Bridging academic science and clinical research in the search for novel targeted anti-cancer agents

Alex Matter, M.D.
Experimental Therapeutics Centre & D3, A*STAR
ESMO Conference, Singapore
19th December 2015
Debating points

- Who we are – Mission & strategies
- Collaborative model between public sector and industry
- Two projects producing clinical candidates: Mnk/Abl, Wnt/porcupine
- Possible role of Public Sector R&D in drug discovery in Asia
Mission of ETC/D3 – Capturing the Opportunities!

- To build bridges between basic science and the clinic – translational R&D
- To guide early-stage scientific discoveries towards Proof-of-Concept clinical trials in man
- To serve unmet medical needs in Singapore and the region through innovative product candidates
- To generate economic benefit
From Drug Target to Proof-of-Concept in man!
Pathway for a small-molecular weight compound, as an example

Legend
- GCP, Good Clinical Practice
- GMP, Good Manufacturing Practice
- GLP, Good Laboratory Practice
- DLT, Dose-limiting toxicity
- ADME, Absorption, Distribution, Metabolism, Excretion

IND/CTC, Permission to start clinical trials
MTD, Maximally tolerated dose
PDC, Preclinical Development Candidate
PoC, Proof-of-Concept
Comprehensive Capabilities and Resources
Primary focus on Oncology and Infectious Diseases

Skill bases & Technologies
- Cell-based Assay Development
- Protein Biochemistry
- High Throughput Screening
- Medicinal Chemistry
- Antibody Technologies
- Molecular Diagnostics
- Analytics – Mass Spectrometry
- High end NMR
- Bioinformatics
- Preclinical Pharmacology

Priority Therapeutic & Product Focus
- Oncology
- Infectious Diseases
- Other Indications
- Products
  - Drug Candidates
  - Vaccine Candidates
  - Diagnostics & Biomarker Candidates

Resources of ETC & D3
- ETC: 88 FTEs, 50% for biochemistry, cell biology, analytics, HTS – 50% for medicinal chemistry and computational chemistry; 24 FTEs outsourced
- D3: small team of experts plus range of specialized consultants
A first example of a drug candidate born in Singapore

A case of inhibiting simultaneously two targets

- one well known and hard to target: BCR-ABL/BCR-ABL\textsuperscript{T315I}
- one with a complex biology: MNK1/2
- Indication: drug-resistant Blast Phase of CML, imatinib-resistant PH1+ALL, DLBCL?

- Collaboration between Prof Tiong S. Ong and Dr Sharon Lim, Duke-NUS and ETC/D3
Blast crisis is the major remaining challenge in the management of Chronic Myelogenous Leukemia (CML).
CML – increased & unregulated growth of myeloid cells in the bone marrow & their accumulation in the blood

β-catenin-mediated self-renewal is an important feature of myeloid blast crisis granulocyte macrophage progenitors
eIF4E overexpression & phosphorylation activates β-catenin in Blast Crisis (BC) Leukemic Stem Cells (LSCs)

Lim et al. PNAS 2013

Targeting of the MNK-eIF4E axis in blast crisis chronic myeloid leukemia inhibits leukemia stem cell function

Chronic Phase

Blast Crisis

S209

MNK1/2

eIF4E

P

β-catenin activity

AKT

S552

β-catenin

eIF4E

P
Clinical Hypothesis
While BCR-ABL inhibitors control CP CML, MNK inhibitors by targeting BC LSCs, will control BC CML

Controlled with BCR-ABL inhibitors

Controlled with MNK inhibitors

Enhanced self-renewal

In vitro Serial Replating Assay (SRA)

In vivo Serial Transplantation Assay
Selective Mnk Inhibitors
Profile of ETC-B and ETC-C: *in vitro* selectivity

<table>
<thead>
<tr>
<th>% Inhibition</th>
<th>Number of kinases inhibited</th>
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<tbody>
<tr>
<td>&gt;90</td>
<td>ETC-B: 1</td>
</tr>
<tr>
<td></td>
<td>ETC-C: 3</td>
</tr>
</tbody>
</table>

Screening of 104 kinases @ 1 μM
Combination of Mnk & Bcr-Abl Inhibitors on Primary BC CML LSCs

![Graph showing the effect of Mnk & Bcr-Abl inhibitors on LSCs. The graph compares the serial replating efficiency of LSCs treated with different concentrations of inhibitors, including ETC-B and Imatinib.]
In Vitro Validation of Biomarkers in CML Cell Line K562 After Compound Treatment

Mnk activity

Bcr-Abl activity

Crkl (~39kDa)

Phospho-Crkl (Tyr207) (~39kDa)

Merged

elF4E (25kDa)

elF4E (p-Ser209) (25kDa)

Merged
In Vivo Validation of Biomarkers in K562 Xenograft Model

- Dose dependent inhibition of p-eIF4E observed with both compounds
- ~35% inhibition of p-eIF4E (ser209) is observed in tumor xenograft excised from mice treated with 30MPK of compound treatment
A second example –
Development of Wnt Signaling Inhibitors

Collaboration between Prof David Virshup/Dr Babita Madan, Duke-NUS and ETC/D3

To identify compounds that block Wnt secretion with the potential to act as specific inhibitors of Wnt signaling for anti-cancer drug development
What Wnts do….

- Regulate cell fate, differentiation and morphogenesis during development
- Regulate stem cell proliferation and differentiation throughout life
- Implicated in diverse processes including bone metabolism, inflammation, wound healing, atherosclerosis, angiogenesis, pathologic fibrosis
- Dysregulated in multiple cancers by mutation, epigenetics, miRNAs
Wnt Signaling is really complex

19 Wnts

 Canonical Pathway

Other important things happen
The Wnt secretion pathway

PORCN and WLS are key regulators of global Wnt production

**PORCN**
- Membrane Bound
- O-acyl transferase
  - Transferase palmitate to conserved serine on Wnt

**WLS**
- Transports palmitoleated Wnt to PM
Genetic loss of PORCN abrogates function of all human Wnts

PORCN Null HT1080 cells

Wnt

β-catenin Signaling (TOPFLASH)

Wild type
PORCN Deleted

Essential role for palmitoleate in ligand-receptor interaction

Can we interfere with Wnt Production using small molecules?

**Wnt Pathway Multistep Drug Screen**

**ER**

**PORCN**

O-acyl transferase, transfers palmitoleate to conserved serine on all Wnts

**WLS**

Transports palmitoleated Wnt to Plasma membrane

1. Wnt
2. Wnt
3. Wnt
4. Dvl
5. APC/GSK/Axin
6. @-cat

**Wnt3a**

**PGK**

**Super Top Flash**

**Luciferase**

Gary Coombs; May Ann Lee and Horst Flotow at Experimental Therapeutics Centre
Inhibition of Wnt/β-catenin activity in STF3a Cells

ETC-1922159 IC₅₀: 0.003μM
ETC-1922130 IC₅₀: NI
ETC-1922131 IC₅₀: 0.0004μM

Relative STF Activity vs. Log conc (µM)
Palmitoleation of Wnt3a is inhibited by PORCN inhibitors

- Label cells with alkyne-palmitate
- IP Wnt via V5 tag
- Click with azido-Biotin
- SDS-PAGE
- Probe for Wnt3a-V5, Biotin-palmitate

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>ETC-1922159</th>
<th>ETC-1922130</th>
<th>ETC-1922131</th>
<th>C-59</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkyne-palmitate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Click</td>
<td>+</td>
<td>+</td>
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Wnt3a-V5
Biotin-palmitate
HeLa cells
Interaction of WNTs with WLS is dependent on PORCN-mediated palmitoleation

<table>
<thead>
<tr>
<th>mWnt3a</th>
<th>WT</th>
<th>S209A</th>
</tr>
</thead>
<tbody>
<tr>
<td>hWls-V5</td>
<td>+</td>
<td>+</td>
</tr>
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### IB

- **Wnt-V5**
- **WLS**
- **Wnt-V5**

### Wnt3a-V5

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<th>EV</th>
<th>DMSO</th>
<th>ETC-1922159</th>
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- **IP (WLS)**
- **Lysate**

- IP WLS
- +/- PORCN inhibitor
- Probe for Wnt-V5 and WLS
Do Porcn Inhibitors block the growth of cancers?

*MMTV-Wnt1* mouse: a genetic model of mammary cancer

TgN(Wnt1)1Hev; Varmus and co-workers, Cell 55, 619–625. (1988)
ETC-1922159 is orally bioavailable

Plasma concentration of ETC-1922159 (ng/ml) vs. Time (h)

IC$_{50}$ = 3 nM
ETC-1922159 prevents growth of the teratocarcinoma PA-1 tumors in mice

![Graph showing the prevention of tumor growth by ETC-1922159. The graph plots the average tumor volume (mm³) against the day of treatment. The bars on the right show the relative expression of Axin 2, with Vehicle and ETC-1922159 treatment groups compared. The graph illustrates a significant reduction in tumor volume and expression for the treatment group compared to the control group.](image-url)
New findings in the Wnt pathway reveal predictive biomarkers
Wnt receptors are regulated, too

Loss of function of RNF43/ZNRF3, or Gain of Function of R-Spondins, make cancer cells much more sensitive to Wnts
# Frequency of these mutations in various cancers

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Frequency of mutation</th>
</tr>
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<tbody>
<tr>
<td>Colorectal</td>
<td>~ 10% RSPO translocations</td>
</tr>
<tr>
<td>Colorectal</td>
<td>~ 3-5% RNF43</td>
</tr>
<tr>
<td>Gastric</td>
<td>~ 4-8% RNF43</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>~ 4% RNF43</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>~ 18% NOTCH1</td>
</tr>
<tr>
<td>Ovarian Mucinous</td>
<td>~ 10% RNF43</td>
</tr>
<tr>
<td>Endometrial</td>
<td>~ 22% RNF43</td>
</tr>
</tbody>
</table>

http://www.cbioportal.org/public-portal/
http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/
ETC-1922159 is effective therapy for HPAF-II RNF43 mutant, pancreatic cancer xenografts

E174X in exon 3 of RNF43

Group Mean Tumor Volume (mm$^3$ ± SEM)

Days

Vehicle
ETC-1922159 - 10 mpk
ETC-1922159 - 30 mpk
ETC-1922159 - 100 mpk
Treatment with ETC-1922159 promotes differentiation: HPAF-II, pancreatic cancer xenografts

**Vehicle**

**ETC-1922159**

Mucin (Alcian Blue Staining)
Colorectal Cancer PDX with a PTPRK-RSPO3 fusion gene: efficacy of ETC-1922159

This compound has reached CTC (HSA) and IRB approval and shall enter clinical trials shortly (June 2015). It is now renamed ETC-159.
Wnt/Porcupine project – Conclusions

Small molecule inhibitors of PORCN inhibit secretion of all Wnts and block proliferation of Wnt dependent tumors.

Intestinal homeostasis is not affected by therapeutic doses of PORCN inhibitors.

Small molecule inhibitors of PORCN inhibit secretion of all Wnts and block proliferation of Wnt dependent tumors.

ETC-1922159

LRP
FRZ
CRD

50 μM
Singapore is a role model for collaborative R&D and ETC/D3 can be a catalyst

ETC/D3 creates value through networking with our public research institutions and hospitals, and is a core interface with the pharma & biotech industry
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