Phosphoproteomics predict response to Venetoclax plus Azacitidine in Acute Myeloid Leukemia patients

Josie A. Christopher[1], Luis Nobre[1], Weronika E. Borek[1], Amy E. Campbell[1], David N. Perkins[1], Pedro Moreno-Cardoso[1], Nazrath Nawaz[1], Janet Kelsall[1], Paolo Gallipoli[2], Andrea Arruda[3], Alex Ambinder[5], Andrew Thompson[1], Andrew Williamson[1], Gabriel Ghiaur[5], Sayantanee Dutta[6], Mark D. Minden[3,4], David J. Britton[1], Heinz Sill[6], John G. Gribben[2], Pedro R. Cutillas[1,2], Arran D. Dokal[1]

Background

- Acute myeloid leukaemia (AML) is a haematological malignancy with high heterogeneity and poor prognosis.
- Approximately two thirds of AML patients achieve complete remission (CR) with the treatment venetoclax plus azacitidine (VA), whilst the remainder experience no response (NR).
- Currently, there are no biomarkers for predicting VA response in AML.
- Phosphoproteomics has previously shown promise in patient stratification of AML and other cancers *[1-4]*.

Here, we used routine biopsies taken at diagnosis to find phosphopeptide biomarkers to subsequently build a predictive model for VA response in AML patients.

Experimental cohort

Table 1. Key characteristics of experimental cohort.

Characteristics	Measurement	CR	NR
Patient number	n	40	26
Age (years)	Median (range)	71 (31 - 89)	72 (64 - 87)
Sex	Male	25	16
	Female	18	15
Sample number	n	43	31

- 74 diagnosis bone marrow (BM) and peripheral blood (PB) samples from 66 AML patients subsequently treated with VA.
- Samples were collected from multiple sites and countries.

KScan® workflow & modelling

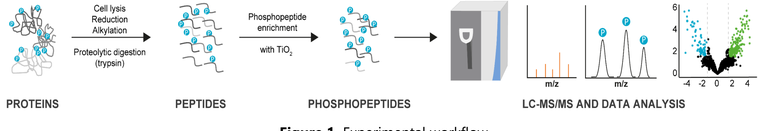
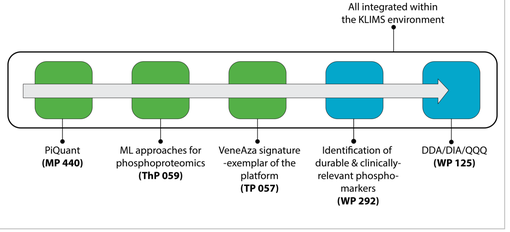


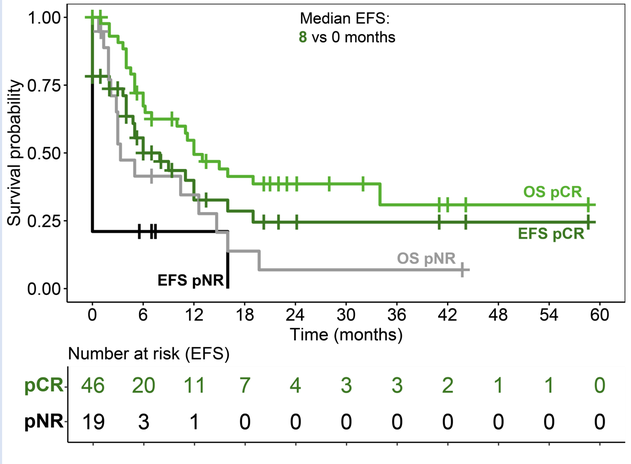
Figure 1. Experimental workflow.

- Tryptic peptides were enriched for phosphopeptides using TiO_2 before analysis on a Thermo Orbitrap Exploris 240 with Vanquish Neo using data-dependent acquisition **(Figure 1).**
- Differential expression analysis and kinase-substrate enrichment analysis (KSEA) performed to gain biological insight.
- Candidate phosphopeptide biomarkers identified for CR vs NR patient grouping using feature selection. Random forest model built using candidate biomarkers and model performance assessed via cross-validation.

Other aspects of the full KScan® workflow are covered in **several other ASMS 2024 posters:**



Phosphoproteomic assay to predict treatment response in blood cancer patients and address unmet clinical need.



EFS Metric	pCR	pNR
Cases	46	19
Events	28	16
RMean (months)	18.8	3.4
SE (months)	4.1	1.5
Median (months)	8	0

HR= 0.31 [95% CI: 0.14-0.67]
p < 2.52e-03

Figure 2. Event-free survival (EFS) and overall survival (OS) of acute myeloid leukaemia (AML) patients predicted to achieve complete remission (CR) and no remission (NR) in response to venetoclax + azacitidine (VA) by phosphoproteomics-based prediction model. Predictions from cross-validation. P-log-rank p(two-sided).

Affiliations
(1) Kinomica Ltd, UK;
(2) Barts Cancer Institute, Queen Mary University of London, UK;
(3) Princess Margaret Cancer Centre, University Health Network, Canada;
(4) Department of Medical Biophysics, University of Toronto, Canada;
(5) Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, USA;
(6) Division of Hematology, Medical University of Graz, Austria.

COI: Authors affiliated with Kinomica Ltd are employees, co-founders and/or shareholders of the company. H.S. is on the advisory board for AbbVie Inc.

Results

- A model built with phosphoproteomics-based features showed promising predictive power in cross-validation for distinguishing between AML patients who would likely achieve CR vs NR. Demonstrating 90% specificity and 60% sensitivity **(Figure 2)**.
- The 25 predictive biomarkers used to build the model were identified from ~7,000 quantified phosphopeptides across 66 patient samples **(Figure 3).**
- These candidate biomarkers, alongside KSEA, indicated known biology of VA resistance and highlight potential therapeutic target strategies, such as modulation of MAPK14/p38a (**Figure 4-5)** and splicing (**Figure 6)** *[5-9]*.

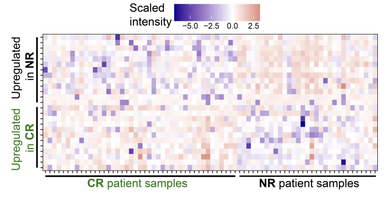


Figure 3. Scaled intensities of the 25 biomarkers used to create model across all samples used for model training.

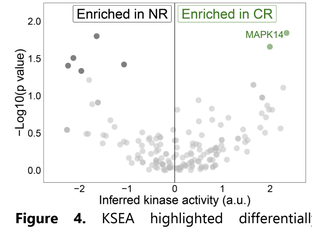


Figure 4. KSEA highlighted differentially modulated kinases in CR vs NR patient groups.

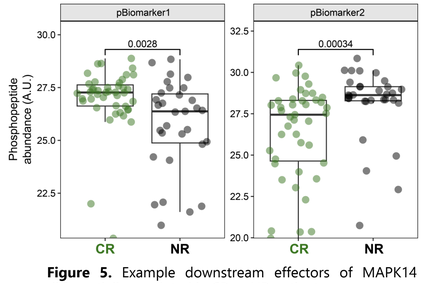


Figure 5. Example downstream effectors of MAPK14 differentially regulated in CR vs NR patient groups.

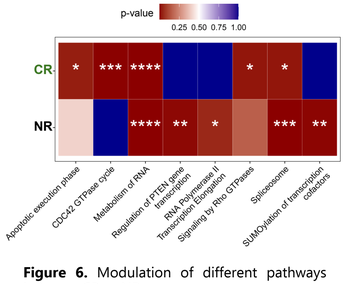


Figure 6. Modulation of different pathways between CR vs NR groups.

Future directions

- Expand model to encompass other commonly used AML treatments.
- Validation and refinement in retrospective cohorts from different geographies is ongoing.
- In parallel to this work, we are building a prognostic model to determine disease severity regardless of administered treatment.
- Broaden assay utility to suggest off-label treatments for patients predicted to be NR (or have early relapse) to currently available drugs or trials.

References:

1. Dokal A., *ASCO Annual Meeting*, 2021.
2. Casado P., *Leukemia*, 2021.
3. Khorsandi S.E., *Cancer Res.*, 2021.
4. Dokal A., *Cancer Res.*, 2021.
5. Kurtz S.E., *Blood*, 2018.
6. Kurtz S.E., *Blood*, 2022.
7. de Camargo Magalhães E.S., *Leukaemia*, 2024.
8. Lachowiez C.A., *Blood Adv.*, 2021.
9. Senapati J., *Blood*, 2023.