

Annexin-A1 as a Potential Therapeutic Target for Solid Tumors

Fiona C Dempsey¹, Hussein Al-Ali², Scott J Crichton¹, Henry C W Hays¹, Christopher N Parris²

¹Medannex Ltd, Edinburgh, UK; ²Anglia Ruskin University, Cambridge, UK.



INTRODUCTION

Annexin-A1 (ANXA1), a member of the annexin protein superfamily, binds acidic phospholipids in a calcium-dependent manner. Normally localized in cell cytoplasm, ANXA1 can be secreted in response to cell activation where it can modulate nearby cell behavior via interactions with formyl peptide receptors (FPR1/2). Current literature suggests ANXA1 can act as a tumor suppressor or an oncogene dependent on cancer indication or subtype. Increased ANXA1 expression has been shown in a range of cancers including triple-negative breast, pancreatic, liver and bladder, and correlates with poor prognosis and decreased overall survival⁽¹⁾. ANXA1 has been demonstrated to influence cancer cell proliferation, angiogenesis, migration and drug resistance^(2,3), and exerts immunomodulatory effects on T-cells, macrophages and dendritic cells^(4,5). We have developed a humanized monoclonal antibody targeting ANXA1 (MDX-124) and here present data showing its anti-proliferative effect on a panel of cancer cell lines. The anti-proliferative activity of MDX-124 correlates with ANXA1 expression and is likely to result from disruption to the cell cycle. Furthermore, we have shown efficacy in a mouse model of triple negative breast cancer.

METHODS

Cell Proliferation: A panel of cancer cell lines was treated with MDX-124, a commercial anti-ANXA1 antibody or IgG isotype control (0-10 μ M) for 72 hours. Cell viability was measured via MTT assay (n=3). Statistical significance was calculated via Mann-Whitney test and indicated by ***p<0.001 and ****p<0.0001 (MDX-124 vs IgG) or °p<0.01 and °°p<0.001 (MDX-124 vs commercial antibody).

Imaging Flow Cytometry: Samples from each cell line were prepared for ImageStreamX® analysis. Multispectral images of cells in brightfield, green (MDX-124) and red (nuclear staining: DRAQ5) were captured. Ideas™ imaging analysis software was used to determine cellular localization of MDX-124 and measure DRAQ5 staining intensity to estimate mean DNA content and provide a preliminary analysis of the G0/G1, S and G2/M phases of the cell cycle.

In-vivo Efficacy Study: MDX-124 was evaluated in a syngeneic orthotopic 4T1-luc TNBC mouse model. BALB/c mice (n=12) were randomized to treatment groups and dosed IV with MDX-124 (1 mg/kg) or vehicle control (PBS). Statistical significance was calculated via 2-tailed unpaired T-test and indicated by *p<0.05 and **p<0.01.

RESULTS

Incubation of a panel of breast, colorectal, pancreatic and ovarian cancer cell lines with MDX-124 (0-10 μ M) for 72 hours resulted in a statistically significant reduction in cell proliferation (Figure 1). MDX-124 was compared to an IgG isotype control or a commercial anti-ANXA1 polyclonal antibody in a selection of cancer cell lines and demonstrated a statistically significant 3-fold increase in potency. Representative data are shown in Figure 2.

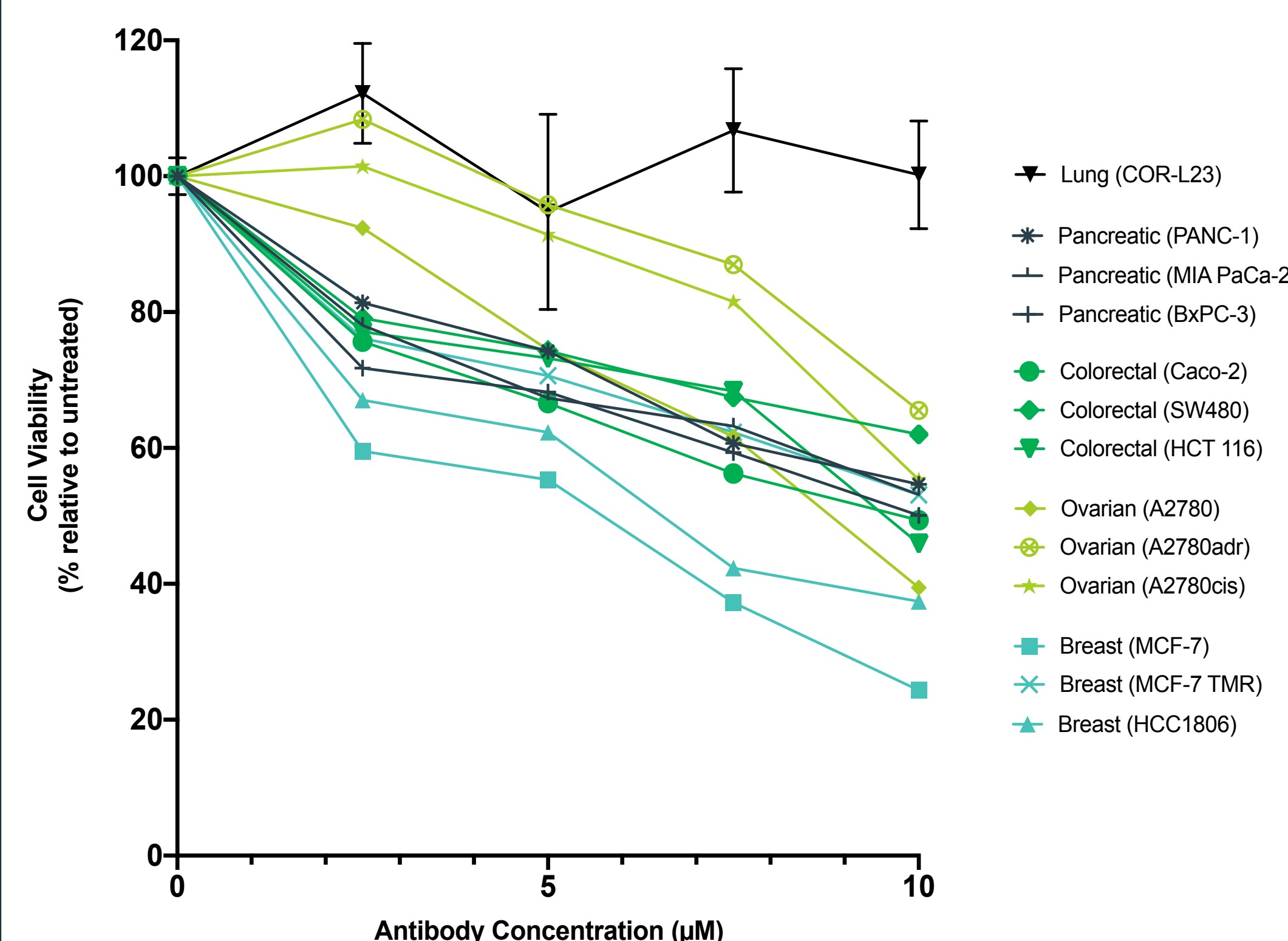


Figure 1. Anti-proliferative effect of MDX-124 in a panel of cancer cell lines.

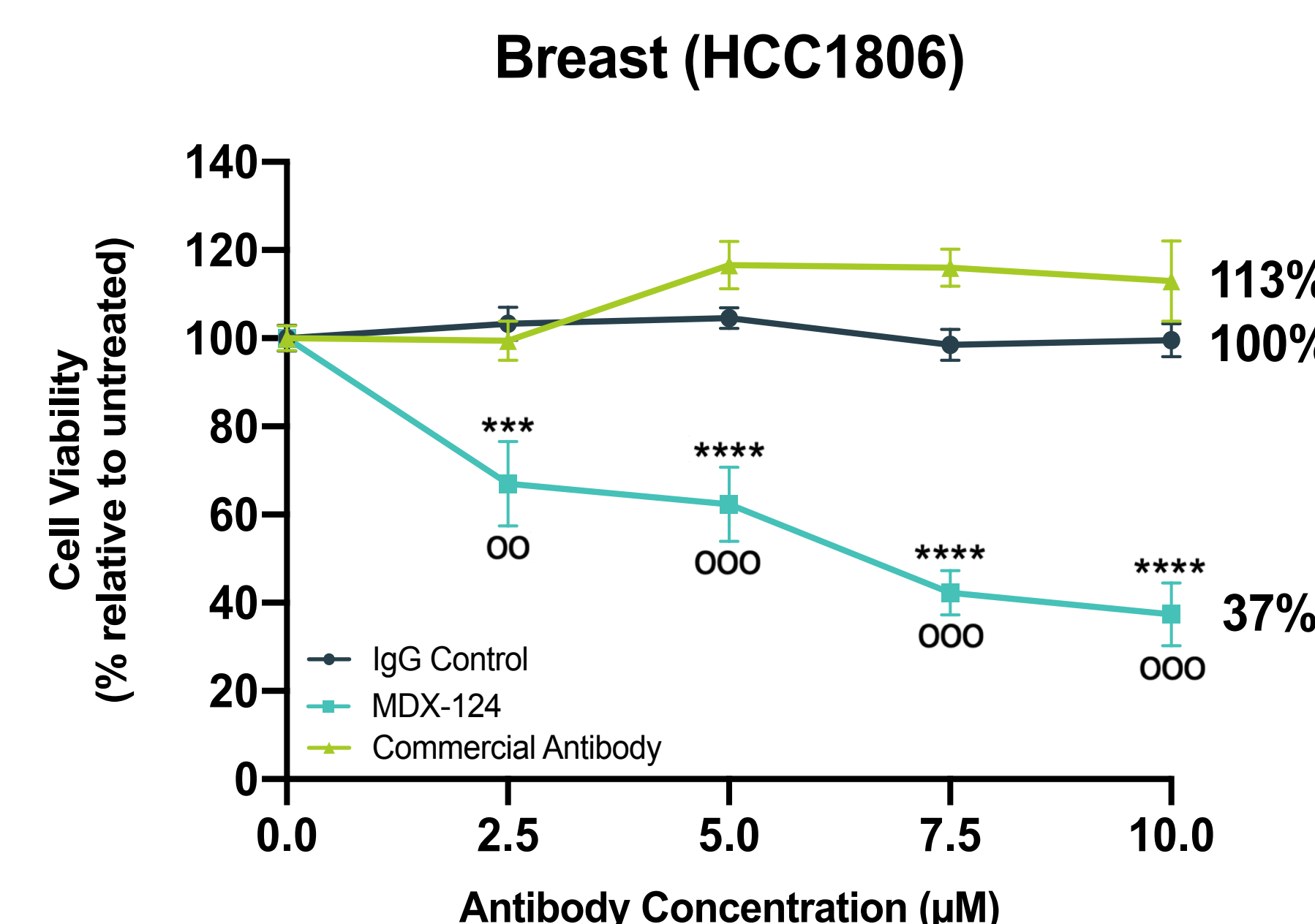


Figure 2. Anti-proliferative effect of MDX-124 in HCC1806 triple-negative breast cancer cells.

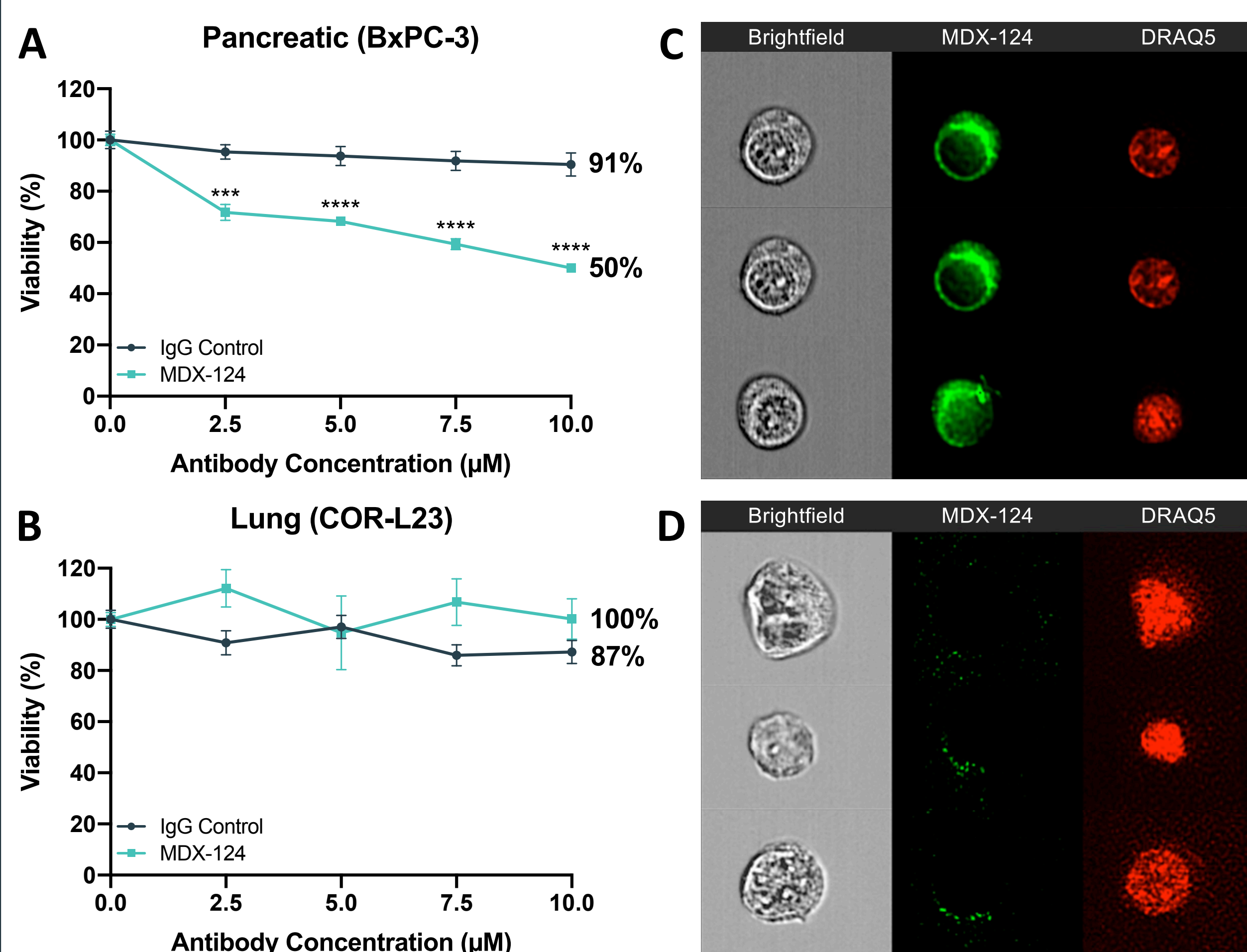


Figure 3. Anti-proliferative effect of MDX-124 correlates with annexin-A1 expression.

RESULTS (continued)

Imaging flow cytometry demonstrated a dose-dependent shift in cell cycle progression in several cancer cell lines sensitive to MDX-124 after 72 hours (Figure 4, representative data). This suggests that the anti-proliferative effect of MDX-124 involves disrupting the cell cycle phase transition.

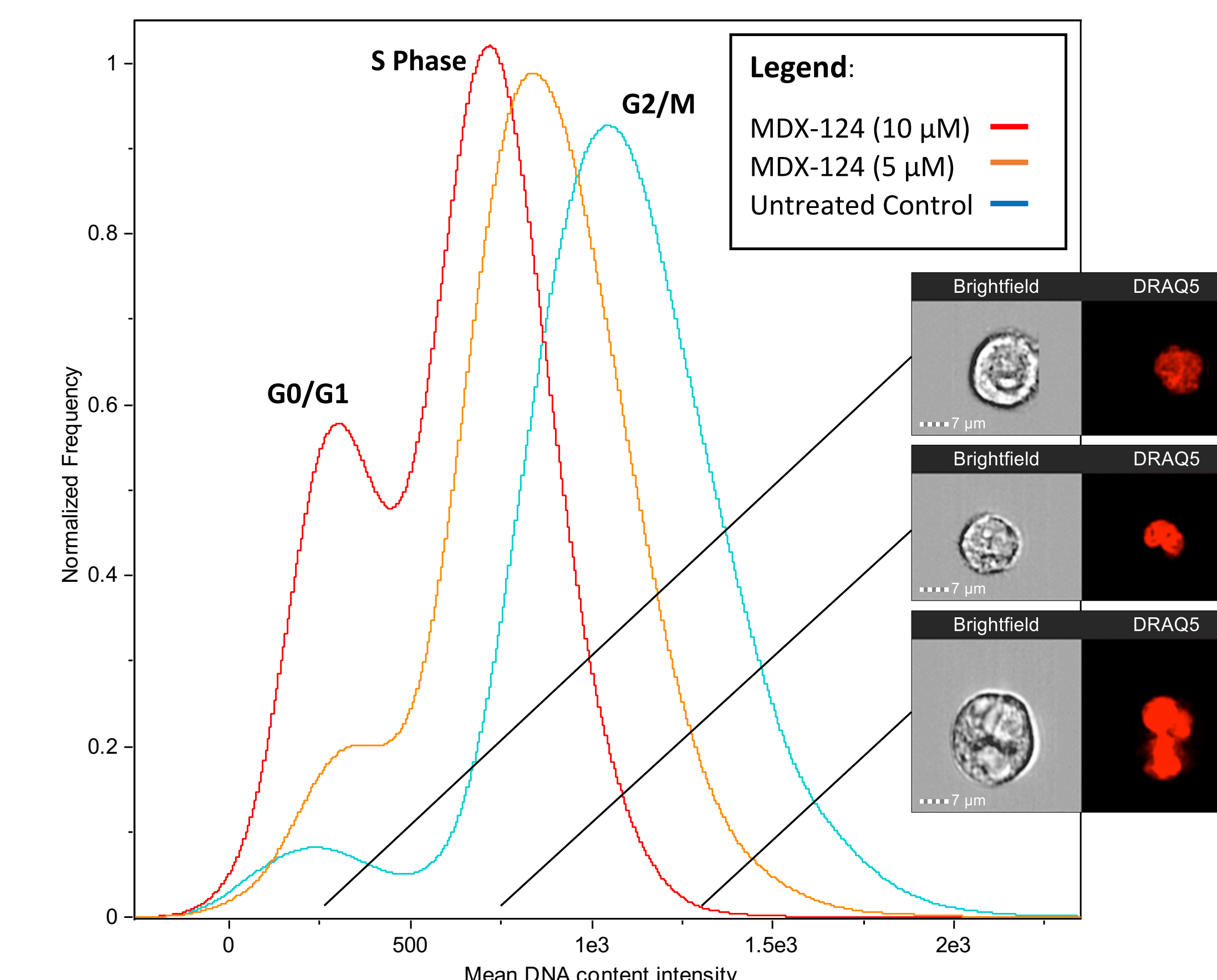


Figure 4. MDX-124 treatment affects cell cycle transition in HCC1806 triple-negative breast cancer cells in a dose-dependent manner.

Further *in-vivo* studies evaluated MDX-124 (1 mg/kg) in the syngeneic, orthotopic, 4T1-luc mouse model of triple-negative breast cancer. Following dosing once per week we identified a statistically significant reduction in tumor volume compared to the vehicle control (*p<0.05 and **p<0.01), without any associated significant change in body weight (Figure 5).

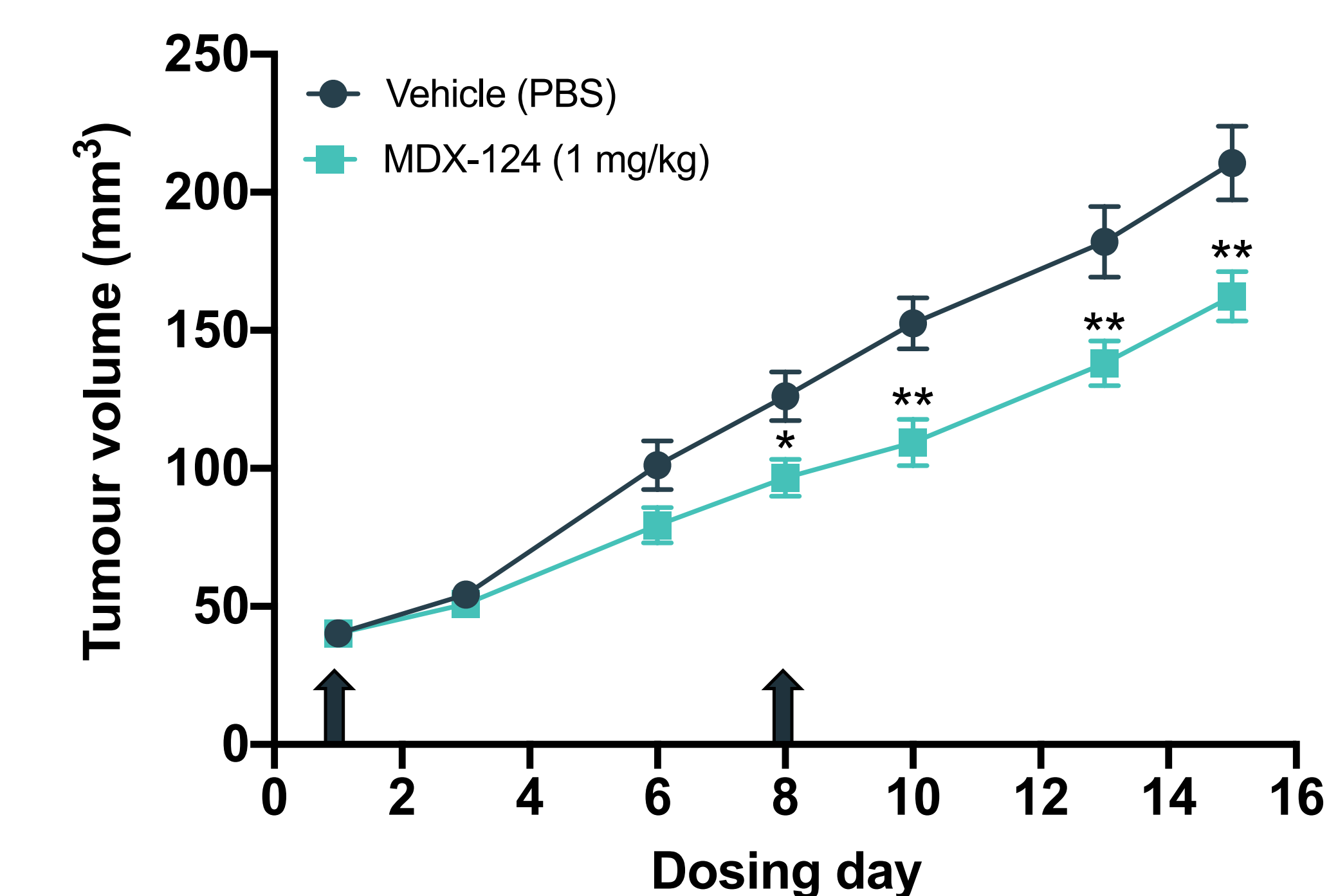


Figure 5. MDX-124 reduces 4T1-luc breast tumour growth. Arrows indicate day of dosing.

CONCLUSION

Our results demonstrate MDX-124 can suppress cancer cell proliferation through targeted inhibition of ANXA1. This suggests MDX-124 is a promising first-in-class monoclonal antibody therapeutic against cancers overexpressing ANXA1. Investigations are continuing to further characterize the mechanism of action of MDX-124 across several cancer subtypes.

References:

- (1) Lin *et al.*, (2014). *Medical Oncology*, 31(327).
- (2) Boudhraa *et al.*, (2016). *Clinical Science*, 130(4): 205–220.
- (3) Onozawa *et al.*, (2017). *Oncology Reports*, 37(1): 235-240.
- (4) Tu *et al.*, (2017). *Pharmacological Research*, 119: 278–288.
- (5) Moraes *et al.*, (2018). *Cell Adhesion & Migration*, 12(5), 417–423.