PARP inhibitors and Temozolamide in Brain Tumors
Until recently, treatment efforts have focussed on maximizing the DNA damage (limited by the toxic effects) that can be inflicted upon tumours, with little consideration of how malignant and normal cells repair DNA damage.

We have the possibility of maximizing the anticancer effects of DNA-damaging therapies (The citotoxicity of radiotherapy and chemotherapy ) by interfering with the DNA-damage response (DDR) of malignant cells.

A number of small molecule inhibitors of the DDR are available and some of them are undergoing clinical assessment in combination with cytotoxic agents.

Among these small molecules, PARP inhibitors are the most developed DDR modifiers.
MECHANISM of ACTION of PARP

- **PARP1**: founding member. The most abundant and best characterized protein within this family.
- **PARP2**: displays almost overlapping functions.
- **PARP3**: DNA repair, mitotic spindle integrity, and telomerase regulation.
These findings emphasize the complexity of PARP-related DNA repair routes and **rationalize the relevance of this enzyme in the safeguard of genomic stability and as therapeutic target.**

Several forms of cancer are more dependent on PARP than regular cells, making PARP an attractive target for cancer therapy.
PARP INHIBITORS (PARPi)

• Group of pharmacological inhibitors of (PARP).

• They are developed for multiple indications (potential treatment for acute life-threatening diseases) but the most important is the treatment of cancer.

Mechanisms of action:

1. PARPi compete with NAD+ at the catalytic site of PARPs inhibiting their enzymatic function and preventing synthesis of PAR→ "PARP1-trapping model"

2. PARP1 inhibited cells accumulate unrepaired SSBs→ DSBs when encountered by the replication machinery. If HR repair is disabled, cells reroute to alternative low fidelity DNA repair pathways, thus hastening genomic instability and cell death.
Supporting notions for targeting PARPs as cancer strategy

RATIONAL FOR COMBINATION THERAPIES

RADIOTHERAPY

PARP inhibitors (PARPi)

1

2
Supporting notions for targeting PARPs as cancer strategy

RATIONAL FOR COMBINATION THERAPIES

CHEMOTHERAPY

MGMT Repair

- Inhibitor of MGMT (O\textsuperscript{6}-BG or PaTrin-2)
- Ubiquitination
- Degradation

B. Mismatch Repair

- Futile Cycling
- DNA Strand breaks
- Apoptosis
- Cytotoxicity

C. Base Excision Repair

- Poly \( \beta \)
- DNA Ligase
- DNA repair complex does not bind
- DNA Strand breaks
- Apoptosis
- Cytotoxicity

PARP inhibitors (PARPi)
Supporting notions for targeting PARPs as cancer strategy

PARPi AS SINGLE THERAPEUTICAL AGENT

SINHTHETIC LETALITY

PTEN DEFICIENCY

"BRCAness"

Biochimica et Biophysica Acta 1846 (2014) 201-215
Can synthetic lethality be achieved in GBM?

Mutations in BRCA1 or 2 are not common in these tumours. Homozygous mutations in other DNA repair proteins have not been described to date.

1.- However, recent evidence indicates that bi-allelic mutations or delections in PTEN gene (30% GBM) are associated with defects in HR and with sensitivity to PARPi. (1,2) - the mechanism responsible for these remains under investigation but might involve downregulation of the key HR protein Rad51 and/or its paralogues.

2.- Glioma cells that overexpress EGFR or bear the constitutively activated EGFRvIII mutation carry a large burden of ROS, accumulate high levels of DNA base damage and exhibit upregulation of proteins involved in repair of these lesions, including PARP.

In line with these observations, glioma cell lines transfected with EGFRvIII constructs showed increased sensitivity to PARPi both as single agents and in combination with radiation. (3)

Although the relevance of these findings to the clinical setting has yet to be determined, it is clear that indirect effects of oncogenes and tumour suppressors genes on DNA damage and repair create opportunities for tumour selective treatments involving PARPi.

(2) Cancer Res., 70(13), 5457-64
(3) PLoS One, 2010, 5(5), e10767
A number of PARP inhibitors are currently being assessed in clinical trials.

<table>
<thead>
<tr>
<th>PARP inhibitor</th>
<th>Target PARP</th>
<th>Route of administration</th>
<th>Findings of key trials, if available, and/or phase of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olaparib (AZD2281)</td>
<td>PARP1/2/3</td>
<td>Oral</td>
<td>Series of phase II trials demonstrated efficacy in BRCA-mutation carriers(^{52,54}). Currently being evaluated in the adjuvant setting in patients with TNBC(^{70}).</td>
</tr>
<tr>
<td>Veliparib (ABT-888)</td>
<td>PARP1/2</td>
<td>Oral</td>
<td>Acceptable safety profile and promising antitumour activity, especially in BRCA-deficient patients with ovarian cancer, was observed in a series of phase I trials(^{71}). Phase II studies are ongoing(^{72}).</td>
</tr>
<tr>
<td>Rucaparib (AG-014,699; CO-338)</td>
<td>PARP1/2</td>
<td>Oral or intravenous</td>
<td>Currently being evaluated in a phase II/III study in BRCA-mutation carriers with mBC or advanced-stage ovarian cancer(^{73,74}).</td>
</tr>
<tr>
<td>BMN-673</td>
<td>PARP1/2</td>
<td>Oral</td>
<td>BMN-673 has shown impressive antitumour activity in patients with BRCA mutations(^{48}). Currently, phase II–III studies in patients with germline BRCA mutations are ongoing(^{75,76}).</td>
</tr>
<tr>
<td>CEP-9722</td>
<td>PARP1/2</td>
<td>Oral</td>
<td>Clear evidence of PARP inhibition has been demonstrated in preclinical studies,(^{77}) and early studies in patients(^{42}).</td>
</tr>
<tr>
<td>Niraparib (MK4827)</td>
<td>PARP1/2</td>
<td>Oral</td>
<td>Phase I–II studies have revealed antitumour activity, especially in patients with germline BRCA mutations(^{49}). At present, phase II–III studies in such patients are ongoing(^{78,79}).</td>
</tr>
</tbody>
</table>
Previous preclinical studies suggested that PARPi, can enhance the efficacy of TMZ in both sensitive and resistant tumors, and also can enhance the efficacy of RT (1,2)

- Robust in vivo TMZ-sensitizing effects with veliparib, limited to TMZ-sensitive primary xenografts lines.
- These effects were lost in derivative-TMZ-resistant xenografts models.

*Effective sensitization of TMZ by ABT-888 is lost with development of TMZ resistance in GBM xenograft lines.*
*Mol Cancer Ther 2009;8(2). Feb 2009*

- Robust sensitization was observed *in vitro* for TMZ-resistant models at supratherapeutic drug concentrations.
- In contrast, using the maximally tolerable *in vivo* regimen robust sensitizing effects with veliparib only were observed in TMZ-sensitive models.
- The lack of efficient chemosensitization in resistant tumors may be related to the biologically achievable/ tolerable concentrations of veliparib.

*Discordant in vitro & in vivo chemopotentiating effects of the PARP inhibitor Veliparib in TMZ sensitive vs resistant GBM xenografts.*
*Clin Cancer Res;20(14) July 15, 2014*

(1) J Natl Cancer Inst 2004;96:56-97
(2) Clin Cancer Res 2000;6:2860-7
The 1st in vitro study (four cell lines) to investigate possible synergy between these 3 agents and to assess the influence of MGMT promoter methylation status on tumour response.

RESULTS:

- ABT-888 enhances the effects of radiation.

- Further sensitization has also been shown when ABT-888 was added to both TMZ and X-rays.

- Although the maximum enhancement in cell killing was obtained in MGMT-methylated cell lines, MGMT expression did not prevent ABT-888 mediated sensitization.

- It is suggested that the MGMT methylation status is not an absolute predictor of response to trimodal treatment.
  (disagreement in the literature on whether ABT-888 mediated sensitization to TMZ is independent of the MGMT) (1,2)

1. Horton TM. Mol Cancer Ther 2009, 8:2232-2242
PARP INHIBITORS (PARPi)

VELIPARIB

Phase I- ABTC trial -> Veriparib+ TMZ+RT for newly diagnosed GBM

Administering ABT-888 bid po in combination with standard TMZ/RT was NOT tolerable in GBM patients as a result of hematologic toxicity

A phase I trial of veliparib (ABT-888) and temozolomide in children with recurrent CNS tumors: a Pediatric Brain Tumor Consortium report†

Jack M. Su, Patrick Thompson, Adekunle Adesina, Xiao-Nan Li, Lindsay Kilburn, Arzu Onar-Thomas, Mehmet Kocak,

Background. A phase I trial of veliparib (ABT-888), an oral poly(ADP-ribose) polymerase (PARP) inhibitor, and temozolomide (TMZ) was conducted in children with recurrent brain tumors to (i) estimate the maximum tolerated doses (MTDs) or recommended phase II doses (RP2Ds) of veliparib and TMZ; (ii) describe the toxicities of this regimen; and (iii) evaluate the plasma pharmacokinetic parameters and extent of PARP inhibition in peripheral blood mononuclear cells (PBMCs) following veliparib.

Methods. TMZ was given once daily and veliparib twice daily for 5 days every 28 days.

Results. Twenty-nine evaluable patients were enrolled. Myelosuppression (grade 4 neutropenia and thrombocytopenia) were dose limiting. The RP2Ds are veliparib 25 mg/m² b.i.d. and TMZ 135 mg/m²/d. Only 2 out of 12 patients treated at RP2Ds experienced dose-limiting toxicities. Although no objective response was observed, 4 patients had stable disease > 6 months in duration, including 1 with glioblastoma multiforme and 1 with ependymoma. At the RP2D of veliparib, pediatric pharmacokinetic parameters were similar to those in adults.

Conclusions. Veliparib and TMZ at the RP2D were well tolerated in children with recurrent brain tumors. A phase I/II trial to evaluate the tolerability and efficacy of veliparib, TMZ, and radiation in children with newly diagnosed brainstem gliomas is in progress.

Neuro-Oncology

Neuro-Oncology 16(12), 1661–1668, 2014
doi:10.1093/neuonc/nou103
Advance Access date 7 June 2014
Background

- Drug delivery is an important obstacle in treatment of GBM
- Tumour PK of small molecules not widely studied in GBM
- Olaparib is an oral PARPi with potential to overcome resistance of GBM to RT and TMZ
- Traditional models of the blood-brain barrier (BBB) may not represent the clinical scenario
- Clinical responses to olaparib have been observed in brain metastases
- No data on penetration of GBM in patients

Hypothesis

Olaparib penetrates GBM despite failing to penetrate the intact BBB
Objectives

1.- Characterize BBB penetration of olaparib
   - **In vitro**: using MDCKII cells expressing -MDR1 (multidrug resistance efflux protein)
   - **In vivo**: using autoradiography showing tissue distribution of $^{14}$C-olaparib

2.- Measure tumour olaparib levels in GBM patient specimens

3.- Correlate tumour olaparib levels with:
   - Olaparib dose and plasma concentrations.
OPARATIC trial stage 1

Results

1. Olaparib is a substrate for MDR1 and does not cross the intact BBB

2. Olaparib does not penetrate the normal CNS in rodent models

Autoradiograph taken 4 hrs after single oral dose of $^{14}\text{C}$-olaparib demonstrated that radioactivity was not measurable in CNS or spinal cord at any time point (1h to 28 days)

Confirmed lack of penetration of the CNS and demonstrated uptake and retention of olaparib in tumour tissue at all time points (6 to 96 h) after a single oral dose.
Results

3.- Olaparib penetrates recurrent GBM at therapeutic concentrations

-8 pts dosed with olaparib for 4 days prior to tumour resection.
  3 pts (200 mg bid) and 5 pts (100 mg once a daily)
  Regions of viable tumour were identified x3 and blood samples
- Tumour and plasma concentrations were determined by liquid chromatography.

Olaparib detected in 24/24 recurrent GBM specimens.
Mean tumour concentrations in same range as those observed in previous breast cancer study (1).
Tumour olaparib concentrations did not correlate with olaparib dose or plasma concentrations.
100 mg once daily oral dosing delivered tumour concentrations associated with clinical responses (1)

4.- Tumour olaparib levels unlikely to be explained by blood levels

Mean tumour olaparib concentrations correlated positively with MRI index of cellular fraction.

These data indicate that tumour olaparib concentrations are determined primarily by intracellular rather than intravascular or extravascular extracellular drug.

(1) Invest New Drugs (2013) 31:949-958
Conclusions

- Olaparib is a substrate for MDR1 and does not cross the intact BBB under normal conditions
- Despite this, olaparib reliably penetrates recurrent GBM at therapeutic concentrations
- Tumour concentrations do not correlate with olaparib dose or plasma concentrations, but may be predicted by tumour cellularity
- Olaparib has therapeutic potential as a radiation and chemo-sensitizer in GBM
- Traditional pre-clinical models of the BBB are not representative of the clinical scenario
What is optimum olaparib schedule in combination with continuous TMZ?

Autoradiograph of nude mouse with subcut. HCT116 xenograft after single oral dose of \([^{14}\text{C}]-\text{olaparib}\)

- **Liver**
- **Small intestine**
- **Bone marrow**
- **Liver**
- **Caecum**

**6 hours**

**72 hours**
Dose escalation of olaparib in combination with continuous low dose temozolomide; 6 weeks on, 2 weeks off

Cohort 1: olaparib 100 mg, TMZ 50 mg/m²
   no DLTs but breaks or dose reductions in 4/6 patients

Cohort 2: olaparib 100 mg d1-5 per week, TMZ 50 mg/m²
   3/3 patients completed at least 1 cycle without DLT

Cohort 3: olaparib 150 mg d1-5 per week, TMZ 50 mg/m²
   no DLTs but break in 1/3 patients: expand to 6 pts
Increase TMZ dose to 75 mg/m² to inform future studies of olaparib in combination with radiotherapy + TMZ

PARADIGM:
Olaparib plus RT (in pts >70 with newly diagnosed GBM 40 Gy in 15#
-Phase I dose escalation of olaparib starting 2 days pre-RT (continue for 4 weeks after RT?)
-Randomised phase II study of RT + olaparib vs RT + placebo

Phase I study in patients <70 with newly diagnosed GBM?
MGMT unmethylated: RT + continuous olaparib?
MGMT methylated: RT + TMZ + intermittent olaparib?
Modulating DNA repair response by selectively inhibiting PARP is a potential therapeutic approach to enhance standard treatment in patients with cancer in general and with GBM in particular.

PARP inhibitors have shown considerable promise in the treatment of several cancers, both in monotherapy and in combination with cytotoxic agents.

Synthetic lethal action of PARP inhibitors has been observed in tumors with mutations in double strand break repair pathways. Further investigation in GBM on this regard is needed.

Identification of the target populations most likely to benefit will be a pivotal step in the ongoing development of this class of drugs. Biomarker studies are needed and are in fact underway to facilitate this.
GRACIAS