Targeting DNA damage repair beyond PARP – further drugs or targets in development

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Disclosure

• I have no conflicts of interest to disclose in relation to the targets discussed in this talk.

• Newcastle University receives research funding for ongoing or previous in projects in this area.
Brief

- Overview of DNA damage response (DDR) pathways
- Lessons from history of DNA repair inhibition and chemotherapy
- Brief description of DDR targets currently in development or late pre-clinical
- Thoughts on trial design
- Opportunities for combinations
DDR Inhibitors in Cancer Treatment

- Radiotherapy and many anticancer drugs act by damaging DNA
  - DNA repair in the tumour may be a cause of resistance
    - DNA repair inhibitors may be chemo- or radiosensitizers

- Some tumours may be more effective at DNA repair than the target normal tissue
  - Inhibiting the DNA repair pathways will level the playing field

- Some tumours lack specific DNA repair pathways (eg, BCRA1/BRCA2, HNPCC, ATM, DNA-PK, Fanconi)
  - Inhibiting alternate repair pathways may be a mechanism for antitumour selectivity ("synthetic lethality")
  - Sensitivity to specific DNA-reactive drugs (eg carboplatin) may occur
MAJOR MECHANISMS OF DNA DAMAGE AND REPAIR

Ionising radiation
Antitumour agents

UV light
Polycyclic aromatic hydrocarbons

Replication errors

Ionising radiation
Oxygen radicals
Spontaneous reactions
Antitumour agents

Alkylating agents

Interstrand crosslink
Double-strand break

(6–4)PP
Bulky adduct
CPD

A–G mismatch
T–C mismatch
Insertion
Deletion

Uracil
Abasic site
8–Oxoguanine
Single-strand break

DNA alkylation
$O^6$–alkylguanine

Recombinational repair (HR, NHEJ)

Nucleotide excision repair

Mismatch repair

Base excision repair

Direct reversal (AGT, MGMT)

Targets under investigation in the DNA Repair Pathways

- DR
  - \(O^6\)-BG
  - Patrin

- BER
  - Glycosylase
  - AGT

- NER
  - APE1
  - AG014699
  - AZD2281
  - BSI-201
  - KU-55933
  - CP466722
  - ATRi
  - SC-202994
  - NU6027

- MMR
  - DSB
  - DNA-PKcs
  - NU7441
  - AMA37
  - IC60211
  - IC86621

- HR
  - MP-470

Modified from Ding et al. Trends in Pharm. Sciences, 2006
With thanks to Mark Kelly
LESSONS FROM CHEMOPOTENTIATION TRIALS WITH DDR MEDIATORS
AGT or MGMT (direct reversal repair)

$\text{AGT} \quad \text{or MGMT (direct reversal repair)}$

$\text{AGT - benzyl}$

$\text{O}^6\text{-methyl}$

$\text{G}$

$\text{AGT}$

$\text{methyl-}$

$\text{AGT}$

$\text{+ BG or BG analogues}$

$\text{AGT}$

$\text{AGT Degradation}$

$\text{O}^6\text{-methyl}$

$\text{G}$

$\text{O}^6\text{-lesion on guanine persists in the DNA.}$
Carmustine Combined with O\textsuperscript{6}-Benzyl guanine

- Recommended dose for combination 40 mg/m\textsuperscript{2} carmustine + 120 mg/m\textsuperscript{2} O\textsuperscript{6}-benzylguanine
- Carmustine dose has to be reduced to 20-25\% of single agent dose

Schilsky et al, Clin Cancer Res 6:3025, 2000
Phase I Trial of 4-BTG & Temozolomide

Depletion of PBMC ATase after IV and PO 4-BTG

ADD of 4-BTG is 10mg/m²
Phase II dose 4-BTG 40mg/day with temozolomide 125mg/m²/day

Ransom, Middleton et al, CCR (2006) 12, 1577-84
<table>
<thead>
<tr>
<th>Agent</th>
<th>Company</th>
<th>Single/Combination therapy</th>
<th>Route of administration</th>
<th>Disease</th>
<th>Clinical status</th>
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</thead>
<tbody>
<tr>
<td>AG014699 (PF0367338)</td>
<td>Pfizer (New York, NY)</td>
<td>Combination and single agent</td>
<td>I.v. And oral</td>
<td>Solid tumors, melanoma</td>
<td>Phase I + II MM complete TMZ, phase II in BRCA pts open, phase I ongoing</td>
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<tr>
<td>KU59436 (AZD2281)</td>
<td>AstraZeneca/ KuDOS (London, United Kingdom)</td>
<td>Single/ Combination ++</td>
<td>Oral</td>
<td>Various</td>
<td>Phase I complete. Numerous phase II studies</td>
</tr>
<tr>
<td>ABT-888 (veliparib)</td>
<td>Abbott Laboratories (North Chicago, IL)</td>
<td>Single/ Combination ++</td>
<td>Oral</td>
<td>Solid tumors and lymphoid malignancies</td>
<td>Phase 0/1 completed Numerous phase II studies RT trials</td>
</tr>
<tr>
<td>BSI-201 (SAR 240550)</td>
<td>BiPar (Brisbane, CA) (SanofiAventis)</td>
<td>Combination with gem carbo, tmz, RT</td>
<td>I.v.</td>
<td>Triple negative breast cancer</td>
<td>Phase II completed Phase III completed</td>
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<tr>
<td>INO-1001</td>
<td>Inotek/ Genentech (Beverly, MA)</td>
<td>Combination with temozolomide, single</td>
<td>I.v.</td>
<td>Melanoma, glioblastoma multiforme</td>
<td>Small phase II in melanoma Reformulation</td>
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<tr>
<td>MK4827</td>
<td>Merck</td>
<td>Single</td>
<td>Oral</td>
<td>Solid, BRCA ovarian</td>
<td>Phase I completed</td>
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<tr>
<td>E7016 (GPI 21016)</td>
<td>Eisai Inc (MGI Pharma )</td>
<td>Combination with temozolomide</td>
<td>Oral</td>
<td>Solid tumors</td>
<td>Phase I ongoing</td>
</tr>
<tr>
<td>CEP-9722</td>
<td>Cephalon</td>
<td>Combination with temozolomide</td>
<td>Oral</td>
<td>Solid tumours</td>
<td>Phase I ongoing</td>
</tr>
<tr>
<td>LT673</td>
<td>Lead Therapeutics/Biomin (Novato, USA)</td>
<td>Single agent and combination</td>
<td>oral</td>
<td>Solid tumours</td>
<td>Phase I planned</td>
</tr>
</tbody>
</table>
PARP inhibitor chemopotentiatiation studies

- AG014699 + TMZ – enhanced myelo-suppression (↓ TMZ 25%)
- AZD2281 + gem/cis (PID 400mg bd)
  - Ola 100 mg days 1-4 cis 50 day 3 gem 400 days 3 and 10
  - Ola 100 mg day 1 only cis 50 day 1 gem 500 day 1 and 8
- ABT888 + topotecan
  - PID in combination with topo 0.6 mg/m² tolerable (d1-5)
- Outlier = BSI-201 gem/carbo or TMZ – no enhancement of myelosuppression
NOVEL TARGETS IN DEVELOPMENT - BER
Consequences of inhibiting Ape1 and BER

Alkylation agent damage

Increased apoptosis
Decreased proliferation
Accumulation of AP sites

DNA Glycosylase (eg MPG)

Cytotoxic & Mutagenic

Block polymerase

Unrepaired AP sites

Binding of Ape1 to the AP site blocked. No Ape activity

Blocked AP site

Methoxyamine (MX)

Ape1

Polymerase β (dRPase & synthesis)

DNA Ligase
Treatment of four ovarian cancer cell lines with TMZ and MX

(MTS assay)

A.

B.

Actin
p53
NOVEL TARGETS IN DEVELOPMENT – DSB REPAIR
Multiple targets of DNA Double and single strand break repair

- DNA SSB
- DNA DSB
- DNA replication
- BER
- NHEJ repair
- HR repair

- PARP
- XRCC1
- Polβ
- Lig III
- Ku 70/80
- DNA-PKcs
- XRCC4
- DNA replication
- HR repair
- G1 arrest
- G2 arrest
- CHK2
- ATM
- ATR
- CHK1
- γH2AX
- MRN
- Claspin
- FANC D2
- RAD52/4
- RPA
- ERCC1
- XRCC3
- BRCA2
- BRCA1
- Rad17
- RAD51
- H2AX
- NHEJ repair
- Predominant in G1
- Predominant in S phase and G2
- HR repair
- BER
- Predominant in G1
DISCOVERY OF THE DNA-PK INHIBITOR NU7441

No activity seen at 10 mM in a screen against 60 diverse kinases

Cells were exposed to drugs for 16 hr prior to seeding for colony formation. DNA-PK<sup>-</sup> cells (V3) are inherently more sensitive to IR and etoposide than DNA-PK<sup>+</sup> cells (V3-Yac). NU7441 potentiates IR and etoposide cytotoxicity in DNA-PK<sup>+</sup> but not DNA-PK<sup>-</sup> cells confirming that DNA-PK is the cellular target of NU7441.

Nicola Curtin and Yan Zhao
Radiopotentiation of human p53 wt and mutant colon cancer cells

Dose Modification Ratio (DMR)

<table>
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<tr>
<th></th>
<th>LOVO</th>
<th>SW620</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Gy</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>6 Gy</td>
<td>38</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Nicola Curtin
CLL data

- Over-expression DNA PK in del 17q cases
- Correlates with drug resistance
- Re-sensitisation by DNA-PK inhibitor

Elaine Wilmore, NICR
DNA-PK inhibition chemosensitises del(17p), p53 mutated cases

**0069 del(17p)**
- Mitoxantrone
- + 0.1μM KU60648

**0085**
- Mitoxantrone
- Doxorubicin

**0066 del(17p)**
- Mitoxantrone
- + 1μM NU7742
- + 0.2μM KU60648

**0025 del(13q)**
- Fludarabine
- + NU7441
Potential Phase I trial design for DNA PKi

- Oral clinical candidate potentially to enter phase I trials in combination with doxorubicin
- MTD and “TID” (target inhibitory dose) to be defined – ex-vivo assay
- PD clinical assay to be validated in PBLs from an established pre-clinical assay
- Measurement of the ability of DNA PK to phosphorylate ser15 on n66p53
IDENTIFICATION OF THE ATM INHIBITOR KU-0055933

Little or no activity seen in a 60 kinase screen at 10 mM

Sensitization of HeLa cells to etoposide and camptothecin by ATM inhibition

Survival: % control vs [etoposide] µM

Survival: % control vs [camptothecin] nM

KU-0055933
IC50 = 13 nM

KU-0058050
IC50 = 3.0 µM
Effect of ATMi in combination with etoposide or irinotecan in the SW620 xenograft model

- **Dosing Days**
  - Vehicle
  - Etoposide 10mg/kg dx5
  - ATMi (2x25mg)
  - Etoposide + ATMi
  - ATMi 20mg/kg dx5
  - CPT-11 2.5mg/kg dx5
  - ATMi + CPT11 20mg/kg + 2.5mg/kg dx5

- **Relative Tumour Volume**
  - Days: 0 to 30
  - Relative Tumour Volume: 0 to 20
ATM inhibitors in pre-clinical development

Chemical structure of CP466722 [2-(6,7-dimethoxyquinazolin-4-yl)-5-(pyridin-2-yl)-2H-1,2,4-triazol-3-amine].

ATMi and radiosensitisation

Pharmacodynamic markers for ATM

- ATM recruited to and signals DNA DSB by phosphorylation of key proteins
- 2 candidate biomarkers:
  - phosphorylation of $\gamma$H2AX as marker of ATM activation
  - RAD51 foci formation as an indication of active HR
- Challenge remains HR active in cells in S phase –
  - Hair follicles, skin punch biopsies, CTCs, leukaemic cells
ATRi – NU6027

- ATR inhibitor – IC$_{50}$ < 5µM
- Potentiates the cytotoxicity of hydroxyurea, cisplatin, temozolomide, doxorubicin and camptothecins
- CM847-KD cells
  - ATR inhibition (via induction of kinase dead or chemical inhibition) increases cytotoxicity of AG014699 – synthetic lethality by dual inhibition
- ATR inhibitors are in late preclinical development and likely to enter clinic in 2011
THOUGHTS ON TRIAL DESIGN AND POSSIBLE COMBINATIONS
Trial design with DDRi

- Combination with DNA damaging agent
  - as potentiator
    - Chemotherapy
    - Radiotherapy

- Synthetically lethal use in appropriate tumour type
  - Molecular biomarkers needed to ensure a therapeutic index

- Combination with other active single agents
  - Scheduling!!!!!
Are combinations of DDRi possible?

Predominant in G1

- DNA-PKcs
- XRCC4
- Ligase IV
- Ku 70/80

NHEJ repair

G1 arrest

- CHK2
- ATM
- ATR

DNA replication

DNA SSB

- PARP
- XRCC1
- Polβ
- Lig III

BER

G2 arrest

- CHK1

FA core complex

- FANC D2

HR repair

- BRCA2
- Rad51
- RPA
- Rad 52/4
- ERCC1
- XRCC3

Predominant in S phase and G2

- DNA DSB
- Claspin
- MRN
- Rad17
- γH2AX

Predominant in G1 and G2

- DNA SSB
- DNA DSB
- DNA replication
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