Statins inhibit proliferation and induce apoptosis in triple-negative breast cancer cells

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Abstract
TP53 (p53) is mutated in 80–90% of cases of triple-negative breast cancer (TNBC). Statins, which are widely used to treat elevated cholesterol, have recently been shown to degrade mutant p53 protein and exhibit anti-cancer activity. The aim of this work was to evaluate the potential of statins in the treatment of TNBC. The anti-proliferative effects of 2 widely used statins were investigated on a panel of 15 cell lines representing the different molecular subtypes of breast cancer. Significantly lower IC50 values were found in triple-negative (TN) than in non-TN cell lines (atorvastatin, \( p < 0.01 \); simvastatin \( p < 0.05 \)) indicating greater sensitivity. Furthermore, cell lines containing mutant p53 were more responsive to both statins than cell lines expressing wild-type p53, suggesting that the mutational status of p53 is a potential predictive biomarker for statin response. In addition to inhibiting proliferation, simvastatin was also found to promote cell cycle arrest and induce apoptosis. Using an apoptosis array capable of detecting 43 apoptosis-associated proteins, a novel protein shown to be upregulated by simvastatin was the IGF-signalling modulator, IGBP4, a finding we confirmed by Western blotting. Finally, we found synergistic growth inhibition between simvastatin and the IGF-1R inhibitor, OSI-906 as well as between simvastatin and doxorubicin or docetaxel. Our work suggests repurposing of statins for clinical trials in patients with TNBC. Based on our findings, we suggest that these trials investigate statins in combination with either doxorubicin or docetaxel and include p53 mutational status as a potential predictive biomarker.

Keywords Statins · Breast cancer · Triple-negative · p53 · Treatment

Introduction
Patients with triple-negative breast cancer (TNBC) lack estrogen receptors, progesterone receptors as well as HER2 gene amplification/overexpression. Consequently, these patients cannot be treated with 2 of the most effective therapies currently available for breast cancer, i.e. endocrine and anti-HER2 therapy. Indeed, until recently, the only form of systemic therapy available for patients with TNBC was cytotoxic chemotherapy [1]. Recently, however, several non-cytotoxic treatments have been approved for TNBC including the monoclonal antibody-conjugate, sacituzumab govitecan; immunotherapy with immune checkpoint such as atezolizumab or pembrolizumab and PARP inhibitors (olaparib, talazoparib) for the approximate 10–20% of TNBC patients with germline BRCA1/2 mutations [2, 3]. Although these therapies are improving outcome in some patients with TNBC, all are efficacious in only a proportion of patients with the disease. Clearly, therefore, additional forms of treatment are necessary for patients with TNBC [2, 3].

One of the most successful forms of anti-cancer treatment introduced in recent years is the use of drugs for targeting cancer driver genes. The most frequently occurring driver gene in TNBC is mutations in p53 which are present in 80–90% of these patients [4, 5]. Thus, mutant p53 is a highly attractive target for new anti-cancer drugs for the treatment of patients with TNBC.

Historically, however, mutant p53 has proved difficult to target and was thus frequently regarded as “undruggable” [6,
This traditional viewpoint, however, is changing as several new strategies have recently been identified for targeting the mutant protein [8, 9]. Two of the most promising strategies include degradation of the mutant protein and reactivation of mutant protein back to its wild-type form (for reviews, see refs. [8, 9]).

While mutant p53-reactivating compounds have been widely investigated [8], less work has been devoted to compounds that promote degradation of the mutant protein. For mutant p53 to exert its oncogenic activity, it must be stabilized [10]. Stabilization is achieved by interaction with heat shock proteins (HSP), especially HSP40, HSP70 and HSP90 [11]. Preventing these interactions might be expected to result in mutant p53 degradation and thus suppression of cancer growth. Early evidence that destabilization of mutant p53 had anti-cancer activity was obtained with HSP inhibitors in a range of experimental models [12]. However, in clinical trials, HSP inhibitors were found to be relatively toxic and lack efficacy [13, 14]. Consequently, these trials were largely abandoned.

In addition to HSP inhibitors, a group of commonly used drugs known as statins, have also been shown to degrade mutant p53 but not WT p53 [15, 16]. Statins which are widely used to treat patients with high levels of cholesterol act by inhibiting HMG-CoA, the rate-limiting enzyme in biosynthesis of the lipid [16]. In addition to reducing cholesterol levels, treatment with statins also lowers levels of other intermediate metabolites in the cholesterol biosynthetic pathway, especially mevalonate phosphate (MVP). Recently, Parrales et al. [15] reported that decreased MVP levels resulting from treatment with the statin, lovastatin led to reduced binding of mutant p53 to a specific form of HSP40 known as DNAJA1. As a result of the decreased binding to DNAJA1, mutant p53 underwent degradation with the ubiquitin ligase CHIP. Consistent with its ability to degrade mutant p53, lovastatin was found to inhibit the in vitro and in vivo growth of tumour cells expressing mutant p53 (conformational mutations) but not cells with wild-type p53, cell lines null for p53 or cell lines containing p53 contact mutations [15]. In a more recent study, treatment with a different statin, i.e. cerivastatin resulted in the dissociation of mutant p53 from HSP90 and degradation by MDM2 [16].

Based on the high prevalence of p53 mutations in TNBC, the ability of statins to degrade mutant p53, and thereby inhibit tumour cell proliferation, the aim of our study was to test the hypothesis that these drugs might provide a new treatment for patients with TNBC.

### Materials and methods

#### Cell culture

All cell lines were originally sourced from the American Type Culture Collection (ATCC), apart from Hs578Ts(i8) which was supplied by Dr. Susan McDonnell, University College, Dublin. All cell lines were maintained in RPMI, supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. All cell culture reagents were purchased from Biosciences (Dun Laoghaire, Ireland) and Sigma-Aldrich (Arklow, Ireland). Cells were maintained at 37 °C in a 5% CO2 environment. The p53 mutation status of these cell lines is shown in Table 1.

#### Proliferation assays

Cell response to statins was assessed by seeding 96-well plates with 5,000 cells per well and allowing to adhere overnight. The following day, fresh media were added, containing statins at the indicated concentration or DMSO. Cells were incubated for 5 days, at which point viability was assessed by adding 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) to 10% of media volume and incubating for 2 h. Absorbance was measured at 570 nm. In some experiments, mevalonate was added at a concentration of 100 or 200 µM.

#### Cell cycle analysis

Cells treated with statins for 24 h were harvested and fixed by resuspending in 500 µL PBS followed by slowly adding

### Table 1 p53 mutational status of cell lines used in this study

| Cell line | p53 status | Mutation |
|-----------|------------|----------|
| MCF-7     | WT         | –        |
| T47D      | MUT        | L194F    |
| ZR-75-1   | WT         | –        |
| CAMA1     | MUT        | R280T    |
| SKBR3     | MUT        | R175H    |
| JIMT-1    | MUT        | R248W    |
| Hs578T    | MUT        | V157F    |
| Hs578Ts(i)8 | MUT        | V157F    |
| MDA-MB-231 | MUT      | R280K    |
| MDA-MB-453 | MUT      | H368delinsG |
| MDA-MB-468 | MUT      | R273H    |
| HCC1143   | MUT        | R248Q    |
| BT-549    | MUT        | R249S    |
| SUM159    | MUT        | R273H    |
| CAL-85-1  | MUT        | L132E    |
4.5 mL ice-cold 70% ethanol. Cells were prepared for cell cycle analysis using FxCycle™ PI/RNase Staining Solution (Thermo-Fisher), following manufacturer’s protocol. Fluorescence was measured on a BD FACSCanto II, using a 488 nm excitation source and a 576 nm emission filter. Cell cycle percentages were calculated using Flowjo software.

**Apoptosis detection**

Cells treated with statins for 48 h were harvested and prepared for analysis using the FITC Annexin V Apoptosis Detection Kit with 7-AAD (Medical Supply Company, Mulhuddart, Ireland) following manufacturer’s guidelines. Fluorescence intensity of FITC and 7-AAD was measured on a BD FACSCanto II, using a 488 nm excitation source and a 525 nm or 695 nm emission filter, respectively.

**Membrane array for detection of apoptosis-associated proteins**

Expression of apoptotic proteins was analysed using the Raybiotech Human Apoptosis Array following manufacturer’s guidelines. Briefly, protein was isolated from SUM159 cells following treatment for 24 h with 10 µM simvastatin or vehicle control, using the provided lysis buffer. Membranes were blocked and incubated with protein samples overnight at 4 ºC. After washing with the provided buffers, membranes were incubated overnight again at 4 ºC with detection antibody. Membranes were incubated at room temperature with HRP-Streptavidin for 2 h. Following a final wash stage, membranes were incubated with detection buffer and imaged using a Licor C-DiGit Blot Scanner. ImageJ software was used to generate densitometry values which were then normalised to the internal controls on the membrane.

**Western blotting**

Protein was isolated and prepared for analysis by Western blotting as previously described [17]. Primary incubations were carried out overnight at 4 ºC with the following antibodies; IGFBP4 (R&D Systems, Abingdon, UK. 1:1000), GAPDH (Sigma, 1:4000). Secondary incubation was performed for 1 h at room temperature using HRP-conjugated IgG kappa binding protein (Santa-Cruz Biotech, Heidelberg, Germany. 1:5000). Blots were imaged using SuperSignal West Pico PLUS substrate (Biosciences) and a Licor C-DiGit Blot Scanner.

**Drug combination assays**

Efficacy of drug combinations was performed following the same protocol as proliferation assays. Drugs were tested as both single agents and in combination. Combination index (CI) values were calculated using Compusyn software [18]. CI values below 1 are indicative of drug synergy.

**Statistics**

All experiments were repeated at least 3 times. All statistical analysis was performed using GraphPad Prism 8 software. Significance was determined using Student t tests or ANOVA post-hoc analysis. A p value of less than 0.05 was deemed statistically significant.

**Results**

**Statins reduce breast cancer cell line proliferation**

We initially tested the effect of 2 widely used statins on the viability of a panel of 15 breast cancer cell lines, representative of the major breast cancer molecular subtypes; luminal (4 cell lines), HER2 (2 cell lines) and TNBC (9 cell lines). Both statins reduced cell viability in a dose-dependent and cell type-dependent manner (Fig. 1A and B). The IC50 values for both statins were highly variable across the panel, ranging from 0.4 to 61 µM for atorvastatin, and from 0.2 to 50 µM for simvastatin (Fig. 1C). Although the IC50 values for the 2 statins were highly correlated across the panel (r = 0.94, p < 0.0001) (Fig. 1D), significantly lower values were found with simvastatin compared with atorvastatin (p < 0.01) (Fig. 1E). Of potential clinical significance, we found significantly lower IC50 values for both statins in TNBC versus non-TNBC cell lines (atorvastatin, p < 0.01; simvastatin p < 0.05) (Fig. 1F). The enhanced efficacy of statins in TNBC cell lines may be due to the high prevalence of p53 mutations in this subtype [19]. Indeed, we found lower IC50 values for both atorvastatin and simvastatin in cell lines harbouring mutant versus WT-p53 alleles (Fig. 1G). As mentioned previously, statins have been shown to exert some of their anti-tumour effect through degradation of mutant p53 [15, 20]. Although a previous study found that only cell lines harbouring conformational p53 mutations were responsive to statins [15], we did not observe any difference in IC50 values between cell lines with conformational versus contact-type mutations (data not shown).

**Anti-proliferative effects of statins are abrogated by mevalonate**

Although statins decrease cholesterol production by inhibiting the rate-limiting enzyme in its biosynthesis (HMGCR), it also reduces levels of the intermediate product, MVA [21]. To confirm that the anti-proliferative effect was via MVA reduction and not an unexpected off-target effect, we selected 4 TNBC cell lines previously observed to be
statin-sensitive and assessed the effect of exogenous MVA on statin-induced growth inhibition. Addition of MVA by itself had no effect on viability except in the SUM159 cell line, where an approximately 30% decrease was observed for both 100 and 200 µM (Fig. 2B). In all tested cell lines, both atorvastatin and simvastatin were again found to effectively reduce viability at either 5 or 10 µM (Fig. 2). This reduction was reversed by the addition of either 100 or 200 µM MVA, which completely restored normal levels of cell viability in 3 cell lines (MDA-MB-231, HS578T and BT-549, Fig. 2A, C, D, respectively) and lead to partial restoration in the SUM159 cell line (Fig. 2B). Although it was not clear why proliferation remained suppressed in the SUM159 cell line, we previously found this cell line to be highly statin-sensitive (Fig. 1A, B), which may explain the limited restoration of viability at MVA dosage used. Abrogation of the

Fig. 1  Anti-proliferative effect of statins in breast cancer. Viability of a panel of breast cancer cell lines was assessed by MTT assay following 5 days incubation with either atorvastatin (A) or simvastatin (B). Dose–response curves were used to calculate IC50 values (C). Cell sensitivity to the two statins was found to be correlated (D) although significantly greater for simvastatin (E). TNBC cell lines were found to be more sensitive to statin treatment than non-TNBC cell lines (F). Presence of mutant p53 allele was also found to influence statin response (G). All figures represent mean of 4 independent experiments. *p < 0.05, **p < 0.01.
antiproliferative effect of statins by MVA further illustrates the importance of this pathway in breast cancer cell viability.

**Effect of simvastatin on cell cycle arrest**

As simvastatin was the more potent of the two statins investigated, we focused on it for the rest of our study. To investigate if simvastatin could induce cell cycle arrest in TNBC cells, we measured DNA content by propidium iodide staining and flow-cytometry in 4 TNBC cell lines following 24 h treatment. Representative graphs of simvastatin-treated MDA-MB-231 cells are shown below (Fig. 3A). Simvastatin induced significant decreases in S-phase in 3 of 4 tested cell lines (Fig. 3B, D, E). In contrast, no significant effect was observed in SUM159 cells (Fig. 3C). It is unclear why this one cell line did not undergo cell cycle arrest. A possible reason is that we limited our analysis to 24 h post-treatment and a longer time-point may have yielded different results.

**Apoptotic effect of simvastatin in TNBC**

We next investigated if the loss of viability seen in statin-treated breast cancer cells was due to activation of apoptotic processes. We treated 3 TNBC and 1 non-TNBC cell lines with escalating doses of simvastatin for 48 h and measured apoptosis by flow-cytometry. All 3 TNBC cell lines had significantly increased levels of annexin-V staining at both 5 and 10 µM simvastatin. In MDA-MB-231 (Fig. 4A), 5 and 10 µM simvastatin led to 38% (p < 0.01) and 46% (p < 0.001) cells staining positive for annexin-V, respectively. Similar levels of annexin-V positivity were seen in Hs578T (Fig. 4C) cells. SUM159 cells were particularly sensitive to apoptosis, with 65% and 68%
In all cases, the increase in apoptotic cells was accompanied by a statistically significant decrease in live cells. In contrast to our findings with TNBC, mutant-p53 cell lines, there was no induction of apoptosis in the luminal cell line MCF-7 at any tested concentrations (Fig. 4D). This inability to induce apoptosis in MCF-7 cells may be related to its WT-p53 status.

To further study the mechanism of apoptosis in statin-treated TNBC cells, we incubated SUM159 cells with 10 µM simvastatin for 24 h and analysed expression of a panel of apoptotic markers using a commercially available antibody array. Several key apoptotic proteins including caspase-3, caspase-8 and Bid were found to be increased following 24 treatments with simvastatin (Fig. 4E, F, G). The highest level of upregulation was seen in the apoptosis executioner caspase-3 (fourfold), followed by the pro-apoptotic Bcl-2 family member, Bid (threefold). A novel finding was the upregulation of IGF4. Enlarged images of highlighted proteins are shown (Fig. 4G).

Statins synergise with IGF pathway inhibition

As IGF4 was not previously reported to be regulated by a statin, we validated this finding via Western blots. As shown in Fig. 5A, both SUM159 and MDA-MB-231 cells had significantly upregulated IGFBP4 in response to 24 h simvastatin treatment. Since IGFBP4 plays a role in regulating the IGF pathway, we assessed if inhibition of IGF signalling could synergise with statins by comparing viability of 4 TNBC cell lines treated with statins alone or in combination with the IGF1-R inhibitor, OSI-906. In agreement with prior studies [22, 23], OSI-906 as a single agent had limited effects on TNBC cell proliferation (Fig. 5B, C, D, E). However, the addition of OSI-906 enhanced its anti-proliferative effect in a synergistic manner i.e. CI values for MDA-MB-231, SUM159 and BT549 cell lines (Fig. 5B, C, D, E).
D) were 0.67, 0.91 and 0.45, respectively. A CI value could not be calculated for Hs578T cells (Fig. 5E), as no tested concentration of OSI-906 had an observable effect on proliferation in this cell line.

Finally, we tested the combination of simvastatin and OSI-906 on apoptosis. MDA-MB-231 and SUM159 cells were treated with a low dose of simvastatin (1 µM) that, by itself, did not significantly induce apoptosis in either cell line (Fig. 4A, B). Similar to results observed in our proliferation assays, OSI-906 as a single agent had no significant effect on the rate of apoptosis (data not shown) but markedly enhanced the apoptotic effect of simvastatin in both cell lines. In MDA-MB-231 cells, combination of 10 µM OSI-906 and 1 µM simvastatin lead to a twofold increase in apoptotic cells versus 1 µM simvastatin alone (Fig. 5F, p < 0.05). The combination was also effective in SUM159 cells (Fig. 5G), where the addition of either 5 µM or 10 µM OSI-906 increased apoptosis relative to simvastatin alone (1.7-fold, p < 0.05; 3.7-fold, p < 0.0001, respectively).

**Statins synergise with chemotherapy drugs**

As statins are unlikely to be used alone in cancer treatment, we also tested the anti-proliferative effect of combined treatment with a statin and standard chemotherapy drugs used for the treatment of breast cancer. As shown in Table 2, synergistic growth inhibition was observed when simvastatin was combined with either doxorubicin or docetaxel in MDA-MB-231 and SUM159 cells.
**A.** SUM159 vs. MDA-MB-231

| Simvastatin (µM) | 0 | 1 | 5 | 10 |
|------------------|---|---|---|----|
| SUM159 (µM)     | 0 | 1 | 5 | 10 |
| MDA-MB-231 (µM) | 0 | 1 | 5 | 10 |

**B.** MDA-MB-231

CI = 0.67

**C.** SUM159

CI = 0.91

**D.** BT549

CI = 0.45

**E.** Hs578T

**F.** MDA-MB-231

| % apoptosis |
|-------------|
| Control     | 0 |
| 1 µM Sim    | 10 |
| 1 µM Sim + 1 µM OSI | 20 |
| 1 µM Sim + 5 µM OSI | 30 |
| 1 µM Sim + 10 µM OSI | 40 |

**G.** SUM159

| % apoptosis |
|-------------|
| Control     | 0 |
| 1 µM Sim    | 10 |
| 1 µM Sim + 1 µM OSI | 20 |
| 1 µM Sim + 5 µM OSI | 30 |
| 1 µM Sim + 10 µM OSI | 40 |

* p < 0.05

**Notes:**
- **IGFBP4** and **GAPDH** images are shown below the tables.
- The graphs represent relative viability and apoptosis rates for the indicated cell lines and treatment combinations.
IGFBP4 binds and sequesters IGF-1 ligands, thus acting implication for statin actions, previous studies showed that investigate the mechanism of this upregulation or its possible upregulated levels of IGBP4. Although we did not inves-
tinated, suggesting that combined treatment with simvasta-
tin and docetaxel or doxorubicin might be investigated in a 
tigated, implying that simvastatin with two frequently used drugs to treat 
t TNBC, i.e. docetaxel and doxorubicin. Both these cyto-
toxic drugs in combination with simvastatin synergistically 
enhanced growth inhibition in the 2 TNBC cell lines inves-
tigated, suggesting that combined treatment with simvasta-
tin and docetaxel or doxorubicin might be investigated in a 
clinical trial.

A novel finding in this report was that simvastatin 
upregulated levels of IGBP4. Although we did not inves-
tigate the mechanism of this upregulation or its possible 
implication for statin actions, previous studies showed that 
IGFBP4 binds and sequesters IGF-1 ligands, thus acting to dampen IGFR1 signalling [24]. Consistent with this 
observation, administration of a high dose of atorvasta-
tin decreased serum IGF-1 levels in diabetic patients [25, 
26]. Furthermore, statins were reported to downregulate 
expression of the IGFR1 [27, 28]. Theoretically, there-
fore, statin-induced upregulation of IGBP4 could lead to sequestration of IGFR1 which in turn could downregulate 
IGFR1 signalling. Decreased IGFR1 signalling might be 
expected to result in inhibition of cell line growth and/or 
promotion of apoptosis as this signalling system has been 
has been shown to promote breast cancer cell proliferation and survival [29]. Modulation of IGBP4 expression has 
previously been suggested as a therapeutic strategy, with 
a degradation-resistant form of the protein demonstrating 
significant effects on tumour growth and angiogenesis in 
both in vitro and in vivo models [30, 31].

We should state however, that there are several other 
mechanisms other than via upregulation of IGBP4 by 
which statins could decrease cell proliferation or promote 
apoptosis. These include a reduction in different cell sig-
alling systems such as from RAS, RHO, Hedgehog, YAP 
or TAZ (for review, see Ref. [32]). Furthermore, statins 
have been shown to reduce N-glycosylation of specific 
membrane proteins and suppress epithelial–mesenchymal 
transition (EMT) [33]. Indeed, the reduced levels of cho-
lesterol following statin treatment have also been associ-
ated with decreased cancer cell growth [34]. All of these 
actions of statins, like that of mutant p53 degradation, 
appear to result from inhibition of HMG-CoA and block-
age of the MVA pathway [32].

Consistent with our preclinical studies, several clinical 
studies have shown that the use of statins was associated 
with improved outcome in breast cancer patients. Thus 
following a systematic review of the literature and meta-
analysis, Manthravadi et al. [35] identified 10 studies con-
taining 75,684 women with breast, that compared outcome 
in statin-users versus non-users. Statin use was found to 
be associated with both improved recurrence-free survival 
(HR 0.64; 95% CI 0.53–0.79), improved overall survival 
(HR 0.66; 95% CI 0.44–0.99) and improved cancer-specific 
(OH 0.70; 95% CI 0.46–1.06). Furthermore, in a 
large population-based study carried out in Denmark, sta-
tin use was associated with a reduced risk of breast cancer 
recurrence in postmenopausal women receiving adjuvant 
aromatase inhibitors (following multivariate analysis, HR 
0.72; 95% CI 0.50–1.04) [36]. Also, in a large randomized 
phase III double blind clinical trial (BIG 1–98) in which hor-
mone receptor-positive patients were undergoing endocrine 
treatment, receipt of statins was related to longer disease-
free-survival (HR 0.79; 95% CI 0.66–0.95; p = 0.01), longer 
breast cancer-free interval (HR 0.76; 95% CI 0.60–0.97; 
p = 0.02) and longer distant recurrence-free interval (HR 
0.74; 95% CI 0.56–0.97; p = 0.03) [37].
Of particular relevance to our study was the recent report showing a significant benefit of statins in patients with TNBC but not in those with non-triple-negative disease [38]. Thus, in a large population-based study that included women with stage I-III breast cancer, Nowakowska et al. found that use of statins improved breast cancer-specific survival (HR 0.42; 95% CI 0.20–0.88; \( p = 0.022 \)) and overall survival (HR 0.70; 95% CI 0.50–0.99; \( p = 0.046 \)) in patients with TNBC (\( n = 1534 \)). In contrast, there was no association with breast cancer-specific survival (HR 0.99; 95% CI 0.71–1.39; \( p = 0.97 \)) or overall survival (HR 1.04; 95% CI 0.92–1.17; \( p = 0.55 \)) in those without TN disease (\( n = 15,979 \)). Since cell lines containing mutant p53 appear to be more sensitive to statins than p53 wild-type cells [20] theoretically, the benefit of statins in the tripe-negative cohort relative to the non-TN patients could be a least partly due to the considerable greater prevalence of p53 mutations in the former subtype of breast cancer [5]. In addition to breast cancer, use of statins has also been associated with improved outcome in several other types of malignancy, although contradictory data have also been published including contradictory results in breast cancer (for review, see ref. [39]).

Despite the multiplicity of preclinical and epidemiological studies linking statin use with anti-cancer activity across different types of cancer, there are little data from randomized clinical trials that treatment with these drugs improves outcome for patients with cancer. Statins however, are one of the most commonly prescribed classes of drug worldwide, with well-established safety and dosage profiles [40]. Their limited toxicity, low cost, and ease of use make them an ideal choice for repurposing as anti-cancer drugs.

In conclusion, our data described in this article may be informative with respect to the design of clinical trials involving statins for the treatment of breast cancer. We show that the anti-proliferative effect of simvastatin is enhanced by combination with doxorubicin or docetaxel. Furthermore, our preliminary finding when combined with that of others [20] suggests that statins are more potent cell-growth inhibitors in mutant than in wild-type p53 cells, implying that the mutational status of p53 might be a predictive biomarker for statin sensitivity. Thus, the mutational status of p53 should be investigated in any clinical trial using statins. Finally, while this work was in progress, 2 clinical trials investigating statins in the neoadjuvant treatment of TNBC began recruiting patients (ClinicalTrials.gov Identifier: NCT03358017 and NCT03872388).

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**Declarations**

**Conflict of interest** MJD and SO’G have no conflicts of interest. JC has received honoraria from Eisai, Amgen, Puma Biotechnology, Seattle Genetics, Boehringer Ingelheim, Pfizer, Vertex and Genomic Health. He has acted in an advisory/consulting role to Eisai, Puma Biotechnology, Boehringer Ingelheim, Pfizer, Vertex, Roche. He also serves on the Speakers’ Bureau for Pfizer, Eisai and Genomic Health and has received Research Funding from Roche, Eisai, Boehringer Ingelheim and Puma Biotechnology. In addition, he has received travel, accommodations, expenses from MSD, Pfizer, Roche, AstraZeneca, Abbvie and Novartis. Finally, he is an employee of OncoMark, has stocks in OncoMark and is named on patent WO2020011770 (A1)—A method of predicting response to treatment in cancer patients.

**Ethical approval** This study utilised commercially available cell lines. No human or animal subjects were used in this study.

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