Annexin-A1: A New Immunomodulation / Checkpoint Pathway in Cancer

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INTRODUCTION

Annexin-A1 (ANXA1) is a member of the annexin protein superfamily that binds acidic phospholipids in a calcium-dependent manner. Increased expression of ANXA1 has been shown in a range of cancers including pancreatic, renal, triple-negative breast (TNBC) and bladder. ANXA1 has been demonstrated to influence cancer cell proliferation, angiogenesis and migration(1), as well exerting immunomodulatory effects on T-cells, macrophages and dendritic cells(2,3). We have developed a humanized monoclonal antibody targeting ANXA1 (MDX-124) and here we present data showing its anti-proliferative effect on a panel of cancer cell lines which correlates with ANXA1 expression. Furthermore, we have shown efficacy in a mouse model of triple negative breast cancer.

METHODS

Cell Proliferation: A panel of cancer cell lines were treated with MDX-124, a commercial anti-ANXA1 antibody or IgG isotype control (0-10 µM) 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 ^{oo}p<0.01 and ^{ooo}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. Cellular localization of MDX-124 was determined using the Ideas™ imaging analysis software.

***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 with vehicle control (PBS) or MDX-124 (1 mg/kg) by IV injection. 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 µM) for 72 hours resulted in a statistically significant reduction in cell proliferation (Figure 1). MDX-124 was then compared to an IgG isotype control or a commercial anti-ANXA1 polyclonal antibody in a selection of cancer cell lines. MDX-124 demonstrated a statistically significant 3-fold increase in potency versus control or commercial antibody. Representative data obtained are shown in Figure 2.

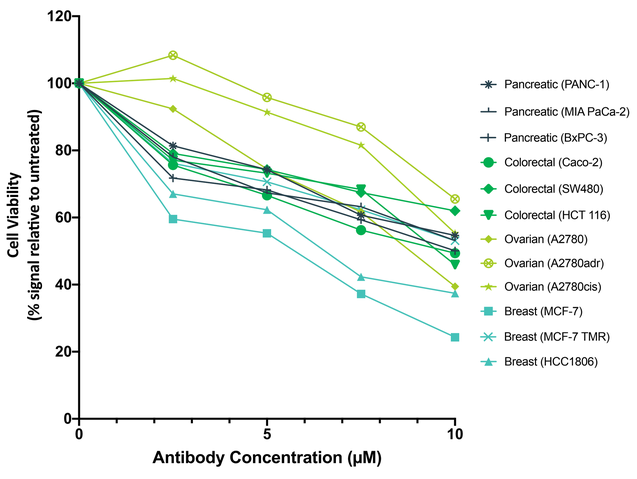

Figure 1. Anti-proliferative effect of MDX-124 in a panel of cancer cell lines.

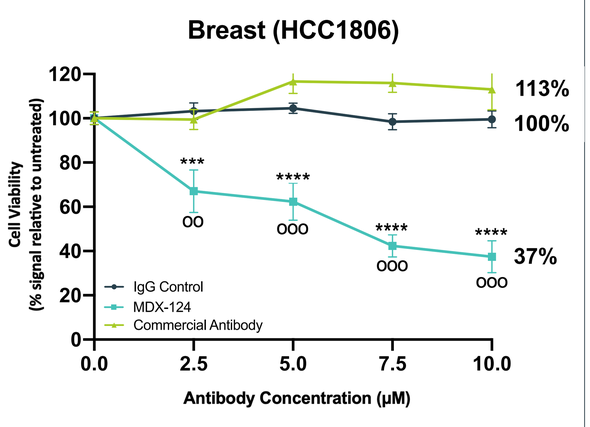

Figure 2. Anti-proliferative effect of MDX-124 in HCC1806 triple negative breast cancer cells.

RESULTS

MDX-124 has been assessed for binding affinity to ANXA1 by Biacore and found to have a KD of 3.98 nM. The antibody has demonstrated strong binding to ANXA1, but poor binding to the most structurally similar annexin protein (ANXA2) by ELISA, indicating specificity to the target molecule.

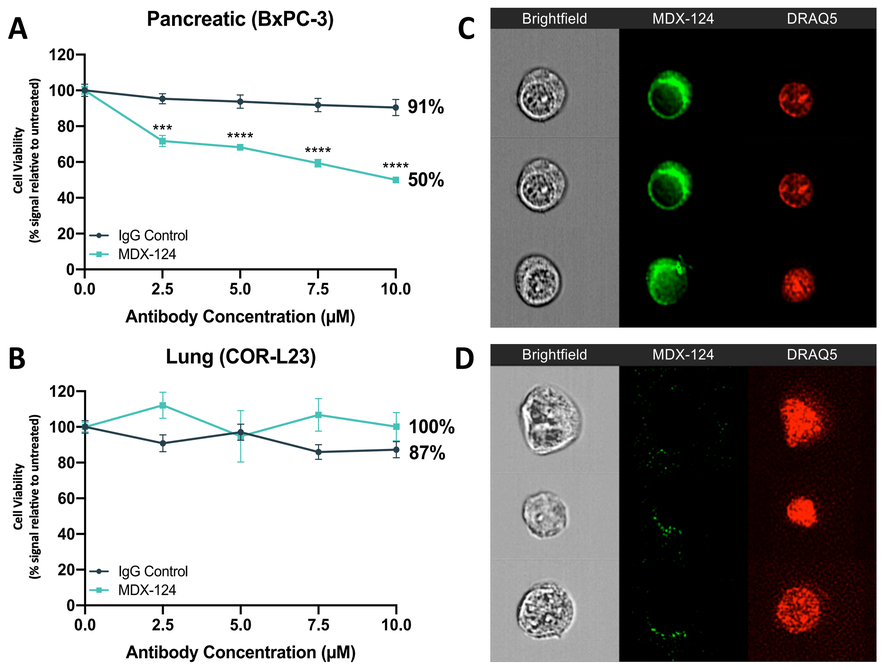

Figure 3. Anti-proliferative effect of MDX-124 correlates with annexin-A1 expression.

Imaging flow cytometry was used to quantitate the level of ANXA1 protein expression in different cellular compartments of cancer cells treated with MDX-124 (5 µM) for 72 hours. An MDX-124 sensitive cell line (Figure 3A) showed significant ANXA1 protein expression, particularly at the plasma membrane (Figure 3C). This was not observed in an MDX-124 refractory cell line (Figure 3B), which showed little to no observable ANXA1 protein expression (Figure 3D). These data indicate the anti-proliferative effect of MDX-124 correlated with levels of ANXA1 protein expression, particularly at the plasma membrane.

Further *in-vivo* studies evaluated MDX-124 (1 mg/kg) in the syngeneic, orthotopic, 4T1-luc triple-negative breast cancer model. 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 4).

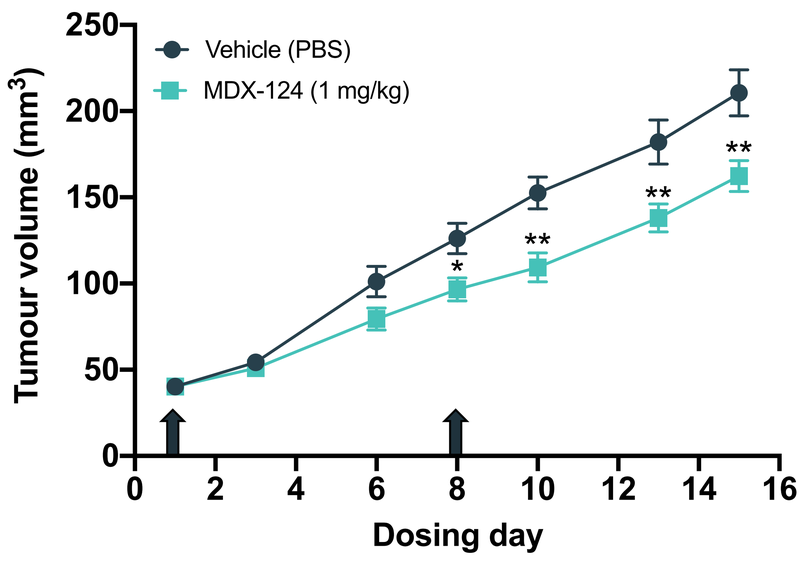

Figure 4. MDX-124 reduces 4T1-luc breast tumour growth. Arrows indicate day of dosing.

CONCLUSIONS

Our results demonstrate that MDX-124 can suppress cancer cell growth through targeted inhibition of ANXA1. This indicates that MDX-124 is a promising first-in-class monoclonal antibody therapeutic against cancers overexpressing ANXA1. Further studies are under way to understand the mechanism of action of MDX-124.

References: (1) Boudhraa *et al.,* (2016). *Clinical Science,* 130 (4): 205–220. **(2)** Tu *et al.,* (2017). *Pharmacological Research,* 119: 278–288. **(3)** Moraes *et al.,* (2018). *Cell Adhesion & Migration,* 12 (5), 417–423.

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