#2631: Identification of a predictive phosphoproteomic signature of response to atezolizumab and bevacizumab (AB) in patients with advanced hepatocellular carcinoma (aHCC)

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Background

- aHCC is the **third most common** cause of cancer-related deaths worldwide.
- There are **no validated predictive biomarkers** to guide systemic aHCC therapy selection.
- While AB is the global standard of care for aHCC, most AB-treated patients progress by 12 months.
- We have previously established phosphoproteomics as a **rich source of biomarkers** in several other cancer types (**AML:** Dokal A., *ASCO Annual Meeting 2021*; Casado P., *Leukemia*, 2021; **Cholangiocarcinoma:** Khorsandi S.E., *Cancer Res.,* 2021; **NSCLC:** Dokal A., *Cancer Res*., 2021).

Here, we identify biomarkers of AB response from phosphoproteomic data from formalin-fixed and paraffin-embedded (FFPE) resected and Tru-Cut liver biopsies, and build a preliminary model predicting AB response.

Methods

FFPE resected and Tru-Cut samples from aHCC patients (n=30; including two mixed HCC-cholangiocarcinoma, **Table 1**) were processed as shown in **Figure 1**. Patients were stratified into 'good responder' (**GRG**, n=20, duration of response (DoR)>7.5 months) and 'poor responder' (**PRG**, n=10, DoR<7.5 month) groups. Phosphoproteomic biomarkers distinguishing the two groups were used to train a random forest response prediction model, which was assessed via cross-validation.

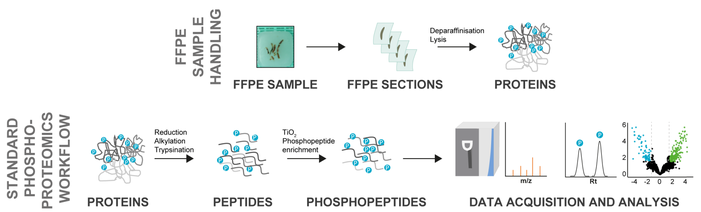

Figure 1. Experimental workflow.

Table 1. Cohort characteristics.

Characteristics	Measurement	PRG	GRG
Patient number	n	10	20
Age in Years	Median (range)	71 (37-83)	64 (35-81)
Sex	Male	8	17
	Female	2	3
BCLC Stage at diagnosis	A	4	9
	B	4	7
	C	2	4
Baseline AFP	Mean (+/-s.d., kIU/L)	344 (+/-550)	5867 (+/-15215)
	>350 kIU/L	2	7
Aetiology	Viral	7	7
	Non-viral	3	13

Using data from routine biopsy samples, phosphoproteomics can potentially identify hepatocellular carcinoma (HCC) patients likely to respond to atezolizumab + bevacizumab

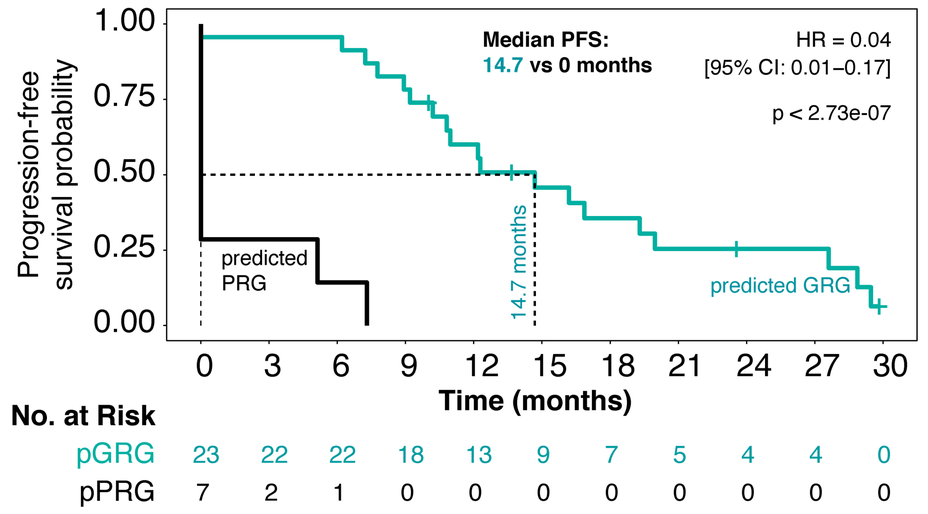

Figure 2. Progression-free survival of patients predicted to fall into PRG and GRG groups by the phosphoproteomics-based AB response prediction model (**cross-validation**). p – log-rank p (two-sided).

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Results

- Forty phosphopeptides, including previously-detected pGSTA1-3^{S202} and pHSPB1^{S9} (**Figure 3**) were used in the predictive model (Negroni, 2014, *MCP*; Bian, 2014, *J Proteomics*).
- In cross-validation, which allows testing predictive models in the absence of validation data, the model correctly predicted the outcomes of all GRG (20/20) and of 7/10 PRG patients (**Figure 2**), demonstrating 100% sensitivity, 87% precision and 70% specificity.
- Kinase substrate enrichment analysis revealed significant modulation of MAP kinases, PRKCI and others, between responder groups (**Figure 4**).
- A subgroup of PRG patients displayed increased activity of the RAF-MEK-ERK pathway, suggesting potential sensitivity to drugs such as sorafenib (**Figure 5**).

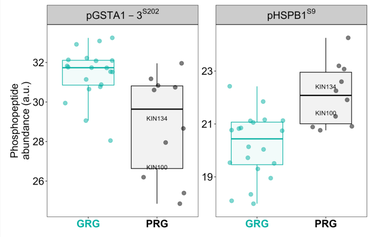

Figure 3. Cellular abundance of phosphoproteomic biomarkers correlates with AB response.

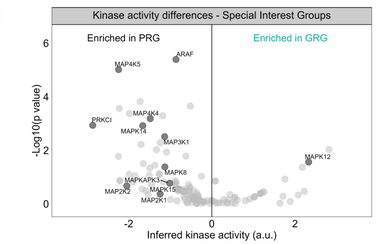

Figure 4. Several kinases are differentially modulated in PRG vs GRG patients.

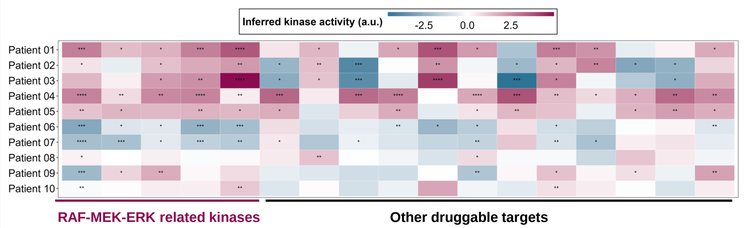

Figure 5. Kinase activation in PRG patients.

Future directions

- Predictive model validation and refinement in larger retrospective cohorts across different geographical region is ongoing. **We welcome new collaborators.**
- In parallel to this work, we are also developing phosphoproteomic signatures of response to the tyrosine kinase inhibitors sorafenib and lenvatinib.
- In the future, we aim to construct a unified phosphoproteomic model allowing selection of most-effective first-line treatment for aHCC.`