Trial setting: advanced, recurrent gynaecological cancers
  clear cell, endometrioid, carcinosarcoma (ovarian and endometrial),
  cervical carcinoma
Study Design: non-randomised, phase II 2 stage design
Sponsor(s): Institute of Cancer Research
Planned No. of patients: 40-116
Current accrual: not open
Other important information: ARID1A analysis
ATARI (ATR inhibitor in combination with olaparib in gynaecological cancer with ARID1A loss)-
A Phase II Proof of Concept Study to assess the Activity of an Ataxia Telangiectasia and Rad3-related (ATR) inhibitor (AZD6738) as a Single Agent and in Combination with Olaparib in ARID1A Stratified Gynaecological Cancers

Cl: Susana Banerjee, Royal Marsden, NCRI
Synthetic lethality with ATR inhibition

ARID1A required for localisation of TOP2A to DNA

TOP2A causes DSBs in DNA and facilitates transport of one double helix through another

ATR activity normally invoked when TOP2A defective

ARID1A defect = TOP2A defect = activation of ATR signaling cascade = delayed progression through S and G2

ARID1A defect plus ATRi = cells fail to adequately respond to TOP2A defect = premature cytokinesis in the face of unresolved DNA structures

Summary – ARID1A mutant tumour cells are sensitive to small molecule ATR inhibitors, both \textit{in vitro} and \textit{in vivo}

\textbf{ATR small molecule inhibitor \textit{in vitro}}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{ATR_in_vitro}
\caption{Surviving Fraction vs. AZ20 (uM) for ARID1A +/- cells. ANOVA $p<0.0001$.}
\end{figure}

\textbf{ATR small molecule inhibitor \textit{in vivo}}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{ATR_in_vivo_WT}
\caption{Tumour Volume vs. Time (days) for ARID1A WT xenografts.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{ATR_in_vivo_DEF}
\caption{Tumour Volume vs. Time (days) for ARID1A DEFICIENT xenografts. ANOVA $p=0.015$.}
\end{figure}

Williamson \ldots Lord Nature Comm. Dec 2016
ARID1A mutant cells show sensitivity to PARP inhibitors

ARID1A mutant cells showed sensitivity to PARP inhibitors, which cause failure of SSB repair and PARP trapping on DNA, leading to potential DSB.

Both ARID1A wildtype and mutant cells showed increased apoptosis with combination treatment.

ARID1A mutant cells show sensitivity to ATR inhibitor and PARP inhibitor combination

- **HCT116 ARID1A mutant**
- **HCT116 ARID1A normal**
  - Drug screening in triplicate
  - 80 drugs ± ATR inhibitor
  - 5 days continuous drug exposure
  - Cellular viability measured
  - Identification of synthetic lethal drugs in combination with ATR inhibitor

**Z scores at 100nM for PARP inhibitors**

- **PARPi 1**
- **PARPi 2**
- **PARPi 3**

Combination therapy (PARPi & ATRi) showed a statistically significant loss of viability in vitro, in the ARID1A deficient setting in all 3 PARPi tested

*Poster at AACR Ovarian Cancer Conference 2016; Saira Khalique, Chris Lord, Rachael Natraj and Susana Banerjee*
## Loss of ARID1A in Gynaecological Cancers

<table>
<thead>
<tr>
<th>Histology</th>
<th>Mutation</th>
<th>Protein loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian clear cell</td>
<td>46-75%</td>
<td>55-73%</td>
</tr>
<tr>
<td>Ovarian endometrioid</td>
<td>30%</td>
<td>50%</td>
</tr>
<tr>
<td>Uterine endometrioid (low-grade)</td>
<td>30%-46.7%</td>
<td>26%</td>
</tr>
<tr>
<td>Uterine endometrioid (high grade)</td>
<td>60%</td>
<td>39%</td>
</tr>
<tr>
<td>Cervix adenocarcinoma</td>
<td>8%</td>
<td>9-60%</td>
</tr>
<tr>
<td>Cervix squamous</td>
<td>8%</td>
<td>6-19%</td>
</tr>
<tr>
<td>Mesonephric cervix</td>
<td>31%</td>
<td>-</td>
</tr>
<tr>
<td>Carcinosarcomas</td>
<td>13-36%</td>
<td>7%</td>
</tr>
</tbody>
</table>
Good Concordance between ARID1A IHC and ARID1A mutational status in 39 Gynaecological Cancers: identification of a reliable antibody

Each case underwent targeted next generation sequencing and IHC using 3 antibodies, scored by 3 pathologists.

<table>
<thead>
<tr>
<th>Antibody 1</th>
<th>Antibody 2</th>
<th>Antibody 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 468 (ARID1A wild type)</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>Score</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Patient 464 (ARID1A mutant)</td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
</tr>
<tr>
<td>Score</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Immunoreactive score** = % of positive tumour cells x staining intensity
(0 = no positive tumour cells staining and 12 = >80% of cells, stain strongly)

Stromal cells stain brown and serve as internal control (Imesch, Modern Pathology (2012))
Proof of concept trial- ATR and PARP inhibition in ARID1A stratified Gynaecological Cancers

Primary objective
To determine whether AZD6738 has clinical activity as a single agent and in combination with olaparib in patients with ARID1A-deficient (‘loss’) relapsed gynaecological cancers (main cohort is clear cell)

Secondary objectives
1. To assess the efficacy and safety of AZD6738 in combination with olaparib in ARID1A no loss relapsed gynaecological cancers
2. To assess the toxicity profile of AZD6738 and AZD6738 in combination with olaparib

Exploratory Objectives
To investigate markers predictive of response and tolerability to AZD6738 and the combination of AZD6738 and olaparib in ARID1A- stratified gynaecological cancers that may be observed in tumour and/or blood from treated patients. This will include the analysis of tumour and circulating biomarkers, such as DNA, mRNA, proteins or metabolites
**STAGE 1**

Notes: trial is based on an optimal Simon two-stage design with \( p_0 = 10\% \), \( p_1 = 30\% \), one-sided alpha 0.05 and 80\% power. In both arms, therapy continues until progression or withdrawal from study.

Notes: If monotherapy clear cell ARID1A loss does not meet criteria for activity in stage II, to enroll patients to combination

Relapsed ovarian and endometrial clear cell carcinoma
Upfront IHC to assess ARID1A status

ARID1A IHC loss

**ARID1A IHC no loss**

AZD6738 monotherapy
\( n=10 \)

AZD6738 and olaparib combination therapy
\( n=10 \)

**STAGE 2**

*numbers of patients dependent on Stage 1 responses*

ASSESSMENT:
Objective Response Rate: CT (RECIST v 1.1) at 8 weeks

If >1 response
Continue recruitment until \( n \) (monotherapy)=29

If <=1 response to monotherapy
AZD6738 and olaparib combination therapy
\( n=10 \)

If <=1 response to combination
Stop

If >1 response to combination
Continue recruitment until \( n \) (combination therapy)=29

If >1 response
Continue recruitment until \( n=29 \)

If <=1 response
Stop

If >1 response
Continue recruitment until \( n=29 \)

If <=1 response
Stop

If >1 response
Continue recruitment until \( n=29 \)

If <=1 response
Stop

Relapsed other gynaecological cancers

Endometrioid (ovarian and endometrial)
Carcinosarcoma (ovarian and endometrial)
Cervical carcinoma (squamous and adeno)
Proof of concept trial- ATR and PARP inhibition in ARID1A stratified Gynaecological Cancers (key inclusion criteria)

1. Progressive or recurrent gynaecological carcinomas of the following histological subtypes:
   - Clear cell (ovarian and endometrial)
   - Endometrioid (ovarian and endometrial)
   - Cervical- Adenocarcinomas, squamous and adenosquamous of the cervix, carcinosarcoma

2. Histological tissue specimen (tissue block or 8-10 unstained slides) must be available (specimen can be the sample at diagnosis or taken at relapse). All patients will have ARID1A IHC assessment for trial entry

3. Failure after ≥1 prior platinum containing regimen. Platinum-based therapy does not need to be the last treatment prior to study entry.

4. Measurable disease (by RECIST criteria v1.1). Patients with CA125 progression in the absence of measurable disease will NOT be eligible.

Translational research- including IHC ARID1A. whole exome sequencing
Archival samples, biopsies (non-mandatory), circulating DNA
Status: Proof of concept trial- ATR and PARP inhibition in ARID1A stratified Gynaecological Cancers

- **Solid pre-clinical data** package demonstrating both *in vitro* and *in vivo* synthetic lethality between ARID1A defects in OCCC and ATR small molecule inhibition

- Completed study confirming 100 % concordance between ARID1A mutation in OCCC and loss of ARID1A IHC signal

- **Astrazeneca – confirmed support for academic sponsored study**

- **NCRI- confirmed support** (discussed/worked up Sept 2016, Feb and Sept 2017)
  - Academic Trial- ICR sponsored (ICR-CTSU)
  - Presented and developed at ECCO-AACR-EORTC-ESMO Workshop on Methods in Clinical Cancer Research 2017
  - Confirmed Interest: NCRI, GINECO, Princess Margaret Consortium, Leuven

- **Translational Research**: Prof Chris Lord (ICR) (and international investigators taking part in the trial) On-going pre-clinical studies (Lord, Natrajan @ICR) assessing mechanisms of ATRi sensitivity and resistance in OCCC

Contact PI: susana.banerjee@rmh.nhs.uk
ICR-CTSU Trial team manager: sophie.perry@icr.ac.uk