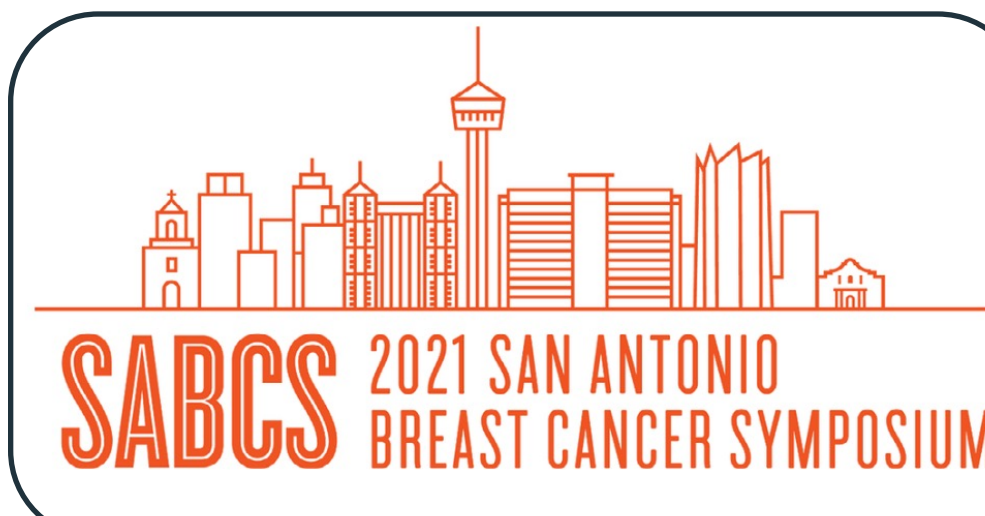


MDX-124, a novel annexin-A1 antibody, shows anti-tumor efficacy in several preclinical models of triple-negative breast cancer



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INTRODUCTION

Annexin-A1 (ANXA1) is a phospholipid binding protein secreted in response to several physiological stimuli where it activates formyl peptide receptors (FPR1/2) triggering multiple oncogenic processes⁽¹⁾. Overexpression of ANXA1 by triple-negative breast cancer (TNBC) cells has been shown to promote several cancer-related processes including cell growth⁽²⁾, cell cycle progression⁽²⁾, angiogenesis⁽³⁾, migration and invasion⁽⁴⁾. ANXA1 has also been shown to influence the tumor microenvironment and have immunomodulatory effects on T-cells⁽⁵⁾, macrophages⁽⁶⁾ and dendritic cells⁽⁷⁾ supporting cancer progression. Furthermore, high tumoral expression of ANXA1 correlates with poor overall and progression-free survival in TNBC^(5,8), indicating ANXA1 is an important new therapeutic target in TNBC. MDX-124 is a humanized antibody targeting ANXA1, and we have previously demonstrated its significant anti-proliferative activity. Here we present additional data showing the efficacy of MDX-124 in preclinical models of TNBC.

METHODS

Cell Cycle Analysis: MDA-MB-231 cells (1×10^6) incubated for 24 h with MDX-124 (10 μ M or 25 mM). Cells harvested and labelled with Cytophase violet dye (8 mM) before incubation at 37 °C for 30 min. Flow cytometry analysis was conducted (CytoFLEX) and analyzed using FlowJo™ (BD Life Sciences) to determine percentage of cells in each phase of cell cycle (G1, S and G2).

Cell Viability: HCC1806 cells (1×10^5) incubated for 72 h with IgG isotype control (0-10 μ M), MDX-124 (0-10 μ M), MDX-124 + IC₅₀ cisplatin (0.65 μ M) or MDX-124 + IC₂₀ paclitaxel (0.4 nM). Cell viability measured via MTT assay. Statistical significance calculated using an unpaired t-test (GraphPad Prism 9.0), where ****p<0.0001 (MDX-124 vs MDX-124 + cisplatin or paclitaxel) or *p<0.05, ***p<0.001 and ****p<0.0001 (MDX-124 vs IgG isotype control).

Proteomic Analysis: HCC1806 cells (5×10^6) incubated with MDX-124 (10 μ M) for 72 h. Cell lysates obtained with relative levels of protein kinase phosphorylation determined versus untreated control cells using a Proteome Profiler™ human phospho-kinase array kit as per manufacturer's instructions (R&D Systems).

In-vivo Efficacy Study: BALB/c mice (n=10) inoculated subcutaneously with EMT6 breast cancer cells (5×10^5) and randomized to treatment groups once tumor volumes reached ~100 mm³. Mice treated with vehicle control (PBS), anti-PD-1 (10 mg/kg, BIW) or anti-PD-1 (10 mg/kg, BIW) + MDX-001 (10 mg/kg, BIW). Tumor volumes expressed in mm³ using formula: $V = (L \times W \times W)/2$.

References: (1) Boudhraa et al., (2016). *Clinical Science*, **130**, 205-220. (2) Vecchi et al., (2018). *BBA Molecular Cell Research*, **1865**(9), 1368-1382. (3) Okano et al., (2019). *Int. J. Mol. Sci.*, **20**(17), 4197. (4) Okano et al., (2015). *Oncol. Rep.*, **33**(3), 1064-1070. (5) Bai et al., (2020). *J. Immunother. Cancer*, **8**(1), 1-9. (6) Moraes et al., (2017). *Scientific Reports*, **7**(1), 1-12. (7) Weyd et al., (2013). *PLoS ONE*, **8**(4), e62449. (8) Gibbs and Vishwanatha, (2018). *Oncotarget*, **9**(2), 2697-2704.

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RESULTS

Incubation of MDX-124 with MDA-MB-231 TNBC cells significantly decreased the proportion of cells in S-phase by 29.1% and increased cells in G1 phase by 33.5% versus untreated controls (**Figure 1**). This occurred in a dose-dependent manner and is consistent with an MDX-124 mediated increase in cell cycle arrest.

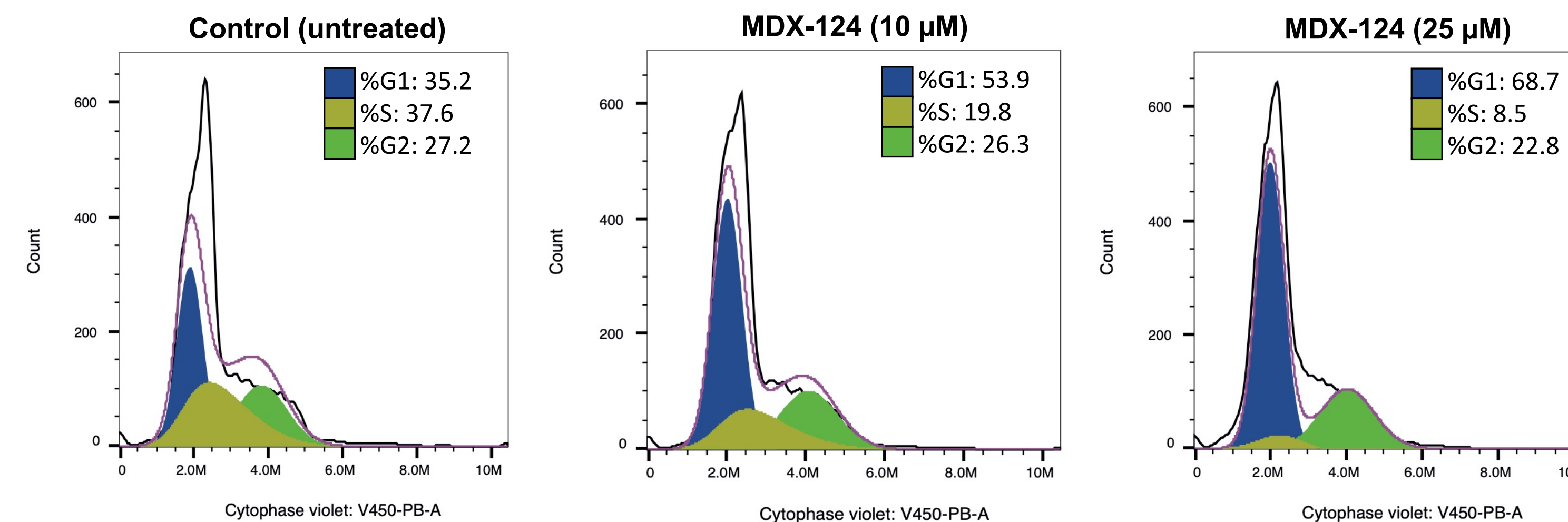


Figure 1. MDX-124 induces cell cycle arrest in MDA-MB-231 TNBC cells.

HCC1806 TNBC cell viability was significantly reduced after 72 h incubation with MDX-124 alone versus an IgG isotype control (p<0.001). The combination of MDX-124 with cisplatin (IC₅₀) or paclitaxel (IC₂₀) demonstrated significant synergy, with cell viability reduced by up to 87% versus untreated control cells (**Figure 2**).

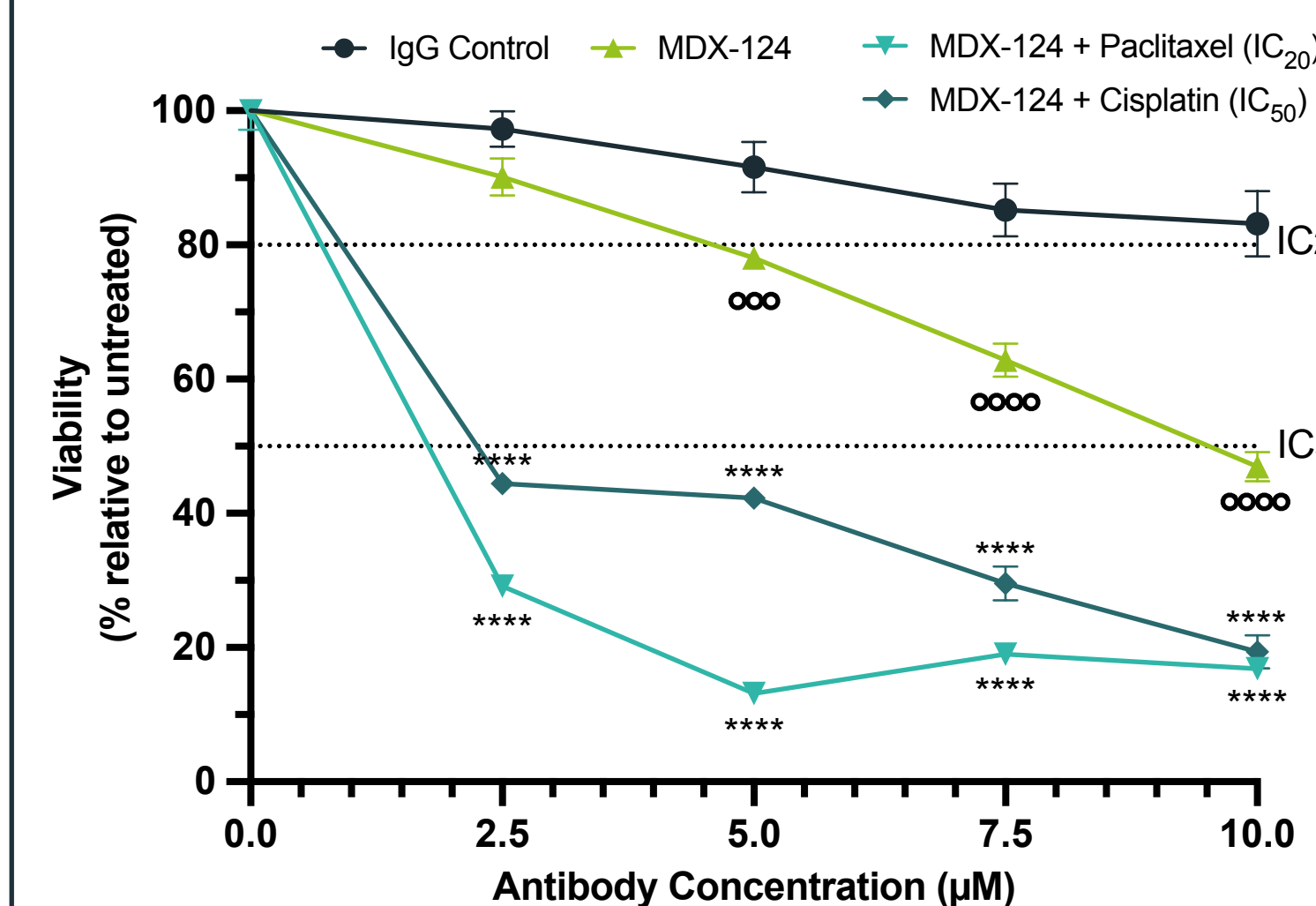


Figure 2. MDX-124 significantly reduces HCC1806 TNBC cell viability alone and in combination with cisplatin and paclitaxel.

Treatment of HCC1806 TNBC cells with MDX-124 altered the phosphorylation of several kinases that regulate cell signaling pathways involved in proliferation, survival, and migration (**Figure 3**). Notably, MDX-124 reduced the phosphorylation of ERK1/2 by 7-fold, p53 (S392) by 4-fold and AKT1/2/3 (S473 and T308) by 4-fold and 2-fold respectively versus untreated control cells.

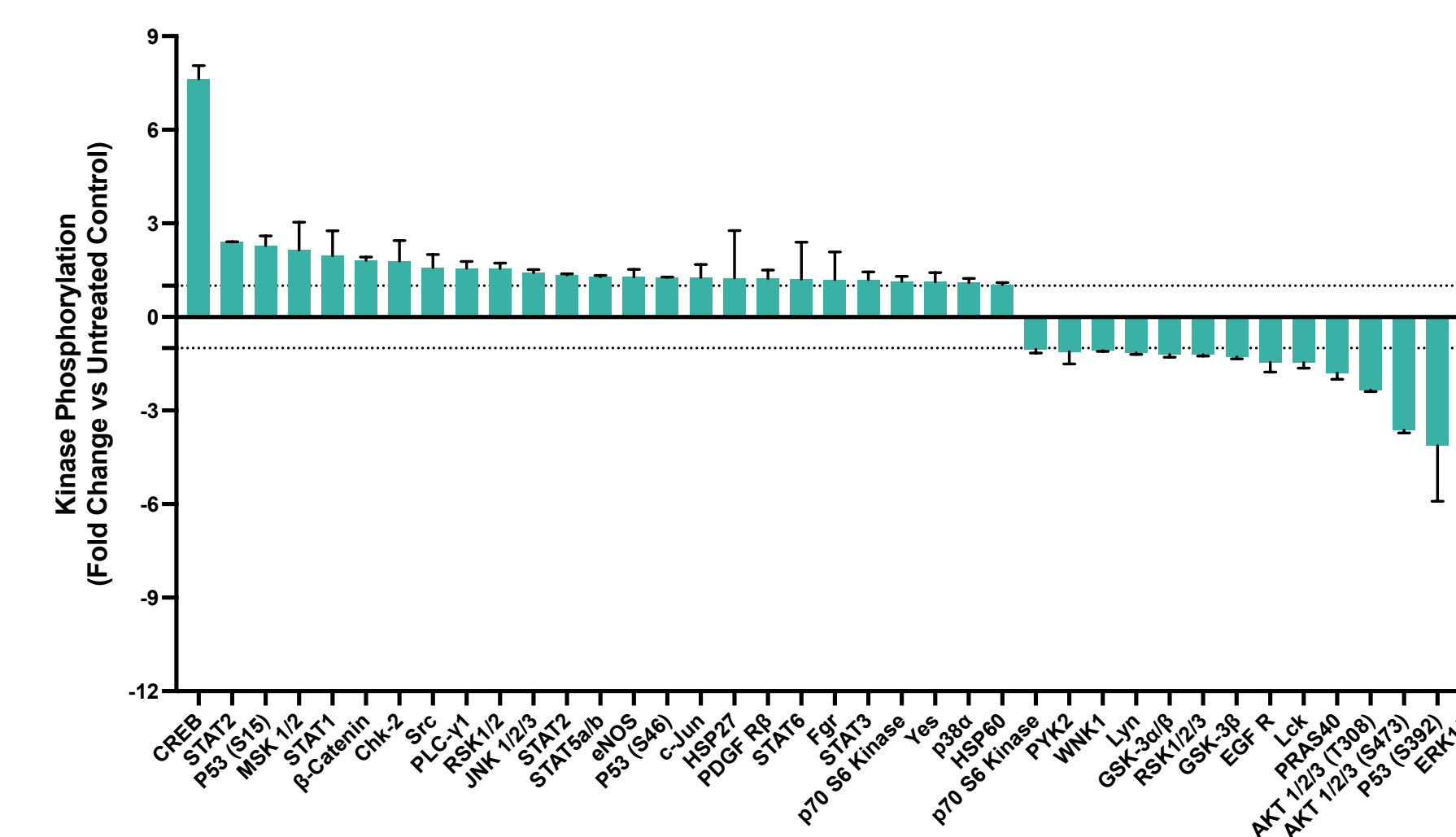


Figure 3. MDX-124 alters kinase phosphorylation in HCC1806 TNBC cells.

RESULTS

In the EMT6 syngeneic mouse model of TNBC, the murine analog of MDX-124 (MDX-001) potentiated mean tumor growth inhibition of single agent anti-PD-1 treatment by 15% (**Figure 4**). Additionally, 30% of treated mice showed tumor regression in the MDX-001 combination therapy group versus 10% in the single agent anti-PD-1 group (**Figure 5**). This suggests that anti-ANXA1 antibody therapy potentiates anti-PD-1 immunotherapy.

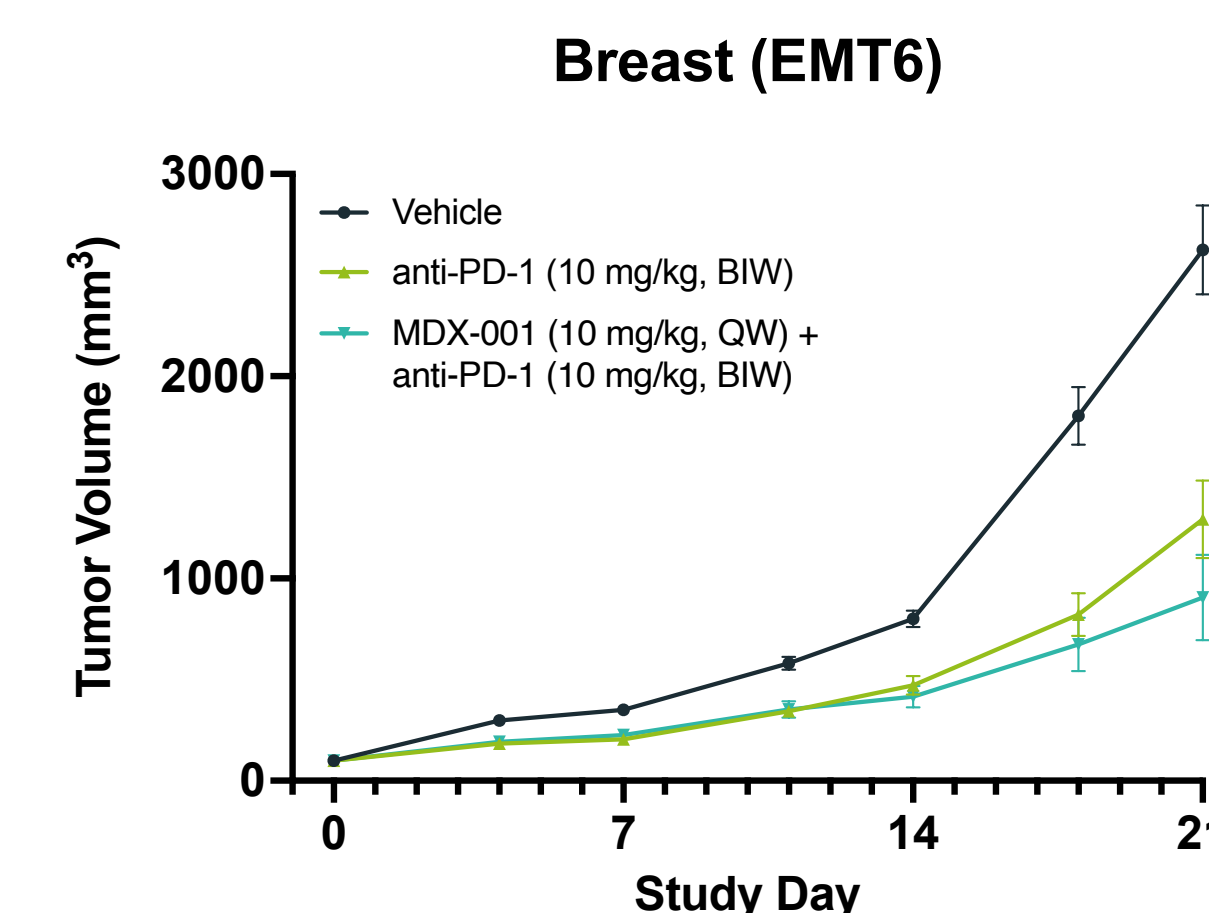


Figure 4. MDX-001 treatment potentiates anti-PD-1 induced breast tumor growth inhibition.

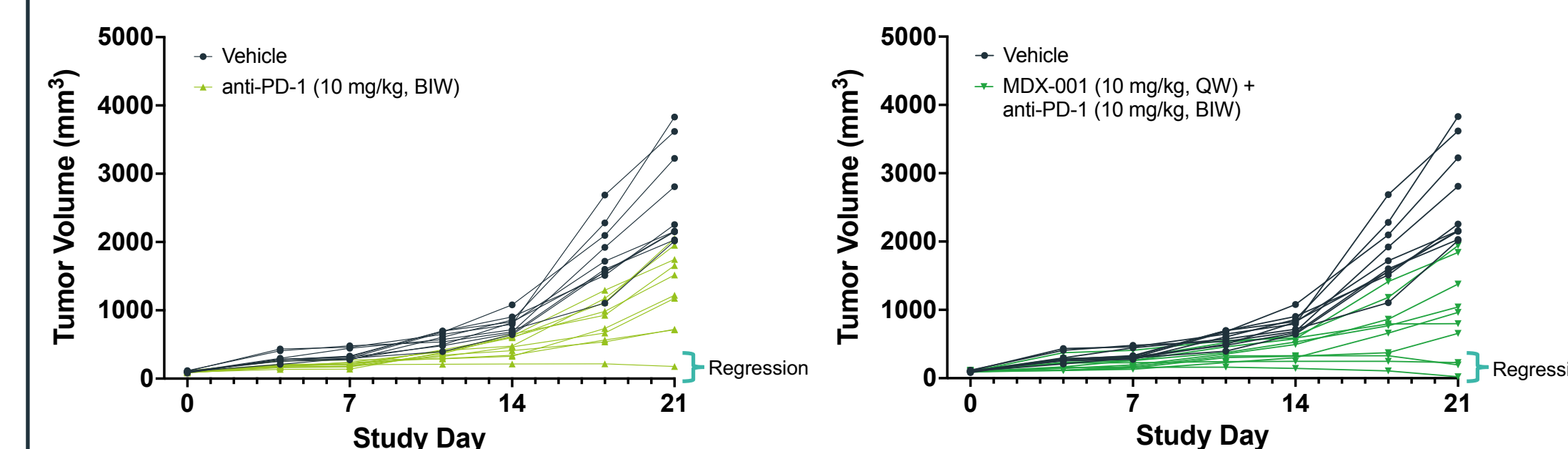


Figure 5. MDX-001 + anti-PD-1 combination therapy leads to increased tumor regression.

CONCLUSION

In conclusion, MDX-124 binds to secreted and extracellular ANXA1, which disrupts interactions with FPR1/2. This results in decreased cell growth via cell cycle arrest and reduced phosphorylation of several key kinases that activate oncogenic signaling pathways and promote cancer progression. MDX-124 has demonstrated anti-tumor efficacy in several *in-vitro* and *in-vivo* TNBC models, as both a single agent and in combination with other cancer therapies including cisplatin, paclitaxel and anti-PD-1 immunotherapy. Medannex initiated a First-In-Human study in Q4 2021 to evaluate MDX-124 in solid malignancies, including TNBC.

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