Immuno-Oncology Platform targeting nFP2X7
A Broadly Occurring and High Specificity Cancer Target
nfP2X7 - A cancer specific therapeutic target

- P2X7 / nfP2X7 introduction
- Targeting nfP2X7
- Induction of nfP2X7
- nfP2X7 expression in tumour samples
- Mode of action of nfP2X7 targeted therapeutics
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P2X$_7$ / nfP2X$_7$ introduction
P2X$_7$ plays an important role in cancer

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$_7$. 

Di Virgilio and Adinolfi Oncogene 2016
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
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
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 P2X7

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

P2X₇ 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.

Many non-functional cell lines express P2X$_7$ at mRNA level

* 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
### nfP2X7 mediates Survival in cancer cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tumour origin</th>
<th>Large pore opening</th>
<th>Reduced P2X7 induces cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC3</td>
<td>Prostate</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Du145</td>
<td>Prostate</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>LNCaP</td>
<td>Prostate</td>
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<td>Yes</td>
</tr>
<tr>
<td>SK-MEL-28</td>
<td>Melanoma</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>SK-MEL-5</td>
<td>Melanoma</td>
<td>No *</td>
<td>No</td>
</tr>
<tr>
<td>PC 9</td>
<td>Lung</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>H460</td>
<td>Lung</td>
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</tr>
<tr>
<td>H520</td>
<td>Lung</td>
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<tr>
<td>MCF7</td>
<td>Breast</td>
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<td>Yes</td>
</tr>
<tr>
<td>SK-N-AS</td>
<td>Neuroblastoma</td>
<td>No</td>
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</tr>
<tr>
<td>Kelly</td>
<td>Neuroblastoma</td>
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<td>Yes</td>
</tr>
<tr>
<td>MiaPaCa2</td>
<td>Pancreas</td>
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<tr>
<td>HCT116</td>
<td>Colorectal</td>
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<td>Yes</td>
</tr>
<tr>
<td>HT1080</td>
<td>Fibrosarcoma</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

For 12 out of 14 cell lines, across 7 different cancer indications, reducing P2X7 levels via siRNA induced cell death.

nfP2X7 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

- P2X$_7$ Normal trimer conformation
  - non-specific ion channel
  - Large pore opening
  - Survival and proliferation signal
  - Cell death due to large pore opening

- nfP2X$_7$ Abnormal trimer conformation
  - non-specific ion channel
  - Large pore opening
  - Survival and proliferation signal only
Targeting nfP2X₇
Biosceptre’s approach

- Our founder hypothesised the exposure of the E200 epitope on nfP2X7 but not P2X7
- Antibodies have been produced that target E200 selectively
- nfP2X7 is found specifically at the surface of cancer cells and plays a critical role in cancer cell survival
- Targeting of nfP2X7 has potential in a broad range of cancers including (but not limited to):
  - Lung, Breast, Prostate, Colorectal

Biosceptre’s 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 P2X₇ and nfP2X₇

nfP2X₇ (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 P2X₇ targeting antibody
- **L4** - Mouse monoclonal anti human P2X₇
  - Made by immunisation of mice with P2X₇ transfected cells
  - Strong binding to functional cells and cells transfected with P2X₇
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
Antibodies target nfP2X<sub>7</sub> receptors via their E200 binding domains

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

L4

P2X₇
Normal trimer conformation

non-specific ion channel

Large pore opening

Survival and proliferation signal
Cell death due to large pore opening

BIL03s

nfP2X₇
Abnormal trimer conformation

non-specific ion channel

Large pore opening

Survival and proliferation signal only
Induction of nP2X$_7$
Tumour microenvironment contains high levels of extracellular ATP

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

Patrizia Pellegatti¹*, Lizzia Raffaghello²*, Giovanna Bianchi³, Federica Piccardi³, Vito Pistoia³, Francesco Di Virgilio⁴

ATP in the tumour microenvironment drives a switch from P2X\(_7\) to nfP2X\(_7\)
nfP2X₇ expression in tumour samples
Binding of nfP2X$_7$ antibody in healthy versus cancerous prostate section

Healthy prostate section stained with BPM09

Cancerous prostate section stained with BPM09

Slater et al. Histopathology 2004
nfP2X7 is found on multiple tumours

- Cervical cancer
- Bowel cancer
- Liver cancer
- Breast cancer (Ductal carcinoma in situ)
nfP2X$_7$ is found on multiple tumours

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

Number of samples tested for a given cancer shown above bars
Mode of action of nfP2X_7 targeted therapeutics
nfP2X7 has broad therapeutic potential via multiple modalities

**Antibody**
- BIL03s Domain Ab
- BIL010t Polyclonal
- BIL03n ADC
- Bispecific

**Vaccine**
- BPM09 Diagnostic Ab
- BIL06v Peptide Conjugate

*Current Biosceptre Activity*
# Systemic Antibody

**BIL03s**

- Binds selectively to nfP2X7
- 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
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

Waterfall plot of percentage change in tumour area

01-003
03-005
01-007
02-008
02-009
01-001
01-010
02-008
01-006
03-003
01-005
03-002
02-002
02-001
02-004
03-006

% change in tumour area

Patient number

Gilbert Br J Dermatol. 2017 Feb
Pathology analysis confirms infiltration of lymphocytes and stromal reaction

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

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

H&E x40
Arrows point to lymphocytes, post-treatment

H&E x40
Arrows point to stromal reaction, 20% tumor reduction, post-treatment
<table>
<thead>
<tr>
<th><strong>Topical Therapeutic</strong></th>
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<tbody>
<tr>
<td><strong>BIL010t</strong></td>
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<tr>
<td><strong>Strong indication of efficacy from informed consent and Phase I studies</strong></td>
<td></td>
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<tr>
<td><strong>Excellent safety profile from preclinical studies, informed consent patients and in phase I trial</strong></td>
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<tr>
<td><strong>BCC and advanced BCC provide validated disease targets</strong></td>
<td></td>
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<tr>
<td><strong>Shown to cause immune cell infiltration</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical plan in development</strong></td>
<td></td>
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</tbody>
</table>
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

## CLINICAL PROGRAMS

<table>
<thead>
<tr>
<th>Product</th>
<th>Approach</th>
<th>Indication</th>
<th>Discovery</th>
<th>Pre-clinical</th>
<th>Phase I</th>
<th>Phase II</th>
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<td>Solid tumours</td>
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## DISCOVERY PROGRAMS

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<th>Phase II</th>
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<td>BiL011t</td>
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<td>BiL04s</td>
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<tr>
<td>BiL07v</td>
<td>Next gen. peptide vaccine</td>
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<td>BiL03n</td>
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<td>BPM09</td>
<td>IHC Antibody [Diagnostics]</td>
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</tbody>
</table>
• 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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