Targeted Anticancer Therapies

Synthetic Lethality in Cancer Treatment: Current Role of PARP Inhibitors

Hilary Calvert
UCL Cancer Institute
University College London Partners
UK

Funding
Disclosures

• Inventors reward scheme, AG014699 / PF-01367338
• Consultancy
  – Agouron / Pfizer
  – AstraZeneca
  – BiPar
  – Eli Lilly
  – Kudos
  – LEAD
• All PARP inhibitors are investigational at the present time
Problems with targeted anticancer agents

• The target may be present and have a function in normal tissues
  – Side effects limit the dose and therefore the anticancer efficacy of the drug

• Inhibiting the target may have little effect on the tumour
  – Alternative proliferative pathways
Ideal targeted agents

• Target a fusion protein unique to the tumour cell and responsible for its malignant transformation
  – Imatinib, CML
  – ALK inhibitors, lung cancer

• Target a mutation unique to the tumour
  – BRAF, melanoma

• Target a gene that is uniquely amplified in the tumour
  – Her2, trastuzumab

• Synthetic lethality
DNA Repair – a process essential to cell survival

<table>
<thead>
<tr>
<th>How long is a piece of DNA?</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA length per cell</td>
<td>2 meters</td>
</tr>
<tr>
<td>Cells per human</td>
<td>$2 \times 10^{13}$</td>
</tr>
<tr>
<td>DNA length per human</td>
<td>$4 \times 10^{13}$ meters</td>
</tr>
<tr>
<td>Distance from the Earth to the Sun</td>
<td>$1.49 \times 10^{11}$ meters</td>
</tr>
<tr>
<td>Number of return trips to the Sun</td>
<td>134</td>
</tr>
</tbody>
</table>

- Each cell sustains 10,000 to 30,000 episodes of DNA damage per day
- 5 Basic types of DNA damage – repair pathways
- Redundancy
  - Different pathways
  - 2 Alleles
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

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)

DNA PKi

ATMi

Nucleotide excision repair

Mismatch repair

Base excision repair

PARPi

Direct reversal (AGT, MGMT)

$O^6$BG
PaTrin

Catalytic Activity of PARP

PARP forms ADP-ribose polymers attached to histone proteins and to itself. PARP uses NAD as a substrate.
Mechanism of Action of PARP in Base Excision Repair

Damage-induced DNA single-strand break → Nick protection → PARP-1 → Poly(ADP-ribose) synthesis → NAD$^+$ → DNA repair → PARP-1 and chromatin dissociation
Poly(ADP-ribose)polymerase (PARP): A truly cancer-specific target?

- 17 isoforms – PARP-1 is the best characterised
- Present in high activity in most tissues
- Activated by DNA strand breaks and involved with single-strand break repair
  - “Housekeeping” function
- Utilises NAD as a substrate to form APD-ribose polymers on histone proteins and itself (automodification)
- Involved in numerous other processes
  - Epigenetic regulation of chromatin structure and gene expression
  - Interacts with transcription factors and co-factors (NF)-kB, PAX6, AP-2, b-Myb, TEF1
  - Interacts with kinetochore proteins
  - etc, etc
We were fortunate in 1990 that we knew only about PARP-1
PARP Inhibitor Programme, Newcastle Anticancer Drug Development Initiative, 1990

• Rationale
  – Inhibition of PARP (PARP-1) known to potentiate in vitro
    • Monomethylating agents (temozolomide, nitrosoureas)
    • Topoisomerase 1 inhibitors (topotecan, irinotecan (SN38))
    • Radiation therapy

• Objective
  – To generate high affinity PARP inhibitors for in-vivo / clinical use

• Note
  – BRCA1 identified 1994, BRCA2 identified 1995
Development of High-Affinity PARP Inhibitors (Newcastle / Agouron)

3-aminobenzamide
Ki = 4μM

PD128763
Ki = 70nM
Hypothermia

NU 1085
Ki = 10nM

AG14699
Ki = < 5 nM
Phase I 2003, In clinical development by Pfizer

Agouron collaboration - crystal structure
Constraining the carboxamide ring in a seven membered ring maintained the interactions with the active site.

$\text{Ki} < 5\text{nM}$ purified full length rhPARP-1

Increased
Structures of PARP Inhibitors

Nicotinamide
Component of NAD

NU 1085
(Newcastle University)

AG014699
Agouron/Pfizer

Iniparib
BSI 201
Bipar / Sanofi-Aventis

MK4827
Merck & Co

Olaparib
AstraZeneca

Veliparib (ABT888)
Abbott
**PARP Inhibitors in Cancer Treatment**

- **Role 1**
  - Potentiate specific cytotoxic drugs
    - Monomethylating agents (e.g., temozolomide)
    - Topoisomerase 1 inhibitors (e.g., topotecan)

- **Role 2**
  - Potentiate radiation therapy

- **Role 3 “Synthetic lethality”**
  - Single agent activity in tumors deficient in homologous recombination repair (HR) (e.g., BRCA1, BRCA2)

- **Role 4**
  - In combination with drugs known to be active in tumors which have HR defects (e.g., carboplatin / ovarian). Specific potentiation of these drugs not consistently seen in vitro

- **(Role 5**
  - Chemoprevention in BRCA carriers)
Phase 0 / 1 Trial of AG014699
Day 1-5 Schedule with temozolomide

• Substantial (≥90%) PARP inhibition seen in peripheral blood mononuclear cells and tumour biopsies
• No significant toxicities attributable to the PARP inhibitor as a single agent
• No dose-reduction for temozolomide
• Clinical activity observed

PARP Inhibitory Dose established using PD assay (immunoblot for polymer in PBLs)

PARP inhibition in PBLs
2 mg/m² AG014699

PARP inhibition in PBLs
12 mg/m² AG014699
Mean tumour PARP activity at 6 hours after a single dose of AG014699
## Comparison of response data with TMZ single agent phase III

<table>
<thead>
<tr>
<th>Efficacy Endpoint</th>
<th>*AG014699 + Temozolomide n 46 pts</th>
<th>M.R. Middleton, Phase III DTIC vs. TMZ (temozolomide arm) n 156 pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>--</td>
<td>2.6 % (95% CI N/A)</td>
</tr>
<tr>
<td>PR</td>
<td>17.4% (95% CI 7.9%-31.6%)</td>
<td>10.9 % (95% CI N/A)</td>
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<tr>
<td>SD ≥ 24 wks</td>
<td>17.4% (95% CI 7.9%-31.6%)</td>
<td>Not Available</td>
</tr>
<tr>
<td>PFS</td>
<td>3.5 months (95% CI 2.0-6.2)</td>
<td>1.9 months (95% CI N/A)</td>
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<tr>
<td>OS</td>
<td>9.9 months (95% CI 6.2-14.7)</td>
<td>7.7 months (95% CI N/A)</td>
</tr>
</tbody>
</table>

*6 UK sites: Newcastle, Belfast, Oxford, Glasgow, Manchester, Birmingham (Plummer, Wilson, Middleton, Evans, Lorigan, Steven)
PARP Inhibitors in Cancer Treatment

• Role 1
  – Potentiate specific cytotoxic drugs
    • Monomethylating agents (eg temozolomide)
    • Topoisomerase 1 inhibitors (eg topotecan)

• Role 2
  – Potentiate radiation therapy

• Role 3 “Synthetic lethality”
  – Single agent activity in tumours deficient in homologous recombination repair (HR) (eg BRCA1, BRCA2)

• Role 4
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• (Role 5
  – Chemoprevention in BRCA carriers)
## PARP Inhibitors in Development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Company</th>
<th>Route</th>
<th>Clinical Status</th>
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<tbody>
<tr>
<td>AG014699</td>
<td>Pfizer</td>
<td>Iv / oral</td>
<td>Phase I/II Combos</td>
</tr>
<tr>
<td>PF-01367338</td>
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<td></td>
<td></td>
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<tr>
<td>KU59436</td>
<td>AstraZeneca/Kudos</td>
<td>oral</td>
<td>Phase II/III Combos</td>
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<tr>
<td>AZD2281 Olaparib</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Veliparib</td>
<td>Abbott</td>
<td>oral</td>
<td>Phase I/II Combos</td>
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<tr>
<td>ABT888</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Iniparib</td>
<td>BiPar/Sanofi-Aventis</td>
<td>Iv Prodrug?</td>
<td>Phase II/III Combos</td>
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<tr>
<td>BSI-201</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INO-1001</td>
<td>Inotek</td>
<td>iv</td>
<td>Phase Ib complete</td>
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<tr>
<td>GPI21016</td>
<td>MGI Pharma/Eisai</td>
<td>oral</td>
<td>Phase I</td>
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<td>CEP-9722</td>
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<td>Phase I</td>
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<tr>
<td>MK4827</td>
<td>Merck &amp; Co</td>
<td>oral</td>
<td>Phase I</td>
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<tr>
<td>BMN-673</td>
<td>Biomarin/LEAD Pharmaceuticals</td>
<td></td>
<td>Preclinical</td>
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</tbody>
</table>
Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase

Helen E. Bryant¹, Niklas Schultz², Huw D. Thomas³, Kayan M. Parker¹, Dan Flower¹, Elena Lopez¹, Suzanne Kyle³, Mark Meuth¹, Nicola J. Curtin³ & Thomas Helleday¹,²

Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy

Hannah Farmer¹,², Nuala McCabe¹,², Christopher J. Lord², Andrew N. J. Tutt²,³, Damian A. Johnson², Tobias B. Richardson², Manuela Santarosa², Krystyna J. Dillon⁴, Ian Hickson⁴, Charlotte Knights⁴, Niall M. B. Martin⁴, Stephen P. Jackson⁴,⁵, Graeme C. M. Smith⁴ & Alan Ashworth¹,²

Nature 2005; 434:913-917 (Newcastle / Sheffield)

BRCA2-deficient cell lines are hypersensitive to PARP inhibitors (Newcastle / Pfizer Compounds)

“Therapeutic ratio” ~ 250

AG014699 (clinical development)

AG014361 (this expt)

Mutation in BRCA1 or BRCA2 Results in Extreme Sensitivity to PARP Inhibition (Kudos/AZ Compounds)

- Active IC50 3.2 nM
- Active IC50 3.4 nM
- Inactive Analogue IC50 730 nM

Adapted from:
Farmer et al. Nature 434, 917-921, 2005
BRCA1 and BRCA2 Cancer Predisposition Genes

- Mutation carriers are predisposed to breast, ovarian, prostate, pancreatic and other cancers
- BRCA1 and BRCA2 are involved in homologous recombination repair – error-free repair of double strand breaks
- Carriers have one allele carrying a mutant, non-functioning gene. Damage to the functioning copy results in error-prone DNA repair and is oncogenic
BRCA Carriers and Cancer Susceptibility

Normal

Allele 1

Allele 2

HR Repair
Error Free

HR

HR

DNA Damage

BRCA Carrier

Allele 2

Allele 1
BRCA Mutation

HR

HR

NHEJ Repair
Error Prone

HR

HR

Unstable Genome

Further Mutations

Oncogenesis
Properties of Cancers Arising in BRCA1/2 Carriers

- The cancer has lost the ability to carry out HR (homologous recombination repair)

- Ovarian cancers are typically more sensitive to platinum treatment than sporadic (non-BRCA) cases.
Proposed Mechanism of Synthetic Lethality of PARP Inhibitors to BRCA1 or 2-Deficient Cells

Without a PARP inhibitor repair occurs

Persistent single strand break leads to double strand break during replication

Collapsed Replication Fork

BRCA related tumours have lost the second allele and cannot perform homologous recombination

Normal tissues in BRCA carrier patients have one functioning allele for BRCA

Homologous Recombination Error-free
Olaparib – Kudos / AstraZeneca

- Orally available PARP inhibitor generated responses in hereditary cancers in Phase I*
- Phase II results in patients with BRCA1 or 2 related breast and ovarian cancer presented at ASCO 2009

Olaparib Phase II in Breast Cancer
Best % change from baseline in target lesions by genotype

400 mg twice daily

100 mg twice daily

Olaparib Phase II in Ovarian Cancer
Best % change from baseline in target lesions

A

B

400 mg twice daily

100 mg twice daily

Resistance to PARP Inhibitors

  - BRCA2 deficient CAPAN1 cells were rendered PARP-inhibitor resistant and showed a re-activating intragenic deletion of BRCA2
  - Similar mechanism in cell lines derived from platinum-resistant patients
  - Not known whether this mechanism occurs in patients treated with PARP Inhibitors

  - 4 of 6 platinum-resistant BRCA1 tumours had acquired secondary genetic changes
  - 0 of 3 platinum-sensitive BRCA1 tumours had secondary genetic changes
Proposed mechanism and therapeutic potential

- Endogenously formed SSB are normally repaired by PARP-dependent BER.
- If PARP is inhibited SSB persist.
- SSB form DSB at replication, which are repaired by HR.
- If HR is defective the breaks are not repaired and the cell dies.
- This is the first exploitation of synthetic lethality in cancer therapy.
- Tumour selective.
Best % Change from Baseline in Target Lesion: High Grade Serous Ovarian/undifferentiated Tubo-ovarian; Unknown or BRCA –ve at Entry

Karen Gelmon, ASCO 2010

Best change in target lesion size is maximum reduction from baseline or minimum increase in absence of reduction.
PARP Inhibitors in Cancer Treatment

• Role 4
  - In combination with drugs known to be active in tumours which have HR defects (e.g. carboplatin / ovarian). Specific potentiation of these drugs not consistently seen in vitro.
Phase 2 mTNBC Study: Treatment Schema

Metastatic TNBC
N = 120

RANDOMIZE

**Gemcitabine** (1000 mg/m², IV, d 1, 8)
**Carboplatin** (AUC 2, IV, d 1, 8)

21-Day Cycle

**Iniparib** (5.6 mg/kg, IV, d 1, 4, 8, 11)
**Gemcitabine** (1000 mg/m², IV, d 1, 8)
**Carboplatin** (AUC 2, IV, d 1, 8)

RESTAGING
Post-Cycle 2 & every 6-8 wks

* Patients randomized to gem/carbo alone could crossover to receive gem/carbo + iniparib at disease progression
Iniparib Phase II in Triple Negative Breast Cancer: Progression-free and Overall Survival Rates

BiPar breast cancer drug fails late-stage trial

A promising breast cancer treatment developed by BiPar Pharmaceuticals Inc. fizzled in a major late-stage study.

The drug, iniparib or BSI-201, did not hit either of the main goals — overall survival and progression-free survival — in a study of 519 women with metastatic triple-negative breast cancer, parent company Sanofi-Aventis said.

Iniparib has been closely watched for scientific and business reasons. For one, it belongs to a class of cancer drugs known as PARP inhibitors. Also, Sanofi made a splash when it bought tiny, South San Francisco-based BiPar in 2009 for as much as $500 million in total payouts, depending on iniparib’s level of success.
Structures of PARP Inhibitors

NU 1085
(Newcastle University)

AG014699
Agouron/Pfizer

Iniparib
BSI 201
Bipar / Sanofi-Aventis

Iniparib itself is not a PARP Inhibitor – an active metabolite is proposed

MK4827
Merck & Co

Olaparib
AstraZeneca

Veliparib (ABT888)
Abbott
Identification of HR Deficient Tumours

- γH2AX, RAD51
  - Mukhopadhyay et al, Clin Cancer Res; 16(8):2344, 2010

- PTEN
  - Mendes-Pereira et al, EMBO Mol Med 1, 315–322, 2009

- BRCA1 Expression
  - Carser, JCO 27:15s, 2009 abstract 5527
PARP-BRCA Summary

- PARP inhibitors provide specific therapy for tumours arising in patients who are BRCA1 or 2 mutation carriers
- PARP Inhibitors also show single agent activity in non-BRCA tumours likely to have a HR defect
- PARP inhibitors will probably potentiate chemotherapy agents – probably preferably in tumours with low homologous recombination repair capability
- Biomarkers are required to select tumours which will be sensitive to PARP inhibitors: Possible potential markers
  - RAD51 focus formation
  - PTEN deficiency
  - BRCA1 Expression
  - ……..
Newcastle Anticancer Drug Development Initiative, 1990

Barbara Durkacz  Roger Griffin  Bernard Golding  Herbie Newell  Nicola Curtin  Ruth Plummer

Discovered PARP1  Medicinal Chemistry  Preclinical Pharmacology  DNA repair Biology  Clinical Trials

The PARP Team
Acknowledgements

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Research nurses

Ruth Plummer
Bernard Golding
Roger Griffin
Nicola Curtin
Barbara Durkacz
David Newell

Plus the other clinical investigators

Pfizer team

Heidi Steinfeldt
Zdenek Hostomsky
Raz Dewji

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