Preclinical Tumour Models: Their Role in our MISSION from Drug Discovery to Clinical Practice

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MISSION Therapeutics Ltd.
MISSION Therapeutics:

Selectively targeting ubiquitin pathways to treat disease

- Company started in July 2011
- Major funding of £26m
- Finance to develop first-in-class inhibitors of de-ubiquitylating (DUB) enzymes
- Target based drug discovery
- Core biology in DNA damage response (DDR) and synthetic lethality (ex-KuDOS)
- Creating a DUB drug discovery platform
- 30 employees + 30 synthetic chemists, CRO network

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Talk outline

- Problems facing oncology drug discovery
- Choosing the right model (in order to ask the right question)
  - Olaparib/SOLO as a case study; what lessons can we learn?
- MISSION: Building the right models for success: the path from bench to bedside (and the models in between)
Problems Facing Drug Discovery
Oncology drug discovery

- Highest discovery activity of all the therapeutic areas
- Clinical trials conducted in heavily pre-treated or late-stage disease
- Tumour heterogeneity
- Traditionally, pre-clinical modelling has used cell line xenografts
  - Relatively easy to establish
  - Well characterised/widely used
  - Inexpensive
  - Homogeneous, clonal, lack of stromal component
  - Lack of a fully functional immune system
- High attrition rates in drug discovery/development
Analysed 359 suspended phase III & 95 new drug/biologic programmes

Lack of efficacy identified as the root-cause of clinical failures

Are the tumour models responsible? Are they sufficiently predictive?
Problem: Lack of historical correlation

**PRECLINICAL**

- NSCLC: Gefitinib + chemo
- NSCLC: Vandetanib + chemo

**CLINICAL**

- INTACT PhIII Gem-Cis +/- Gefitinib
- ZODIAC PhIII Docetaxel +/- Vandetanib

**Problem:**

Lack of historical correlation
In fact, multiple myeloma models have a reasonably good degree of predictivity of clinical activity.
Choosing the Right Models to Ask the Right Questions
How do we analyse the data?
47 ovarian cancer cell lines analysed (CN, mut & mRNA expression) and compared to tumours

The most commonly used* cell line models: SK-OV-3, A2780, OVCAR-3, CAOV3 & IGROV1, do not map onto the profile of ovarian tumours

Identified several rarely used cell lines that more closely resemble cognate tumour profiles

Propose mathematical cell line suitability score

*As quantified by PubMed citations (i.e. 90% of publications mentioning at least one of the 47 CCLE ovarian cancer cell lines)
Pre-clinical models used in olaparib R&D

Additional models to further validate results...
Cancer Therapy: Preclinical

Tumor Growth Inhibition by Olaparib in BRCA2 Germline-Mutated Patient-Derived Ovarian Cancer Tissue Xenografts

Ursula Kortmann, Jessica N. McAlpine, Hui Xue, Jun Guan, Gavin Ha, Sophie Tully, Sharaz Shafait, Alan Lau, Aaron N. Cranston, Mark J. O’Connor, David G. Huntsman, Yuzhuo Wang, and C. Blake Gilks

Clin Cancer Res; 17(4); 783–91. ©2010 AACR.

Poly(ADP-Ribose) Polymerase-1 Inhibitor Treatment Regresses Autochthonous Brca2/p53-Mutant Mammary Tumors In vivo and Delays Tumor Relapse in Combination with Carboplatin


Cancer Res 2009; 69: (9). May 1, 2009
How can we use our learning effectively?

- Understand the biology
- Develop a biomarker (patient stratification & response)
- Identify the ‘right’ models
- Utilise all models at your disposal
- Important that clinical teams take the learning from pre-clinical models to aid in the design of clinical trials

Fig. 1 PFS results from the subset of patients with a BRCA mutation
Fig. 1 PFS results from the subset of patients with a BRCA mutation

- Substantially better benefit in the mutant BRCA patient population (HR=0.18)
- Median PFS = 11.2 mo
- Median PFS = 4.3 mo

High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs


Olaparib increases survival of mice bearing authochthonous mammary tumours
MISSION: Building Models for Success
From bench to bedside and the models in between
MISSION: developing innovative effective cancer medicines

- First-in-class deubiquitylating (DUB) inhibitors
- Monotherapy
- Combination with standard-of-care
- Novel/novel combinations

- Unmet clinical need cancers
- Characterisation of biomarkers for tumour selection
- Personalised medicine

- New oncology targets and other disease opportunities
- Selectivity profiling
- Unprecedented hit finding cascade
- Chemical equity (probes & tools)
- Proprietary DUB database

MISSION will target unmet need cancers through inhibiting DUBs and will create world-class expertise to drug DUBs across different therapeutic areas
MISSION: targeting DUBs in cancer

Jacq et al., Cell Biochem & Biophysics (2013)

- DUBs involved in the DNA damage response for synthetic lethality interactions
- DUBs involved in tumour resistance to ‘standard-of-care’ e.g. platinums
- Oncogene DUBs driving tumorigenesis
- Opportunities: Non-oncology DUBs with high disease linkage (e.g. infection, neurodegeneration, muscle wasting)

DUB target through MISSION’s platforms
Approx. 44,000 women in EU each year will develop ovarian cancer
In the majority, the disease is advanced and surgery is not effective
Treatment for late-stage ovarian cancer involves platinum-based chemotherapy
In 25% of these women the disease will recur 1-6 months after end of treatment → platinum resistant disease
Unmet need: platinum resistance

- Approx. 44,000 women in EU each year will develop ovarian cancer
- In the majority, the disease is advanced and surgery is not effective
- Treatment for late-stage ovarian cancer involves platinum-based chemotherapy
- In 25% of these women the disease will recur 1-6 months after end of treatment → platinum resistant disease
- Ovarian cancer has a high mortality rate with 2/3 women not surviving 5 years (33,000 deaths in EU each year)

Platinum resistant ovarian cancer – an area of high unmet need
Identifying DUB targets for platinum-resistant disease

siRNA screen for the identification of DUBs that selectively affect proliferation of platinum-resistant ovarian cancer
Targeting USP11 in ovarian cancer with acquired platinum resistance

- PEO1 & PEO4: matched pre-/post-treatment cell lines (Cooke et al., Oncogene 2014)

Colony Formation Assay

siRNA against USP11 is effective in killing clinically relevant platinum resistant cells
Small molecule inhibition of USP11

- Small molecules recapitulate the phenotypes identified in the initial screens

Strong sensitisation observed in both BRCA2 and platinum resistant backgrounds
Cell assays used in our drug discovery workflow

- In vitro assays
  - siRNA assays
  - 3D spheroid assays
  - Synthetic lethality screens
  - Panel screens (broad and focussed)
  - Combination PF_{50} assays
  - ‘Scratch’ invasion assays
  - Cell EC_{50} assays

Animal models
Cell assays – focussed panels

- Initial screens identified Multiple Myeloma as sensitive to MTX inhibitors

Consistent sub-μM cell activity across MM cell lines
Scratch assay (time-lapse)

- Looking at ability of cells to invade over time +/- cpd

Blue = invasive margin; Gold = plastic substrate

MTX cpd prevents invasion of human cancer cell lines
3D tumour spheroid assay

- Altered proliferation and cell morphology
- Changes in gene expression and cell behaviour
- Reveals a more realistic drug response → more predictive of *in vivo* response?

Growth of cells in spheroids results in differential zones of proliferation due to oxygen, nutrient, and waste gradients, Lin and Chang, 2008.
3D tumour spheroid assay

**2D**

Effect of MTX cpd on cancer cells (2D)

<table>
<thead>
<tr>
<th>MTX cpd (uM)</th>
<th>RLU normalised</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.5</td>
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<tr>
<td>0.01</td>
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<td>0.1</td>
<td>0.5</td>
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IC50 = 0.2473

**3D**

Effect of MTX cpd on cancer cells (3D)

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IC50 = 1.332

3D EC₅₀ is 5.3 times higher than 2D EC₅₀

Cpds show a drop-off in potency in 3D spheroids vs. 2D cell cultures; is there a further drop-off *in vivo*?
Multiple myeloma xenografts

Can achieve \textit{in vivo} tumour growth inhibition and delay with an early hit oral inhibitor

Quantitative analysis of tumours \textit{ex vivo} to confirm MoA and identify biomarkers of response
IHC and whole slide quantitative image analysis (CC3)

Original Image showing CC3 IHC in brown

ROI image overlay showing viable tumour in orange

Detection of CC3 IHC marker area (yellow overlay)

<table>
<thead>
<tr>
<th>Slide name</th>
<th>ROI name</th>
<th>CC3 Marker Area (µm²)</th>
<th>No Stain Area (µm²)</th>
<th>% Marker Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>G3-33 Level 1</td>
<td>Tumour</td>
<td>15,160,638</td>
<td>57,448,708</td>
<td>20.88</td>
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</tbody>
</table>

Stats: Mann-Whitney non-parametric test

$p=0.0399$
IHC and whole slide quantitative image analysis (c-Myc)

Original c-Myc IHC tissue image

Original c-Myc IHC (magnified area)

c-Myc nuclei IHC detection and stain intensity classification

<table>
<thead>
<tr>
<th>Slide name</th>
<th># All Nucleus</th>
<th># Nucleus Negative</th>
<th># Nucleus Positive</th>
<th>Positive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1-23 Level 1</td>
<td>495,660</td>
<td>70,954</td>
<td>424,706</td>
<td>0.86</td>
</tr>
</tbody>
</table>

IHC Intensity
- -ve Nuclei
- +ve Low
- +ve Med
- +ve High

Viable Tumour
Necrotic Tumour
Artefact
White Space / Host Tissue

Stats: Mann-Whitney non-parametric test
\( p=0.0023 \)
IHC and whole slide quantitative image analysis (p53)

Original p53 IHC tissue image

Analysis ROI overlay image

Slide name | # Nucleus Positive | Viable Tumour Area (µm²) | Positive Nuclei per mm² Viable Tumour
--- | --- | --- | ---
G3-33 Level 2 | 22,621 | 84,855,926 | 267

Stats: Mann-Whitney non-parametric test

\[ p=0.0012 \]
Test leads in defined subset relevant PDX models

- Mutation/pathway absent
- Mutation/pathway present

Group into relevant subsets

No response

Response

Mouse images used with permission from Crown Bioscience
Panels of models to represent drug targets, tumour types, genetic subsets & drug resistance
Summary

Using pre-clinical models to...

- Validate drug targets
- Confirm drug MoA
- Identify/validate biomarkers for drug response
- Identify/validate biomarkers for patient selection/stratification
- Deliver PK/PD modelling
- Anticipate drug resistance mechanisms
- Test combinations
- Cross-validate pre-clinical models
- Give us confidence going into clinical development/trials

*Utilise all the models at your disposal*

All models have their limitations but broad, sweeping generalisations do not apply – many have great utility