_m1), and GAPDH (Hs02758991_g1) as an internal control. All qRT-PCR data were mean ± S.D. from technical triplicate ($n = 3$).

Results

Cell proliferation of two fibroblast strains derived from HGPS patients and the effects of celastrol and AZD1981

In general, primary cells derived from HGPS patients are less proliferative and have a shorter replicative lifespan than those derived from normal donors. However, various factors such as age of patients, methods of strain establishment, and conditions of subsequent cell culture could

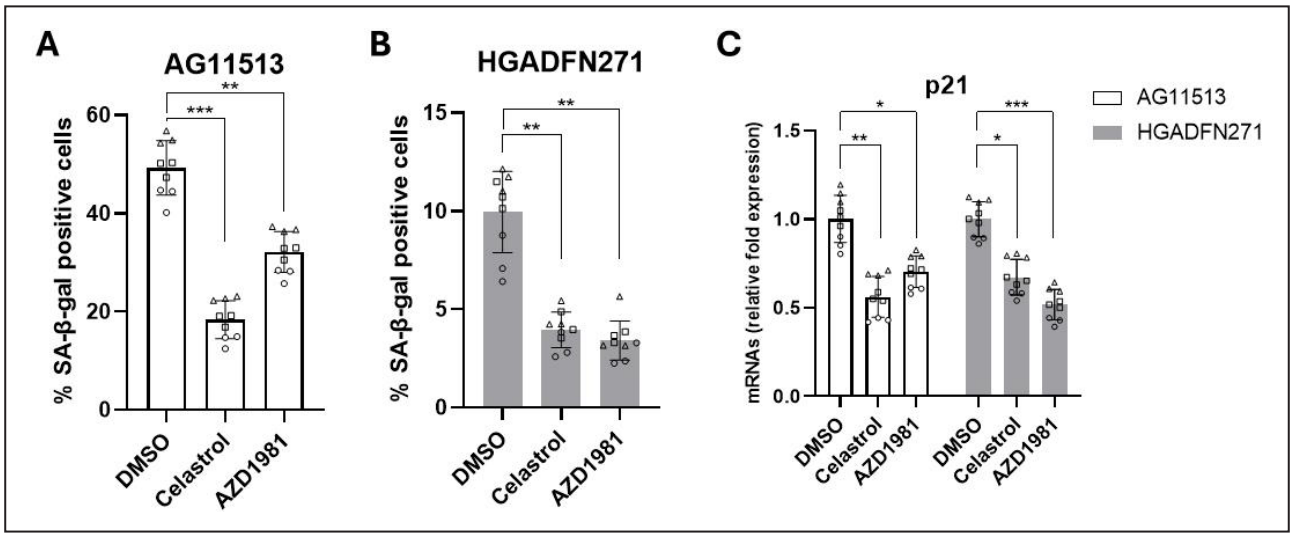

Figure 3. Detection and quantification of senescent cells in HGPS patients-derived fibroblasts treated with celastrol or AZD1981. (**A** & **B**) AG11513 (**A**) and HGADFN271 (**B**) fibroblasts were treated as in Figure 2, followed by 4-day cell culture before staining for SA-β-gal activity. The quantitative data for the percentages of SA-β-gal-positive cells are presented as mean ± SD, which are 49.3 ± 5.6 (DMSO), 18.4 ± 3.9 (celastrol) and 32.1 ± 4.2 (AZD1981) in (**A**), and 9.9 ± 2.1 (DMSO), 4.0 ± 0.9 (celastrol) and 3.4 ± 1.0 (AZD1981) in (**B**). Nine data points with each bar represent 3 technical replicates from each of 3 biological replicates, indicated by circles, triangles, or squares. Note the difference in vertical-axis values between (**A**) and (**B**). Representative images are presented in Supplementary Figure 3. (**C**) qRT-PCR assay of $p21^{WAF1}$ mRNA expression. Relative expression levels to DMSO alone are presented as mean ± SD, which are 1.0 ± 0.13 (DMSO), 0.56 ± 0.12 (celastrol) and 0.7 ± 0.09 (AZD1981) in AG11513, and 1.0 ± 0.1 (DMSO), 0.67 ± 0.1 (celastrol) and 0.52 ± 0.09 (AZD1981) in HGADFN271. Nine data points with each bar represent 3 technical replicates from each of 3 biological replicates, indicated by circles, triangles, or squares. Unpaired 2-tailed Student's t-test: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

significantly affect their proliferative potential and replicative lifespan. We therefore monitored two HGPS-derived fibroblast strains, AG11513 and HGADFN271, for long-term proliferation in cell culture (Figure 1A & B, DMSO alone). These two strains in our laboratory, at the time of use in this study, were different in proliferative capacity and remaining replicative lifespan: AG11513 fibroblasts (derived from an 8-year-old patient) proliferated slowly and only had ~5 population doubling levels (PDLs) before proliferation arrest (Figure 1A, DMSO alone), while HGADFN271 fibroblasts (derived from a 1-year-3-month-old patient) still had ~11 PDLs remaining (Figure 1B, DMSO alone).

Treatment of these fibroblasts with celastrol or AZD1981, added fresh every 3 or 4 days, resulted in continuous proliferation beyond the point at which the control cells became proliferation-arrested (Figure 1A & B, after 42 days). This extension of replicative lifespan appeared more pronounced in AG11513 fibroblasts, which had fewer PDLs remaining.

Celastrol and AZD1981 upregulate endogenous Δ133p53α protein in HGPS-derived fibroblasts

AG11513 and HGADFN271 fibroblasts were treated with celastrol (0.1 μM) or AZD1981 (10 μM) for 24 h, followed by 4 days of cell culture in the absence of compound and then harvested for western blot analysis. Both celastrol and AZD1981 were found to upregulate Δ133p53α protein levels by approximately 5- to 6-fold in these cells (Figure 2 and Supplementary Figure 1), concurrent with decreased expression of $p21^{WAF1}$ (Figure 2), which is known to be repressed by Δ133p53α [2, 7, 15]. Neither compound affected progerin levels (Figure 2). Consistently, the progerin-associated defect in nuclear morphology characteristic of HGPS-derived cells [31] was not corrected by either compound (Supplementary Figure 2).

Celastrol and AZD1981 reduces cellular senescence in HGPS-derived fibroblasts

Consistent with the proliferative characteristics shown in Figure 1, SA-β-gal-positive senescent cells were more abundant in untreated AG11513 fibroblasts (~50%, Figure 3A) than in untreated HGADFN271 fibroblasts (~10%, Figure 3B). Despite this difference in basal senescence levels, treatment with celastrol or AZD1981 significantly reduced the percentage of SA-β-gal-positive senescent cells in both fibroblast strains. In AG11513, celastrol reduced it to ~20% and AZD1981 to ~30% (Figure 3A). In HGADFN271, both compounds resulted in only ~3% of SA-β-gal-positivity (Figure 3B). The mRNA expression levels of $p21^{WAF1}$, a mediator of cellular senescence, were also reduced in these treated fibroblasts (Figure 3C), confirming the protein levels shown in Figure 2. The inhibition of cellular senescence by celastrol and AZD1981 parallels the extension of replicative lifespan by these compounds (Figure 1).

Celastrol and AZD1981 reduces IL-6 secretion in HGPS-derived fibroblasts

In line with the difference in basal senescence levels (Figure 3A & B), the senescence-associated, proinflammatory cytokine IL-6 [32] was secreted into the culture medium at higher levels by untreated AG11513 fibroblasts (Figure 4A) than by untreated HGADFN271 fibroblasts (Figure 4B). Again, despite this difference in basal IL-6 secretion,

celastrol and AZD1981 resulted in ~30% reduction in secreted IL-6 levels in both AG11513 and HGADFN271 fibroblasts (Figure 4A & B). The mRNA expression levels of IL-6 were also reduced in these fibroblasts treated with celastrol or AZD1981 (Figure 4C).

Discussion

This is the first report of testing Δ133p53α-activating compounds in primary human cells from disease patients. Celastrol and AZD1981, when used to treat fibroblasts derived from HGPS patients, were confirmed to upregulate endogenous Δ133p53α levels. These compounds reduced cellular senescence and IL-6 secretion, which are major phenotypes prematurely occurring in these diseased cells, and extended their replicative lifespan. Consistent with our previous findings with the lentiviral expression of Δ133p53α [2, 7, 15], compound-induced upregulation of Δ133p53α led to repressed expression of $p21^{WAF1}$. The inhibition of cellular senescence by celastrol and AZD1981 may be attributed at least in part to this repression of $p21^{WAF1}$, a well-known downstream mediator of p53-induced senescence [33, 34]. Although an increase in $p21^{WAF1}$ protein associated with senescence is assumed to occur in the nucleus [35], it remains to be confirmed whether the compound-induced decrease of $p21^{WAF1}$ in HGPS-derived cells is indeed a nuclear event. Further experiments on the subcellular localization of $p21^{WAF1}$ protein are needed to distinguish between nuclear and cytoplasmic $p21^{WAF1}$, which are functionally distinct [36]. Another mechanism that requires further clarification is how these compounds reduce IL-6 production. IL-6, a component of the senescence-associated secretory phenotype, is regulated by both the p53 and NF-kB signaling pathways [9, 37, 38]. The Δ133p53α/p53-mediated effects of celastrol and AZD1981 (suggested in this current study), their NF-kB-mediated effects on IL-6 regulation, and the functional interplay between these mechanisms warrant further investigation. Indeed, celastrol has been reported to inhibit broad inflammatory responses by suppressing the NF-kB signaling pathway [39, 40].

It should be noted that, as we previously observed with lentiviral expression of Δ133p53α [7], celastrol and AZD1981 neither affected the expression level of progerin nor corrected the progerin-associated defective nuclear morphology. Considering that currently available therapies primarily target the synthesis, modification and protein turnover of progerin [41-43], celastrol and AZD1981, acting independently of progerin regulation, may synergize with these therapies. Future studies will also address whether celastrol, AZD1981, or vector-driven expression of Δ133p53α can correct HGPS-associated deficiencies in histone modifications and chromatin structure [44, 45].

Two fibroblast strains used in this study showed different proliferative potentials and senescence levels. A possible reason for these phenotypic differences might be the difference in age of patients from whom the strains were derived: the less proliferative, more senescent strain AG11513 was from the older patient (8 years old), while the other strain HGADFN271 was from the younger (1 year and 3 months old). However, other donor- and strain-specific genetic and environmental factors could influence not only their proliferation and senescence phenotypes but also their responses to the compounds. A premature interpretation from the effects regardless of baseline levels of

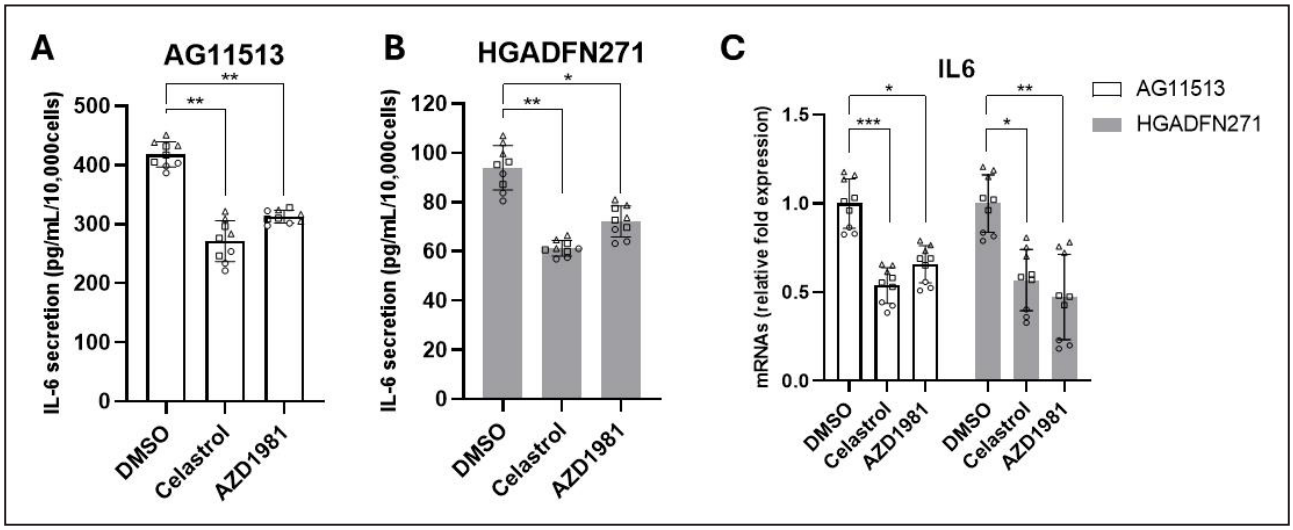

Figure 4. Quantification of IL-6 secretion (A & B) and IL-6 mRNA expression (C) in HGPS patients-derived fibroblasts AG11513 and HGADFN271 treated with celastrol or AZD1981. The cells were treated as in Figure 2. (**A** & **B**) Following 4-day cell culture, culture media were collected for IL-6 ELISA and cell numbers were counted for normalization. The concentrations of secreted IL-6 (pg/mL) were normalized to cell numbers (per 1 x 10^4) and are presented as mean ± S.D., which are 418.4 ± 21.2 (DMSO), 271.5 ± 34.7 (celastrol) and 312.9 ± 10.6 (AZD1981) in (**A**), and 94.0 ± 9.0 (DMSO), 61.3 ± 3.1 (celastrol) and 72.2 ± 6.3 (AZD1981) in (**B**). Nine data points with each bar represent 3 technical replicates from each of 3 biological replicates, indicated by circles, triangles, or squares. Note the difference in vertical-axis values between AG11513 (**A**) and HGADFN271 (**B**). (**C**) qRT-PCR assay of IL-6 mRNA expression. Relative expression levels to DMSO alone are presented as mean ± SD, which are 1.0 ± 0.14 (DMSO), 0.54 ± 0.1 (celastrol) and 0.66 ± 0.1 (AZD1981) in AG11513, and 1.0 ± 0.16 (DMSO), 0.57 ± 0.17 (celastrol) and 0.47 ± 0.24 (AZD1981) in HGADFN271. Nine data points with each bar represent 3 technical replicates from each of 3 biological replicates, indicated by circles, triangles, or squares. Unpaired 2-tailed Student's t-test: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

cell proliferation and senescence may be that these compounds act on both actively dividing and proliferation-arrested cells. To justify this interpretation, however, further experiments using a larger number of strains, more relevant cell types such as vascular smooth muscle cells [46], and more complex *ex vivo* and *in vivo* models (as described below) will certainly be required.

A major limitation of this study is that the data were obtained only from *in vitro* cell culture experiments. Further studies using *in vivo* and *ex vivo* models that are more relevant to human disease conditions and pharmacodynamics will be critically needed for future clinical translation. Since only humans and primates endogenously express Δ133p53α [5, 14], current mouse models of HGPS are unsuitable for directly testing celastrol or AZD1981. A human p53 knock-in mouse strain, which theoretically expresses endogenous Δ133p53α [47], may enable *in vivo* studies to evaluate the effects of these compounds. Additionally, human cell organoids derived from HGPS patients [48] would provide a valuable *ex vivo* model for testing these compounds.

Our previous cell culture studies showed that Δ133p53α, even when overexpressed from the retroviral or lentiviral vector in normal human cells, does not cause cell immortalization or transformation *in vitro* [2, 3, 5, 7, 15]. This is explained by its preferential inhibition of p53-mediated senescence with its DNA repair activity maintained [11, 14, 15]. On the other hand, increased expression of Δ133p53α has been linked to human cancer such as colon cancer [2, 49]. It is therefore critical to assess the potential risk of carcinogenesis *in vivo* when considering clinical translations of the vector-based expression or pharmacologic activation of Δ133p53α in the future. Our recent generation of Δ133p53α-transgenic mice has allowed us to find that these mice are not prone to spontaneous tumorigenesis as of 24 months of age [50], in marked contrast to p53-knockout mice developing spontaneous tumors by 6 months of age [12]. Nevertheless, further assessments will be required to accumulate more valid safety data, including carcinogen-induced tumorigenesis in Δ133p53α-transgenic mice and long-term monitoring of the above-mentioned human cell organoids. Such long-term experiments *in vitro*, *ex vivo* and *in vivo* will also address potential cellular adaptation, development of resistance, and other adverse effects associated with prolonged activation of Δ133p53α.

Both celastrol and AZD1981 have been suggested to increase Δ133p53α protein levels without altering mRNA levels [16]. AZD1981, originally developed as a drug to treat asthma, functions as an antagonist of the prostaglandin DP2 receptor (PTGDR2) [22]. However, no direct link has yet been established between this pharmacologic activity and Δ133p53α protein levels. Since PTGDR2 is primarily expressed in immune cells such as eosinophils, mast cells and basophils [51, 52], a pharmacologic action of AZD1981 independent of PTGDR2 in non-immune cells is still worth investigating. The potential roles and activities of PTGDR2 and AZD1981 in senescence- and aging-associated diseases also warrant further study. In contrast, the Hsp70-inducing activity of celastrol [20] aligns with our previous finding that Hsp70 interacts with Δ133p53α to stabilize it from autophagic degradation [4]. Celastrol has also been reported to exert therapeutic effects in mouse and rat models of senescence- and aging-associated diseases and functional decline, such as neurodegenerative diseases [53], osteoarthritis [54], and skeletal muscle atrophy [55]. Since Δ133p53α is present only in humans and primates as mentioned above [5, 14], celastrol could exhibit additional Δ133p53α-dependent therapeutic activities in human diseases beyond those observed in rodent models. A potential synergy between Δ133p53α-activating compounds and senolytic or senomorphic drugs also merits exploration [10].

Conclusions

Celastrol and AZD1981, identified in our previous drug and investigational agent library qHTS, inhibit cellular senescence and IL-6 secretion in fibroblasts derived from HGPS patients, recapitulating the beneficial effects exerted by lentiviral expression of Δ133p53α. These compounds warrant further investigation for potential clinical translation.

Declarations

Acknowledgments: We thank Dr. Jean-Christophe Bourdon for SAPU antibody and Dr. Borivoj Vojtesek for MAP4 antibody.

Authors contributions: Performed data acquisition and analyses: Joruiz SM, Lissa D, von Muhlinen N; Made substantial contributions to conception and design of the study: Dranchak PK, Inglese J, Horikawa I, Harris CC; Wrote the manuscript: Joruiz SM, Lissa D, Horikawa I.

Availability of data and materials: All materials in this study and raw data related to this study are available upon request.

Financial support and sponsorship: This research was supported by the Intramural Research Program of the National Institutes of Health (NIH): ZIA BC 011496 (Harris CC) and ZIA TR 000052 (Inglese J). The contributions of the NIH authors were made as part of their official duties as NIH federal employees, are in compliance with agency policy requirements, and are considered Works of the United States Government. However, the findings and conclusions presented in this paper are those of the authors and do not necessarily reflect the views of the NIH or the U.S. Department of Health and Human Services.

Conflicts of interest: Lissa D is a current employee at AstraZeneca but had no association with the company at the time of this project, and no participation from AstraZeneca was received in any form. All authors declared that there are no conflicts of interest.

Ethical approval and informed consent: Not applicable.

References

1. Joruiz S, & Bourdon J. p53 isoforms: key regulators of the cell fate decision. *Cold Spring Harbor Perspectives in Medicine*, 2016, 6(8): a026039. [Crossref]
2. Fujita K, Mondal A, Horikawa I, Nguyen G, Kumamoto K, Sohn J, *et al*. p53 isoforms Δ133p53α and p53β are endogenous regulators of replicative cellular senescence. *Nature Cell Biology*, 2009, 11(9): 1135-1142. [Crossref]
3. Mondal A, Horikawa I, Pine S, Fujita K, Morgan K, Vera E, *et al*. p53 isoforms regulate aging- and tumor-associated replicative senescence in T lymphocytes. *J Clin Invest*, 2013, 123(12): 5247-5257. [Crossref]
4. Horikawa I, Fujita K, Jenkins L, Hiyoshi Y, Mondal A, Vojtesek B, *et al*. Autophagic degradation of the inhibitory p53 isoform Δ133p53α as a regulatory mechanism for p53-mediated senescence. *Nat Commun*, 2014, 5: 4706. [Crossref]
5. Turnquist C, Horikawa I, Foran E, Major E, Vojtesek B, Lane D, *et al*. p53 isoforms regulate astrocyte-mediated neuroprotection and neurodegeneration. *Cell Death Differ*, 2016, 23(9): 1515-1528. [Crossref]
6. Mondal A, Zhou H, Horikawa I, Suprynowicz F, Li G, Dakic A, *et al*. Δ133p53α, a natural p53 isoform, contributes to conditional reprogramming and long-term proliferation of primary epithelial cells. *Cell Death Dis*, 2018, 9(7): 750. [Crossref]
7. von Muhlinen N, Horikawa I, Alam F, Isogaya K, Lissa D, Vojtesek B, *et al*. p53 isoforms regulate premature aging in human cells. *Oncogene*, 2018, 37(18): 2379-2393. [Crossref]
8. Turnquist C, Beck J, Horikawa I, Obiorah I, von Muhlinen N, Vojtesek B, *et al*. Radiation-induced astrocyte senescence is rescued by Δ133p53α. *Neuro-Oncology*, 2019, 21(4): 474-485. [Crossref]
9. Ungerleider K, Beck J, Lissa D, Joruiz S, Horikawa I, & Harris C. Δ133p53α protects human astrocytes from amyloid-beta induced senescence and neurotoxicity. *Neuroscience*, 2022, 498: 190-202. [Crossref]
10. Horikawa I, Yamada L, Harris B, & Harris C. Δ133p53α-mediated inhibition of astrocyte senescence and neurotoxicity as a possible therapeutic approach for neurodegenerative diseases. *Neuroscience*, 2025, 580: 54-61.
11. Roselle C, Horikawa I, Chen L, Kelly A, Gonzales D, Da T, *et al*. Enhancing chimeric antigen receptor T cell therapy by modulating the p53 signaling network with Δ133p53α. *Proceedings of the National Academy of Sciences of the USA*, 2024, 121(10): e2317735121. [Crossref]
12. Donehower L, Harvey M, Slagle B, McArthur M, Montgomery C, Jr., Butel J, *et al*. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*, 1992, 356(6366): 215-221. [Crossref]
13. Efeyan A, & Serrano M. p53: guardian of the genome and policeman of the oncogenes. *Cell Cycle*, 2007, 6(9): 1006-1010. [Crossref]
14. Horikawa I, & Harris C. Δ133p53α: A p53 isoform enriched in human pluripotent stem cells. *Cell Cycle*, 2017, 16: 1631-1632. [Crossref]
15. Horikawa I, Park K, Isogaya K, Hiyoshi Y, Li H, Anami K, *et al*. Δ133p53 represses p53-inducible senescence genes and enhances the generation of human induced pluripotent stem cells. *Cell Death and Differentiation*, 2017, 24(6): 1017-1028. [Crossref]
16. Lissa D, Joruiz S, Dranchak P, Ungerleider K, Yamada L, Horikawa I, *et al*. A quantitative high-throughput screen identifies compounds that upregulate the p53 isoform Δ133p53α and inhibit cellular senescence. *ACS Pharmacol Transl Sci*, 2025, 8: 2061-2074. [Crossref]
17. Salminen A, Lehtonen M, Paimela T, & Kaarniranta K. Celastrol: molecular targets of thunder god vine. *Biochemical and Biophysical Research Communications*, 2010, 394(3): 439-442. [Crossref]
18. Seo H, Seo W, Pyun B, Lee B, Jin Y, Park K, *et al*. Radiosensitization by celastrol is mediated by modification of antioxidant thiol molecules. *Chemico-Biological Interactions*, 2011, 193(1): 34-42. [Crossref]
19. Trott A, West J, Klaić L, Westerheide S, Silverman R, Morimoto R, *et al*. Activation of heat shock and antioxidant responses by the natural product celastrol: transcriptional signatures of a thiol-targeted molecule. *Molecular Biology of the Cell*, 2008, 19(3): 1104-1112. [Crossref]
20. Westerheide S, Bosman J, Mbadugha B, Kawahara T, Matsumoto G, Kim S, *et al*. Celastrols as inducers of the heat shock response and cytoprotection. *Journal of Biological Chemistry*, 2004, 279(53): 56053-56060. [Crossref]
21. Yang H, Chen D, Cui Q, Yuan X, & Dou Q. Celastrol, a triterpene extracted from the Chinese "thunder of god vine," is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice. *Cancer Research*, 2006, 66(9): 4758-4765. [Crossref]
22. Schmidt J, Bell F, Akam E, Marshall C, Dainty I, Heinemann A, *et al*. Biochemical and pharmacological characterization of AZD1981, an orally available selective DP2 antagonist in clinical development for asthma. *British Journal of Pharmacology*, 2013, 168(7): 1626-1638. [Crossref]
23. Eriksson M, Brown W, Gordon L, Glynn M, Singer J, Scott L, *et al*. Recurrent *de novo* point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*, 2003, 423(6937): 293-298. [Crossref]
24. Hennekam R. Hutchinson-Gilford progeria syndrome: review of the phenotype. *American Journal of Medical Genetics. Part A*, 2006, 140(23): 2603-2624. [Crossref]
25. Ding S, & Shen C. Model of human aging: recent findings on Werner's and Hutchinson-Gilford progeria syndromes. *Clinical Interventions in Aging*, 2008, 3(3): 431-444.
26. Brassard J, Fekete N, Garnier A, & Hoesli C. Hutchinson-Gilford progeria syndrome as a model for vascular aging. *Biogerontology*, 2016, 17(1): 129-145. [Crossref]
27. Wheaton K, Campuzano D, Ma W, Sheinis M, Ho B, Brown G, *et al*. Progerin-induced replication stress facilitates premature senescence in Hutchinson-Gilford Progeria Syndrome. *Molecular and Cellular Biology*, 2017, 37(14): e00659. [Crossref]
28. Xu Q, Mojiri A, Boulahouache L, Morales E, Walther B, & Cooke J. Vascular senescence in progeria: role of endothelial dysfunction. *Eur Heart J Open*, 2022, 2(4): oeac047. [Crossref]
29. Batista N, Desai S, Perez A, Finkelstein A, Radigan R, Singh M, *et al*. The molecular and cellular basis of

Hutchinson-Gilford Progeria Syndrome and potential treatments. *Genes*, 2023, 14(3): 602. [Crossref]
30. Marcel V, Khoury M, Fernandes K, Diot A, Lane D, & Bourdon J. Detecting p53 isoforms at protein level. *Methods in Molecular Biology*, 2013, 962: 15-29. [Crossref]
31. Scaffidi P, & Misteli T. Lamin A-dependent nuclear defects in human aging. *Science*, 2006, 312(5776): 1059-1063. [Crossref]
32. Ungerleider K, Beck J, Lissa D, Turnquist C, Horikawa I, Harris B, *et al*. Astrocyte senescence and SASP in neurodegeneration: tau joins the loop. *Cell Cycle*, 2021, 20(8): 752-764. [Crossref]
33. Brown J, Wei W, & Sedivy J. Bypass of senescence after disruption of $p21^{CIP1/WAF1}$ gene in normal diploid human fibroblasts. *Science*, 1997, 277(5327): 831-834.
34. Jackson J, & Pereira-Smith O. p53 is preferentially recruited to the promoters of growth arrest genes p21 and GADD45 during replicative senescence of normal human fibroblasts. *Cancer Research*, 2006, 66(17): 8356-8360. [Crossref]
35. Ogrodnik M, Carlos Acosta J, Adams P, d'Adda di Fagagna F, Baker D, Bishop C, *et al*. Guidelines for minimal information on cellular senescence experimentation *in vivo*. *Cell*, 2024, 187(16): 4150-4175. [Crossref]
36. Klepacki H, Kowalczuk K, Łepkowska N, & Hermanowicz J. Molecular regulation of SASP in cellular senescence: therapeutic implications and translational challenges. *Cells*, 2025, 14(13): 942-955. [Crossref]
37. Lopes-Paciencia S, Saint-Germain E, Rowell M, Ruiz A, Kalegari P, & Ferbeyre G. The senescence-associated secretory phenotype and its regulation. *Cytokine*, 2019, 117: 15-22. [Crossref]
38. Lowe J, Menendez D, Bushel P, Shatz M, Kirk E, Troester M, *et al*. p53 and NF-κB coregulate proinflammatory gene responses in human macrophages. *Cancer Research*, 2014, 74(8): 2182-2192. [Crossref]
39. Chiang K, Tsui K, Chung L, Yeh C, Chen W, Chang P, *et al*. Celastrol blocks interleukin-6 gene expression via downregulation of NF-κB in prostate carcinoma cells. *PloS One*, 2014, 9(3): e93151. [Crossref]
40. Zhang J, Zhou K, Zhang X, Zhou Y, Li Z, & Shang F. Celastrol ameliorates inflammation in human retinal pigment epithelial cells by suppressing NF-κB signaling. *Journal of Ocular Pharmacology and Therapeutics*, 2019, 35(2): 116-123. [Crossref]
41. Arun A, Nath A, Thankachan B, & Unnikrishnan M. Hutchinson-Gilford progeria syndrome: unraveling the genetic basis, symptoms, and advancements in therapeutic approaches. *Ther Adv Rare Dis*, 2024, 5: 26330040241305144. [Crossref]
42. Desai D, Jyotishi C, Prajapati S, & Gupta R. Assessing the efficacy of small molecule drugs in Hutchinson-Gilford Progeria Syndrome: a review of clinical trials. *Reviews on Recent Clinical Trials*, 2025, [Crossref]
43. Koblan L, Erdos M, Wilson C, Cabral W, Levy J, Xiong Z, *et al*. *In vivo* base editing rescues Hutchinson-Gilford progeria syndrome in mice. *Nature*, 2021, 589(7843): 608-614. [Crossref]
44. Ashapkin V, Kutueva L, Kurchashova S, & Kireev, I. Are there common mechanisms between the Hutchinson-Gilford Progeria Syndrome and Natural Aging? *Front Genet*, 2019, 10: 455-465. [Crossref]
45. Zhang H, Sun L, Wang K, Wu D, Trappio M, Witting C, *et al*. Loss of H3K9me3 correlates with atm activation and histone H2AX phosphorylation deficiencies in Hutchinson-Gilford Progeria Syndrome. *PloS One*, 2016, 11(12): e0167454. [Crossref]
46. Ngubo M, Chen Z, McDonald D, Karimpour R, Shrestha A, Yockell-Lelièvre J, *et al*. Progeria-based vascular model identifies networks associated with cardiovascular aging and disease. *Aging Cell*, 2024, 23(7): e14150. [Crossref]
47. Luo J, Yang Q, Tong W, Hergenhahn M, Wang Z, & Hollstein M. Knock-in mice with a chimeric human/murine p53 gene develop normally and show wild-type p53 responses to DNA damaging agents: a new biomedical research tool. *Oncogene*, 2001, 20(3): 320-328. [Crossref]
48. Jeon S, Park C, Hong J, Kim J, Lee Y, Hur J, *et al.* Patient-derived cortical organoids reveal senescence of neural progenitor cells in Hutchinson-Gilford Progeria Syndrome. *Aging Cell*, 2025: e70143. [Crossref]
49. Zhao L, Punga T, & Sanyal S. Δ133p53α and Δ160p53α isoforms of the tumor suppressor protein p53 exert dominant-negative effect primarily by co-aggregation. *Elife*, 2025, 14: 106469. [Crossref]
50. Nakamichi S, Yamada L, Roselle C, Horikawa I, June C, & Harris C. The senescence-inhibitory p53 isoform Δ133p53α: enhancing cancer immunotherapy and exploring novel therapeutic approaches for senescence-associated diseases. *Geroscience*, 2025. [Crossref]
51. Marone G, Galdiero M, Pecoraro A, Pucino V, Criscuolo G, Triassi M, *et al*. Prostaglandin D_2 receptor antagonists in allergic disorders: safety, efficacy, and future perspectives. *Expert Opin Investig Drugs*, 2019, 28(1): 73-84. [Crossref]
52. Singh D, Ravi A, & Southworth T. CRTH2 antagonists in asthma: current perspectives. *Clinical Pharmacology: Advances and Applications*, 2017, 9: 165-173. [Crossref]
53. Liu D, Zhang Q, Luo P, Gu L, Shen S, Tang H, *et al*. Neuroprotective effects of celastrol in neurodegenerative diseases-unscramble its major mechanisms of action and targets. *Aging and Disease*, 2022, 13(3): 815-836. [Crossref]
54. Yang G, Wang K, Song H, Zhu R, Ding S, Yang H, *et al*. Celastrol ameliorates osteoarthritis via regulating TLR2/NF-κB signaling pathway. *Frontiers in Pharmacology*, 2022, 13: 963506. [Crossref]
55. Yadav A, Yadav S, Singh S, & Dabur R. Natural products: potential therapeutic agents to prevent skeletal muscle atrophy. *European Journal of Pharmacology*, 2022, 925: 174995. [Crossref]

Cite this article as: Joruiz SM, Lissa D, Von Muhlinen N, Dranchak PK, Inglese J, VHorikawa I, *et al.* Pharmacologic activation of Δ133p53α reduces cellular senescence in progeria patients-derived cells. *Aging Pathobiol Ther*, 2025, 7(3): 218-225. doi: 10.31491/APT.2025.09.185

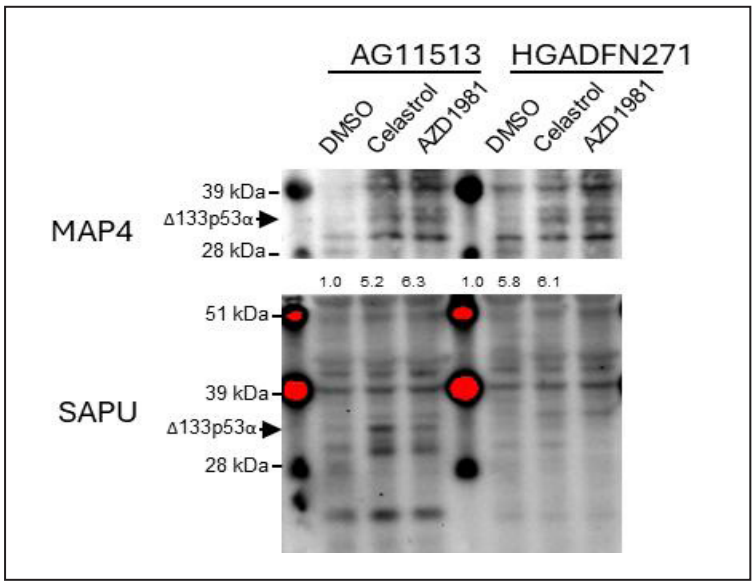

Figure S1. Original western blot images obtained with MAP4 and SAPU antibodies. The images obtained with the rabbit polyclonal antibody MAP4 (above; cropped bands shown in Figure 2) and the sheep polyclonal antibody SAPU (below) both consistently showed bands corresponding to Δ133p53α, which were upregulated by celastrol and AZD1981 (indicated by arrows).

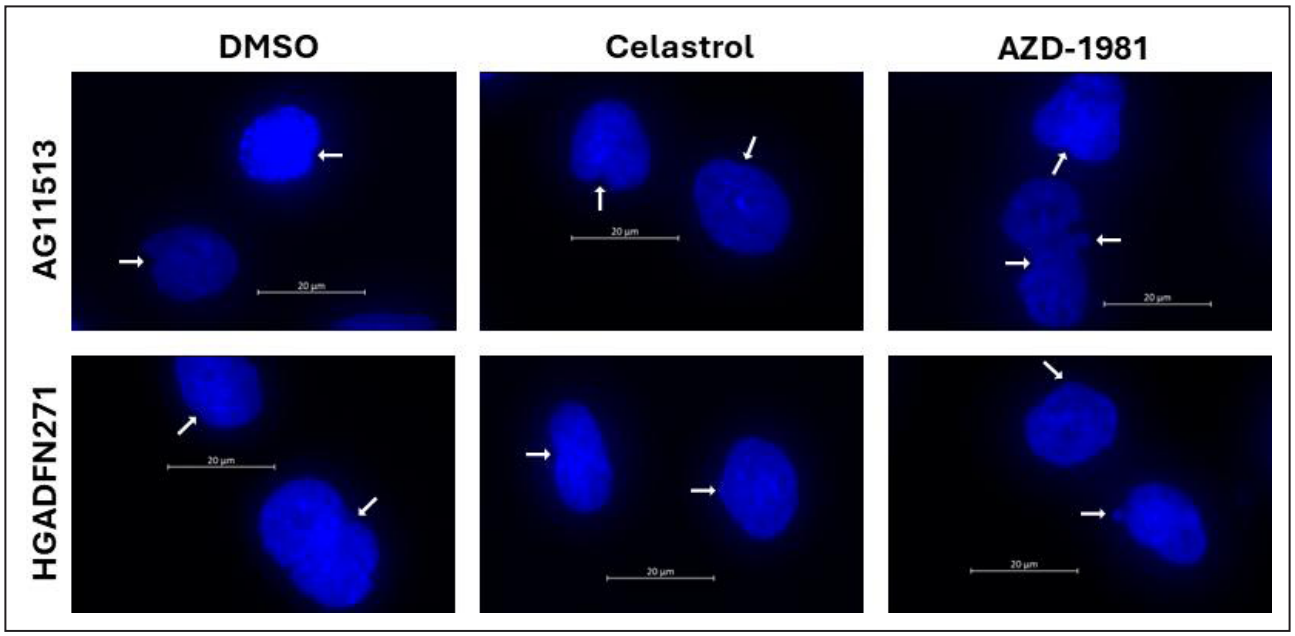

Figure S2. Neither celastrol nor AZD1981 corrects abnormal nuclear morphology in HGPS-derived fibroblasts. DAPI staining was performed. Nuclear lobulations, blebs and irregular outline are indicated. Magnification, 40. Scale bars, 20 μm.

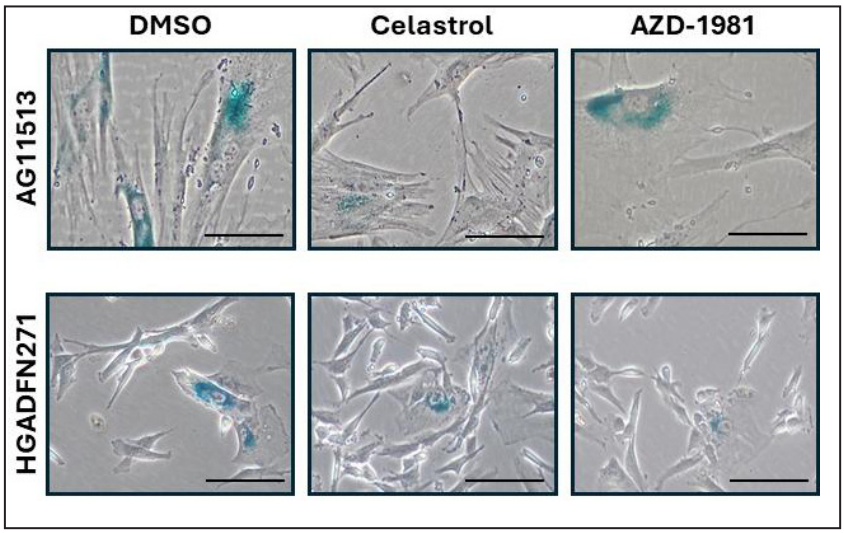

Figure S3. Representative images of SA-β-gal staining. Magnification, 40. Scale bars, 50 μm.