Activity of the Monocarboxylate Transporter 1 Inhibitor AZD3965 in Small Cell Lung Cancer

Fiona Blackhall MD PhD
Medical Oncologist
TAT March 2-4 Paris
Disclosures

Advisory Board
Trial steering committee membership
Research support

Astra Zeneca
Monocarboxylate transporters 1-4 (MCT1-4)

Cairns, Harris, Mak. Regulation of Cancer Cell Metabolism. Nat Rev Cancer 2011; 11, 85-95
Glycolysis, Lactate & MCT1

**Reliance on glycolysis occurs** under both aerobic (Warburg effect) & anaerobic (Pasteur effect) conditions in cancer cells
- a hypoxic microenvironment
- presence of p53 loss and/or MYC amplification
- the clinical utility of FDG-PET scanning is based on the ‘addiction’ of cancers to glucose

**Inhibition of MCT1 will lead to intracellular lactate accumulation, acidosis & cell death**

**Upregulation of MCT1** compared to MCT4
- various cancer cell lines and tumours
- in breast cancer associated with worse outcomes (Boidot et al Cancer Res 2011)

**Inhibition of MCT1**
- cell death in glioma cell lines
- suppresses growth of colon cancer xenografts
AZD3965: oral, selective small molecule inhibitor of MCT1

Originally developed by AstraZeneca for use in transplant rejection/ autoimmune disease
- 10 fold selectivity for MCT1 over MCT2
- Does not inhibit MCT4
- Potent anti-proliferative activity in vitro in cancer cells with high MCT1 and low MCT4
- High MCT4 expression is associated with resistance

Well tolerated in preclinical toxicity studies
- MCT 2-4 will maintain lactate homeostasis in normal tissues
- Glycolytic respiration rare in normal cells

A CR-UK sponsored and funded phase I trial is in progress in patients with solid tumours (NCT01791595) Cl: R Plummer (Newcastle)

Primary objective: Tolerated dose at which MCT1 is inhibited (assessed by at least a four-fold change in lactate levels in PBMCs) and/or the MTD in patients with advanced solid tumours or lymphomas
Small cell lung cancer characteristics
- High proliferation rate (MIB-1/Ki67 >95%), tumour doubling time 30 days
- MYC amplification / p53 mutation
- Highly FDG-PET avid
- Exhibit regions of necrosis & hypoxia
- High unmet clinical need - <5% 5 year survival

Activity of the Monocarboxylate Transporter 1 Inhibitor AZD3965 in Small Cell Lung Cancer

Radosław Polański¹, Cassandra L. Hodgkinson¹, Alberto Fusi⁴, Daisuke Nonaka⁴, Lynsey Priest¹,⁴, Paul Kelly¹, Francesca Trapani¹, Paul W. Bishop⁵, Anne White²,³, Susan E. Critchlow⁶, Paul D. Smith⁶, Fiona Blackhall¹,⁴, Caroline Dive¹, and Christopher J. Morrow¹
AZD3965 activity in Normoxic and Hypoxic SCLC cell lines

72 hours exposure to AZD3965 at 8nmol/L fraction of unaffected cells treated relative to untreated control (SRB / resazurin assay)

Polanski et al CCR 2014
Proof of concept: AZD3965 efficacy is associated with lactate accumulation in vitro & in vivo

Treatment with AZD3965 8nmol/L
- Reduction in cell number
- ~4 fold increase in IC lactate

Polanski et al CCR 2014
Analysis of cell cycle, cleaved casp3, cleaved & total CK-18: Mode of cell death is non-apoptotic

Polanski et al CCR 2014
MCT4 expression is associated with resistance to AZD3965

<table>
<thead>
<tr>
<th></th>
<th>Sensitive</th>
<th></th>
<th>Resistant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COR-L103</td>
<td>NCI-H1048</td>
<td>DMS114</td>
<td>DMS79</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AZD3965</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MCT1</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MCT4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>tubulin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

44 kDa
48 kDa
55 kDa

MCT4
48 kDa
55 kDa

Hypoxic AZD3965 Fraction Unaffected

Fraction Unaffected

MCT4:Tubulin Ratio

p=0.0028

Polanski et al CCR 2014
Resistance to AZD3965 in NCI-H1048 cells engineered to overexpress MCT4

Polanski et al CCR 2014
MCT4 RNAi confers sensitivity to AZD3965 in resistant cell lines

Polanski et al CCR 2014
MCT1,4 and CAIX expression & clinical significance in SCLC tumours : TMA n= 78

Polanski et al CCR 2014
PD & predictive biomarkers for AZD3965 in patients with SCLC: circulating tumour cells, MCT1 & MCT4

Biopsy is possible but invasive - yields ~ 150 cells

Mean CTC# in 7.5mls blood ± sd = 1795 ± 4620 cells
Post 1 treatment cycle CTC# declines (PD biomarker)

Cellsearch assay for analysis of CTCs for MCT1 drug target and MCT4 resistance marker expression

Validation of an Immunofluorescence Method to Determine MCT1 and MCT4 Expression in Circulating Tumour Cells Kershaw, Cummings, Morris, Tugwood, and Dive submitted

© Cancer Research UK Manchester Institute
Preclinical models of SCLC derived from patients’ circulating tumour cells Hogkinson et al Nat Med 2014

- CTC derived explant (CDX) models established using CTCs from patients represent clinical SCLC and are a new platform for drug testing / preclinical development

Models recapitulate different sensitivities to platinum / etoposide chemotherapy observed in patients

Clusters & sheets of densely packed small oval, scant cytoplasm, enlarged hyperchromatic nuclei, inconspicuous nucleoli, speckled chromatin.

Expression of CK, CD56, synaptophysin, chromogranin

High Proliferation indices (~80%)
Summary & Perspectives

- Preclinical data provides a rationale to evaluate AZD3965 in SCLC
- Early clinical development will be enhanced by employing assay of CTCs serially
- Assessment of AZD3965 in novel CDX models will provide further preclinical data to inform development and to test AZD3965 in combination

- TAT 2015
- Hypothesis: ‘A biomarker is needed for patient selection’
- Alternative: ‘We do not always need a biomarker to be successful’

- Early phase trials need to test the biomarker hypothesis – phase III is too late
ACKNOWLEDGEMENTS

Clinical & Experimental Pharmacology Group Lead
Caroline Dive
Preclinical Senior Scientist
Chris Morrow

Post doctoral and clinical Fellows
Radek Polanski, Alberto Fusi

Pathology
Daisuke Nonaka

CTC and GCLP Teams
Cassandra Hodgkinson, Karen Morris, Jeff Cummings

Lung Clinic/Lab liaison
MCRC Biobank Jane Rogan, Gary Ashton
Lynsey Priest

Astra Zeneca Paul Smith
Newcastle ECMC Ruth Plummer
CRUK DDO