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PLEASE SCROLL DOWN FOR TEXT.
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.
Biosensor Assay

• Development of a novel *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

**Key Features of the Assay:**

• 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

• **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...

- PB or BM sample

Patient factors (age, sex...)

- Cytogenetics
- Molecular markers

Leukaemia factors

- Sensitivity

Analyses

3 to 14 days

Functional testing

1 day

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

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

Treatment decision
Biosensor

How does it work?

Difference proportional to Ara-CTP in AML cell

Ara-C → Ara-CTP

Cell damage and death

Cell lysis

Ara-C + Ara-CTP

- phosphatase

+ phosphatase

Ara-CTP → Ara-C

Low Light

Biosensor

Ara-CTP → Ara-C

Biosensor

AML cell

High Light
1. Blast cells isolated from peripheral blood or bone marrow aspirates
2. Cells counted and adjusted to $2 \times 10^6$/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)
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>Plus AP</td>
</tr>
<tr>
<td>Control</td>
<td>Minus AP</td>
<td>Plus AP</td>
</tr>
</tbody>
</table>

Control Sample  
Ara-C Treated Sample

### Resistant patient (no remission)

<table>
<thead>
<tr>
<th>Zero Control</th>
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</tbody>
</table>

Control Sample  
Ara-C Treated Sample
Biosensor assay analysis of patient samples

Sensitive patient (remission after 1\textsuperscript{st} cycle)

Resistant patient (no remission)

Ara-C Sensitivity Index = 33.5%

Ara-C Sensitivity Index = 0%
<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>
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<tr>
<td>Peripheral blood</td>
<td>16</td>
<td></td>
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<tr>
<td>Bone marrow</td>
<td>18</td>
<td></td>
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<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><strong>13/14</strong></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><strong>18/20</strong></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.
Acknowledgments

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

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