Targeting cancer aneuploidy:

Light at the end of the tunnel?

Tak W Mak    Toronto    Canada
Today’s cancer targets: shoot the horses!

Horses:

- Oncogenes
  - EGFR
  - Her2
  - Abl
  - Raf
  - alk
  - Btk
  - Etc.

- Oncogenes and tumour suppressor genes drive the development of tumours
Will Targeted Drugs Hit the Wall Soon?

- **1998**  Her-2, Herceptin etc
- **2001**  Bcr-abl, Imatinib etc
- **2003**  EGFR, Gefitinib etc
- **2003**  Proteasome, Bortezomib
- **2004**  VEGF, Bevacizumab
- **2006**  HDAC, Vorinostat
- **2007**  mTOR, Temsirolimus
- **2010**  Provenge
- **2011**  anti-CTLA4
- **2011**  ALK, Crizotinib
- **2011**  B-raf, Vemurafenib
- **2012**  Hedgehog, Vismodegib
- **2013**  Btk, Ibrutinib
- **2014**  PI3-Kδ, Idelalisib
“There are more paths to developing tumors than there are stars in the sky”  R Weinberg

Galaxy 200 billion
Universe 3,000 million billion

CML, APL, Her-2 (Ovarian, NB, etc)  Pancreas (k-ras p53 p16 smad4)  Breast/Lung/Colon (EGFR ALK mTOR)
Personalised Medicine:

1. Biomarkers (genomics)
2. Drug Combinations
   - pathways knowledge
   - Intuitive deductions
Future: target the carts?

- Oncogenes
- Carts:
  - Immune
  - Metabolism
  - Aneuploidy

The cart is the transformed state of the cell as a consequence of the actions of oncogenes and tumour suppressor genes.

Carts are INDEPENDENT of oncogenes & TSGs
Determinants of the Tumour Metabolic Phenotype

Genetic alterations (affecting p53, MYC, AMPK, PI3K and HIF1)

Tumour microenvironment (hypoxia, pH, nutrients and autophagy)

Abnormal metabolic phenotype

Bioenergetics

Biosynthesis

Redox

NADPH

GS-SG → GSH

ROS
Are Tumours Addicted to Oncogenes or Metabolism?

- Metabolism

Rapamycin

Translation

Growth

Metabolic Addiction?

Oncogene Addiction?

Growth factor
Receptor tyrosine kinase

Ras

MEK 1/2

ERK 1/2

p38

MEK 3/6

AMPK

S6K

4E-BP

mTOR

AKT

PTEN

PI3K

Sos

Grb2

Shc

IRS

Transcription

Translation

Growth

- Metabolism

Rapamycin

Translation

Growth
CPT1C Expression Correlates With Resistance to Rapimycin
211 tumours; 40k probes (AVEO)

PI3 Kinase Index

Cpt1c probe 1

Cpt1c probe 2

Mean Cpt1c

P<0.00001

No correlations with cpt1a/b!!

Zaugg et al G&D 2011
The Role of CPT1c in Tumour Metabolic Adaptation

CPT1a/b

CPT1c (p53 & hypoxia inducible)

Bioenergetics
Biosynthesis
Bioenergetics
Biosynthesis
Redox

Reilly & Mak, Clinical Cancer Research, 2012
Inducers and Scavengers of Reactive Oxygen Species:

**INDUCERS**
- Hypoxia
- Metabolic defects
- ER stress
- Oncogenes

**SCAVENGERS**
- Glutathione
- NADPH
- Nrf2
- Tumor suppressors

**BALANCE**
- Dietary antioxidants

Potential Sources of Reducing Equivalents (NADPH)

\[
\text{GS-SG} \rightarrow \text{GSH} \rightarrow \text{ROS}
\]

Pentose Phosphate Shunt

Glutaminolysis

→ Isocitrate Dehydrogenase 1/2*

*IDH1/2 mutated in GBM, glioma, AML, AITL, Chon-Sa, Chol-Ca
LysM-Knock-in Mice Exhibit Age-dependent Splenomegaly & Decreased BM Cellularity Extra-medullary Hemopoiesis, Increases in progenitors - similar to Myelodysplastic Syndrome

Preliminary Results on AG-221 mIDH2 Inhibitor Ongoing Phase 1 Clinical Trials on Refractory AML

<table>
<thead>
<tr>
<th></th>
<th>2HG</th>
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<tr>
<td>Complete remission</td>
<td>9</td>
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<tr>
<td>Partial remission</td>
<td>5</td>
</tr>
<tr>
<td>Progression disease</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
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Drug well tolerated
No discontinuation due to adverse events
Not reached MTD

Stein et al 2014
Inducers and Scavengers of Reactive Oxygen Species:

**Inducers**
- Hypoxia
- Metabolic defects
- ER stress
- Oncogenes

**Scavengers**
- Glutathione
- NADPH
- Nrf2
- Tumor suppressors
- Dietary antioxidants

**Inducers and Scavengers of Reactive Oxygen Species**

- G6PD
- GCLM
- GPX2
- PGD
- GCLC
- TXN1
- ME1
- GSR
- PRDX1
- IDH1
- XCT
- TXNRD1


**Balance**

- ROS
Can the regulation of ROS levels explain BRCA1 carriers mainly only develop Breast and ovarian cancers?

Lifetime risk

70% breast
40% ovarian
15% prostate
Estrogen-controlled NRF2 activation in BRCA1-related tumorigenesis

BRCA1+/-

Brca1 somatic loss

BRCA1-/-

estrogen

PI3K

mTOR

E2

NRF2

ROS

cell death

OTHER TISSUES

p53 loss/mutation
53BP1 loss/PTEN mutation

cell survival

CANCER

BREAST & OVARY

Basal

Gorrini, Gauthier et al JEM, PNAS 2014
Basal-like Breast Cancers Express Lower Levels of Nrf2 Target Gene NQO1

Chin et al., 2006
n.s.

Pawitan et al., 2005
**
n.s.

Sabatier et al., 2011
n.s.
<table>
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*Nuclear PTEN controls homologous recombination mediated DNA repair and sensitivity to DNA damage*  
Bassi et al Science 2013

Oxidative Stress                          DNA Repair

Chromosomal Instability

Aneuploidy

Genetic Aberrations

Malignancies
Basal cancers: much more re-arrangements

Basal 15%

Non-basal 85%

Triple Negative Breast Cancer mutations. Aparicio et al 2012
The Consequences of Aneuploidy

- Mouse models of mitotic checkpoint dysfunction have shown that aneuploidy can be causative for cancer

→ Cancer is a potential outcome of aneuploidy (70%)

- Aneuploidy acts as tumor suppressor in certain contexts

![Diagram showing low and high levels of aneuploidy leading to tumorigenesis and cell death.](image-url)
Whole kinome SiRNA screens on 14 breast cancer lines

There is no silver bullet!
Limit your target to one cancer subtype!
Kinome siRNA Z-scores of 14 Breast Cancer Cell Lines

PLK1

Aurora A

Aurora B

4 TNBC/basal lines
2 non-malignant basal lines
3 luminal HER2-/ER+
2 HER2+/ER+
3 HER2+/ER−
Kinome siRNA Z-scores of 14 Breast Cancer Cell Lines

PLK1

Aurora A

Aurora B

Mason et al., Cancer Cell 2014

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3 HER2+/ER−
Basal cancers: much more re-arrangements.

Basal 15%

Non-basal 85%

Aneuploid breast cancer cell

>150  46

Triple Negative Breast Cancer mutations. Aparicio et al 2012
PLK4 and TTK are Potential Therapeutic Targets for Triple-negative/Basal-like Breast Cancer

Mason et al., Cancer Cell 2014
Aneuploidy

PLK4 centriole duplication CFI-400945

TTK spindle assembly checkpoint CFI1870
Expression of PLK4 in Breast Cancers and Prognosis

<table>
<thead>
<tr>
<th>All Breast Cancers</th>
<th>Triple-negative/Basal-like</th>
<th>HER2+</th>
<th>Luminal A</th>
<th>Luminal B</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Up</td>
<td>Ratio</td>
<td>p-value</td>
<td>% Up</td>
<td>Ratio</td>
</tr>
<tr>
<td>26</td>
<td>1.46</td>
<td>8.5x10^{-4}</td>
<td>48</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>1.06</td>
</tr>
</tbody>
</table>


NEJM Dataset Survival vs. PLK4, probe NM_014264
Cox regression p-value = 7x10^{-5}, BH FDR = 0.002

--- high PLK4
--- low PLK4

Fraction of Surviving Patients

0 5 10 15
0.6 0.7 0.8 0.9 1.0
Knockdown of PLK4 Inhibits Tumour Growth
Structure-guided Lead Optimization

Screening Hit 11
PLK4 IC₅₀ = 32 μM

New Chemotype 17
PLK4 IC₅₀ = 0.29 μM

Potent PLK4 Inhibitor 50
PLK4 IC₅₀ = 0.0006 μM

PLK1/2/3 = 50μM

Jeremy Squires

TTKi
Pre-clinic

PLK4i
IND approved 11/13
1st patient dosed 4/14

>70 chromosomes

1 compound

2
Contrasting Cellular Phenotypes Induced by Mitotic Kinase Inhibitors

**DMSO**
- PERICENTRIN
- ALPHA-TUBULIN
- DAPI

**CFI-TTK-001**
- Segregation Errors
- Mitotic Acceleration
- Aneuploidy

**CFI-PLK4-002**
- Centrosome Amplification/Multipolar Division
- Failed Cytokinesis/Polyplody

**BI2536 (PLK1i)**
- Monopolar Spindles
- Mitotic Arrest/Death

**MLN8054 (AURKAi)**
- Monopolar Spindles
- Mitotic Delay
- Aneuploidy

**AZD1152 (AURKBi)**
- Misalignments
- Failed Cytokinesis
- Polyploidy
CFI-400945 Causes Centriole Duplication Defects in Cancer Cells
Effects of CFI-400945 on p-HH3 & aberrant mitosis in PDX xenografts.

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Phospho-Histone H3 (Ser10) Positive Cells $^1$</th>
<th>Aberrant Mitoses $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Metaphase with Misaligned Chromosomes</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.1 ± 0.1 %</td>
<td>1.3 ± 0.5 %</td>
</tr>
<tr>
<td>9.4 mg/kg CFI-400945</td>
<td>2.7 ± 0.1 %</td>
<td>5.0 ± 1.2 %</td>
</tr>
</tbody>
</table>
CFI-400945 Increases the Level of PLK4 at Centrioles
Centrosome Duplication Requires PLK4 Degradation

PLK4 auto-phosphorylation needed for degradation

Mónica Bettencourt-Dias ’14 NRMCB
PKL4i converts tumorigogenic initiation role of aneuploidy to inducing cell death

PLK4 degrades
PLK4Kinase pS305
PLK4

PLK4 stabilizes
PLK4Kinase pS305
PLK4

CFI400945

Holland, Cleveland ‘10 ‘12
Gordinho, Pellman ‘09 ‘14
Mónica Bettencourt-Dias, ’10 ‘14
Profound cancer cell killing by CFI-400945

Untreated

Treated with CFI-400945

Densely packed cancer cells

Dead/dying cancer cells

Scar tissue/supporting cells
Antitumor Effects of CFI-400945 on a Carboplatin-sensitive Ovarian PDX

Mason et al., Cancer Cell 2014
CDDP Resistance Does Not Affect Sensitivity of Ovarian Cancer Cells to CFI-400945

<table>
<thead>
<tr>
<th>CELL LINE</th>
<th>GROWTH INHIBITION, GI&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CFI-400945</td>
</tr>
<tr>
<td>OVCAR-5</td>
<td>0.01</td>
</tr>
<tr>
<td>OVCAR-5-CisR</td>
<td>0.007</td>
</tr>
<tr>
<td>SKOV-3</td>
<td>0.01</td>
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<tr>
<td>SKOV-3-cis</td>
<td>0.007</td>
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<tr>
<td>A2780</td>
<td>0.007</td>
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<tr>
<td>A2780/CP70</td>
<td>0.009</td>
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Cells were treated with CFI-400945 for 5 days, and GI<sub>50</sub>s were measured by cell counts, except for A2780 and A2780/CP70 cells which were measured by Alamar Blue assay.
CFI-400945 in a Breast Cancer PDX

Primary Human Breast Cancer (ER$^{\text{low}}$/PR$^{-}$/HER2$^{-}$, PTEN Null)

- **Vehicle**
- **CFI-400945, 13.5 mg/kg PO, 2 Days On/5 Days Off**
- **Carboplatin, 75 mg/kg IP, QWX2**
- **CFI-400945, 13.5 mg/kg PO, 2 Days On/5 Days Off**

Mason et al., Cancer Cell 2014
Confidential


A.

<table>
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<th>PTEN mutant</th>
<th>PTEN wild-type</th>
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<td>PIK4</td>
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B.

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PTEN Z score

SUM44 | MDA-MB-468 | CAL120 | ADAM30 | BT20 | T47D | T47D S | HCC1143 | HCC1143 | MDA-MB-231 | MDA-MB-231 | SUM149 | JIMT-1 | MCF-7 | CAL51 | CAMA1

Box plot showing TTK Z score distribution for PTEN wild-type and PTEN mutant.
Percent Involvement of PTEN, BRCA1, p53 Breast Ca Subtypes

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Response Profile of CFI-400945 Versus Breast Cancer Cell Lines.

P < 0.005
Jeremy Squires

TTKi Pre-clinic

PLK4i
IND approved 11/13
1st patient dosed 4/14

>70 chromosomes

1 compound

2
CFI-400945

Drug Substance
(DSM Pharmaceuticals)

Drug Product
(Pharmatek)

670g GMP CFI-400945 fumarate produced

Sites opened: PMCC, Toronto, UCLA

290g API formulated
- 17,300 1.5 mg tablets
- 22,700 8 mg tablets