Characterising the mode of action of a novel therapeutic antibody to treat a wide variety of human cancers.

A case study in commercial engagement in Life Sciences
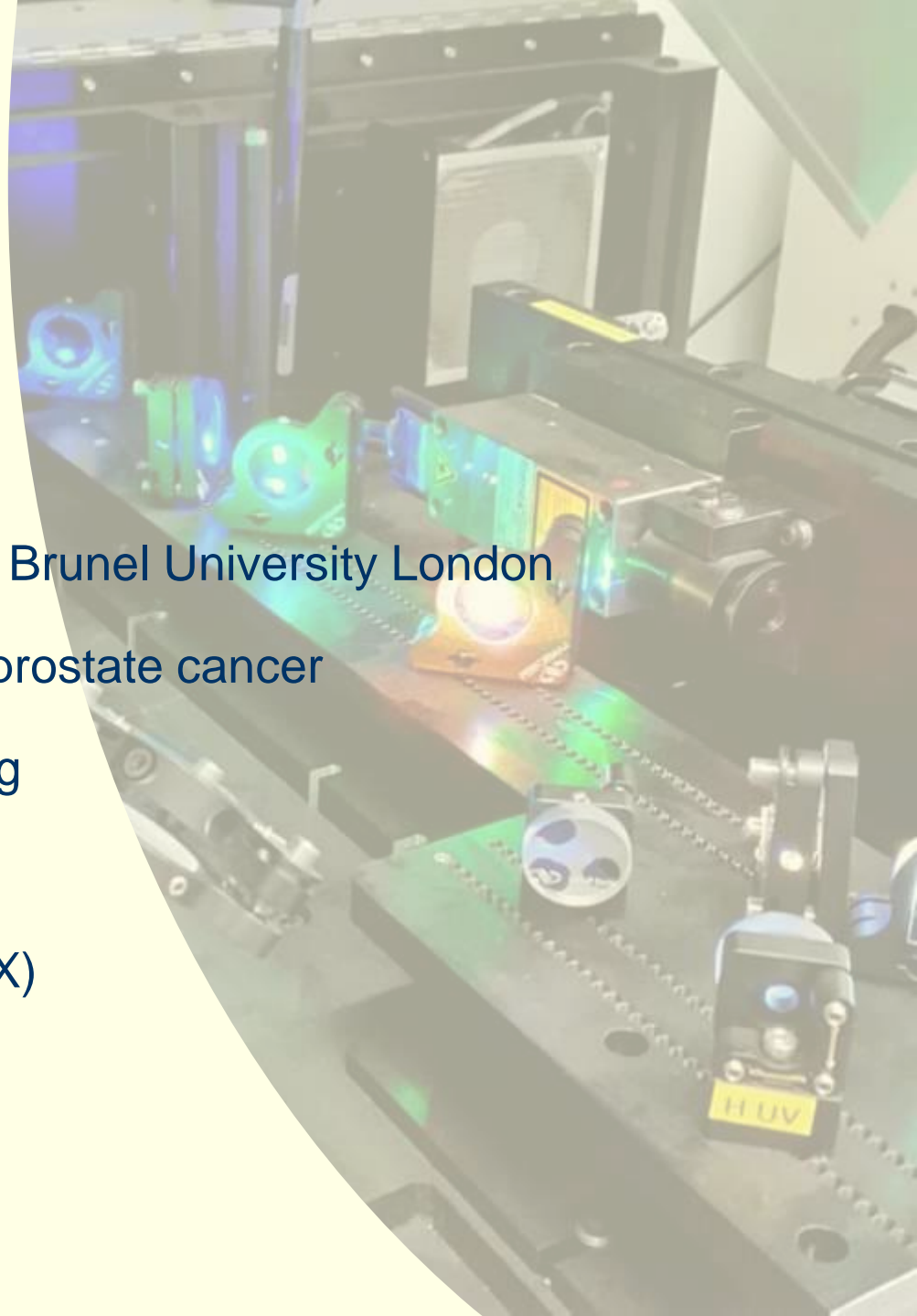
Dr Hussein Nahidh Al-Ali

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Background

- BSc – Biomedical Sciences, Brunel University London

- PhD – CTC identification in prostate cancer

- Immunofluorescence staining

    - EpCAM, CD45, TJ

- Imaging Flow Cytometry (ISX)

Research at ARU

- Commercially sponsored

- MedAnnex Ltd.

  - Biotechnology company based in Edinburgh

  - Specialising in the development of immunomodulatory antibodies

  - Autoimmune diseases

  - Collaboration began through clinical links Chris had developed prior to ARU

  - Other collaborators: University of Glasgow and UCL

- Module leader: Molecular Genetics and Bioinformatics

MDX-124 (Humanised mouse IgG)

- Targets the ANXA1 protein which is associated with mediating immune responses
    - Binds to the antigen's N-terminal domain
- ANXA1 is found to be deregulated in a variety of human cancers
- Linked with:
    - Invasion
    - Metastasis
    - Angiogenesis
    - DNA repair
    - Cell cycle regulation

Aim of research

- MDX-124 currently under investigation as a treatment for autoimmune diseases (e.g. Systemic Lupus Erythematosus)
    - Request to explore its anticancer effect
- Research plan agreed to investigate:
    - Cytotoxicity
    - Antibody localisation
    - Antigen expression
   - Mechanism of cell kill

Cytotoxicity studies

- MTT cell survival assays

- Cancers (cell lines) examined:

  - **Breast**: MCF7, MCF7-TAMR7, HCC1806

  - **Colon**: HCT116, CACO-2, SW480, LoVo, SW1463, HT29

  - **Lung**: H69, NCI-H69, COR-L23, COR-L23/5010

  - **Pancreatic**: Mia PaCa, BxPC-3, PANC-1

  - **Ovarian**: A2780, A2780 adrR, A2780 cisR

- Comparison with:

  - Isotype control antibody (IgG)

  - Polyclonal commercial variant (N-terminus binding)

    - Costs £40,000

    - Limited number of experiments conducted
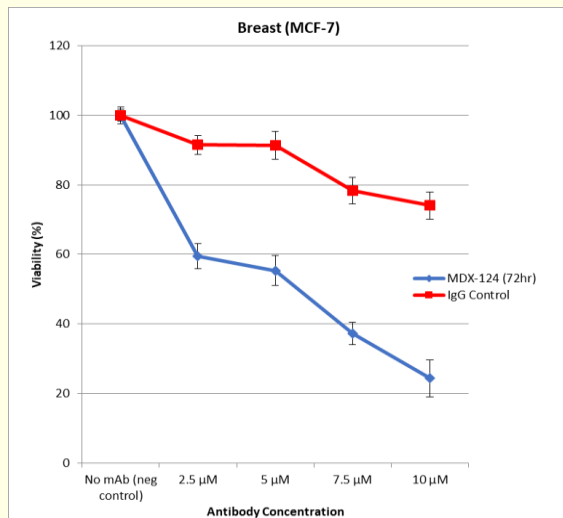   (30 mgs after purification)

MTT assay design

- Cells plated based on their growth properties

- They were exposed to 2.5, 5, 7.5 and 10µM of the MDX-124

- Cell were incubated for 3 days with the antibody

- This was repeated for IgG control and the commercial antibody (n=3)

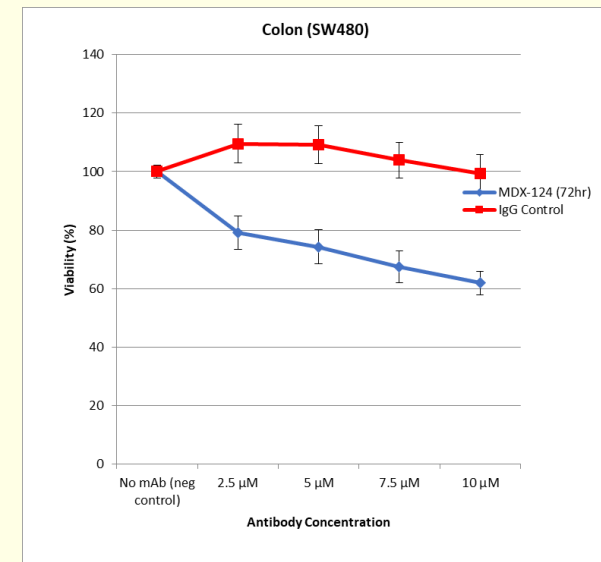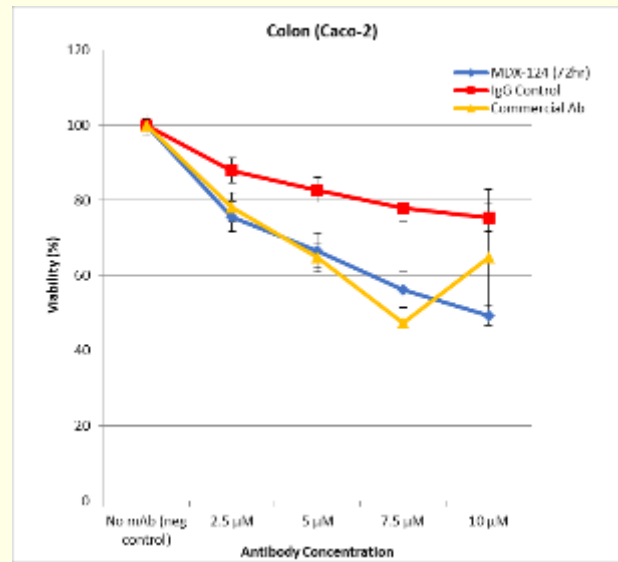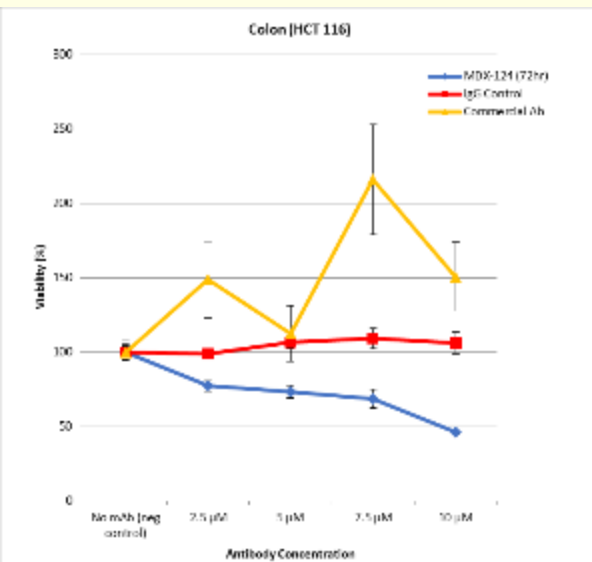- Commercial antibody is polyclonal, targeting the N-Terminus
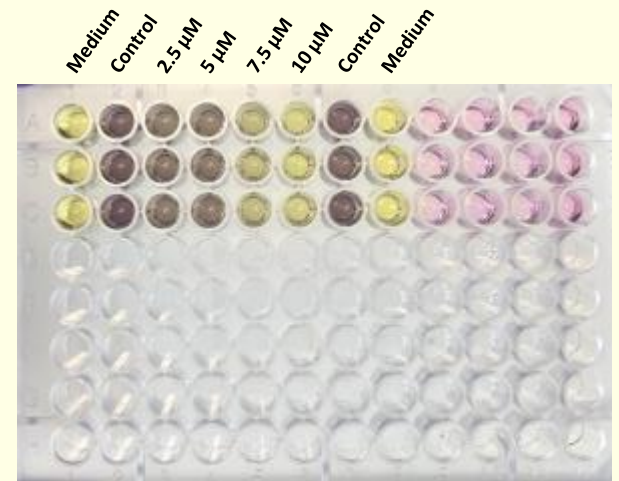
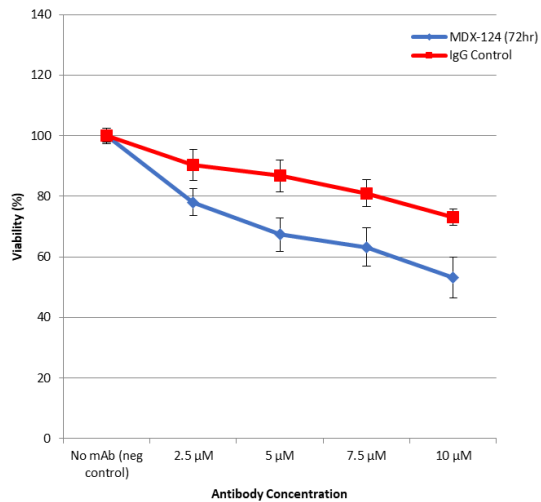# Breast Cancer cell lines results
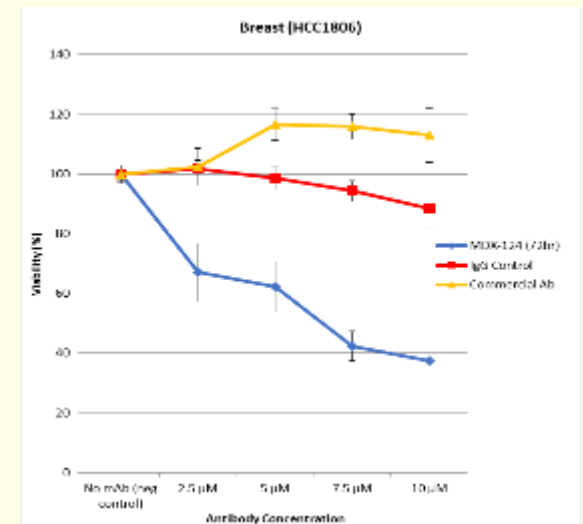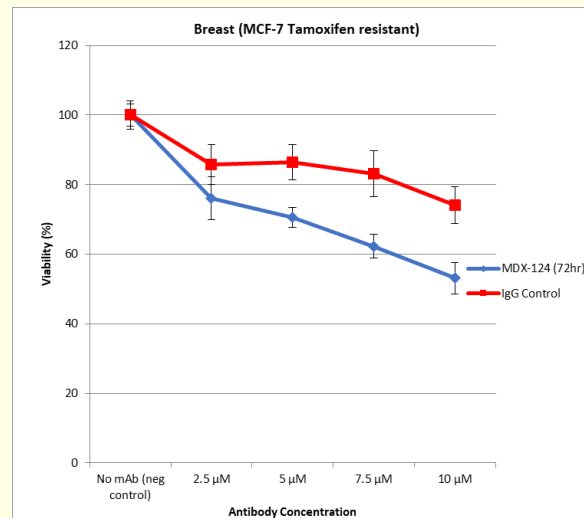
Ovarian Cancer cell lines results
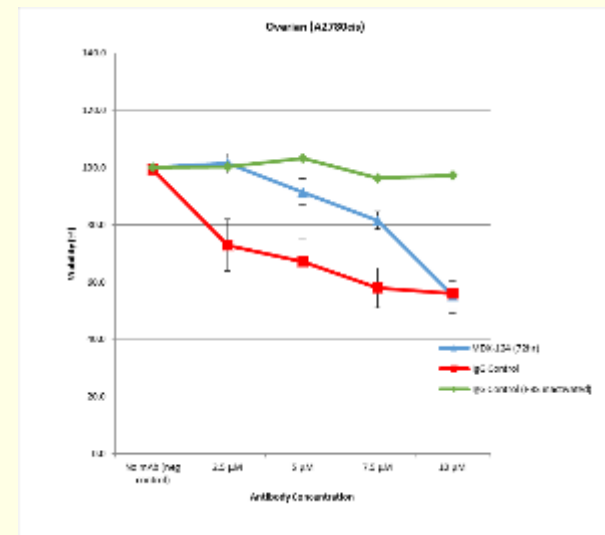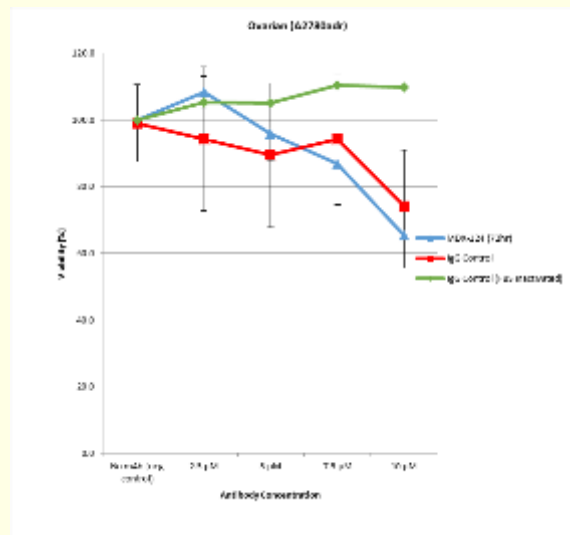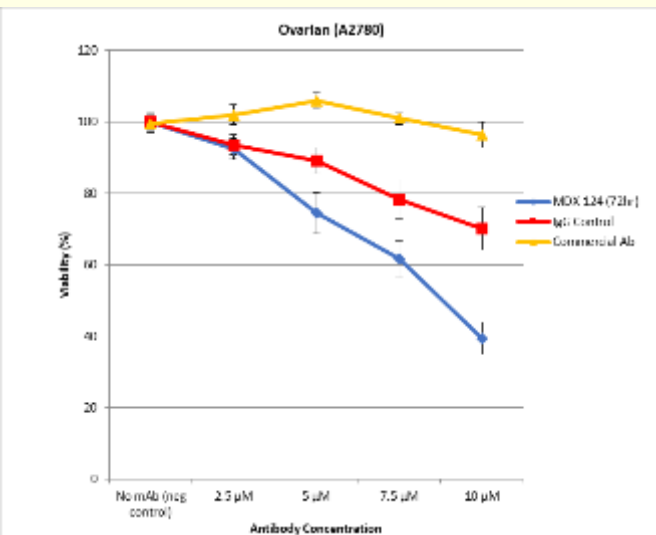
Colon cancer cell lines results

Pancreatic Cancer cell lines results
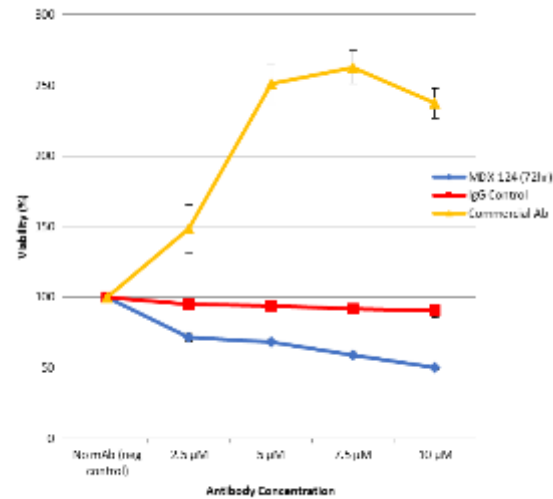
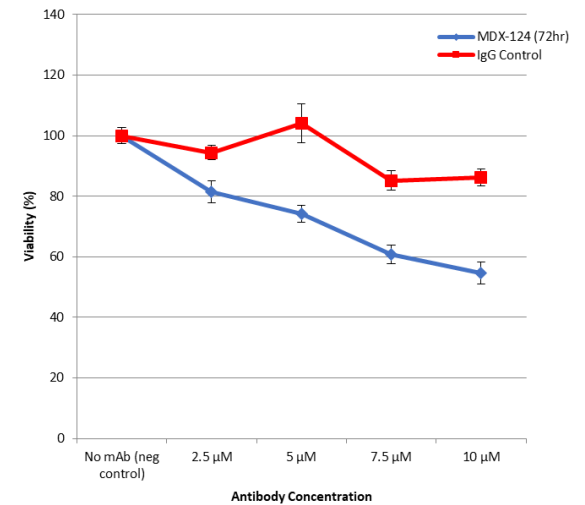# Lung Cancer cell lines results
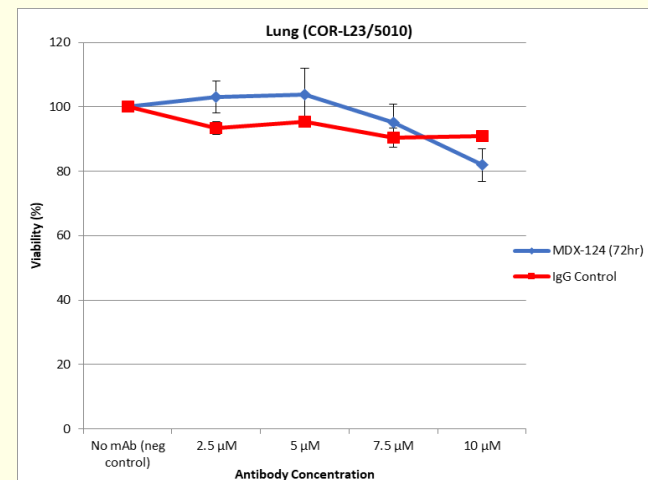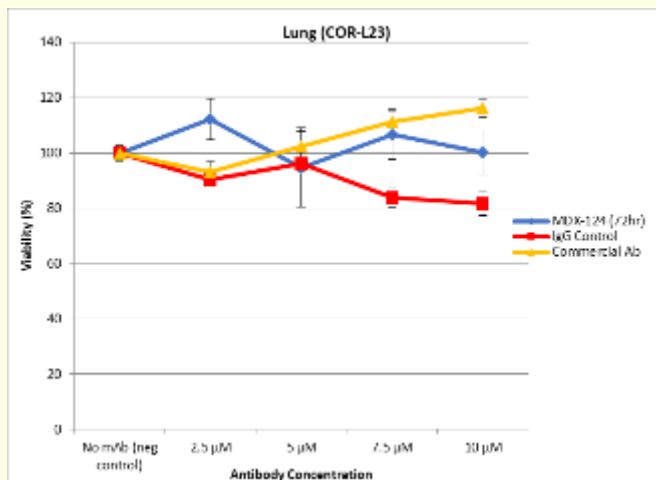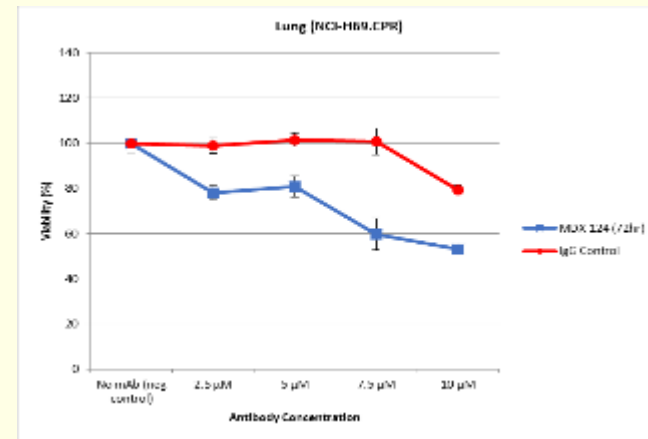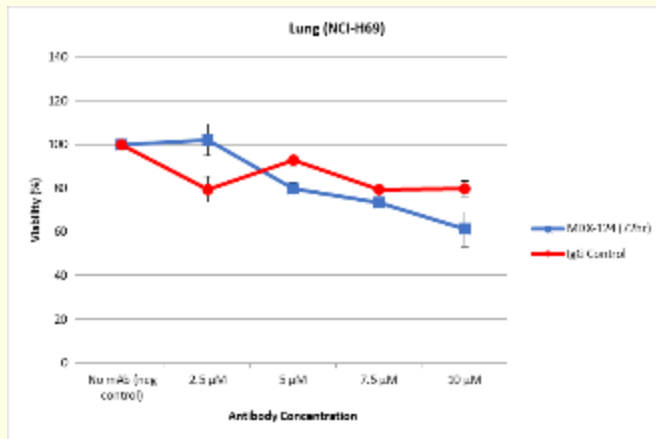
Summary of MTT experimentation

	Indication		Chart Data - Viability (MDX-124)					Experiment Log		
								MDX-124	IgG Contro	Comm
Cancer Type	**Cell Line**	**No mAb (neg control**	**2.5 µM**	**5 µM**	**7.5 µM**	**10 µM**	**n = 72hr**	**n = 72hr**	**n =**
Breast	MCF-7	100	59.5	55.3	37.3	24.4	3	5	
Breast	MCF-7 tamoxifen resistan	100	76	71	62	53	3	3	
Breast	HCC1806	100	67.1	62.3	42.3	37.4	3	4	
Ovarian	A2780	100	92	74	62	39	3*	3	
Ovarian	A2780cis	100	101.4	91.4	81.6	55.3	3*	?	
Ovarian	A2780adr	100	108.4	95.8	86.9	65.4	3*	?	
Lung	COR-L23	100	112	95	107	100	3**	4	
Lung	COR-L23.5010	100	103	104	95	82	3	3	
Lung	NCI-H69	100	102	80	74	61	3	3	
Lung	NCI-H69 CPR	100	78	81	60	53	3	3	
Colorectal	Caco-2	100	76	67	56	49	3	4	
Colorectal	HCT 116	100	77	73	68	46	3	3	
Colorectal	SW480	100	79	74	67	62	3	3	
Pancreatic	MIA PaCa-2	100	78	67	63	53	3	3	
Pancreatic	BxPC-3	100	72	68	59	50	3	3	
Pancreatic	PANC-1	100	81	74	61	55	3	3	

Summary of all indications
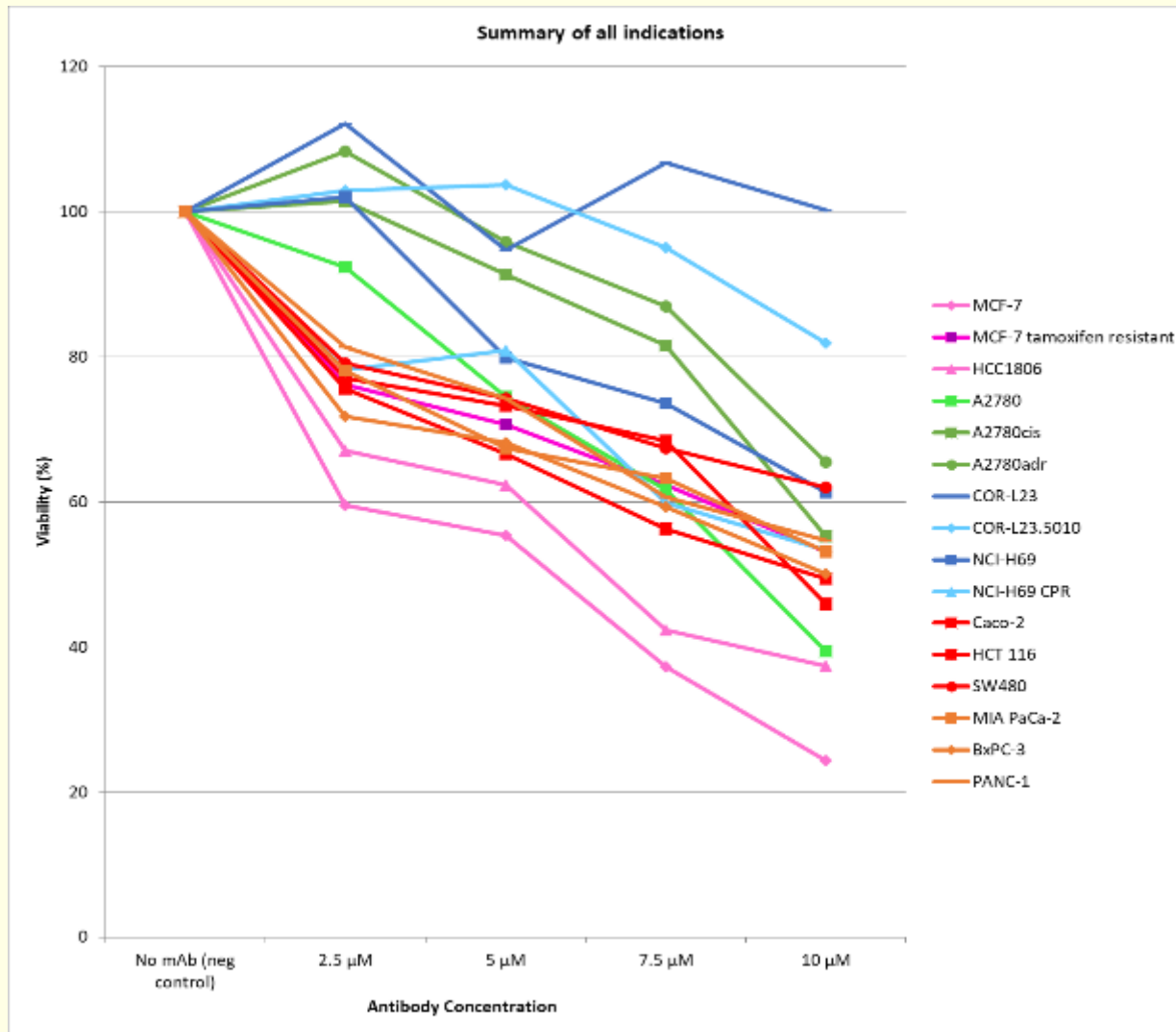
Cell Titer-Glo validation

- Luminescence based cell survival assay

- Relies on the quantification of ATP in viable cells

- Few experiments conducted to validate the MTT data.

HCC1806 response to IgG and MDX-124
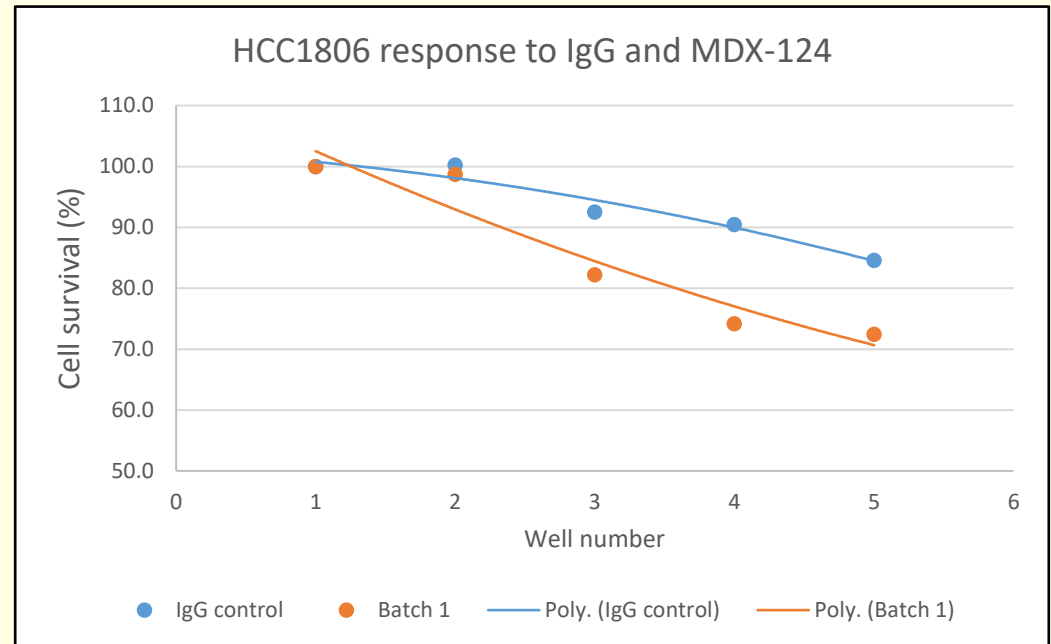
MTT experiments summary

- The MDX-124 antibody caused cell death in:
  - Breast
  - Ovarian
  - Colon and
  - Pancreatic cancer cell lines

- The lung cancer cell lines were not as responsive and relatively resistant to MDX-124.

- With the exception of the lung cancer cell lines, MDX-124 significantly reduced the survival of cancer cells when compared to isotype control antibody.
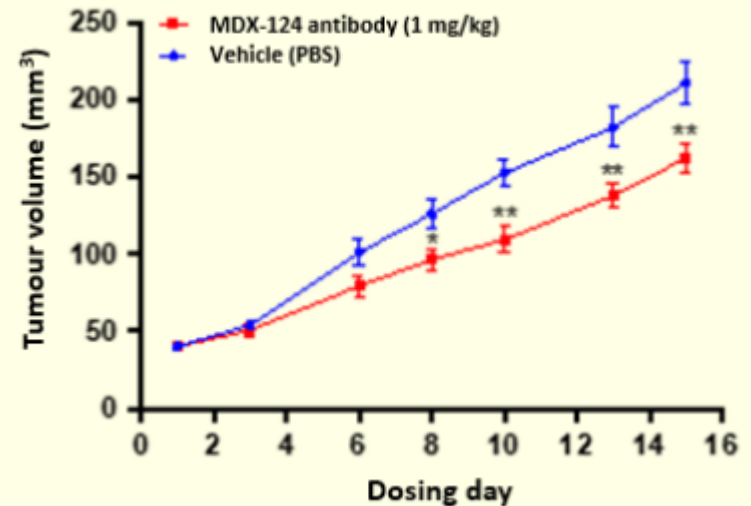
Does the antibody have an anticancer effect in *in vivo*?

Tumour growth *in vivo* study

- MDX-124 data in a breast cancer model in mice

- Proof-of-concept in orthotopic mouse model

- Statistically significant reduction in tumour growth with MDX-124

- Maybe greater effect if dose is increased? More work required – but encouraging initial results.

Antibody reduces the rate of increase in tumor volume compared to vehicle control. Each data point presented as S.E.M. with significance (Welch's T-test) indicated by * p<0.05 and ** p<0.01. (MedAnnex – Crown Bioscience)

Cellular localisation studies

- Using imaging flow cytometry

- ImageStreamX Mark II (Amnis®)

- Previous work include:

    - The PARP-1 inhibitor Olaparib suppresses BRCA1 protein levels, increases apoptosis and causes radiation hypersensitivity in BRCA1 +/- lymphoblastoid cells (Parris, et al. 2017)

    - Enhanced γ-H2AX DNA damage foci detection using multi-magnification and extended depth of field in imaging flow cytometry (Al-Ali et al. 2015)

    - Hypersensitivity of BRCA1 Heterozygote Lymphoblastoid Cells to Gamma Radiation and PARP Inhibitors (Bourton, et al. 2013)

What is Imaging flow cytometry?

- ISX Mark II combines the capabilities of flow cytometry and microscopy

    - Images can be captured at a rate of up to 1000 objects/second

    - Provides up to 250 analysis features

- Previously used to characterise CTCs
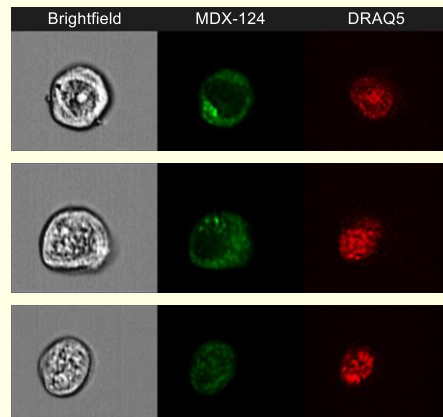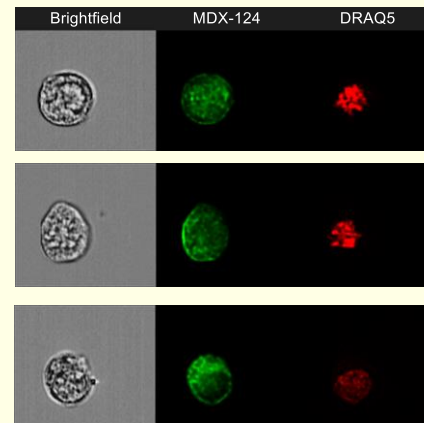
- DNA repair analysis

PC3

PBL

Live cell analysis of a prostate cancer patient sample
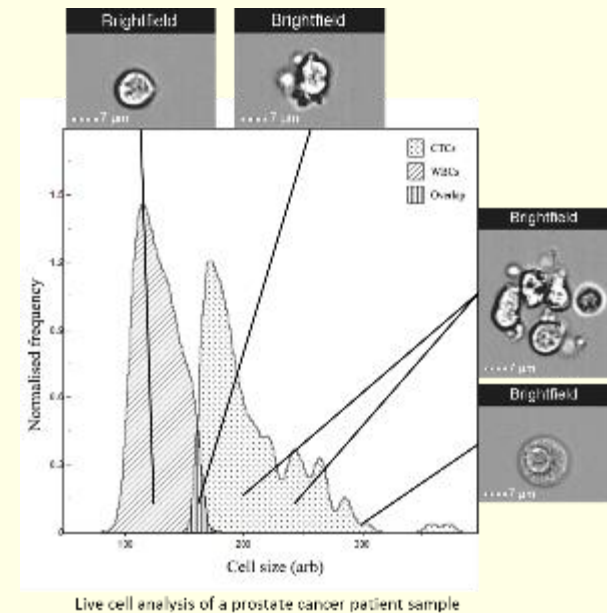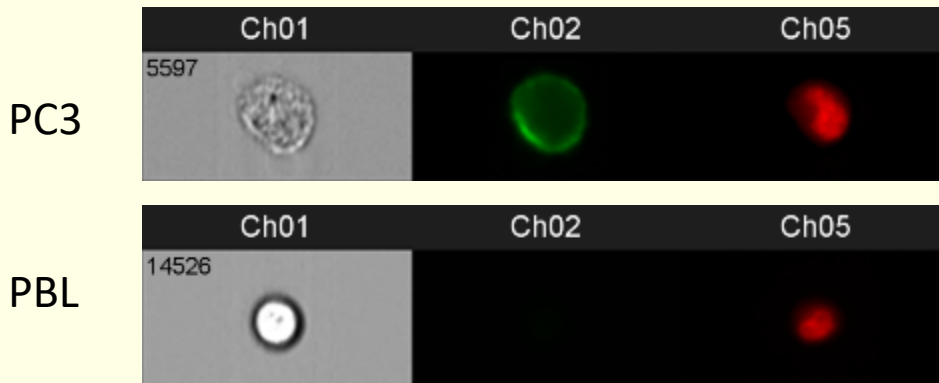
Hypothesis

Cell lines exhibiting a dramatic reduction in cell survival will display high antibody binding compared to those displaying little or no cell kill.
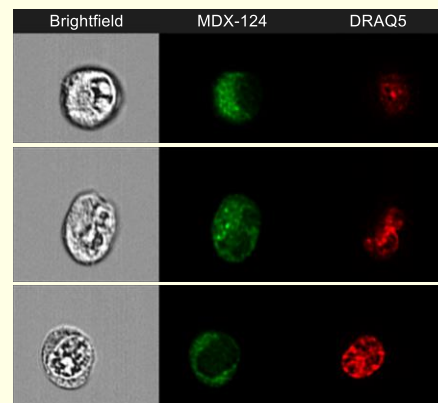
Breast Cancers data

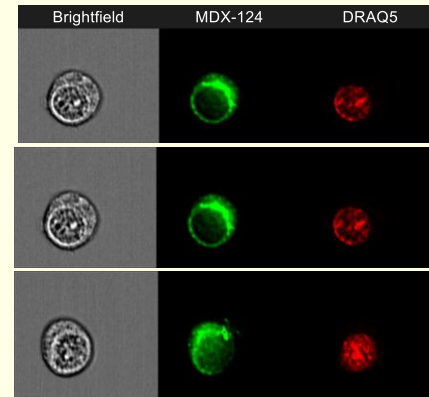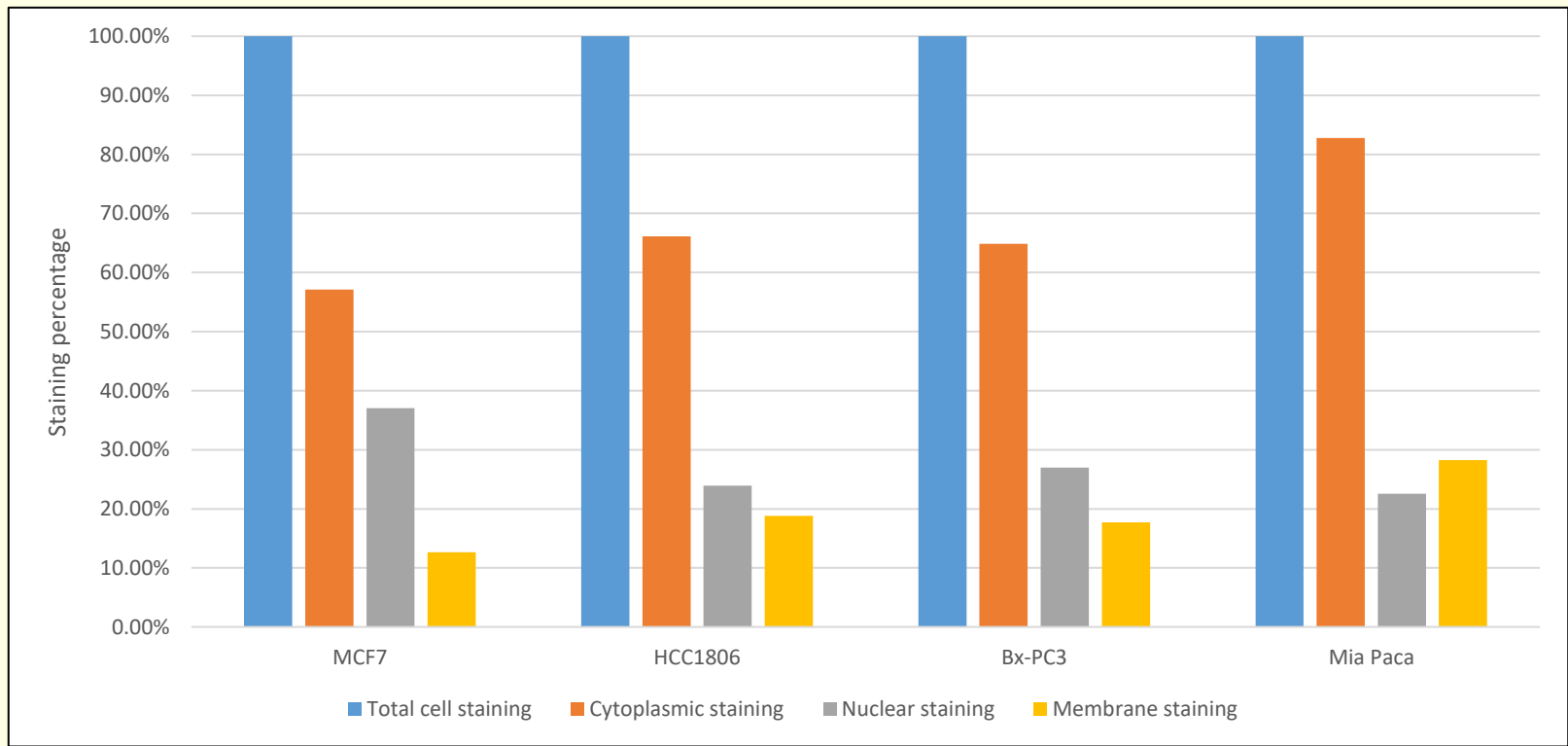**MCF7**   **HCC1806**

Pancreatic Cancers data
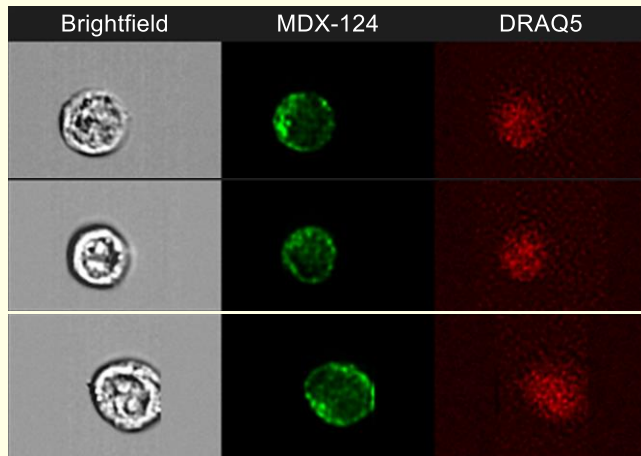
Mia PaCa

BxPC-3

MDX-124 staining intensity and distribution

Ovarian Cancers data

A2780

| Brightfield | MDX-124 | DRAQ5 |

A2780 adrR

| Brightfield | MDX-124 | DRAQ5 |

A2780 cisR

| Brightfield | MDX-124 | DRAQ5 |
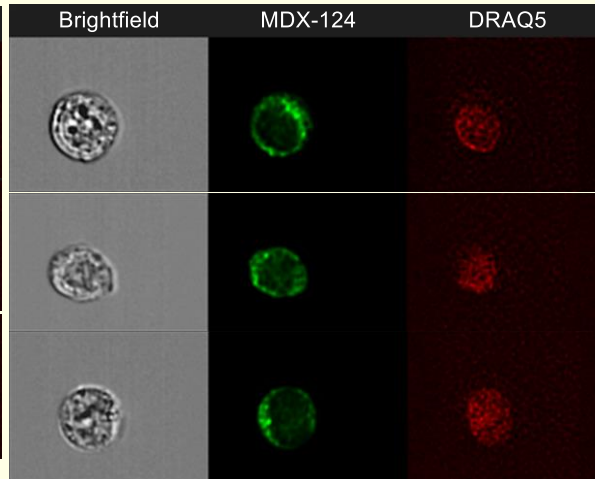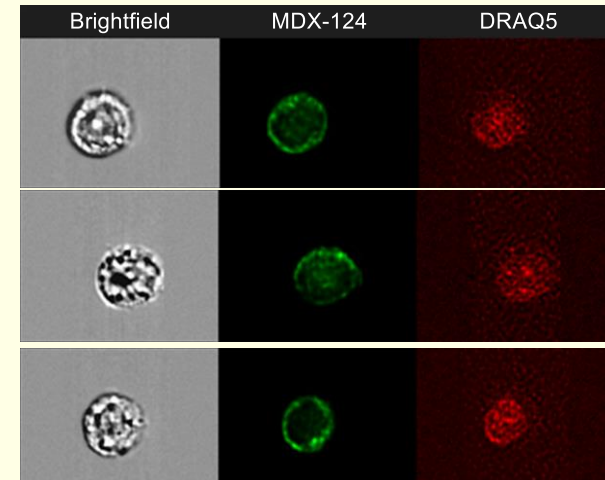
Colon Cancer data

CACO-2

| Brightfield | MDX-124 | DRAQ5 |
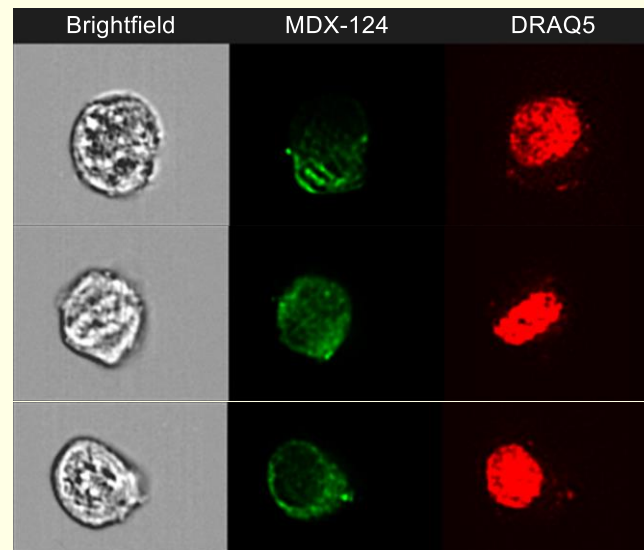
Lung Cancers data

COR-L23
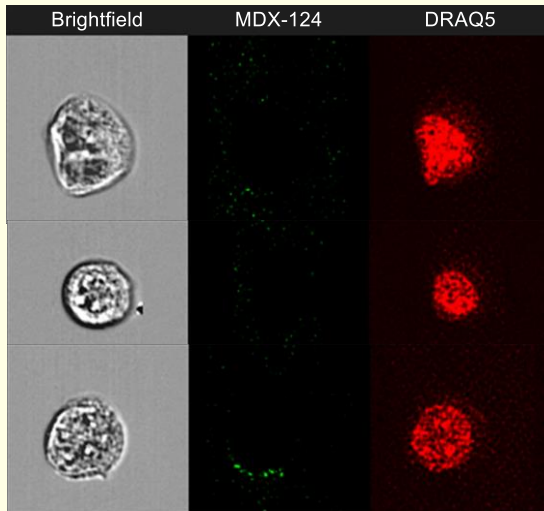
COR-L23/5010
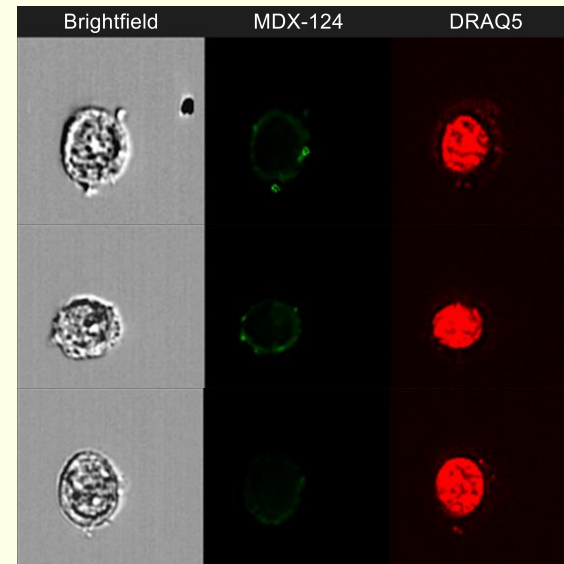
Breast vs Lung
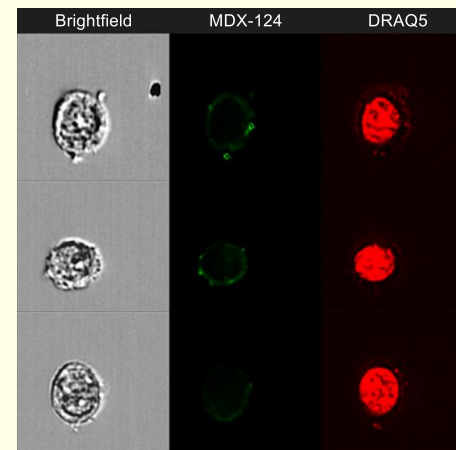
MCF7
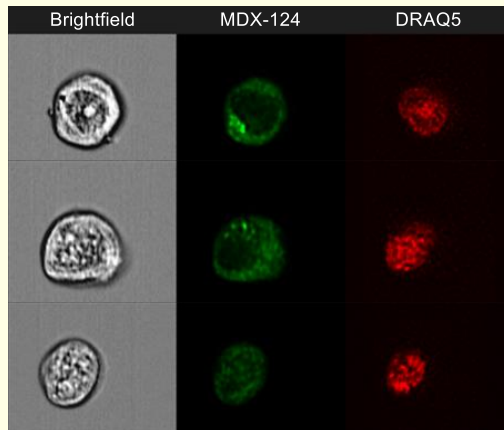
COR-L23
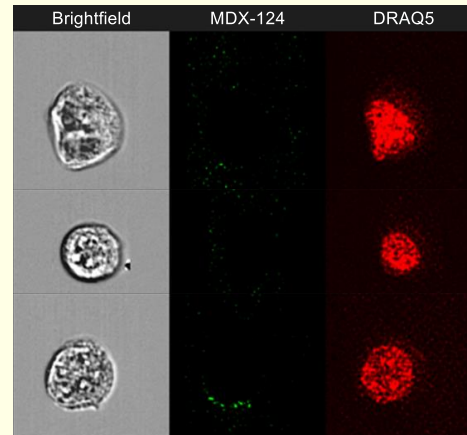
HCC1806
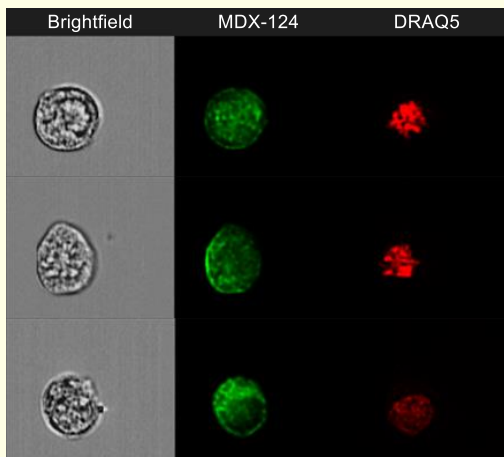

COR-L23/5010

Mechanism of cell kill

- Potential areas to explore:
    - Apoptosis
    - Cell cycle inhibition
    - Cell signalling pathways
    - DNA repair modulation
    - Angiogenesis inhibition?

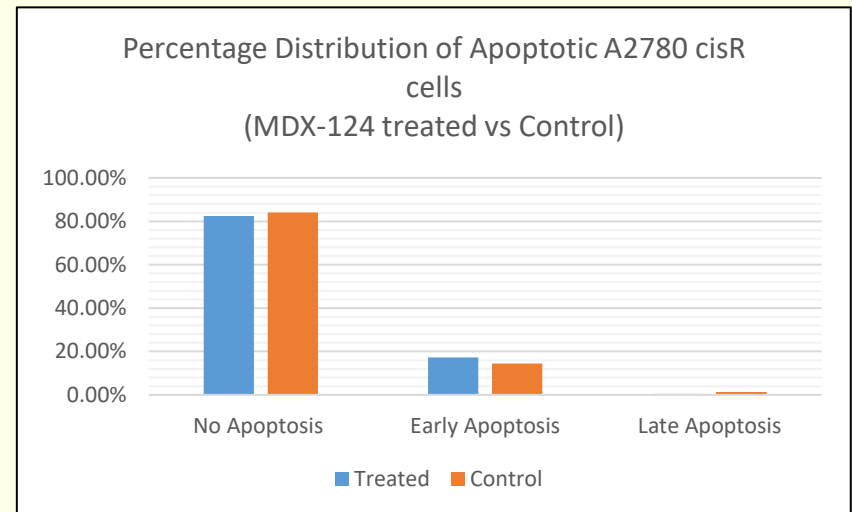- Can this help us enhance the cytotoxic effect of the antibody?

Apoptosis: Annexin V expression

- Phosphatidylserine is expressed on surface of apoptotic cells

- Binds to Annexin V. Membrane staining – early apoptosis

- Cytoplasmic staining – late apoptosis

  - Due to loss of membrane integrity

© 2015 Abcam plc.
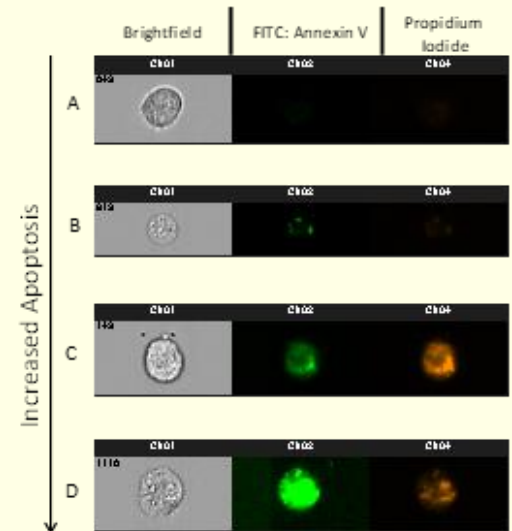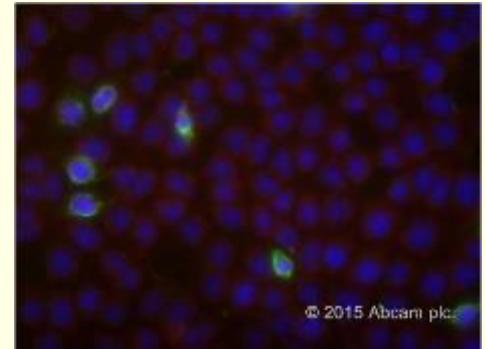
Apoptosis results

Percentage Distribution of Apoptotic MCF7 cells (MDX-124 treated vs Control)

Percentage Distribution of Apoptotic A2780 cells (MDX-124 treated vs Control)

Percentage Distribution of Apoptotic A2780 cisR cells (MDX-124 treated vs Control)
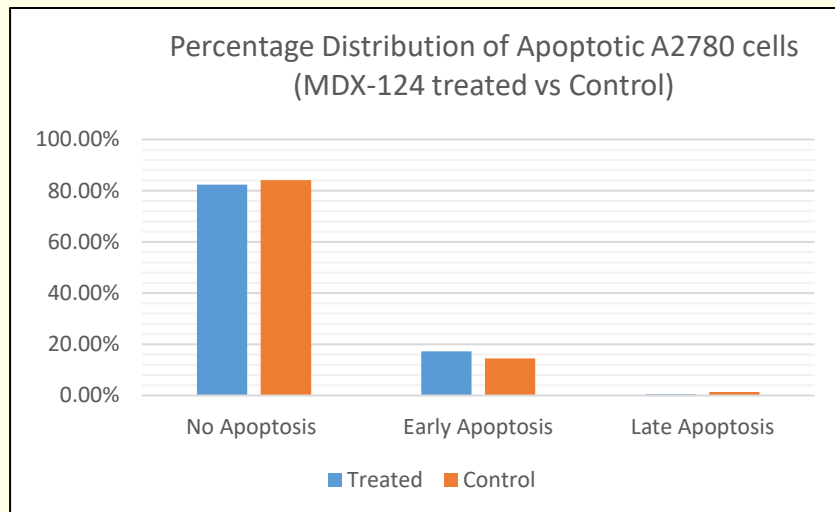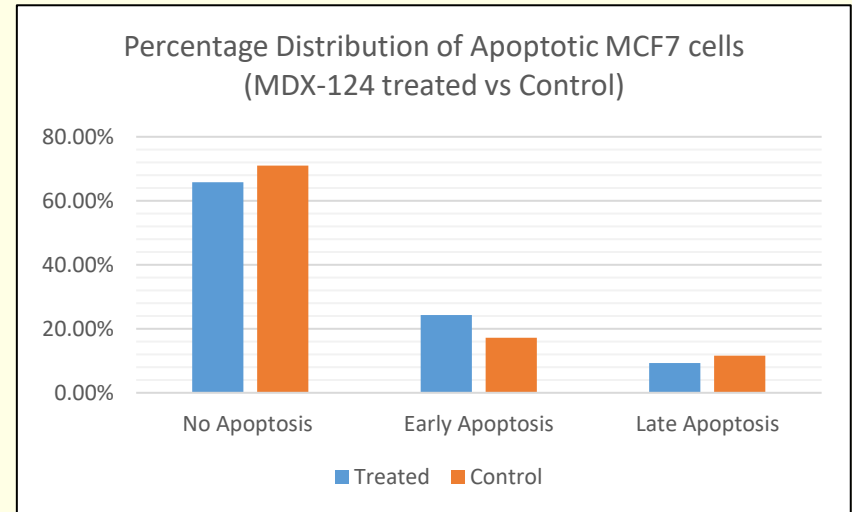
Percentage distribution of Apoptotic CACO-2 cells (MDX-124 treated vs Control)

Percentage of Apoptotic Mia PaCa cells (MDX-124 treated vs Control)
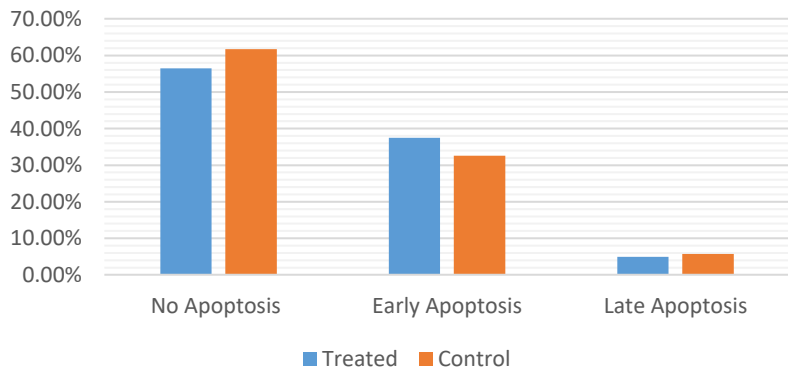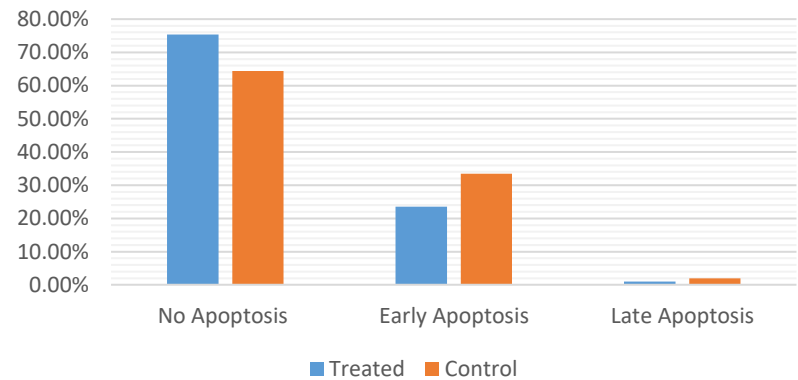
Percentage Distribution of Apoptotic H69 cells (MDX-124 treated vs Control)
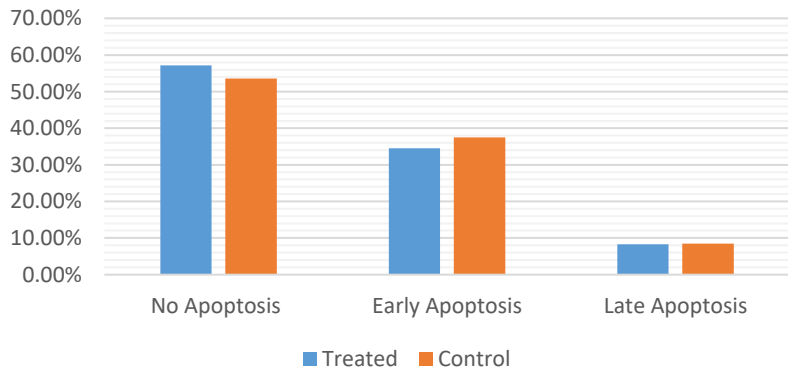
Percentage Distribution of Apoptotic H69-CPR cells (MDX-124 treated vs Control)
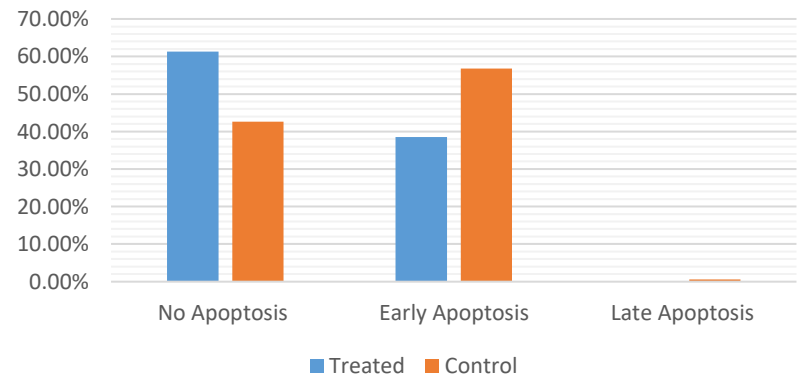
Cell Cycle Inhibition

- Is there a delay in transition through cell cycle?

- Can the MDX-124 cause a cell cycle arrest?

- And if so, will increasing the antibody concentration cause a further delay in the cycle?
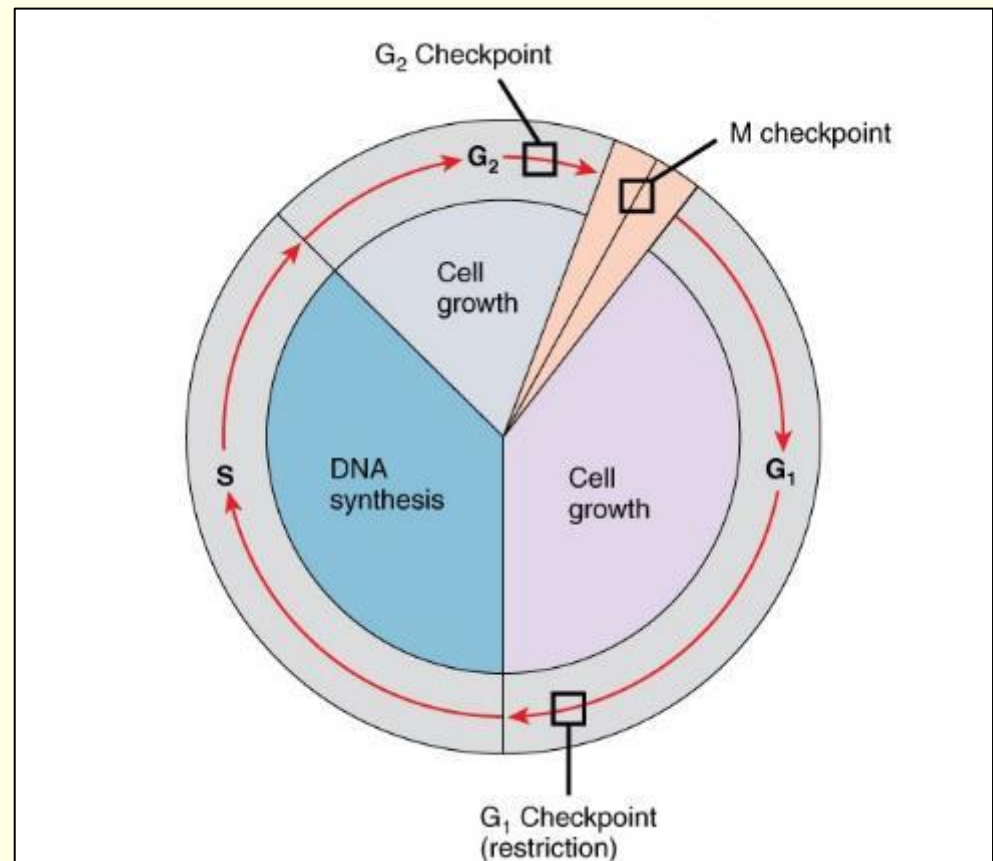
Pancreatic Cancer
(Mia PaCa)

DNA content intensity (arb) comparison between Mi PaCa cells (MDX-124 treated vs Control)

Breast Cancer (HCC1806)

DNA content intensity (arb) comparison between HCC1806 cells (MDX-124 treated vs Control)

Legend:
MDX-124 (10 µM)
MDX-124 (5 µM)
Untreated Control

Normalized Frequency

Mean DNA content intensity
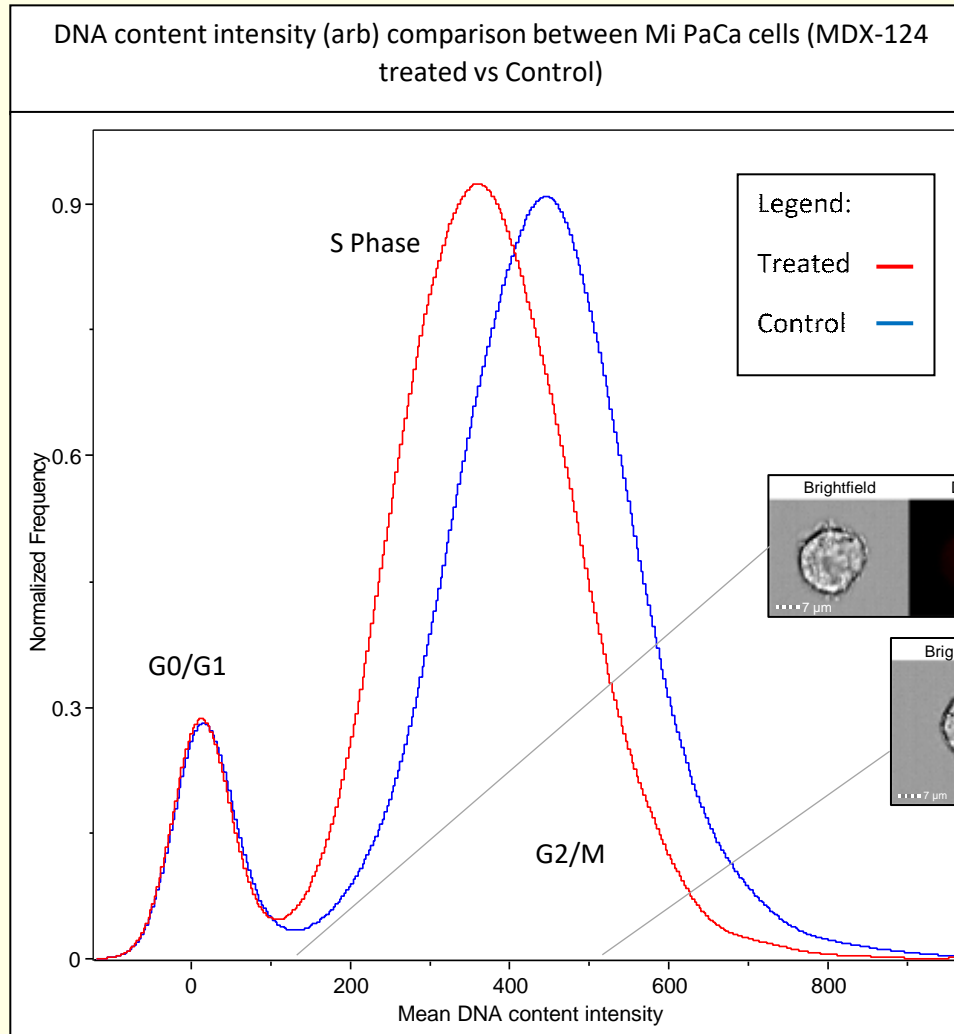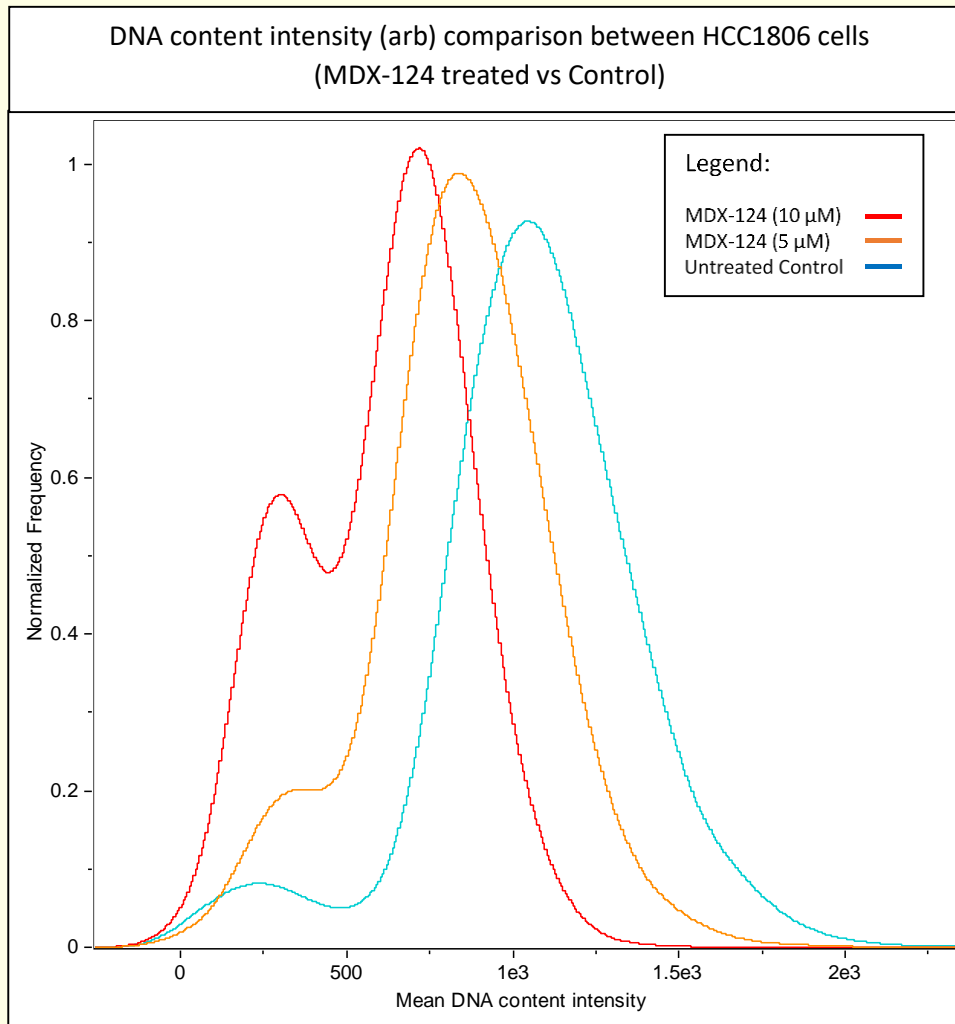
Breast Cancer (HCC1806)
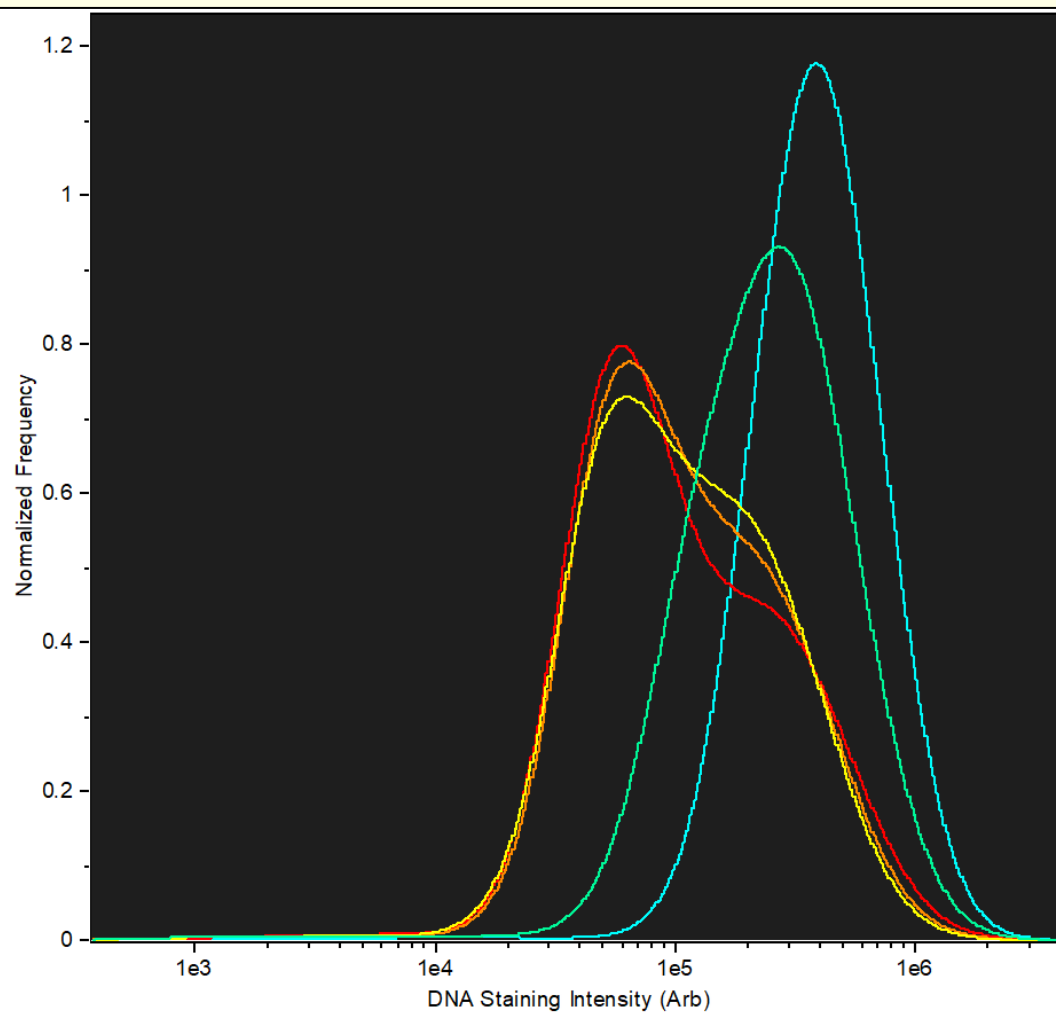
DNA Staining Intensity (Arb)

Population	Count	Mean	Median	Std. Dev.	Geo. Mean	
2.5uM MDX-124	10000	299747.26	251885.59	207353.73	236549.21	
5uM MDX-124	10000	153682.29	98801.64	146246.95	105059.22	
7.5uM MDX-124	10000	158115.29	94337.55	157921.68	106497.97	
10uM MDX-124	10000	165359.19	86829.11	181904.9	104521.18	
Control	10000	433336.02	389456.79	225757.89	375136.58	

DNA content intensity (arb) comparison between H69-CPR cells (MDX-124 treated vs Control)
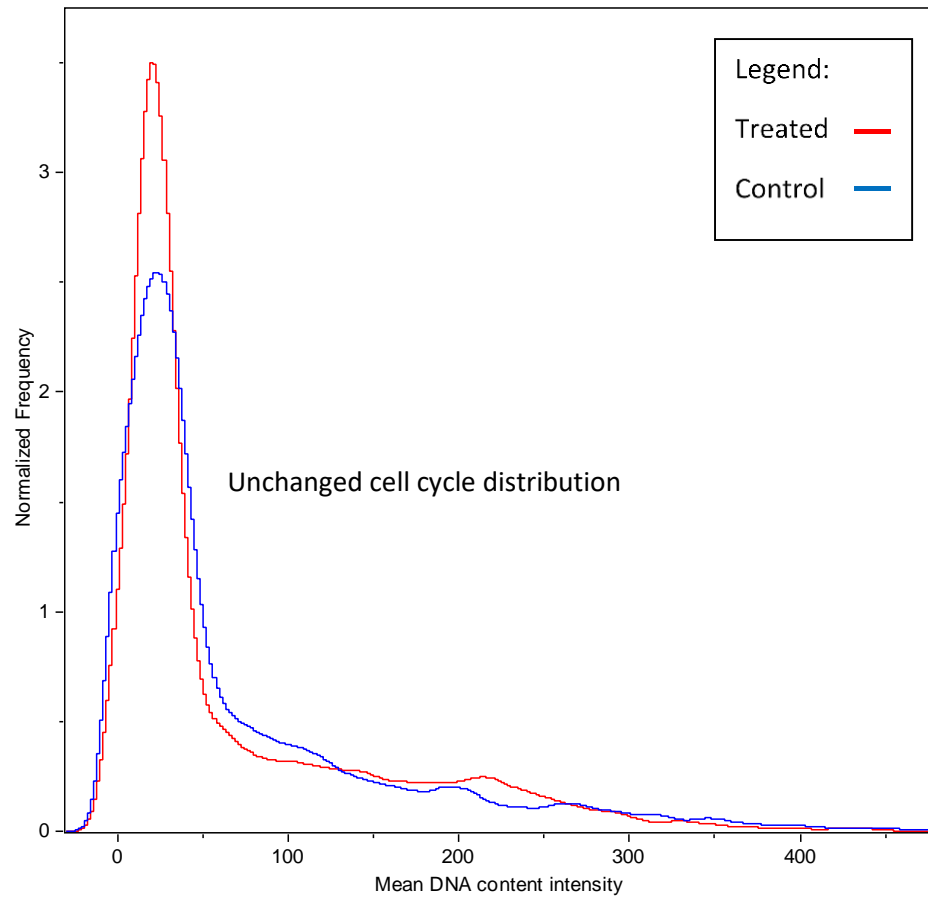
Colorectal Cancer (SW1463)
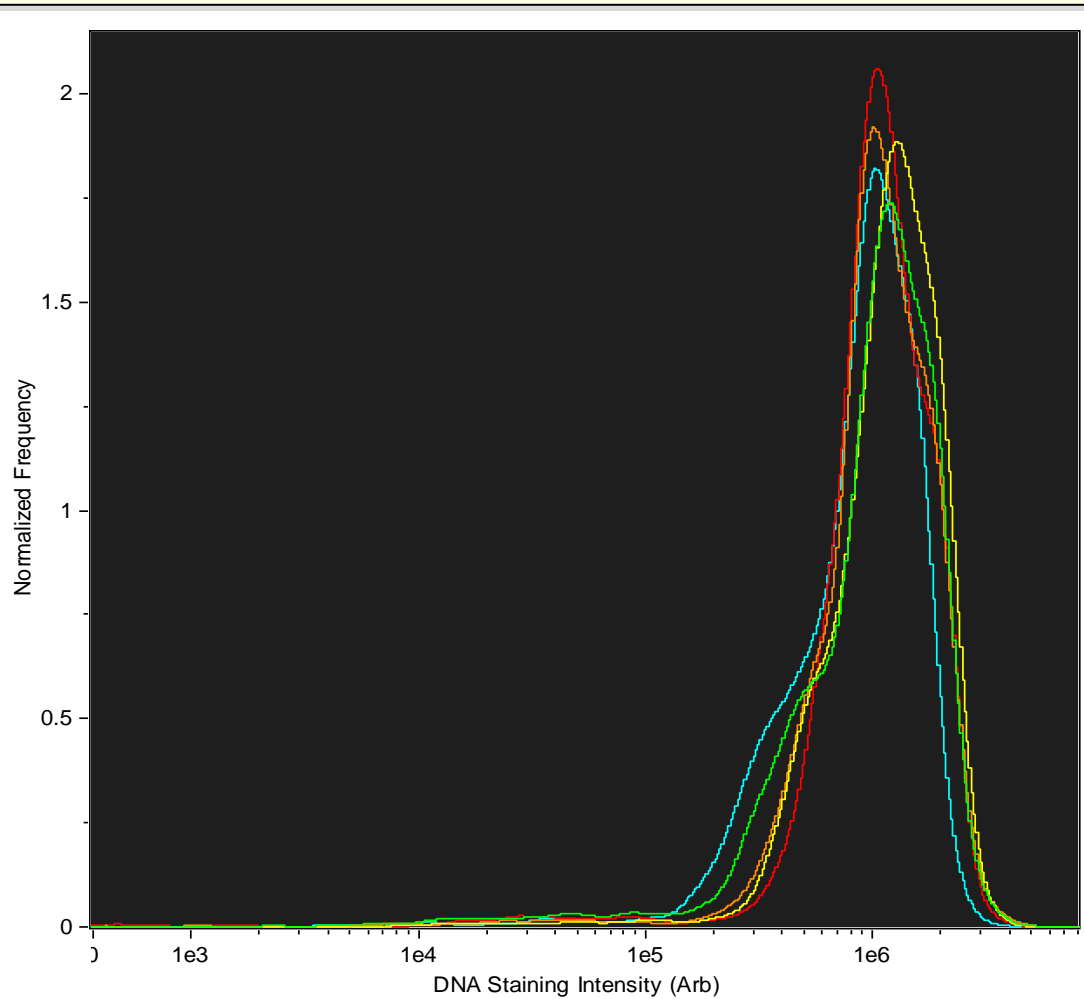
DNA Staining Intensity (Arb)

Population	Count	Mean	Median	Std. Dev.	Geo. Mean	
2.5uM	4058	1151760.11	1114014.35	608500.18	922904.26	
5uM	4078	1277465.1	1228825.42	579871.1	1110254.1	
7.5uM	5880	1176759.88	1080441.25	571937.64	1002748.2	
10uM	3050	1179602.54	1091347.51	536878.71	1003226.13	
Control	5112	976618.99	961276.29	496924.91	813237.59	

Future work

- Work was put on hold due to COVID-19

- We to hope to validate the cell cycle results by the end of January

- Cell signalling pathways

  - Proteome profiling (protein signature)

- Combination studies with anticancer drugs

  - Cisplatin, Adriamycin, Tamoxifen, 5FU, etc…

- Further validation of *in vivo* results

- Leading to clinical trials

MDX-124 and Corona Virus

- COVID-19 patients with severe symptoms may suffer from Acute Respiratory Distress Syndrome (ARDS)

- This is caused by the excessive release of cytokines by WBC

- MDX-124 has demonstrated ability to reduce levels of cytokines through stimulation with lipopolysacchrides

- This is similar to a recently discovered treatment option relying on Dexamethazone to reduce the effects of COVID-19

Acknowledgement

Thank you

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