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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

UWE BRISTOL University of the West of England

Frimley Park Hospital NHS NHS Foundation Trust

University Hospitals Bristol NHS NHS Foundation Trust

The Royal Marsden NHS NHS Foundation Trust

Acute Myeloid Leukaemia

- 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

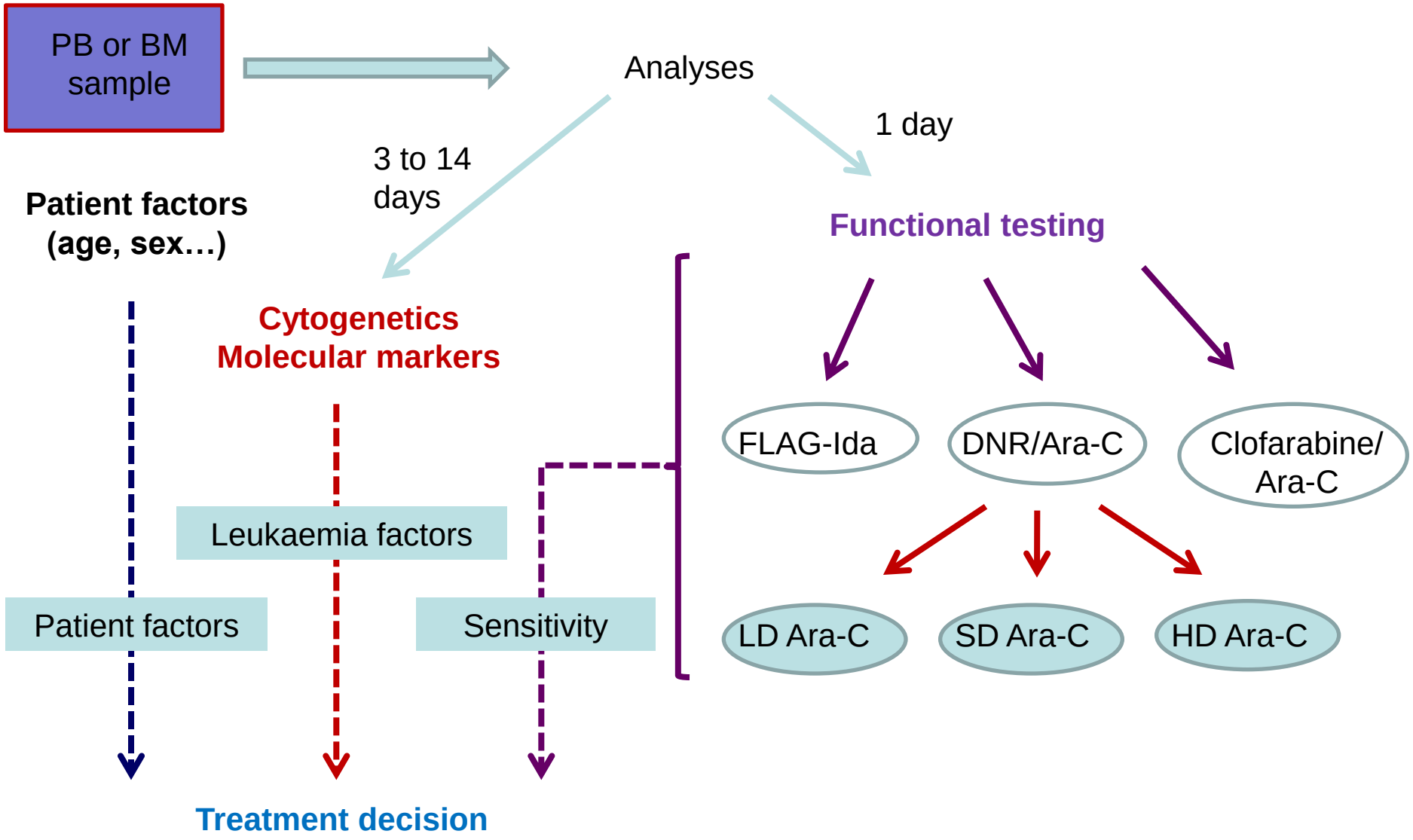
Biosensor Assay

- 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:

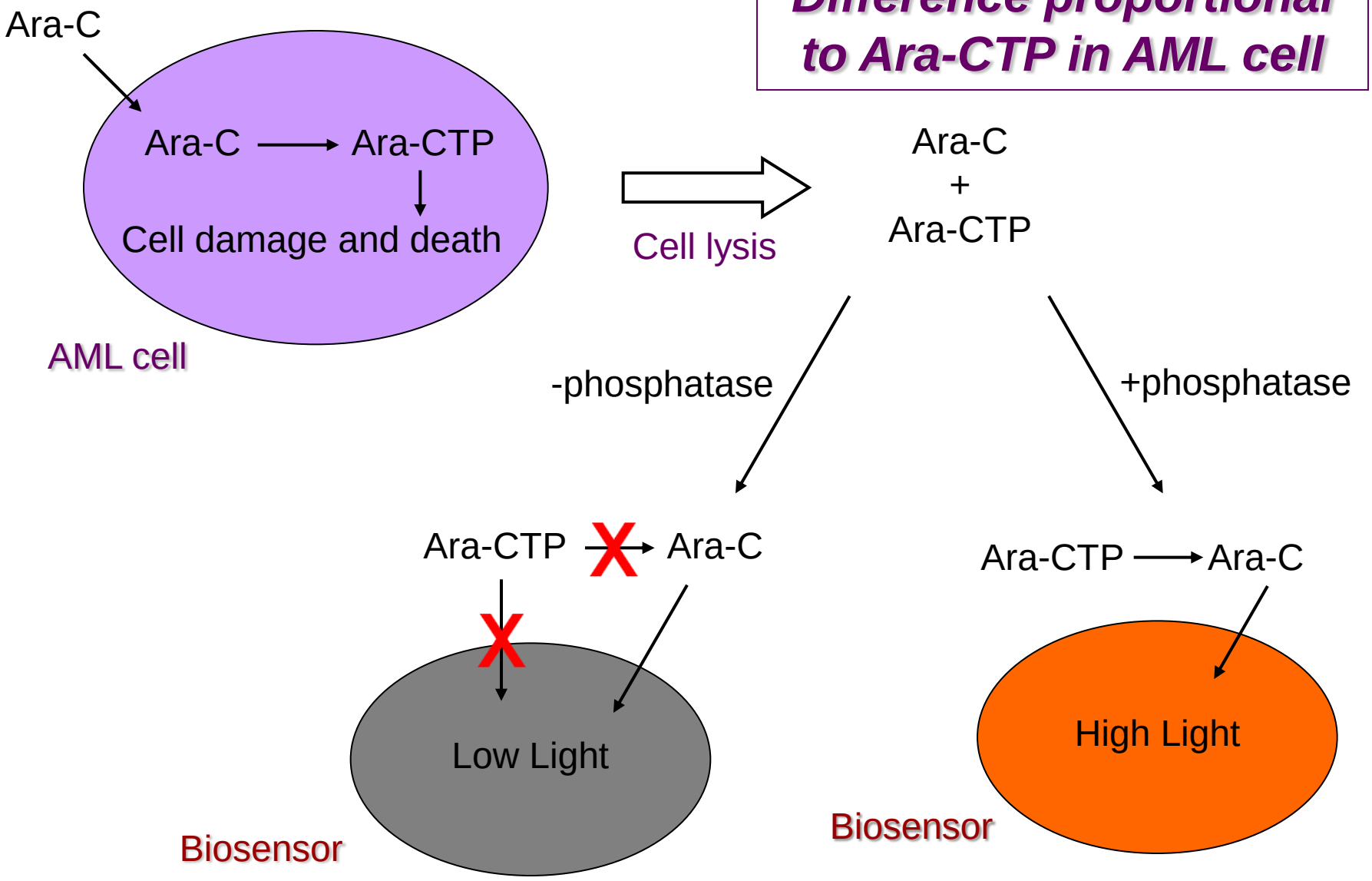
- Predict individual response of a patient to Ara-C prior to treatment, singly or in combination with other agents
- Peripheral blood or bone marrow aspirates
- **Results are obtained in under 1 day**
- Tailor dosing (low, standard or high dose)
- Monitor effectiveness of treatment
- Reduce treatment times and costs
- Increase long term remission
- Increase quality of life by reducing side effects and hospital stays

The scenario…



How does it work?

Difference proportional to Ara-CTP in AML cell



Assay protocol

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)

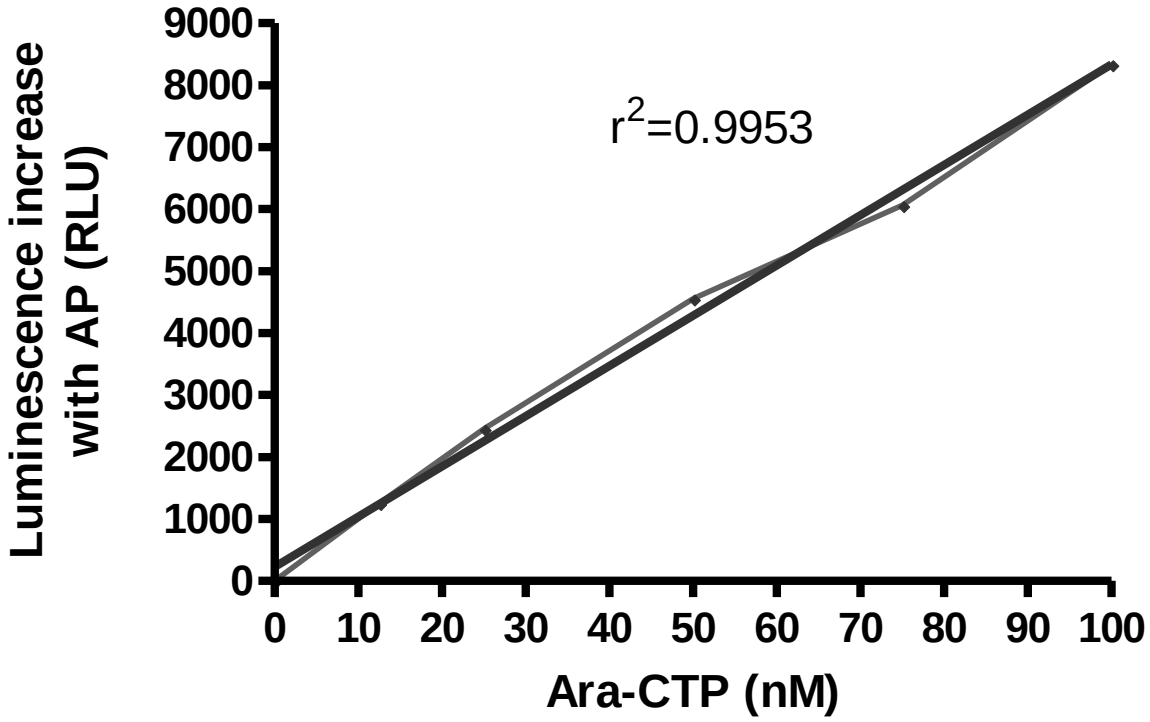
8 hours from cell separation to result!

Sensitivity of the biosensor to Ara-CTP

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 ($p<0.001$)



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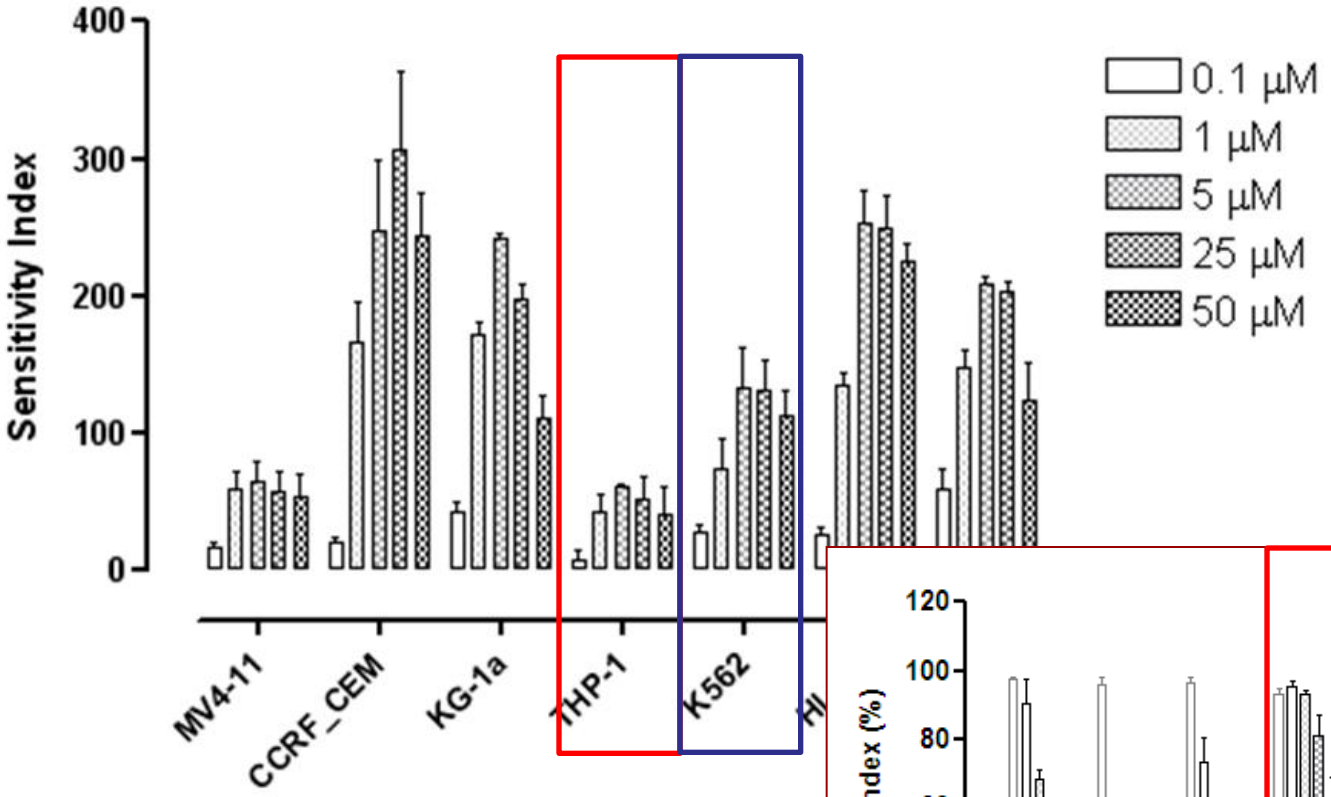
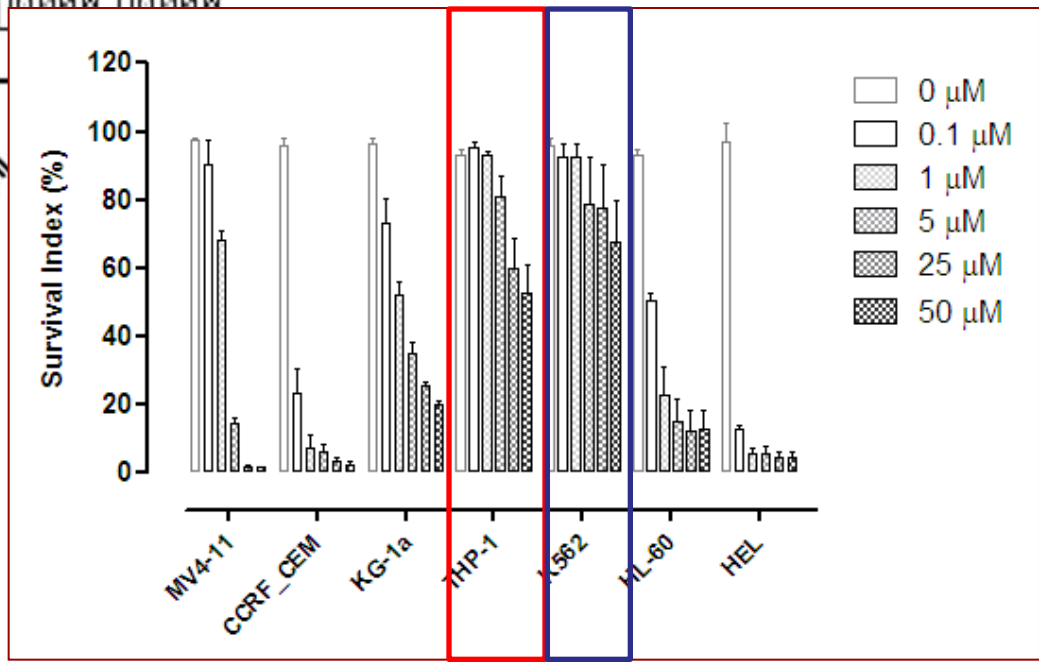
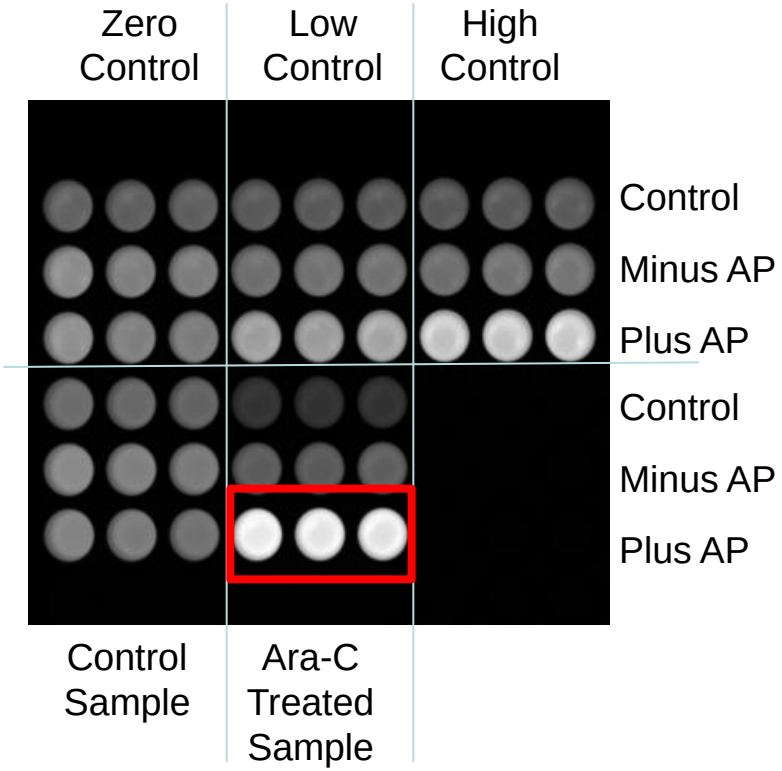
Biosensor assay analysis of cell lines



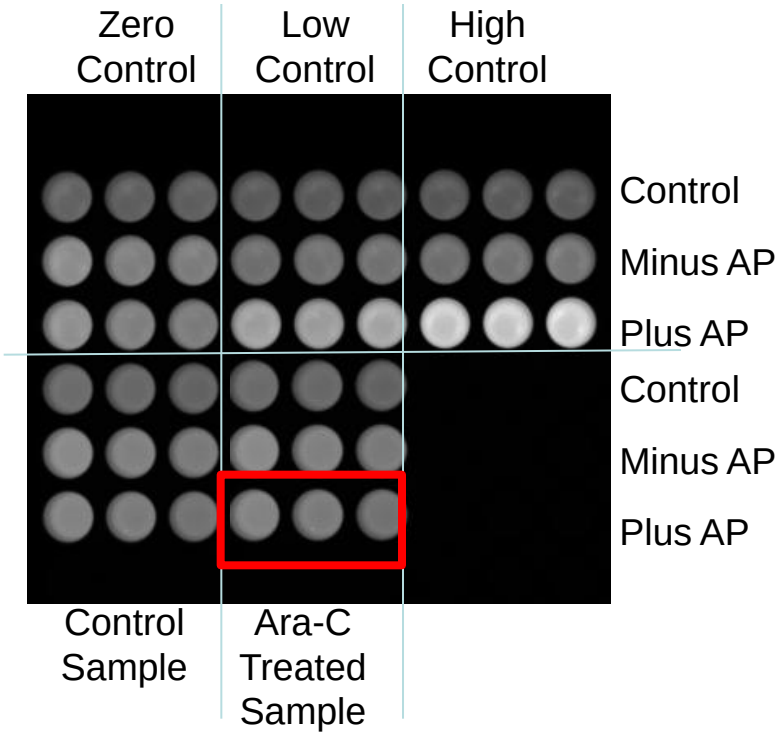


Biosensor assay analysis of patient samples

Sensitive patient (remission after 1st cycle)



Resistant patient (no remission)



Biosensor assay analysis of patient samples

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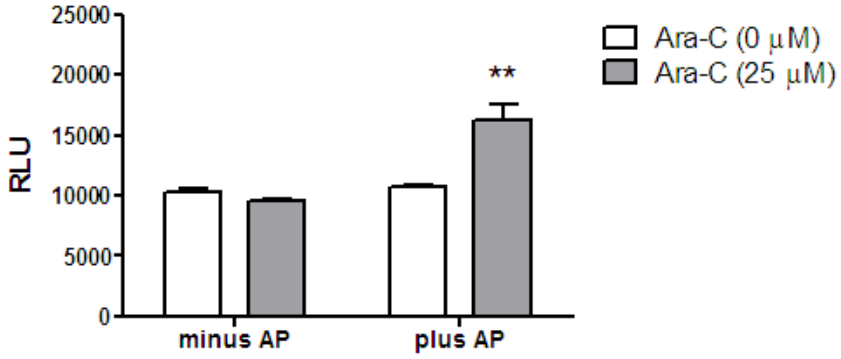
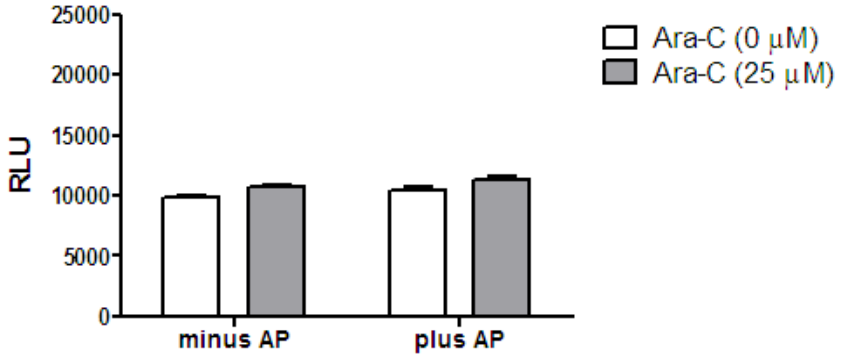


Ara-C Sensitivity Index = **33.5%**

Resistant patient (no remission)



Ara-C Sensitivity Index = **0%**

Data from preliminary testing

ANLL patient samples	Total analysed	56
Clinical outcomes		34
	Peripheral blood	16
	Bone marrow	18
	Correct	31
	Incorrect	3
Complete remission	Total correct	**13/14**
	Sensitivity range (%)	10 to 128
	Median (%)	36
Non-remission	Total correct	**18/20**
	Sensitivity range (%)	-9 to 7
	Median (%)	3.5

Conclusions

- 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

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- Prof Graham Smith, Consultant Haematologist, Frimley Park Hospital, UK
- Dr Priyanka Mehta, Haematology Consultant, University Hospital Bristol, UK
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