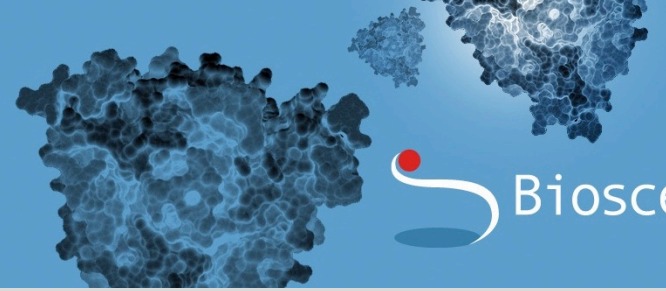


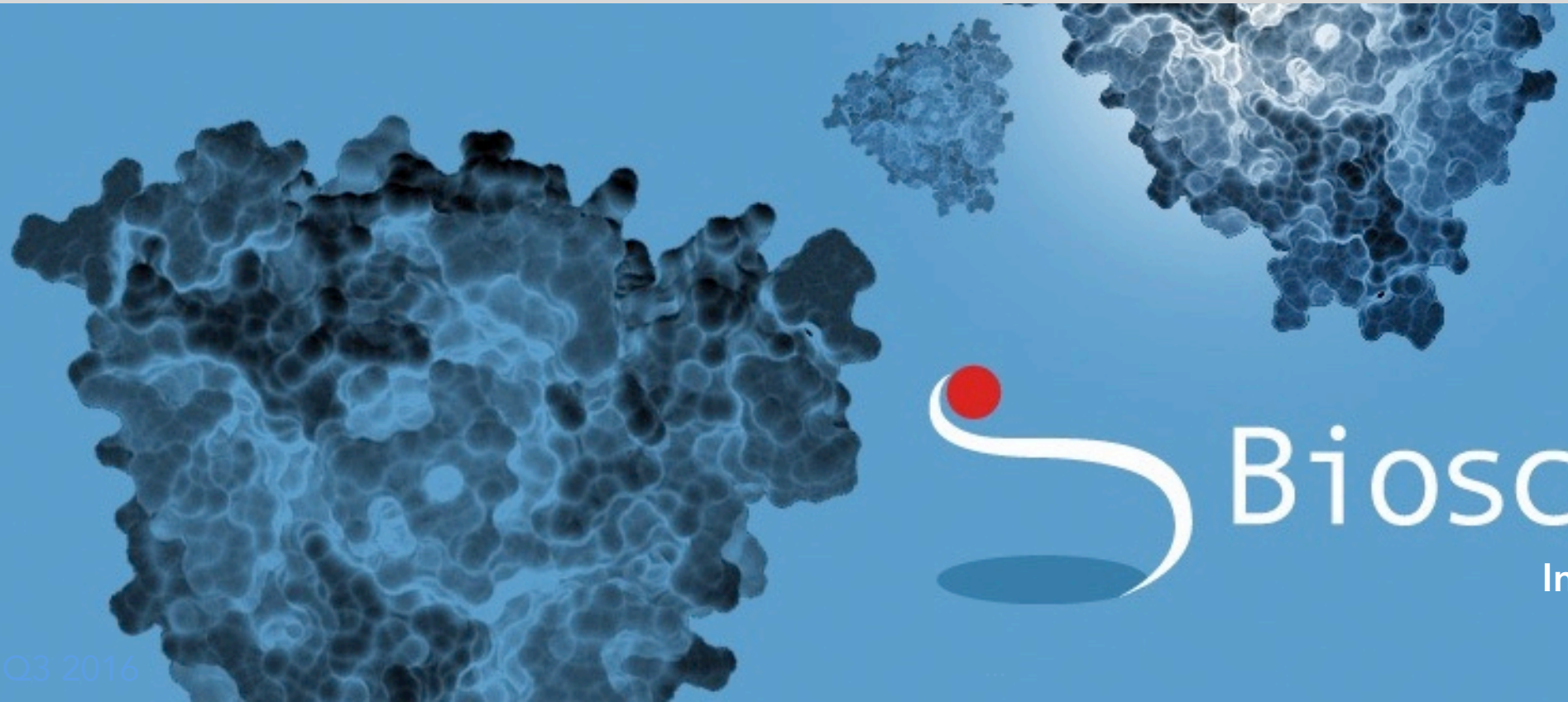
Biosceptre

Keynote Presentation
Fourth COST Action BM1406
23rd-24th of March 2017

Romain Lara

Immuno-Oncology Platform targeting nfP2X$_7$

A Broadly Occurring and High Specificity Cancer Target



Biosceptre

International Limited

$nfP2X_7$ - A cancer specific therapeutic target

- $P2X_7$ / $nfP2X_7$ introduction
- Targeting $nfP2X_7$
- Induction of $nfP2X_7$
- $nfP2X_7$ expression in tumour samples
- Mode of action of $nfP2X_7$ targeted therapeutics

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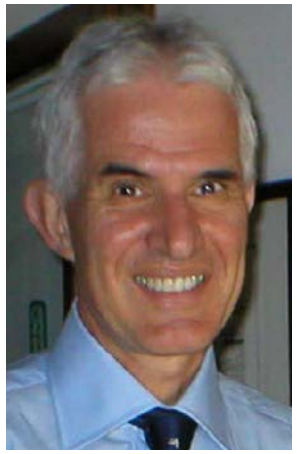

Professor Francesco Di Virgilio

Professor of Clinical Pathology, University of Ferrara

Professor Terence Rabbitts FRS, FMedSci

Professor of Molecular Biology at the University of Oxford

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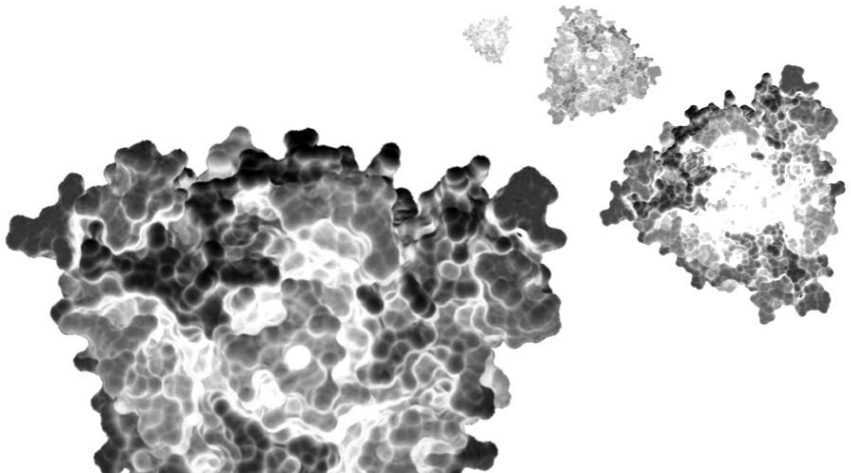
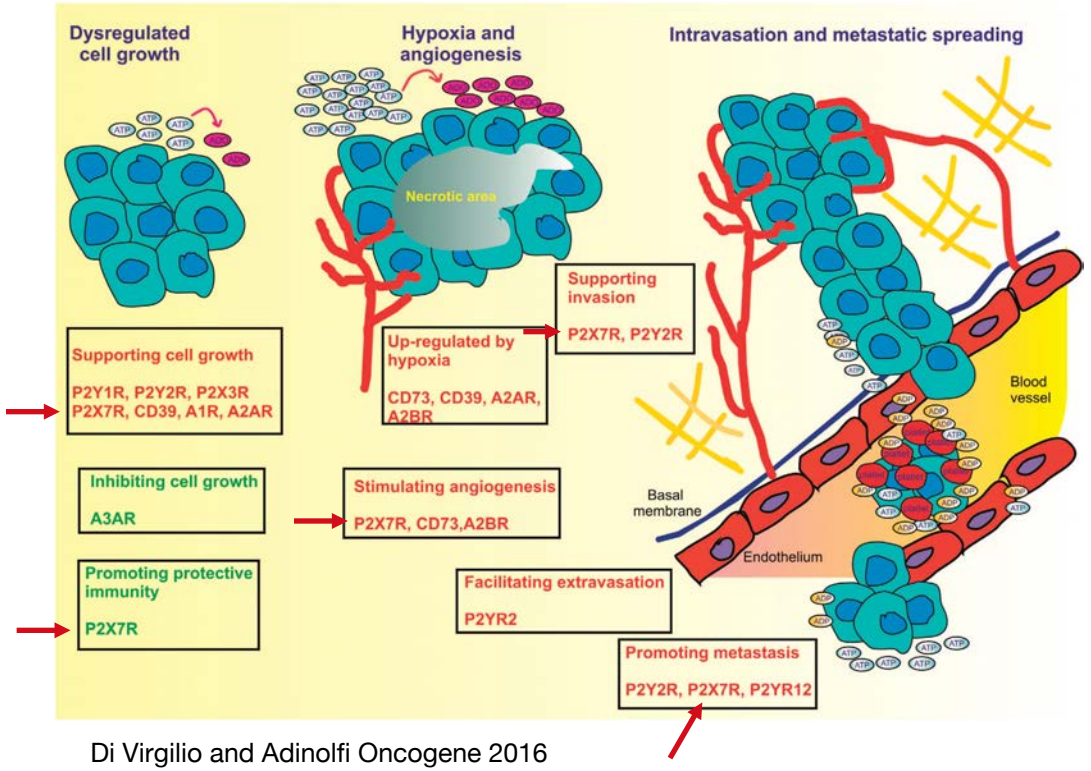
$P2X_7$ / $nfP2X_7$ introduction



$P2X_7$ plays an important role in cancer



Di Virgilio and Adinolfi Oncogene 2016

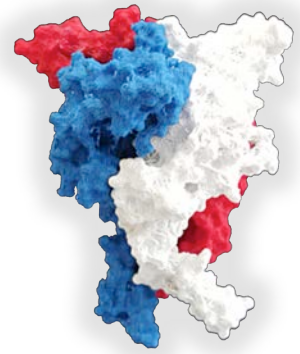
$P2X_7$ and $nfP2X_7$ are upregulated in many malignancies (Roger et al. Biochimica et Biophysica Acta 2015)

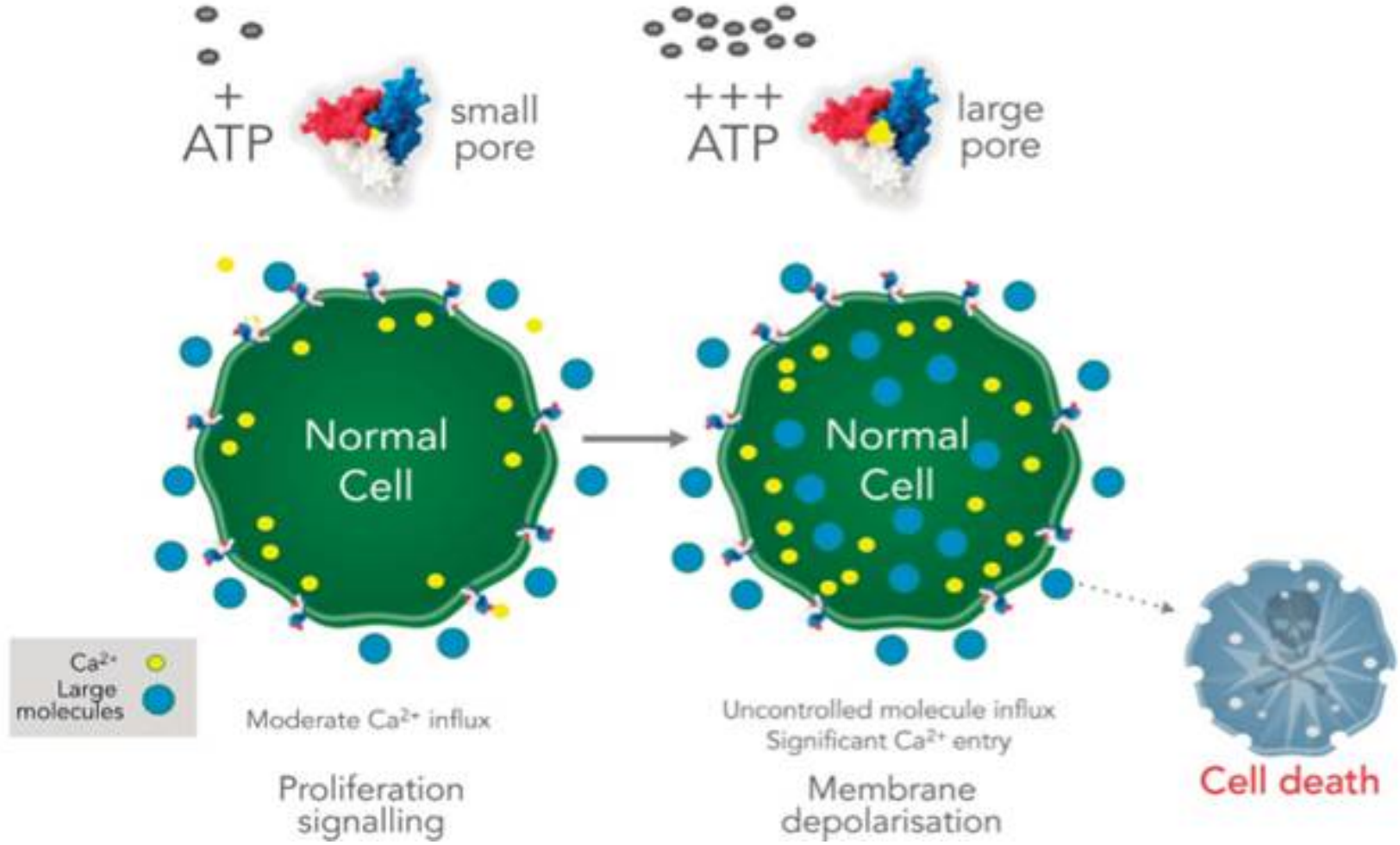
Most studies do not assess functional status of $P2X_7$

$P2X_7$ has 2 open states driven by ATP binding

- A non-specific ion channel in response to rapid ATP stimulation
- A large molecular weight pore in response to prolonged ATP stimulation

$P2X_7$
Normal
trimer
conformation

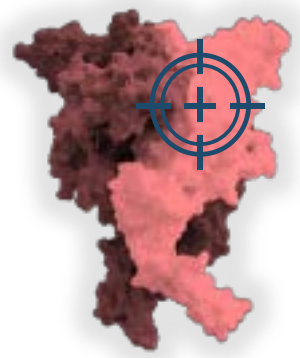


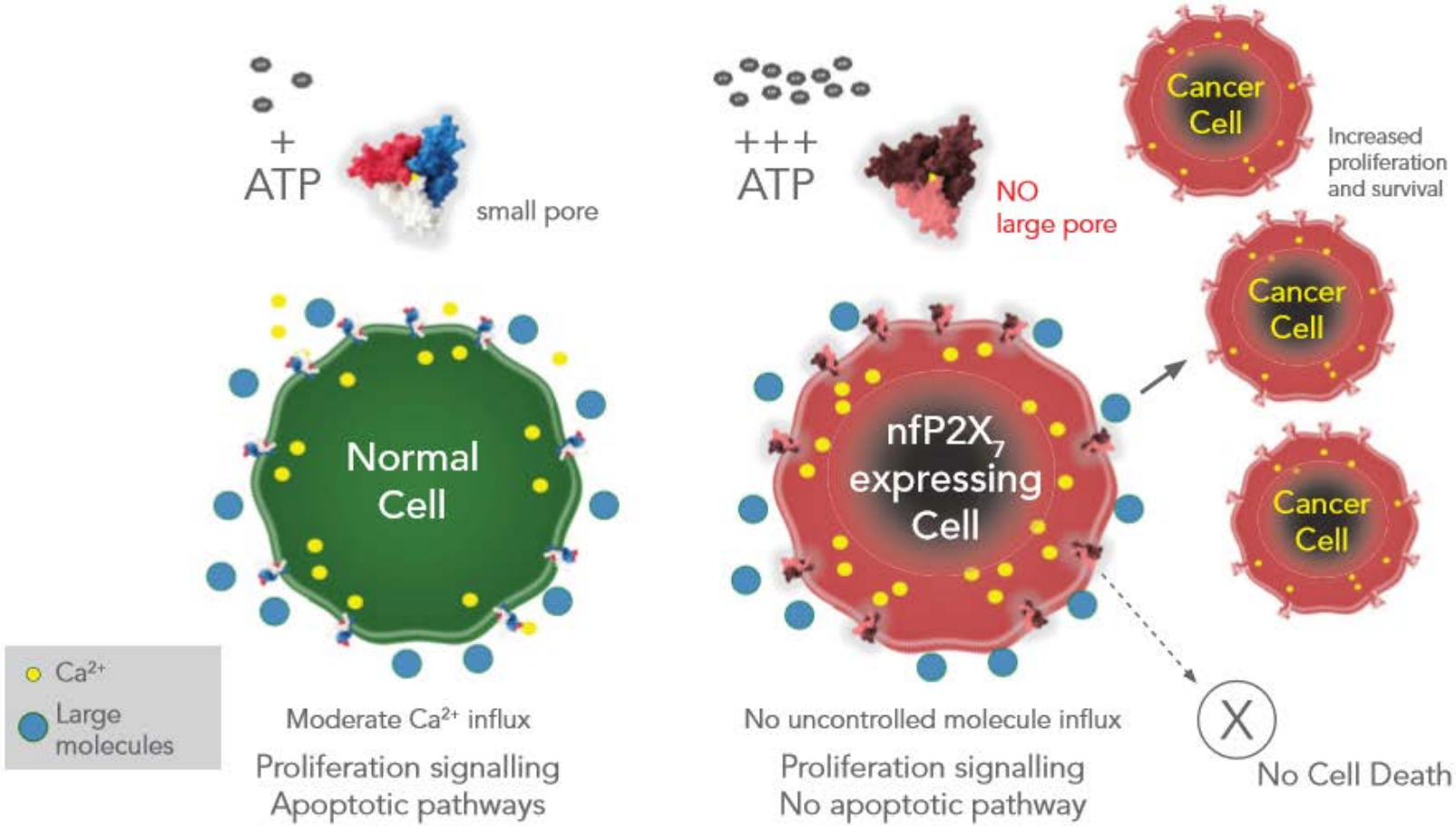


nfP2X$_7$ and Cancer

- Some forms of P2X7 can not form a large molecular weight pore in response to ATP. These we term nfP2X7 (non functional) BUT critical signalling functionality is retained
- In cancer, loss of the P2X7 "pore" response to ATP stimulation confers a positive survival advantage

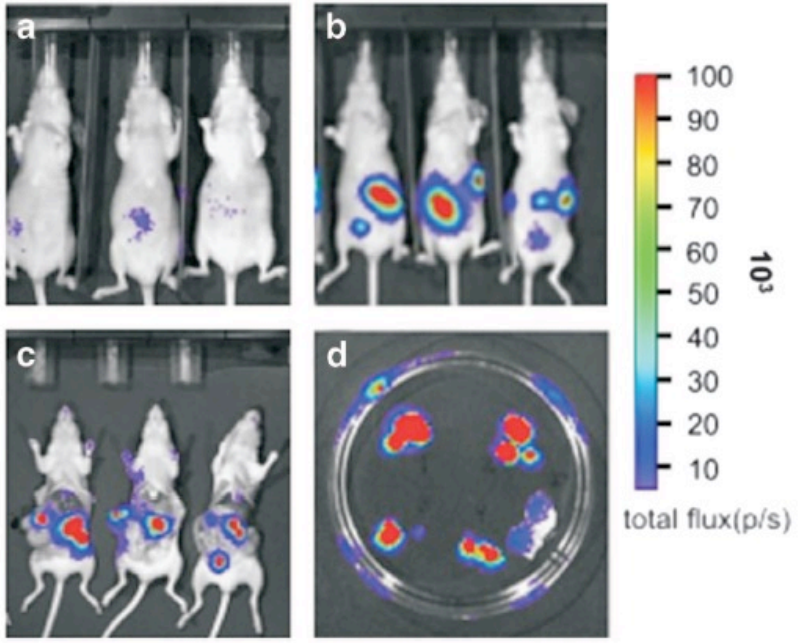
nfP2X$_7$ Abnormal trimer conformation





Tumour microenvironment contains high levels of extracellular ATP

- ATP is secreted by cancer cells and intracellular ATP is released from dying cells
- ATP is present in the hundreds of micro molar range while almost undetectable in healthy tissue

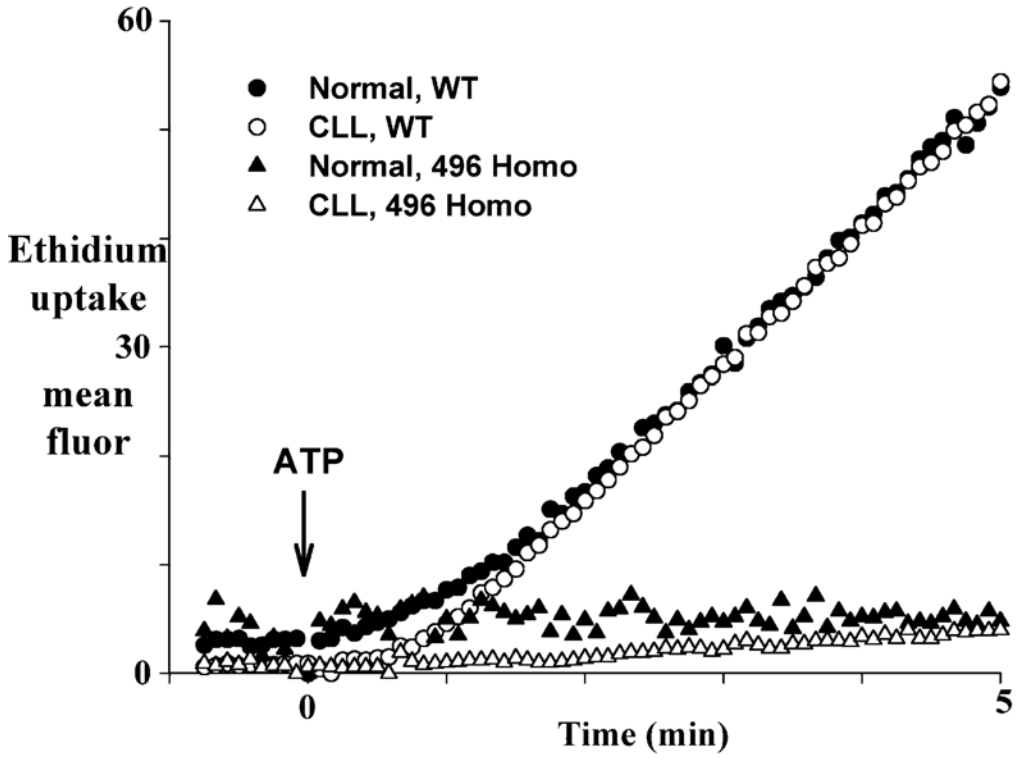


Increase extracellular ATP concentration at tumour sites of healthy (a) or human ovarian carcinoma (OVCAR)-bearing mice (b, c and d)

Di Virgilio and Adinolfi Oncogene 2016

Acquisition of Non-functional P2X7 (nfP2X7) enables tumour cells to survive and proliferate in the presence of high ATP concentrations

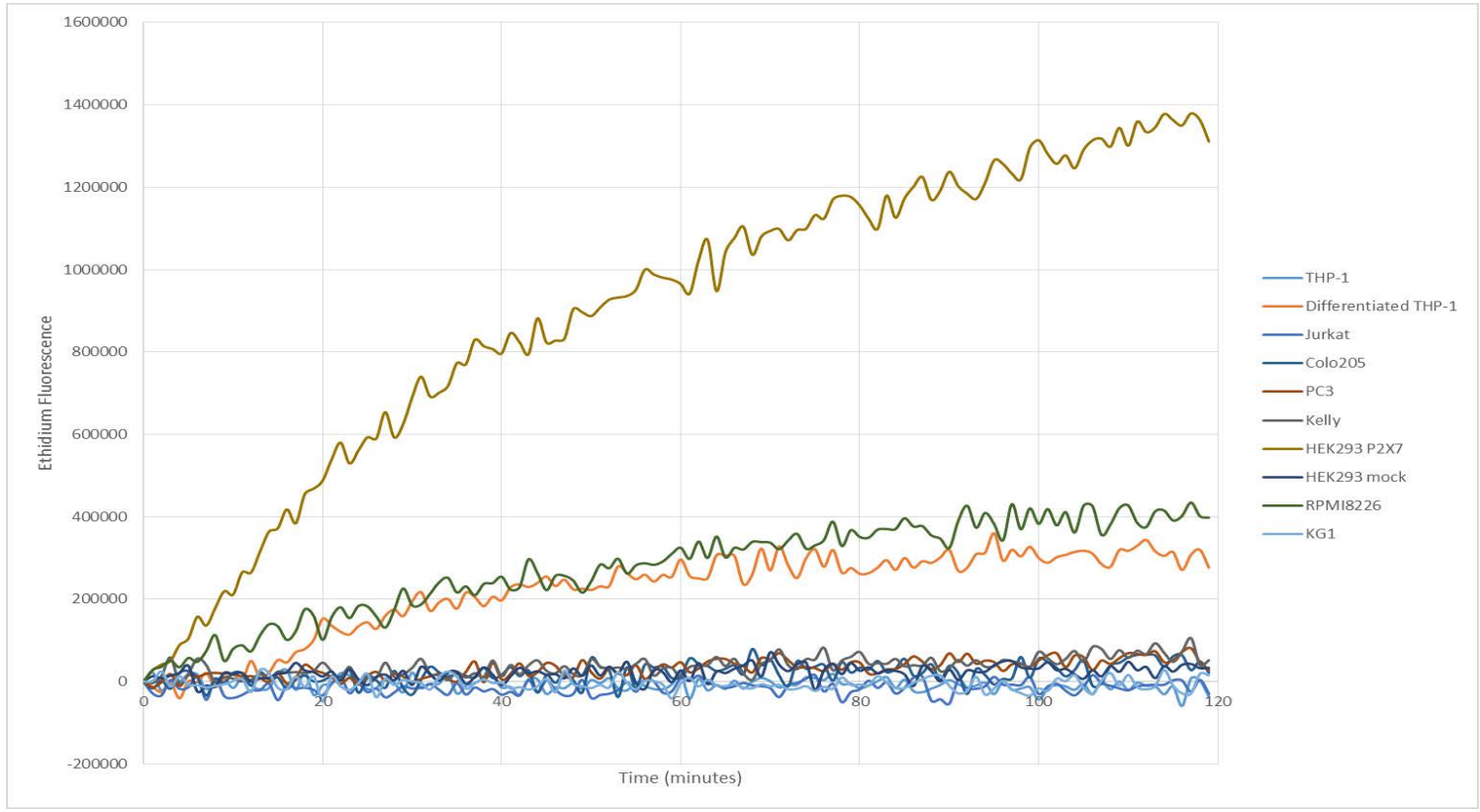
Loss of pore functionality for $P2X_7$



- SNPs / mutations
  (at least 11 reported to impair or abrogate P2X 7 large pore function)
- Splice variants
  (3/10 reported to impair or abrogate P2X7 larg e pore function in human)
- N-glycosylation
- ADP-ribosylation in mouse
- Intracellular binding partners
- Interaction with other P2X family members

Gu et al. J Biol Chem. 2001

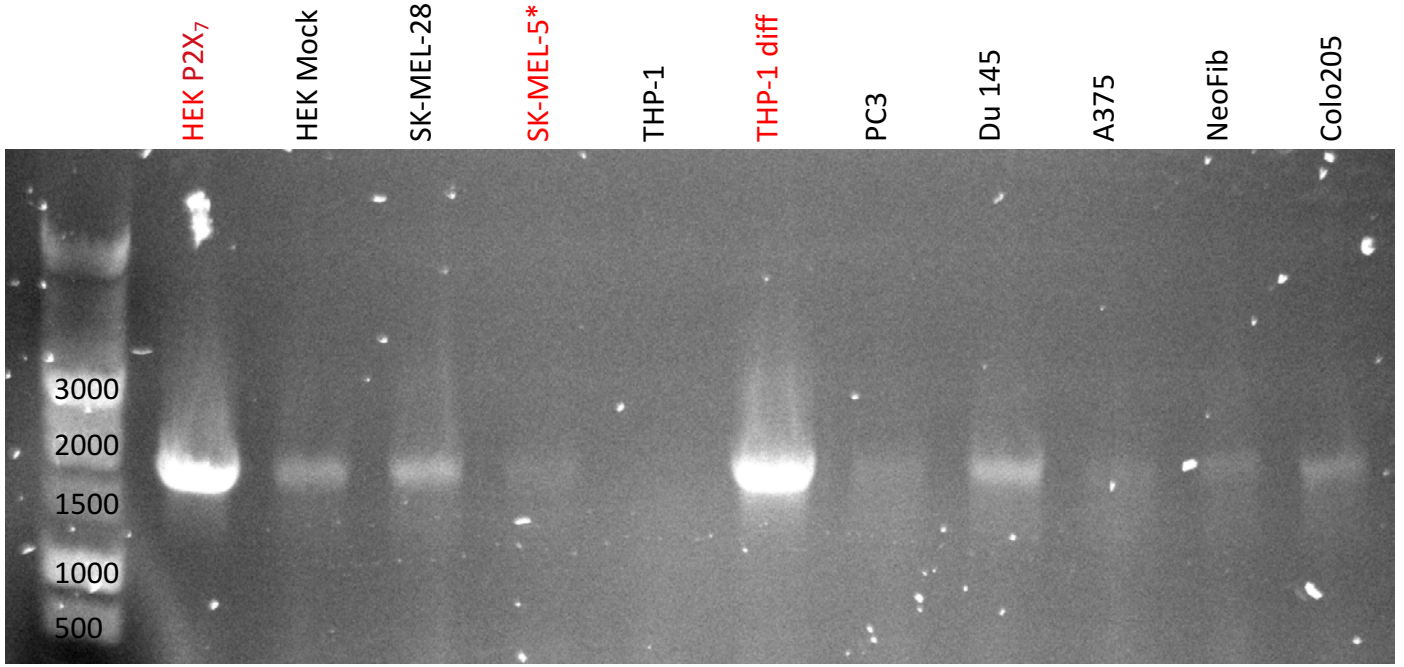
$P2X_7$ function as defined by Ethidium influx



Cell lines with functional P2X7 show Ethidium influx in response to 100μM BzATP

RT-PCR for $P2X_7$ in a panel of cell lines

PCR of cDNA prepared from cell lines using primers to exon 1 and 13 of $P2X_7$ sequence.



* Functional only upon very high ATP stimulation

Functional cell line as defined by ethidium influx in pore assay

Non-functional cell line as defined by the absence of ethidium influx in pore assay

Many non-functional cell lines express $P2X_7$ at mRNA level

nfP2X$_7$ mediates Survival in cancer cell lines

Cell line	Tumour origin	Large pore opening	Reduced P2X$_7$ induces cell death
PC3	Prostate	No	Yes
Du145		No	Yes
LNCaP		No	Yes
SK-MEL-28	Melanoma	No	Yes
SK-MEL-5		No *	No
PC 9	Lung	No	Yes
H460		No	Yes
H520		No	Yes
MCF7	Breast	No	Yes
SK-N-AS	Neuroblastoma	No	Yes
Kelly		No	Yes
MiaPaCa2	Pancreas	No	No
HCT116	Colorectal	No	Yes
HT1080	Fibrosarcoma	No	Yes

* Functional only upon very high ATP stimulation

For 12 out of 14 cell lines, across 7 different cancer indications, reducing P2X$_7$ levels via siRNA induced cell death.

nfP2X$_7$ can mediate survival in a wide range of cancer cell lines.

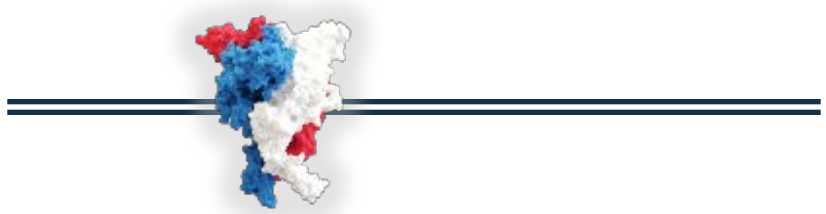
Large pore opening tested using concentration up to 1mM BzATP or 5mM ATP.

Working Hypothesis

Biosceptre

+++
ATP

$P2X_7$
Normal
trimer conformation



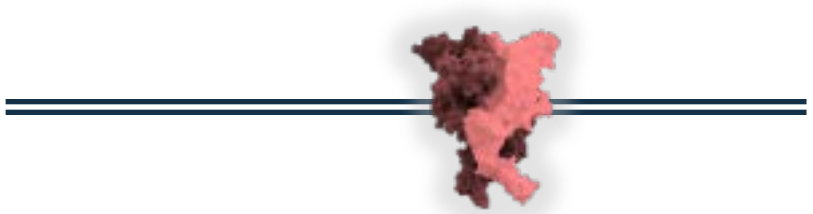
✓ non-specific ion channel

✓ **Large pore opening**

Survival and proliferation signal
Cell death due to large pore opening

+++
ATP

$nfP2X_7$
Abnormal
trimer conformation



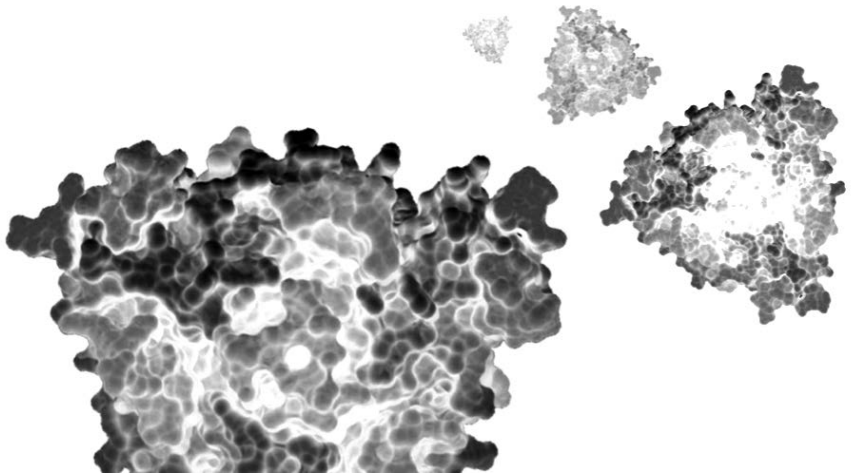
✓ non-specific ion channel

X **Large pore opening**

Survival and proliferation signal only

Biosceptre

Targeting nfP2X$_7$

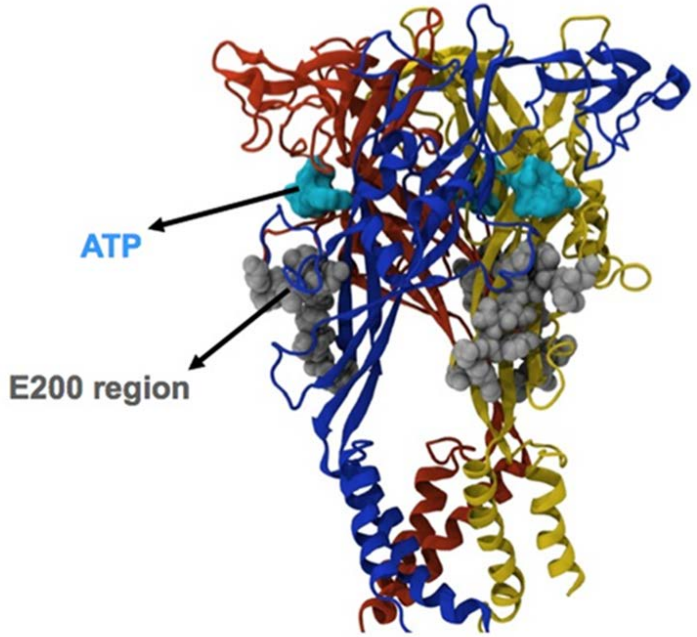


Biosceptre's approach

- Our founder hypothesised the exposure of the E200 epitope on nfP2X$_7$ but not P2X$_7$
- Antibodies have been produced that target E200 selectively
- nfP2X$_7$ is found specifically at the surface of cancer cells and plays a critical role in cancer cell survival
- Targeting of nfP2X$_7$ has potential in a broad range of cancers including (but not limited to):
    - Lung, Breast, Prostate, Colorectal

Biosceptres clinical portfolio :

- **BIL03s** a fully human domain antibody for systemic use
- **BIL010t** polyclonal sheep antisera for topical application
- **BIL06v** peptide-protein conjugate vaccine therapeutic



Antibodies against $P2X_7$ and $nfP2X_7$

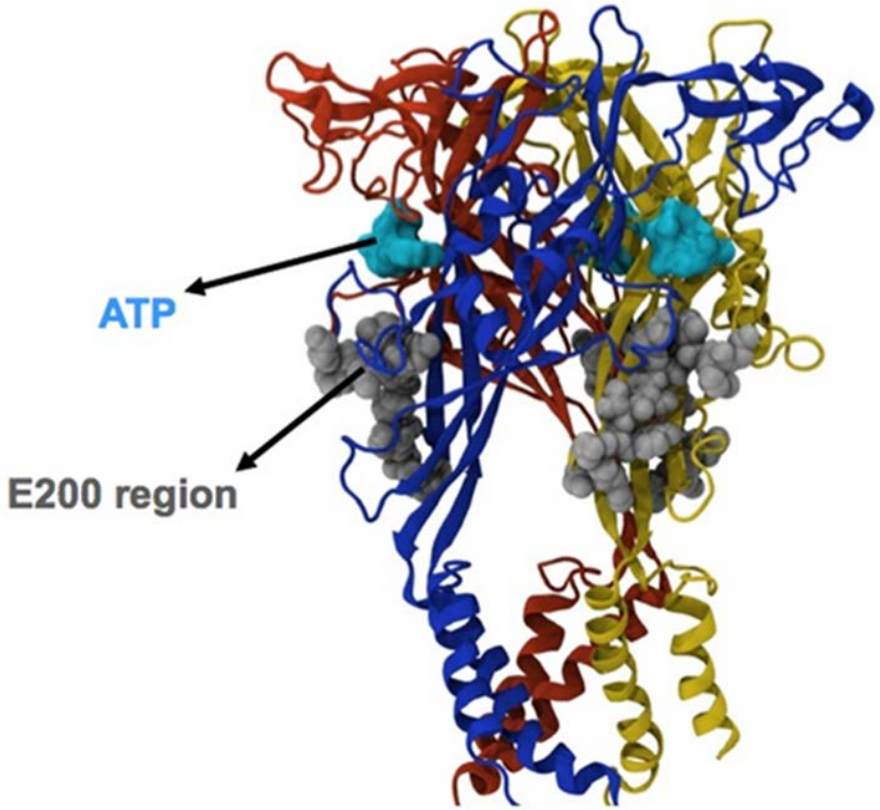
$nfP2X_7$ (E200) targeting antibodies and vaccine

- **BIL03s -** Selected for binding to human E200 epitope by phage display
- **BIL010t -** Polyclonal made by immunising sheep with human E200
- **BPM09 -** Mouse monoclonal antibody raised against human E200
- **BIL06v -** peptide conjugate vaccine based on the E200 sequence

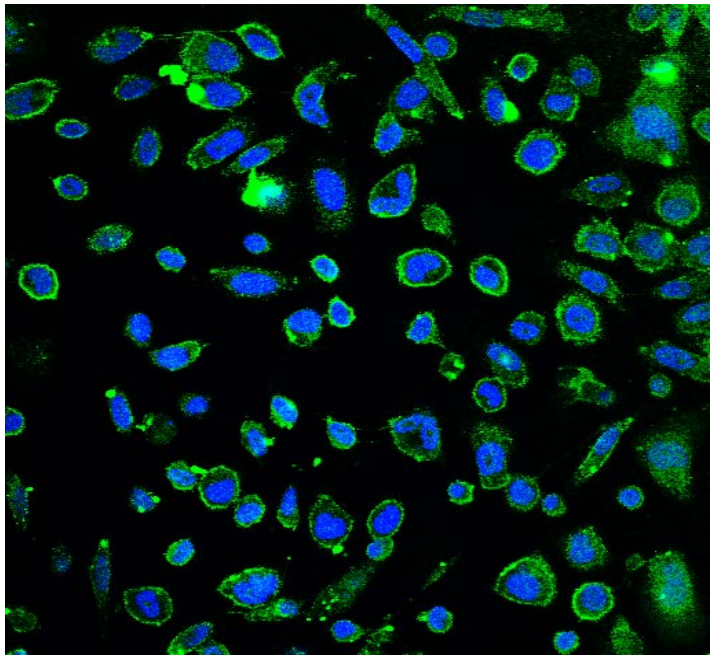
Functional $P2X_7$ targeting antibody

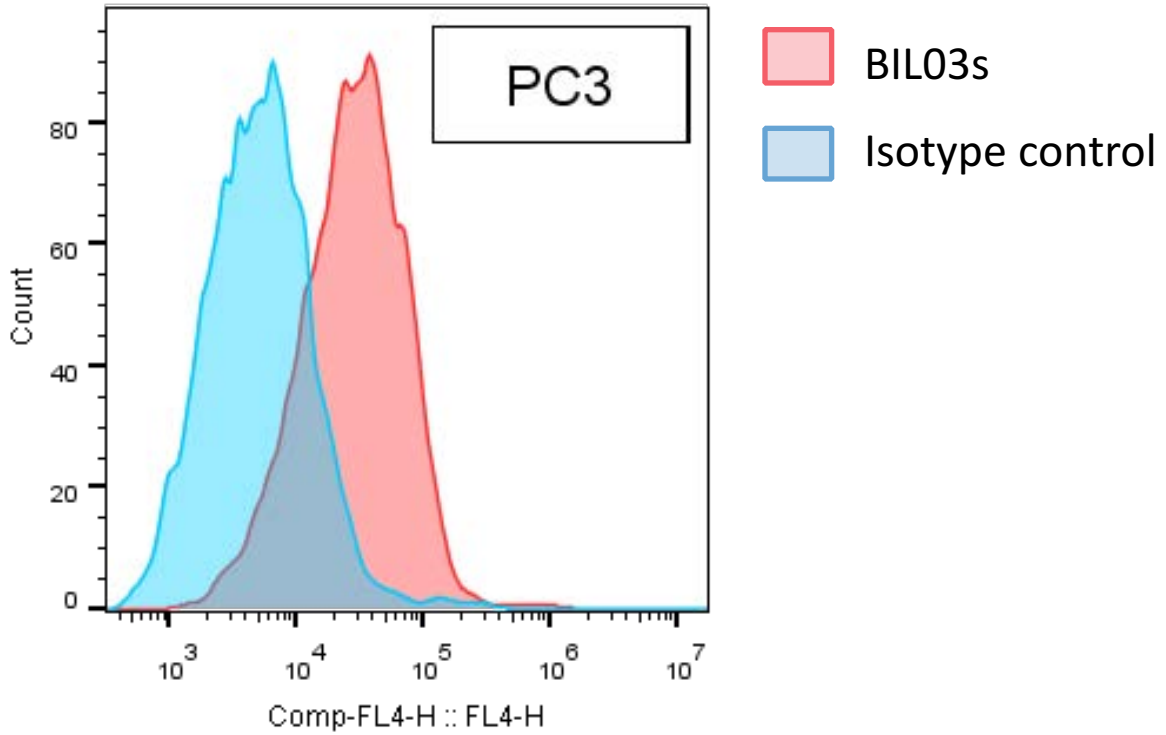
- **L4** - mouse monoclonal anti human $P2X_7$
    - Made by immunisation of mice with $P2X_7$ transfected cells
    - Strong binding to functional cells and cells transfected with $P2X_7$ a

BIL03s was raised against E200 sequence and binds $P2X_7$ extracellular domain



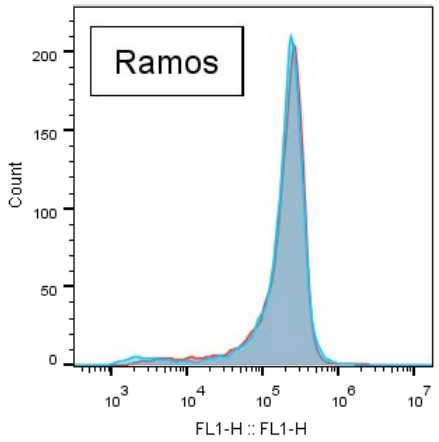
BIL03s binds to the surface of cancer cells

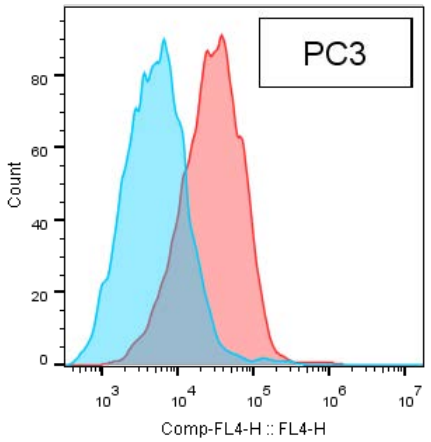


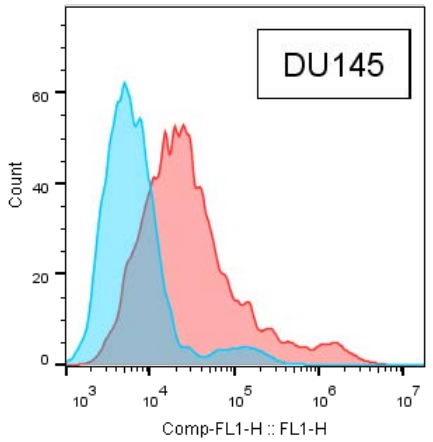


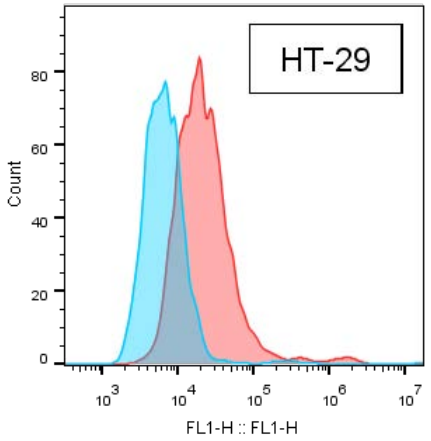
Binding of BIL03s to PC3 cells Immuno-fluorescence and confocal imaging showing binding of BIL03s to non-permeabilized PC3 cells in green, cell nuclei were labelled using Hoescht stain in blue

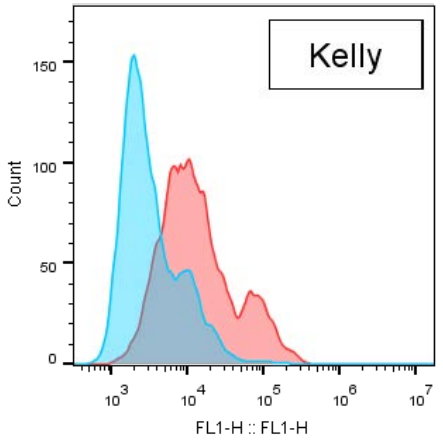
BIL03s shows high binding to most solid tumours









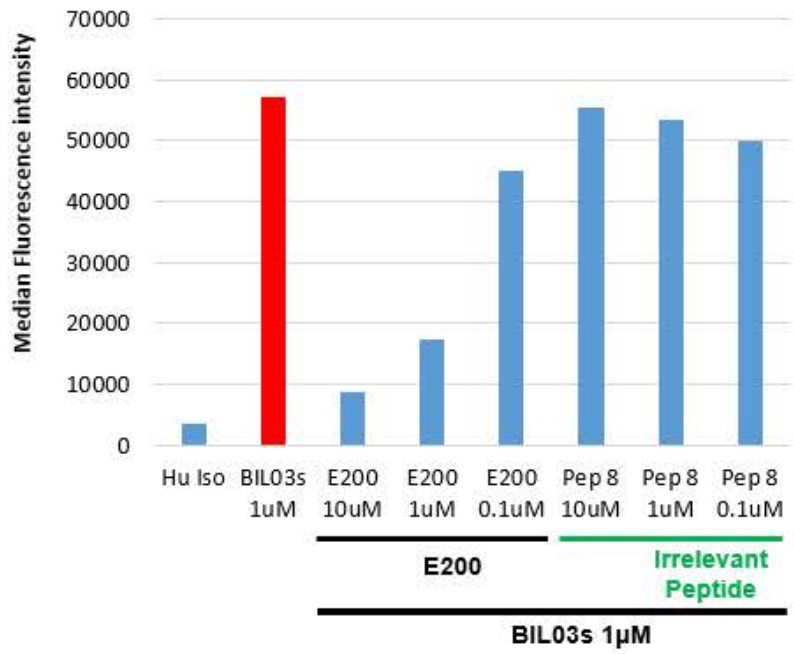


BIL03s

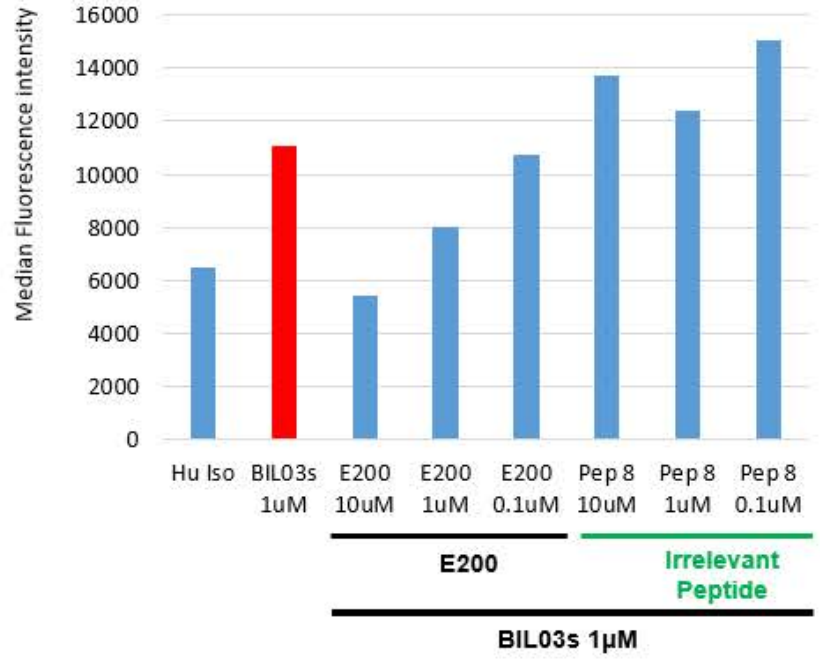
Isotype control

Antibodies target nfP2X$_7$ receptors via their E200 binding domains

PC3

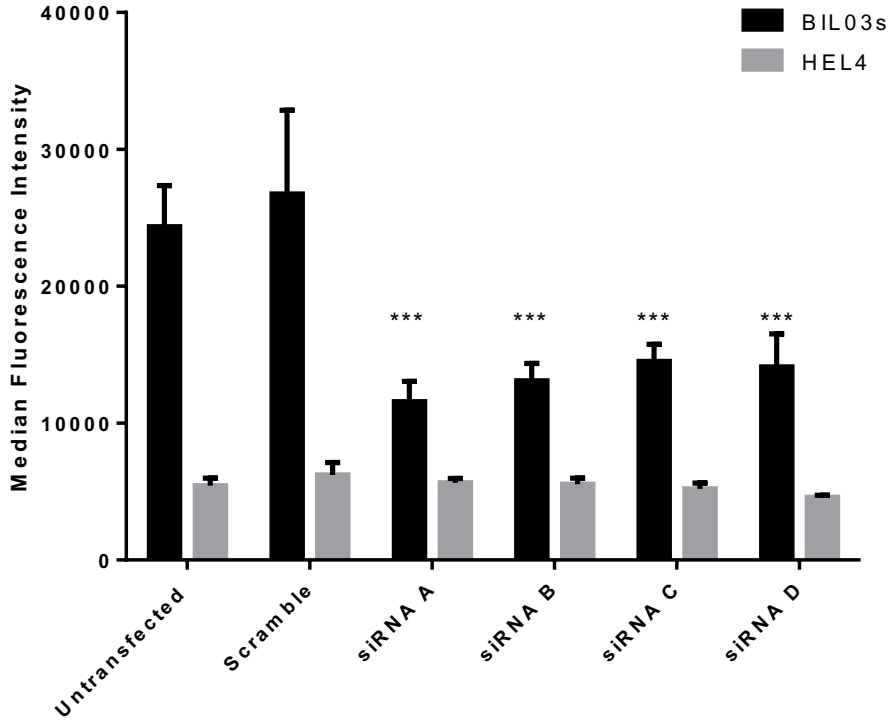


DU145



E200 can compete BIL03s binding demonstrating that it binds to cells via an E200 specific binding site

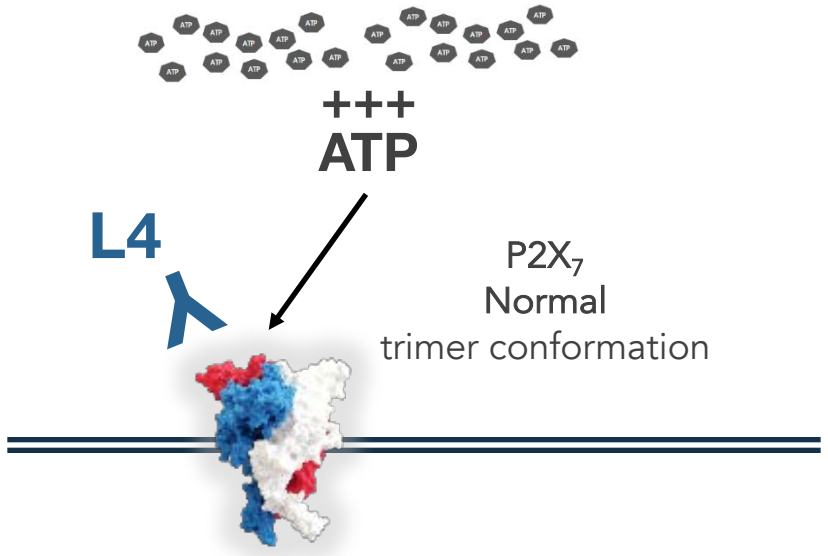
BIL03s binding is specific for $P2X_7$



BIL03s binding to Du145 prostate cancer cells tested 72 hours after transfection with P2X7 targeted siRNAs.

Significant reduction in binding seen for P2X7 targeted siRNA versus scrambled

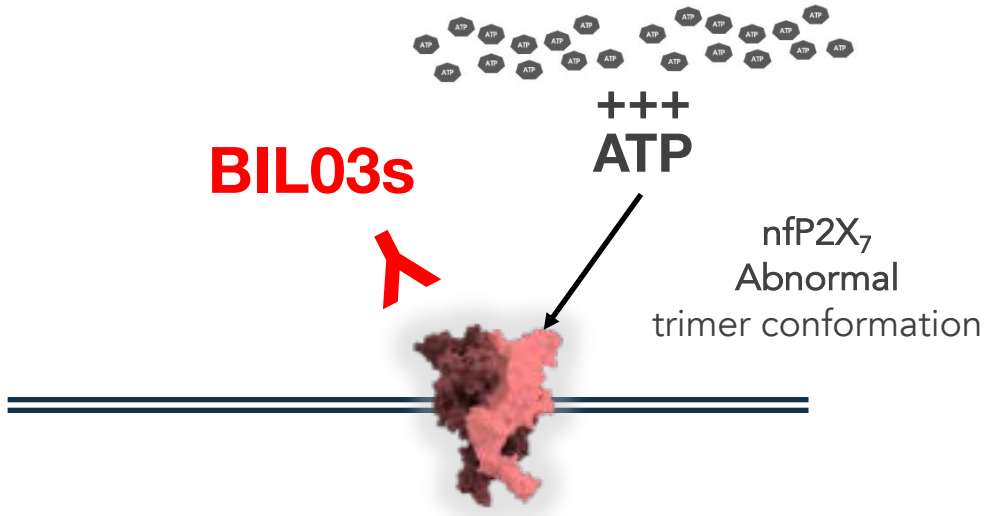
Working Hypothesis



✓ non-specific ion channel

✓ **Large pore opening**

Survival and proliferation signal
Cell death due to large pore opening



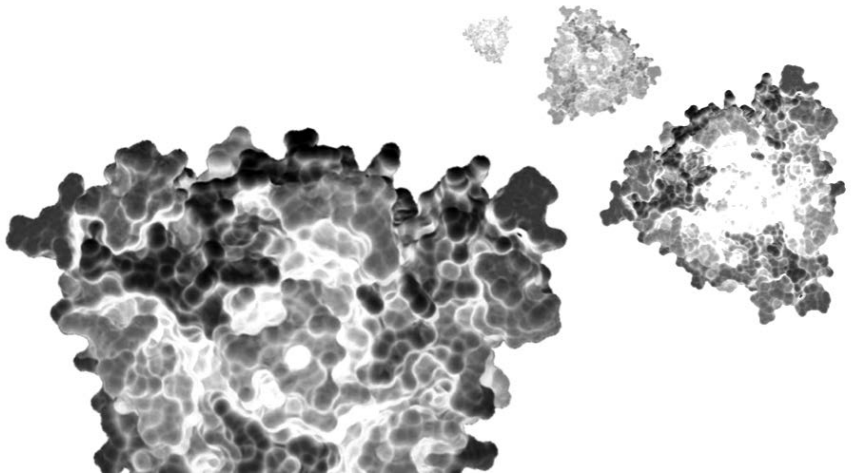
✓ non-specific ion channel

X **Large pore opening**

Survival and proliferation signal only

Biosceptre

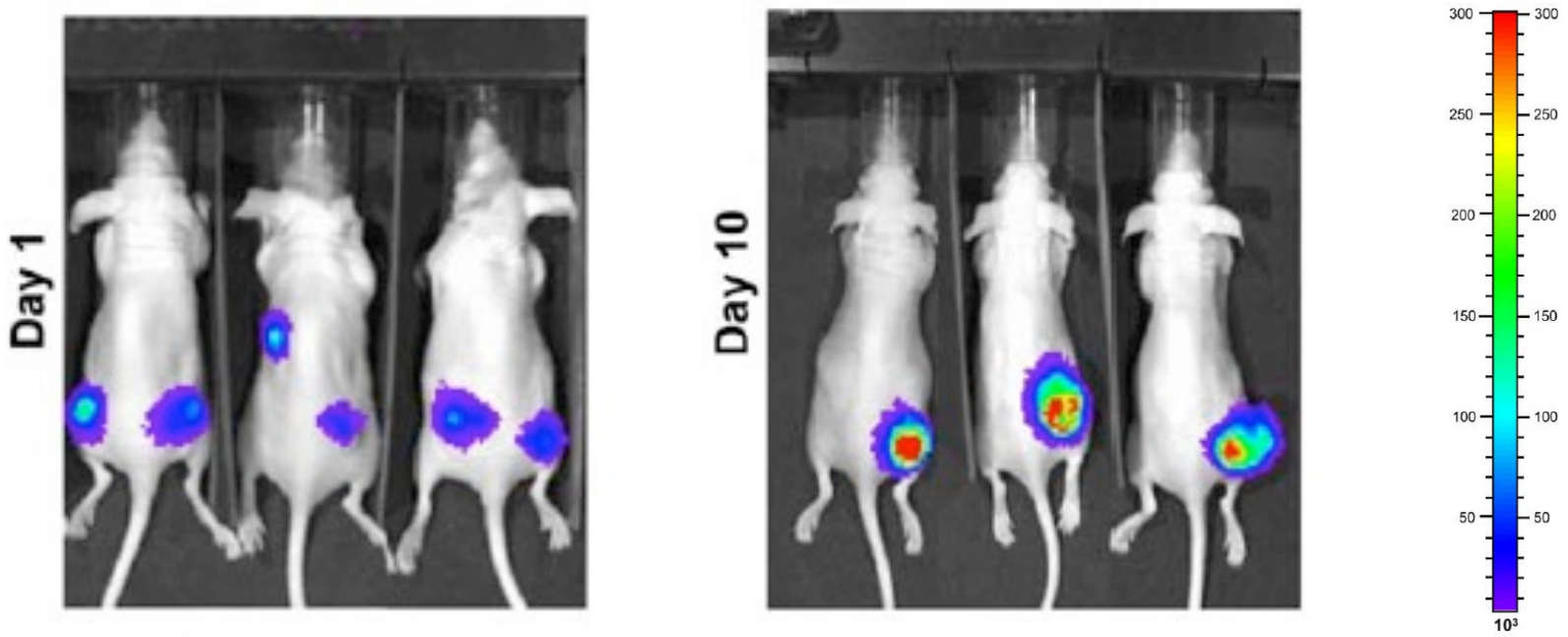
Induction of nfP2X$_7$



Tumour microenvironment contains high levels of extracellular ATP

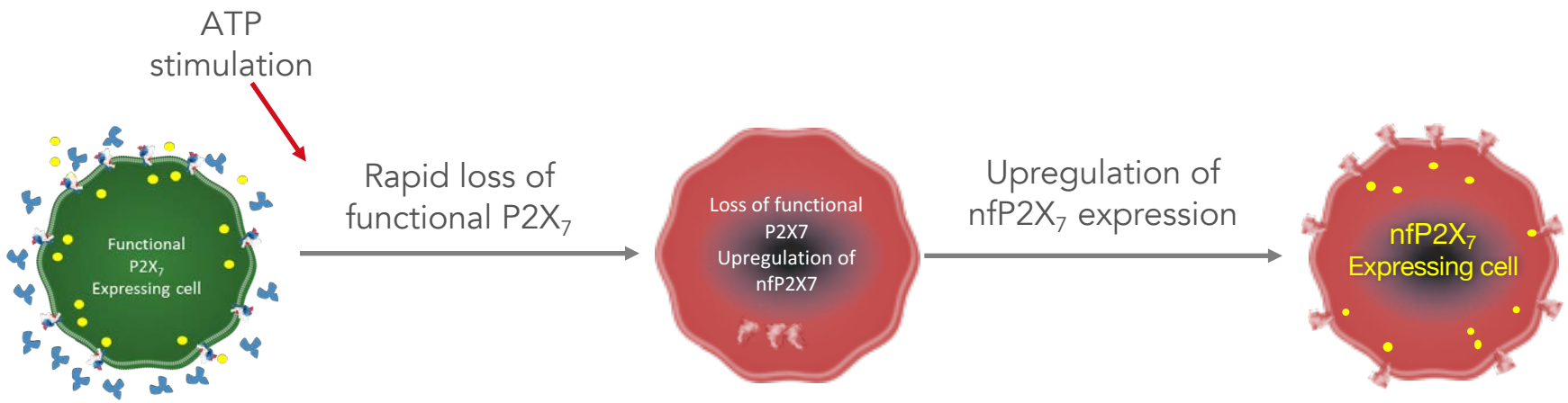
Increased Level of Extracellular ATP at Tumor Sites: *In Vivo* Imaging with Plasma Membrane Luciferase

Patrizia Pellegatti[1☯], Lizzia Raffaghello[2☯], Giovanna Bianchi[2], Federica Piccardi[3], Vito Pistoia[2], Francesco Di Virgilio[1*]



Pellegatti P et al. PLoS One. 2008 Jul 9;3(7):e2599.

ATP in the tumour microenvironment drives a switch from $P2X_7$ to $nfP2X_7$



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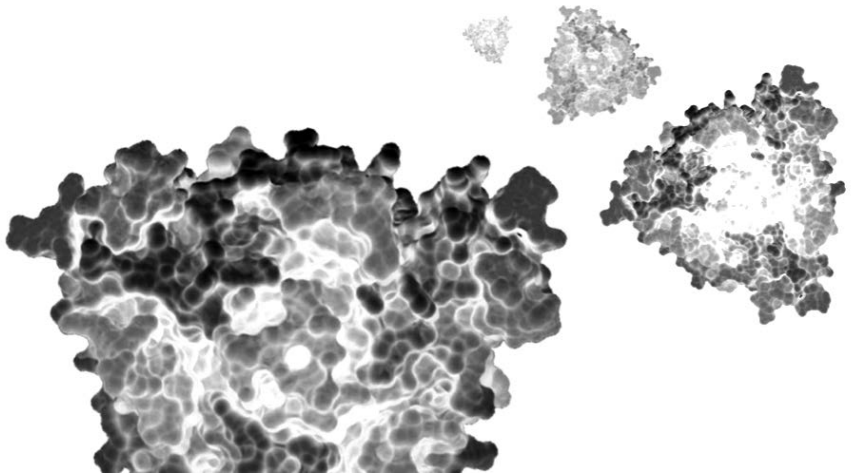
nfP2X$_7$ expression in tumour samples



Binding of nfP2X$_7$ antibody in healthy versus cancerous prostate section

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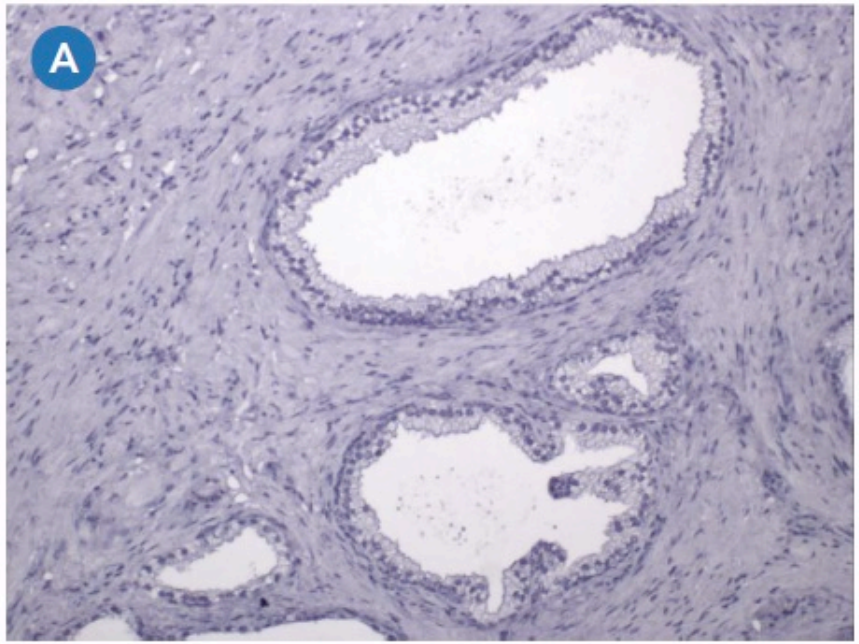
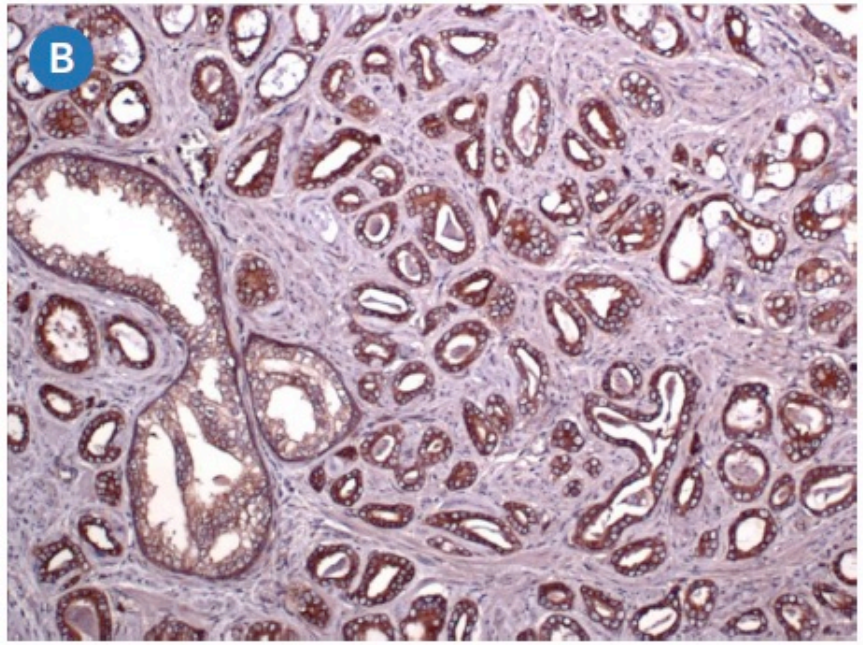
Healthy prostate section stained with BPM09



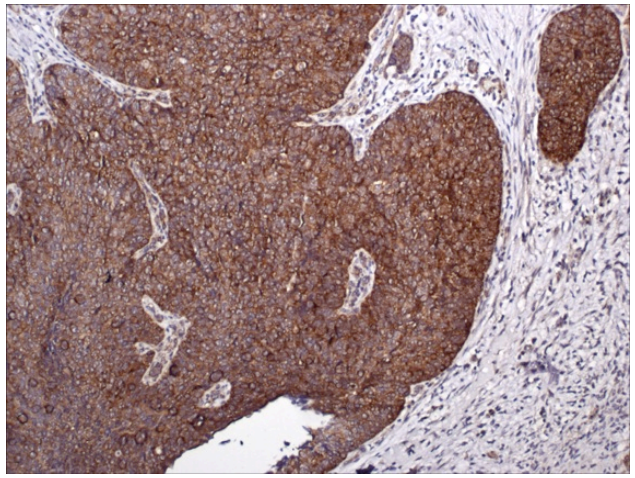
Cancerous prostate section stained with BPM09



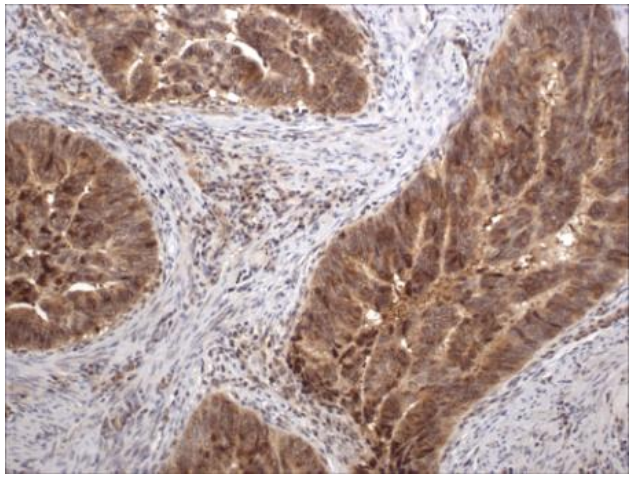
Slater et al. Histopathology 2004

nfP2X$_7$ is found on multiple tumours

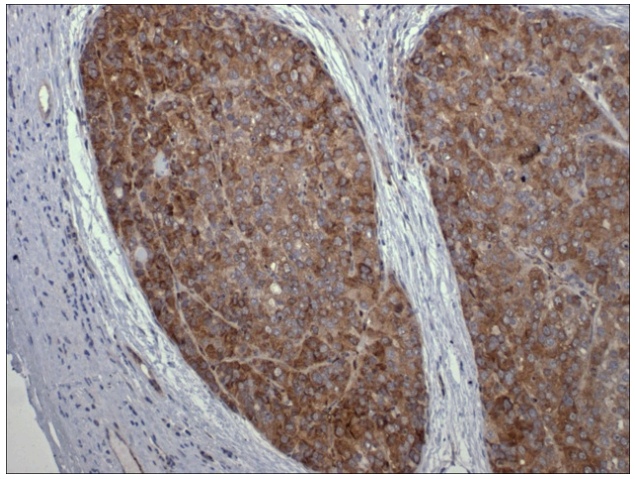
Cervical cancer



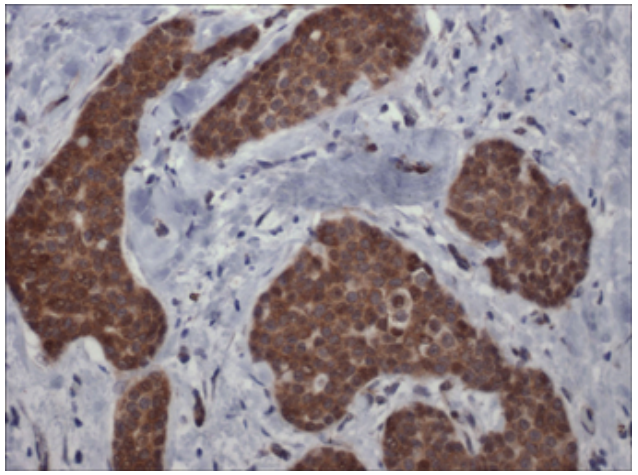
Bowel cancer



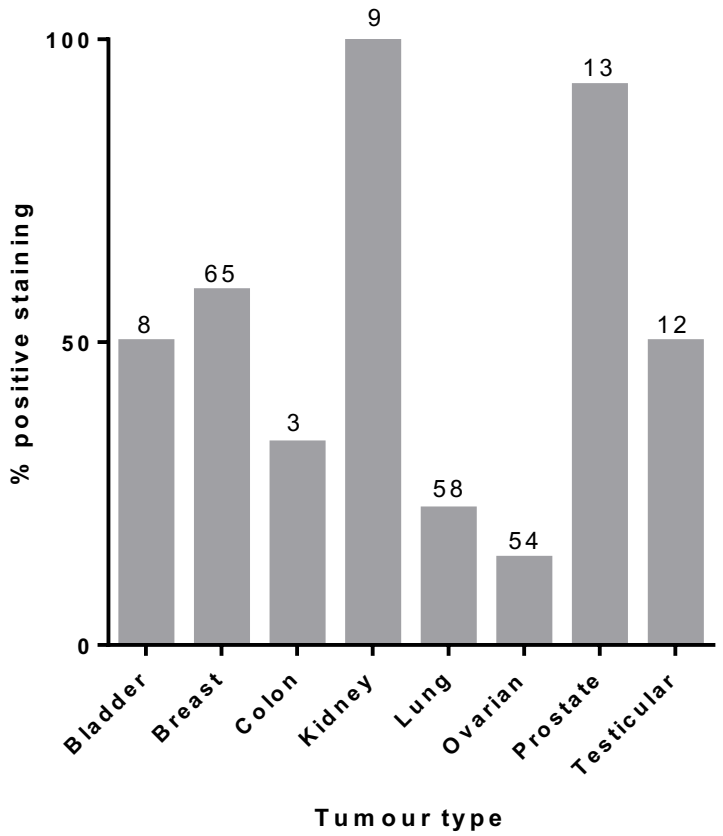
Liver cancer



Breast cancer Ductal carcinoma in situ



nfP2X$_7$ is found on multiple tumours



Number of samples tested for a given cancer shown above bars

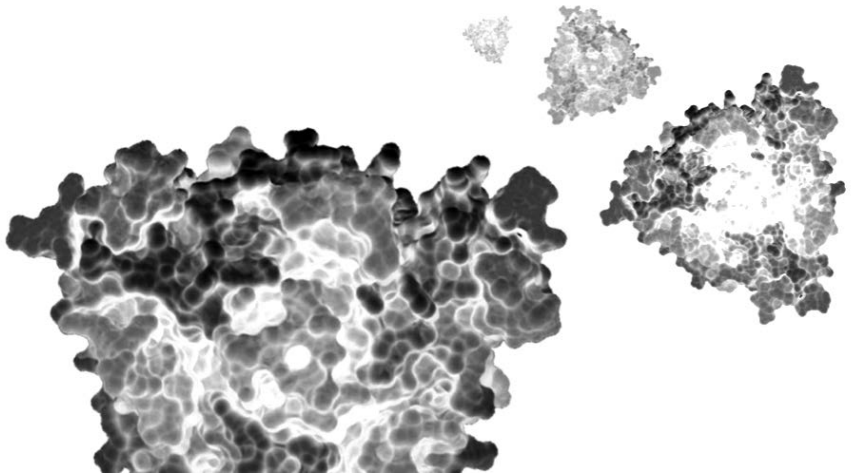
BPM09 is a diagnostic mouse antibody developed for IHC staining of nfP2X$_7$

IHC on FFPE tumour sections
Performed by External Lab
50% of FFPE stained positive

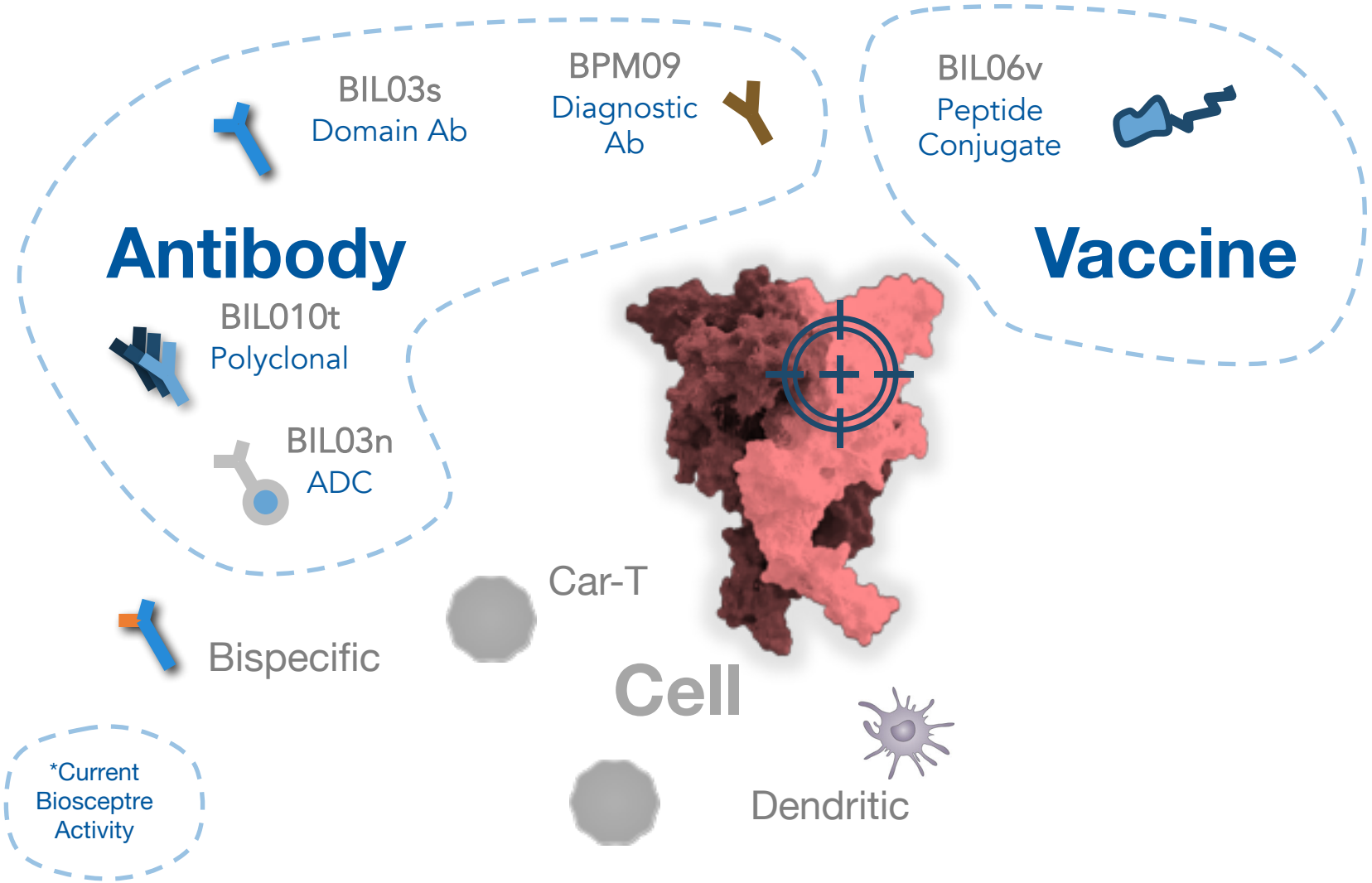
Several external studies have shown no binding of nfP2X7 antibodies to the cell surface in normal tissues including FDA tissue panel

Biosceptre

Mode of action of nfP2X$_7$ targeted therapeutics

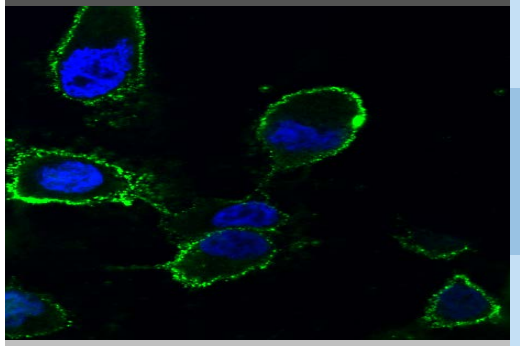


nfP2X$_7$ has broad therapeutic potential via multiple modalities



Systemic Antibody

BIL03s

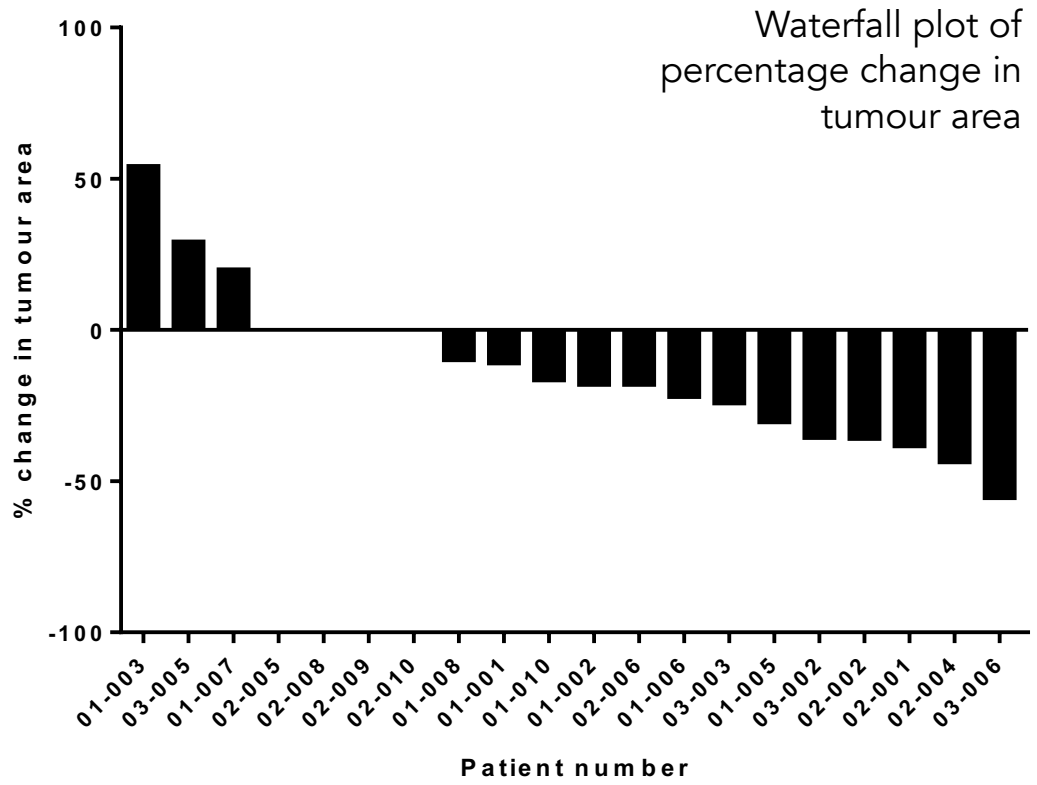


- Binds selectively to nfP2X$_7$
- Indications of efficacy have been shown in mice
- Shown to bind to many cancers including
  - Prostate
  - Kidney
  - Ovarian
  - Melanoma
  - Lung
  - Colorectal
- Safety confirmed in formal toxicology study
- Manufacturing process established and transferred
- Clinical plan established and approved by Ethics Committee

Topical Therapeutic

BIL010t

Phase I FDA clinical trial data



Completed 28 day FDA Phase I study in BCC patients with excellent safety profile

Indication of efficacy:

- 65% of subjects showed regression of surface lesion
- 20% stable
- 15% progression

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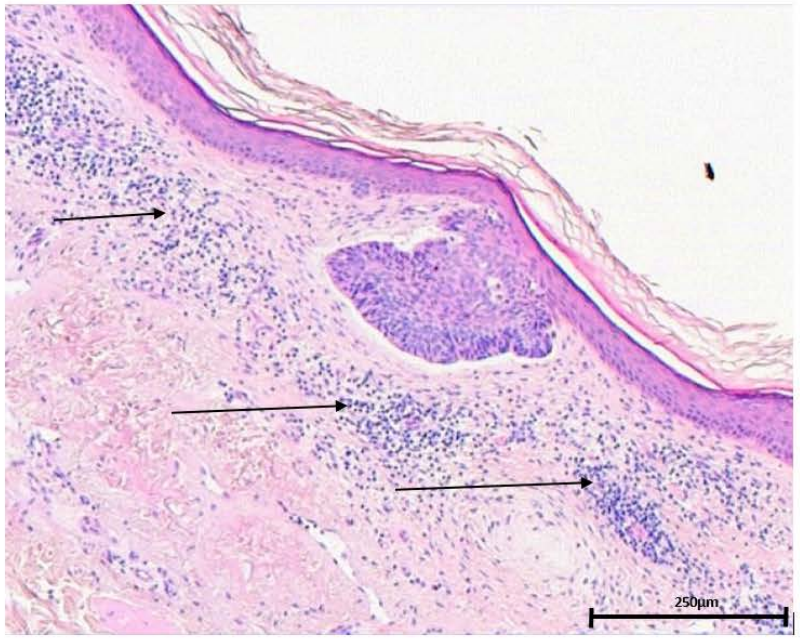
Topical Therapeutic

BIL010t

Pathology analysis confirms infiltration of lymphocytes and stromal reaction

Biosceptre

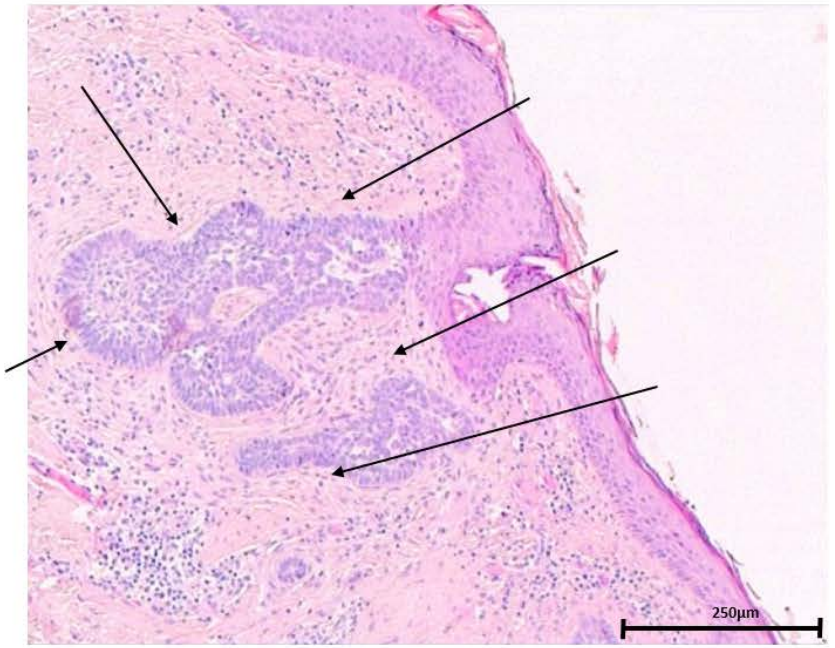
Patient with Nodular BCC post-treatment, tumour margins contained within the cut surfaces.



H&E x40

Arrows point to lymphocytes, post-treatment

Patient with Infiltrative BCC post-treatment, tumour margins contained within the cut surfaces.



H&E x40

Arrows point to stromal reaction, 20% tumor reduction, post-treatment

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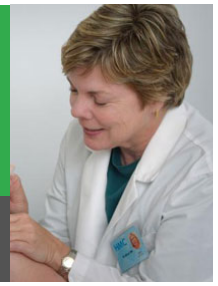
Topical Therapeutic

BIL010t

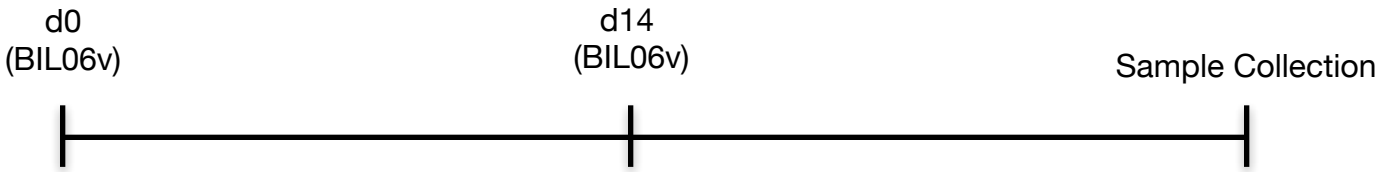
- Strong indication of efficacy from informed consent and Phase I studies
- Excellent safety profile from preclinical studies, informed consent patients and in phase I trial
- BCC and advanced BCC provide validated disease targets
- Shown to cause immune cell infiltration
- Clinical plan in development

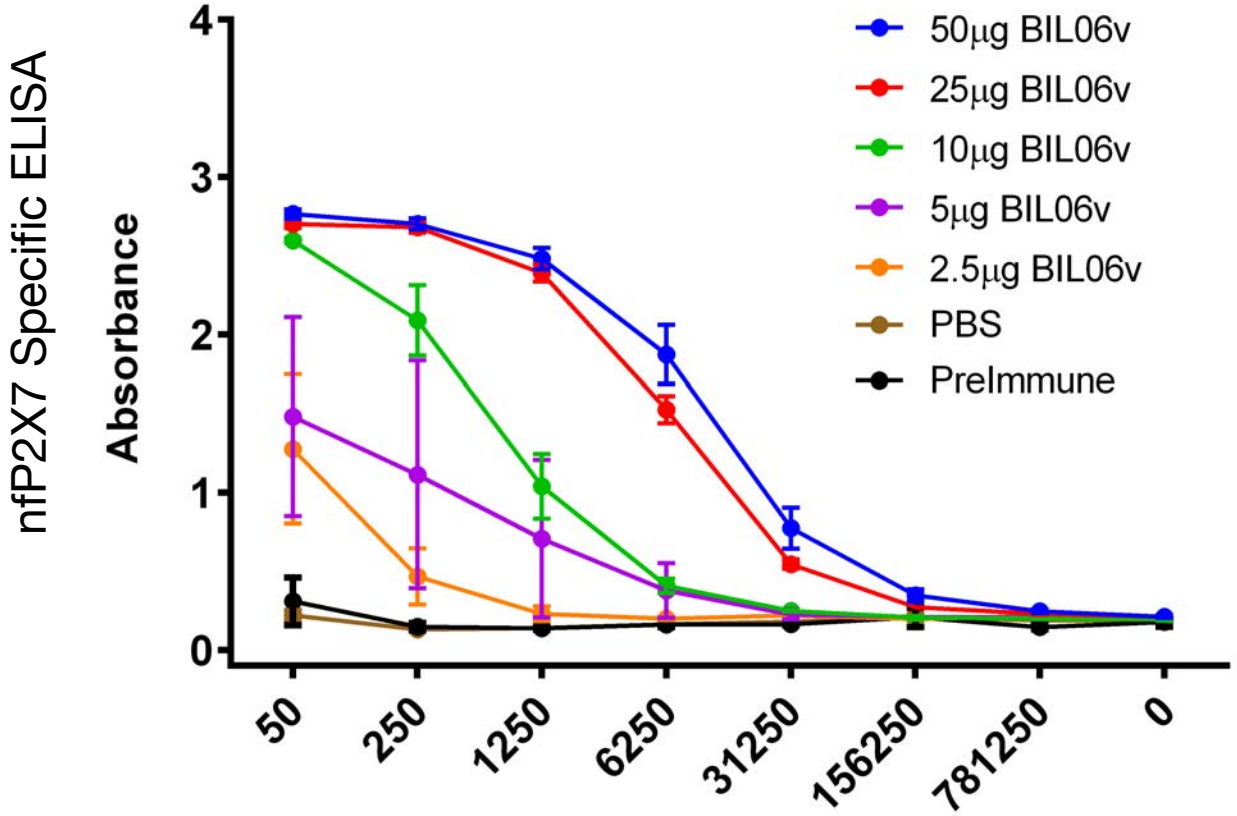
Therapeutic Vaccine

BIL06v



BIL06v is Immunogenic in Mice





BIL06v increases antigen-specific antibody titers in a dose dependent manner.

Therapeutic Vaccine

BIL06v

Shown to break immune tolerance - increased Ab titres in mice and humans and activated T cells in mice

Indications of efficacy have been shown in mice and informed consent patients

A candidate for clinical development has been identified

Process development is on going to support a phase I clinical trial

Additional candidates evaluated in ongoing preclinical studies

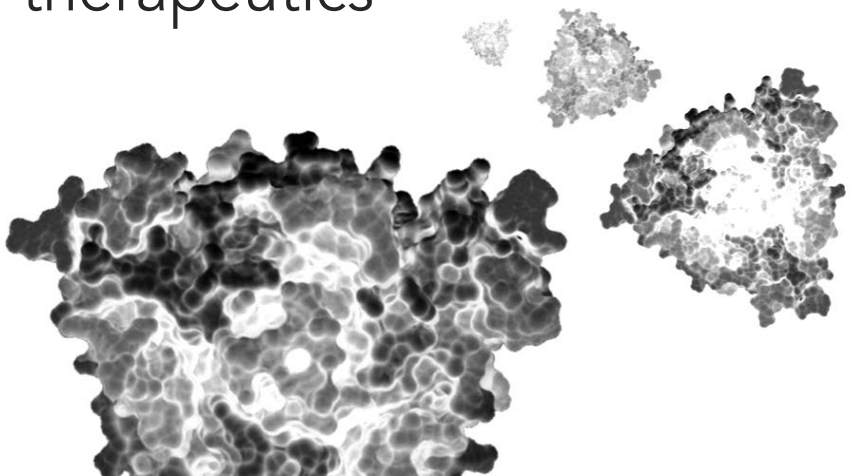
Clinical & Discovery Pipeline

Biosceptre

Product	Approach	Indication	Discovery	Pre-clinical	Phase I	Phase II
CLINICAL PROGRAMS						
BIL03s	Domain Ab	*Solid tumours*				
BIL06v	Peptide Vaccine	*Solid tumours*				
BIL010t	Polyclonal Ab	*Basal cell carcinoma*				
DISCOVERY PROGRAMS						
BIL011t	Topical dAb					
BIL04s	Next gen. systemic monoclonal					
BIL07v	Next gen. peptide vaccine					
BIL03n	Conjugate candidate					
BPM09	IHC Antibody [Diagnostics]					

Conclusion

- Many cancer cell lines express a $P2X_7$ form unable to open a large pore. This form of $P2X_7$ is termed $nfP2X_7$
- Mimicking the high ATP concentration present in the tumour microenvironment drives $nfP2X_7$ expression at the surface of cancer cells
- Biosceptre has designed antibodies and vaccine therapeutics able to specifically target $nfP2X_7$



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