

MDX-124, a novel annexin-A1 antibody, induces an anti-tumor immune response and wide-ranging anti-cancer activity in multiple preclinical models

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INTRODUCTION

Annexin-A1 (ANXA1) is a calcium regulated, phospholipid-dependent membrane binding protein linked to several immunomodulatory and oncogenic processes. In response to a number of cell-activating or environmental stimuli, ANXA1 is secreted into the extracellular environment where it can activate formyl peptide receptors 1 and 2 (FPR1/2), in an autocrine or paracrine manner, triggering several oncogenic pathways. ANXA1 expression is often upregulated in cancerous tissue versus adjacent non-cancerous tissue and is associated with poor patient prognosis in many tumor types including colorectal, liver, pancreatic, triple negative breast, lung and gastric⁽¹⁻⁷⁾. The overexpression of ANXA1 by cancer cells increases cell proliferation⁽⁸⁾, angiogenesis⁽⁹⁾, migration and invasion⁽¹⁰⁾, and drug resistance⁽¹¹⁾. In addition, ANXA1 influences immune cells and the tumor microenvironment by enhancing regulatory T-cell function⁽¹²⁾, promoting the polarisation and activation of M2 tumor-associated macrophages⁽¹³⁾, suppressing dendritic cell activation and impairing CD8⁺ T-cell anti-tumor immunity⁽¹⁴⁾. We are exploring the potential of MDX-124, a novel humanized antibody targeting ANXA1, as a cancer therapeutic and here present data from several pre-clinical models.

METHODS

Cell Proliferation: A panel of human cancer cell lines was treated with MDX-124 (2.05 µg/mL - 2.05 mg/mL) for 72 h. Cell proliferation was measured via the CellTiter-Glo® assay (Promega). Dose-response curves were fitted using a non-linear regression model (GraphPad Prism 9.0).

Cell Cycle Analysis: HCC1806 cells (1x10⁴) were treated with MDX-124 (0-10 µM) for 72 h and analyzed by imaging flow cytometry (ImageStreamX®). Nuclear staining intensity (DRAQ5) of individual cells was captured and analyzed using IDEAS® software to estimate mean DNA content and provide a preliminary analysis of the G0/G1, S and G2/M phases of the cell cycle.

Migration: MDA-MB-231 cells (1x10⁶) were pre-treated with MDX-124 (0.5 µM – 50 mM) for 1 h prior to being transferred into 5µm pore polycarbonate transwell inserts and incubated for 24 h. Migrated and non-migrated cells were harvested and counted via flow cytometry (CytoFLEX LX).

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC): ADCC activity evaluated using an ADCC Reporter Bioassay (Promega). A549 lung cancer target cells (12.5 x10³ cells/well) were pre-treated with PMA (10 nM for 24 h) and dexamethasone (1 µM for 3 h) to stimulate externalisation of ANXA1 to the cell membrane and co-cultured with ADCC bioassay effector cells at E:T of 6:1, in the presence of MDX-124, IgG1 isotype control, or cetuximab for 6 h. ADCC activity measured via luciferase-based luminescence (RLU). Dose-response curves fitted using 4-parameter non-linear regression model (GraphPad Prism 8.0).

RESULTS

Incubation of MDX-124 with a human cancer cell line panel (n=151) covering all major histological and molecular subtypes, showed significantly reduced cancer cell growth in a dose-dependent manner in a number of cell lines including those of lung, pancreatic and ovarian origin (representative data shown in **Figure 1**).

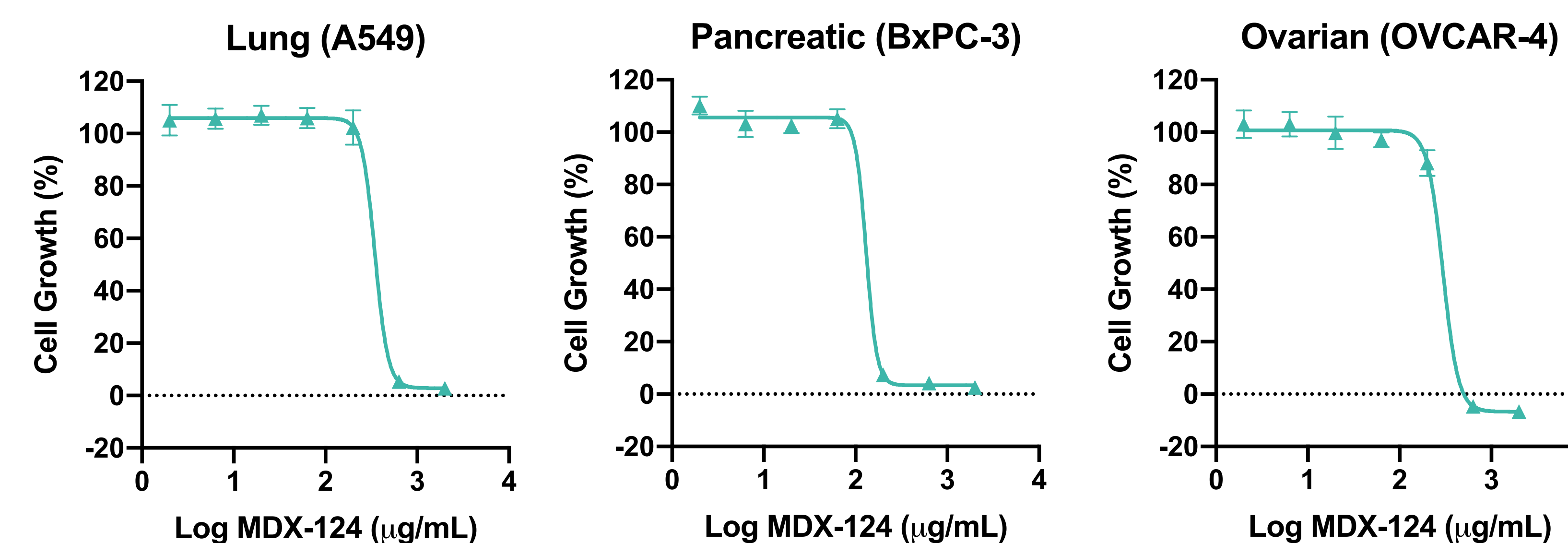


Figure 1. MDX-124 significantly reduces proliferation in multiple cancer cell lines.

Imaging flow cytometry analysis demonstrated a dose-dependent shift in DNA staining intensity in HCC1806 triple-negative breast cancer cells after 72 h treatment with MDX-124 (**Figure 2**). Cells were delayed in transitioning into the G2/M phase and are delayed in the late S/early G2 phase of the cell cycle suggesting that the anti-proliferative effect of MDX-124 involves disrupting the cell cycle. Using a transwell assay, it was shown MDX-124 was able to significantly inhibit the migration of MDA-MB-231 triple-negative breast cancer cells in a dose-dependent manner at concentrations >5 µM after 24 h (**Figure 3**).

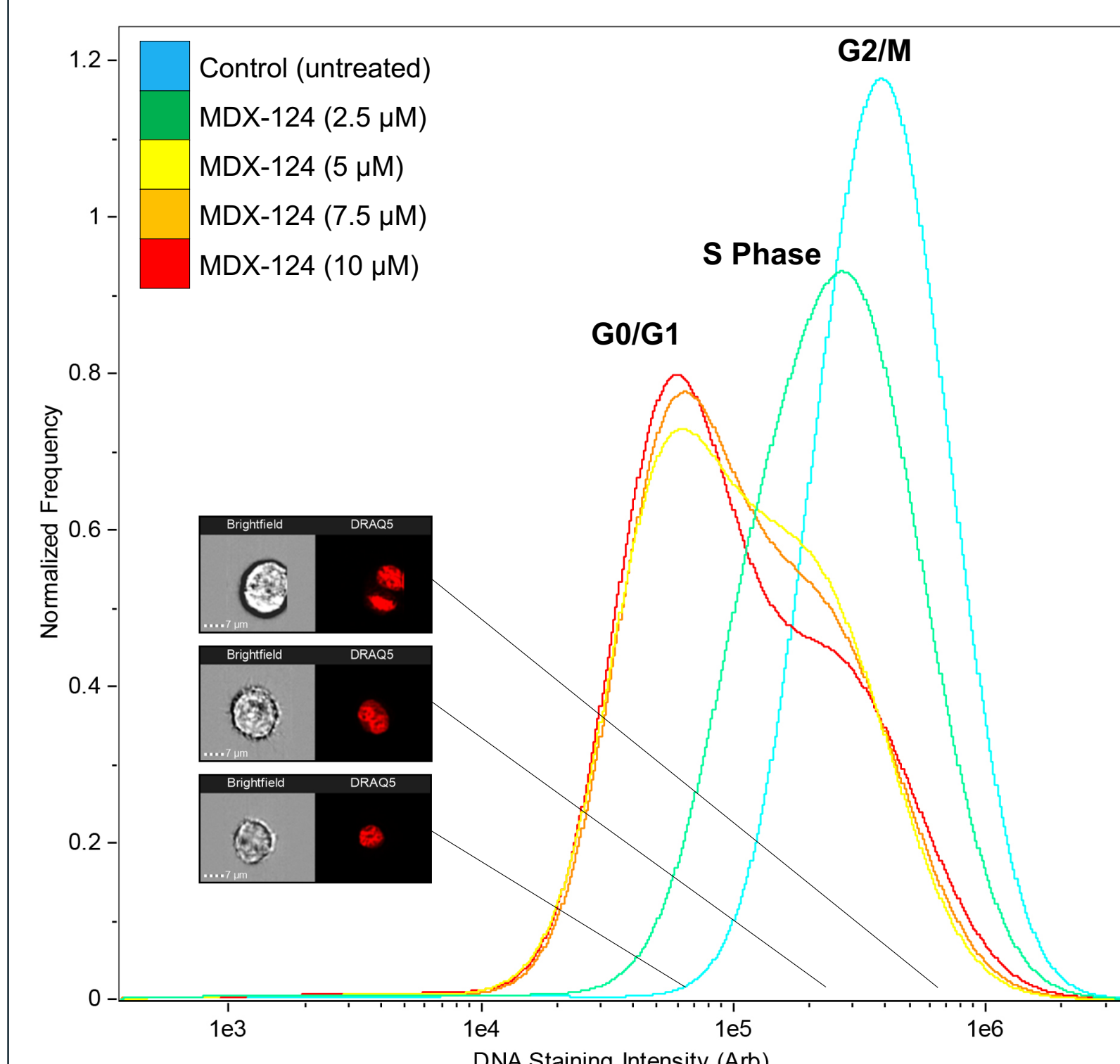


Figure 2. MDX-124 affects cell cycle transition in HCC1806 triple-negative breast cancer cells.

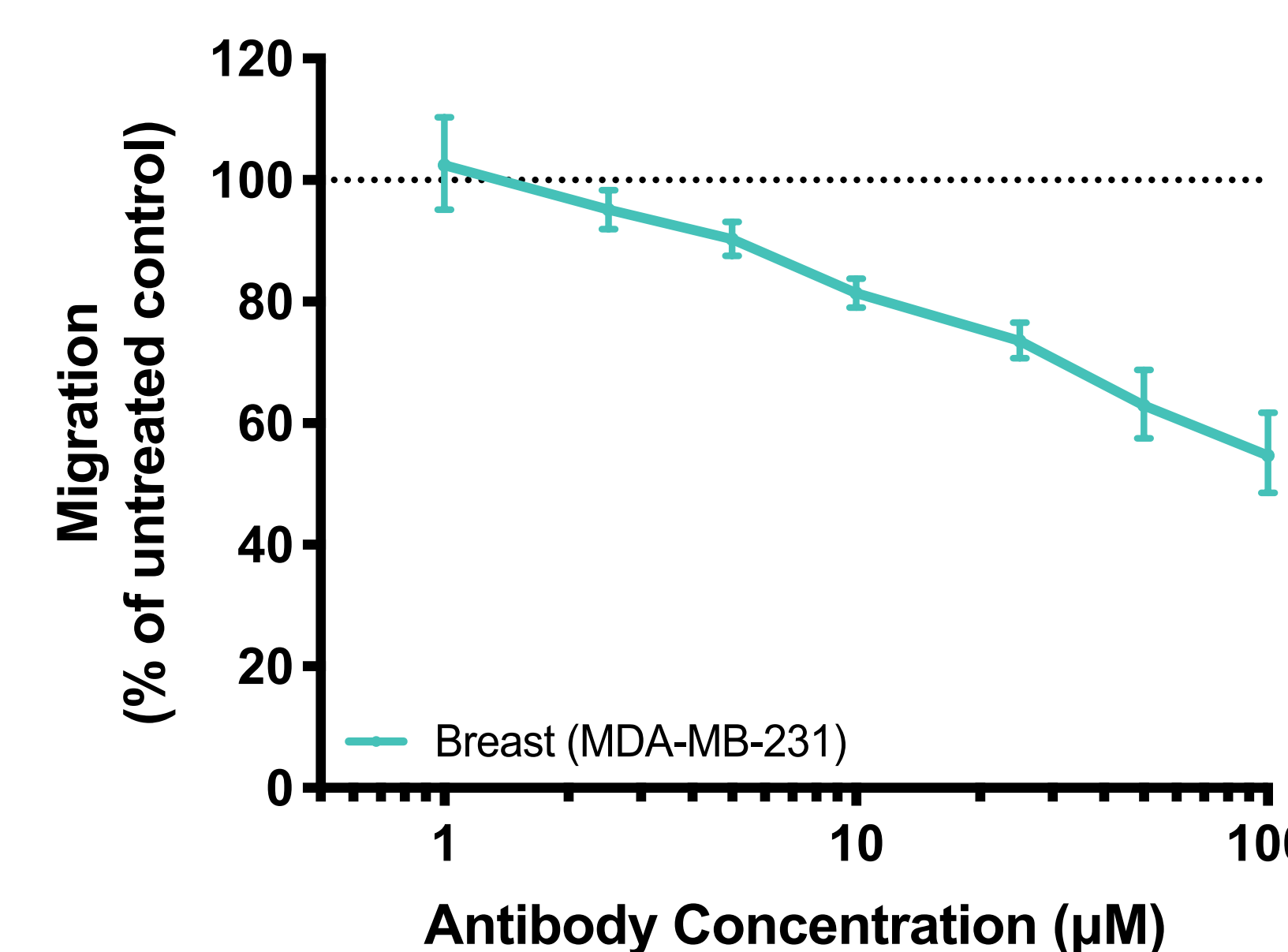


Figure 3. MDX-124 reduces migrative capacity of MDA-MB-231 triple-negative breast cancer cells.

RESULTS (continued)

Within a luminescence reporter bioassay, MDX-124 induced ADCC activity in a dose-dependent manner (EC₅₀ = 0.38 nM). This activity was similar to that observed with cetuximab, an antibody known to induce ADCC activity (assay positive control), whilst the IgG isotype negative control induced no effect (**Figure 4**). These data indicate that MDX-124 is likely to promote target cell lysis in ANXA1-expressing cancer cells.

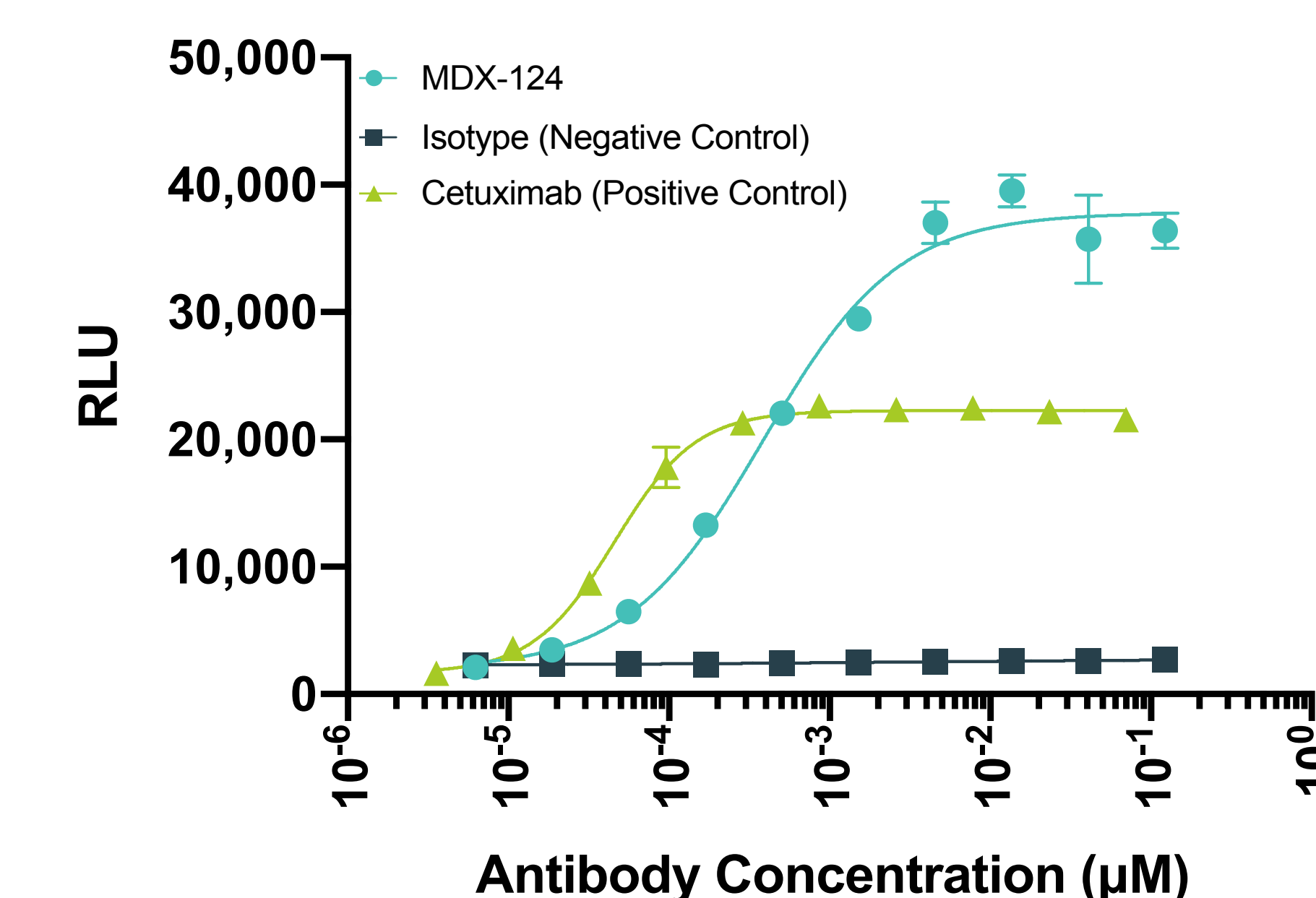


Figure 4. MDX-124 induces ADCC activity in A549 lung cancer cells.

CONCLUSION

In conclusion, MDX-124 binds to secreted and extracellular ANXA1 disrupting interactions with FPR1/2 and preventing the activation of downstream oncogenic processes that promote cancer cell proliferation and migration (**Figure 5**). Furthermore, MDX-124 induces ADCC activity by binding to ANXA1 overexpressed on the surface of cancer cells (**Figure 5**). MDX-124 therefore provides an innovative approach to cancer therapy. Studies are ongoing to further elucidate the mechanism of action of MDX-124. Medannex plan to initiate a First-In-Human study in 2021 evaluating MDX-124 in solid malignancies.

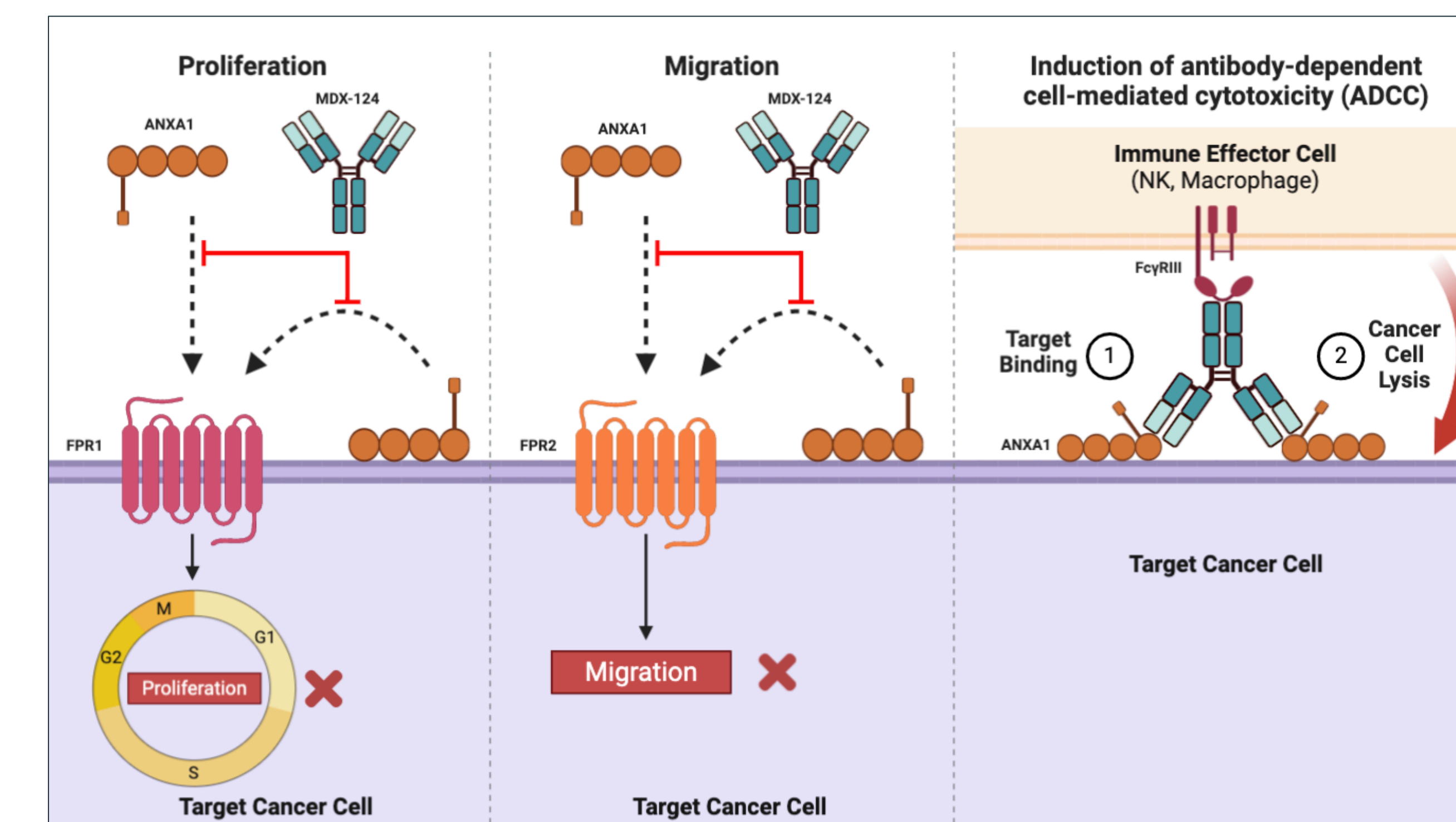


Figure 5. Mechanism of action summary for MDX-124.

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