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

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 oncopogenic 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(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 anti-tumor data showing the efficacy of MDX-124 in preclinical models of TNBC.

METHODS

Cell Cycle Analysis: MDA-MB-231 cells (1x10^6) incubated for 24 h with MDX-124 (10 µM or 25 nM). Cells harvested and labeled with CytoPhase violet dye (8 µM) before incubation at 37 ºC for 30 min. Flow cytometry analysis was conducted (Cytodex®) and analyzed using FlowJo™ (BD Life Sciences) to determine percentage of cells in each phase of cell cycle (G1, S and G2).

Cell Viability: HCC1808 cells (1x10^4) incubated for 72 h with IgG isotype control (0-10 µM), MDX-124 (0-10 µM), MDX-124 + IgG (0.65 µM) or MDX-124 + IgG (0.65 µM). Cell viability measured via MTT assay. Statistical significance calculated using unpaired t-test (GraphPad Prism 9.0), where ***p<0.001 (MDX-124 vs IgG control) or p<0.05, **p<0.01, and *p<0.001 (MDX-124 vs IgG control).

Proteomic Analysis: HCC1808 cells (5x10^5) incubated with MDX-124 (10 µM) for 72 h. Cell lysates obtained with relative proteins 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 (6x10^5) and randomized to treatment groups once tumor volumes reached ~100 mm³. Cells were treated with vehicle control (PBS), anti-PD-1 (10 mg/kg, BIW) or anti-PD-1 (10 mg/kg, BIW) + MDX-124 (10 mg/kg, BIW). Tumor volumes expressed in mm³ using formula: V = L x W x W/2.

RESULTS

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

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

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 (IC50) demonstrated significant synergy, with cell viability reduced by up to 87% versus untreated control cells (Figure 2).

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

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) by 4-fold and 2-fold respectively versus untreated control cells.

Figure 4. MDX-101 treatment potentiates anti-PD-1 induced breast tumor growth inhibition.

In the EMT6 syngeneic mouse model of TNBC, the murine analog of MDX-124 (MDX-101) preclinical 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 specific combination therapy potentiates anti-PD-1 immunotherapy.

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 kinases that promote 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 the 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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