A novel bacterial-based bioluminescent assay for the rapid pre-screening of chemotherapy efficacy

Ashley Martin, Mark Ruddock, Elizabeth Anderson, Habib Alloush, Vyv Salisbury, Priyanka Mehta, Ann Smith, Graham Smith, John Lamont
• AML is a condition affecting the adult population with a median age at presentation of 67 years. AML accounts for approximately 80% of acute leukaemia diagnosed in adults

• Cytarabine (Ara-C) is the first line of treatment for AML even though 30-40% of patients fail to respond to initial treatment

• Treatment with Ara-C is given without any pre-screening to determine sensitivity

Require the development of a rapid assay for pre-screening of patient prior to Ara-C chemotherapy
Key Features of the Assay:

• Development of a novel \textit{in vitro} bioluminescent biosensor assay which is capable of identifying sensitivity or resistance to Ara-C via the formation of the active metabolite Ara-CTP

• Predict individual response of a patient to Ara-C prior to treatment, singly or in combination with other agents

• Peripheral blood or bone marrow aspirates

• \textbf{Results are obtained in under 1 day}

• Tailor dosing (low, standard or high dose)

• Monitor effectiveness of treatment

• Reduce treatment times and costs

• Increase long term remission

• Increase quality of life by reducing side effects and hospital stays
The scenario…

Patient factors (age, sex…)

PB or BM sample

Analyses

3 to 14 days

Cytogenetics
Molecular markers

Patient factors
Leukaemia factors
Sensitivity

Functional testing

1 day

DNR/Ara-C
FLAG-Ida
Clofarabine/Ara-C

LD Ara-C
SD Ara-C
HD Ara-C

Treatment decision
How does it work?

**Difference proportional to Ara-CTP in AML cell**

Ara-C → Ara-CTP

Cell damage and death

AML cell

Ara-C + Ara-CTP

Cell lysis

Ara-CTP → Ara-C

Phosphatase

Biosensor

Low Light

High Light

Biosensor

Ara-CTP → Ara-C

+ phosphatase

- phosphatase

Ara-C → Ara-CTP

Ara-C

How does it work?
1. Blast cells isolated from peripheral blood or bone marrow aspirates
2. Cells counted and adjusted to 2x10^6/mL
3. Cell suspension treated with:
   - Ara-C (25 µM) for 30 minutes
   - Vehicle control for 30 minutes
4. Cells are washed to remove traces of drug and lysed
5. Lysates are applied to the biosensor in the presence/absence of IPTG and Alkaline Phosphatase (AP)
6. Luminescence is recorded using a CCD camera system at the peak max (t = 5.25 hours)

8 hours from cell separation to result!
Biosensor tested across a range of concentrations of Ara-CTP

Results for light output following exposure to lysate spiked with Ara-CTP in the presence and absence of alkaline phosphatase (AP)

Limit of detection was 25 nM Ara-CTP ($p<0.001$)

$r^2=0.9953$
Biosensor assay analysis of cell lines
### Sensitive patient (remission after 1\textsuperscript{st} cycle)

<table>
<thead>
<tr>
<th>Zero Control</th>
<th>Low Control</th>
<th>High Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Minus AP</td>
<td>Control</td>
</tr>
<tr>
<td>Low</td>
<td>plus AP</td>
<td>Low</td>
</tr>
<tr>
<td>Ara-C Sample</td>
<td>Treated</td>
<td>Ara-C Sample</td>
</tr>
</tbody>
</table>

### Resistant patient (no remission)

<table>
<thead>
<tr>
<th>Zero Control</th>
<th>Low Control</th>
<th>High Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Minus AP</td>
<td>Control</td>
</tr>
<tr>
<td>Low</td>
<td>Plus AP</td>
<td>Low</td>
</tr>
<tr>
<td>Ara-C Sample</td>
<td>Treated</td>
<td>Ara-C Sample</td>
</tr>
</tbody>
</table>

Biosensor assay analysis of patient samples
Biosensor assay analysis of patient samples

Sensitive patient (remission after 1st cycle)

Resistant patient (no remission)

Ara-C Sensitivity Index = 33.5%

Ara-C Sensitivity Index = 0%
### Data from preliminary testing

<table>
<thead>
<tr>
<th>ANLL patient samples</th>
<th>Total analysed</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Correct</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Incorrect</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Complete remission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td>13/14</td>
<td></td>
</tr>
<tr>
<td>Sensitivity range (%)</td>
<td>10 to 128</td>
<td></td>
</tr>
<tr>
<td>Median (%)</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Non-remission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total correct</td>
<td>18/20</td>
<td></td>
</tr>
<tr>
<td>Sensitivity range (%)</td>
<td>-9 to 7</td>
<td></td>
</tr>
<tr>
<td>Median (%)</td>
<td>3.5</td>
<td></td>
</tr>
</tbody>
</table>
• This rapid and robust assay simply and accurately determines sensitivity to Ara-C in under 8-hours of receipt of the patient sample

• Proof of principle analysis has shown 85% efficiency (correlation with clinical outcome and CellTiterGlo® assay) for 34 clinical samples analysed to date (p=0.052)

• Represents the first assay of this type, allowing oncologists to obtain a chemosensitivity profile of a patient prior to commencement of chemotherapy with Ara-C alone or in combination

**Current activities:**
Retrospective testing in larger patient cohort in collaboration with National Cancer Research Institute (NCRI) UK

Testing on alternative dosing regimes used in treatment of leukaemia, including daunorubicin/Ara-C, fludarabine/Ara-C and clofarabine/Ara-C
Collaborators

- Prof Vyv Salisbury, University of the West of England, Bristol, UK
- Dr Ann Smith, Scientific Director of Stem Cell Transplant Lab, Royal Marsden, UK
- Prof Graham Smith, Consultant Haematologist, Frimley Park Hospital, UK
- Dr Priyanka Mehta, Haematology Consultant, University Hospital Bristol, UK
- Dr Habib Alloush, American University of Beirut, Lebanon
- Dr Steve Knapper, Haematology Consultant, University Hospital of Wales, UK

Funding

- Randox Laboratories Ltd, UK
- BBSRC (with Dr Phil Hill, University of Nottingham)
- UK Technology Strategy Board
- National Institute for Health Research (NIHR), UK