Predicting Response to Neoadjuvant Therapy in Oesophageal Adenocarcinoma Pre-Treatment Biopsies

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Background: Less than a third of patients with locally advanced adenocarcinoma of the oesophagus gain a clinically meaningful benefit from neo-adjuvant therapy[1]. We urgently require tools to predict response to NAT prior to treatment. This pilot study aims to identify differentially expressed genes in formalin fixed paraffin embedded (FFPE) pre-treatment biopsies between responders and non-responders to NAT.

Method: Diagnostic FFPE tissue from 26 responders (Mandard Tumour Regression Grade[2] (TRG) 1-2) and 30 non-responders (TRG 4-5) underwent gene expression profiling with two nuclease protection assays (EdgeSeq (Figure 1), HTG – Oncology Biomarker Panel (2568 genes) and Precision Immuno-Oncology Panel (1410 genes)). Sequencing was performed on the NextSeq500 (Illumina). Differentially expressed genes and pathways were assessed in "R" to determine the most predictive model of response to NAT. Differences in corresponding clinical characteristics between the two groups were also analysed.

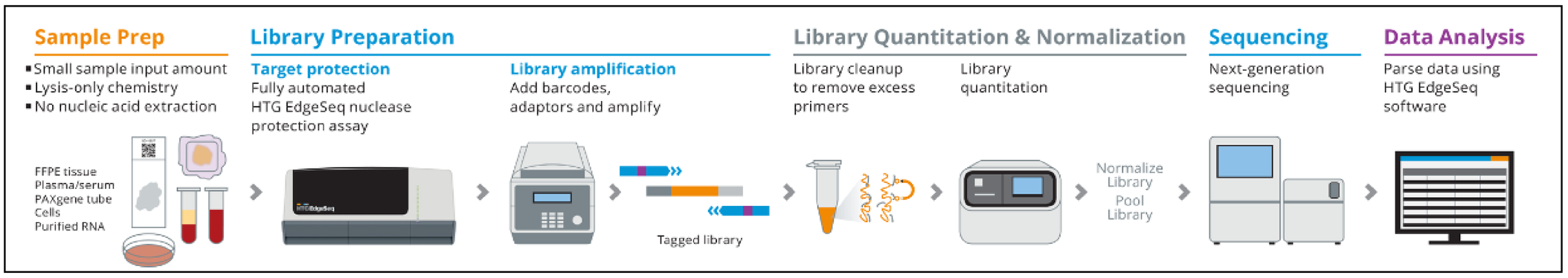

Figure 1. HTG EdgeSeq Workflow. From www.htgmolecular.com/systems/edgeseq

Results:

Patient Demographics: There were no statistically significant differences in age, gender, pre-treatment TNM stage or ASA grade between responders and non-responders. Responders had better overall survival than non-responders (responders, mean overall survival 3.76 years versus non-responders, mean overall survival 2.95 years, $p = 0.047$)

Panel Correlation: 731 genes are included in both panels with excellent correlation (Figure 2, $R = 0.82$, $p < 0.0001$), validating the EdgeSeq platform.

Differentially expressed genes: Genes significantly ($p < 0.05$) up-regulated in responders were involved in processes including apoptosis and cell cycling (Figure 3A). Conversely, genes significantly upregulated in non-responders were involved in cytokine signalling and the immune response (Figure 3B). To identify the most differentially expressed genes between responders and non-responders, we applied a threshold of log2 fold change >1 and $p < 0.05$. This identified 26 differentially expressed genes from the Oncology Biomarker Panel (Figure 4A) and 11 differentially expressed genes from the Precision Immuno-Oncology Panel (Figure 4B) between responders and non-responders.

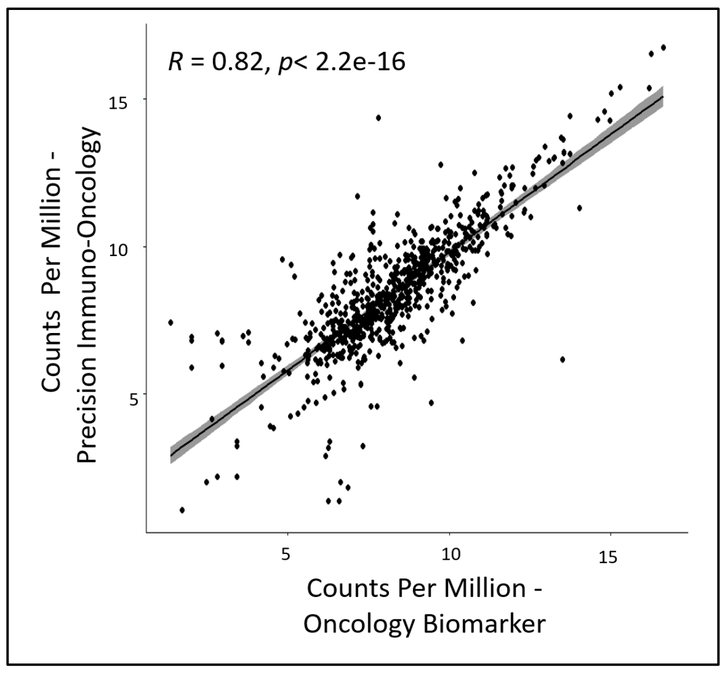

Figure 2. Correlation between Precision Immuno-Oncology and Oncology Biomarker EdgeSeq Panels

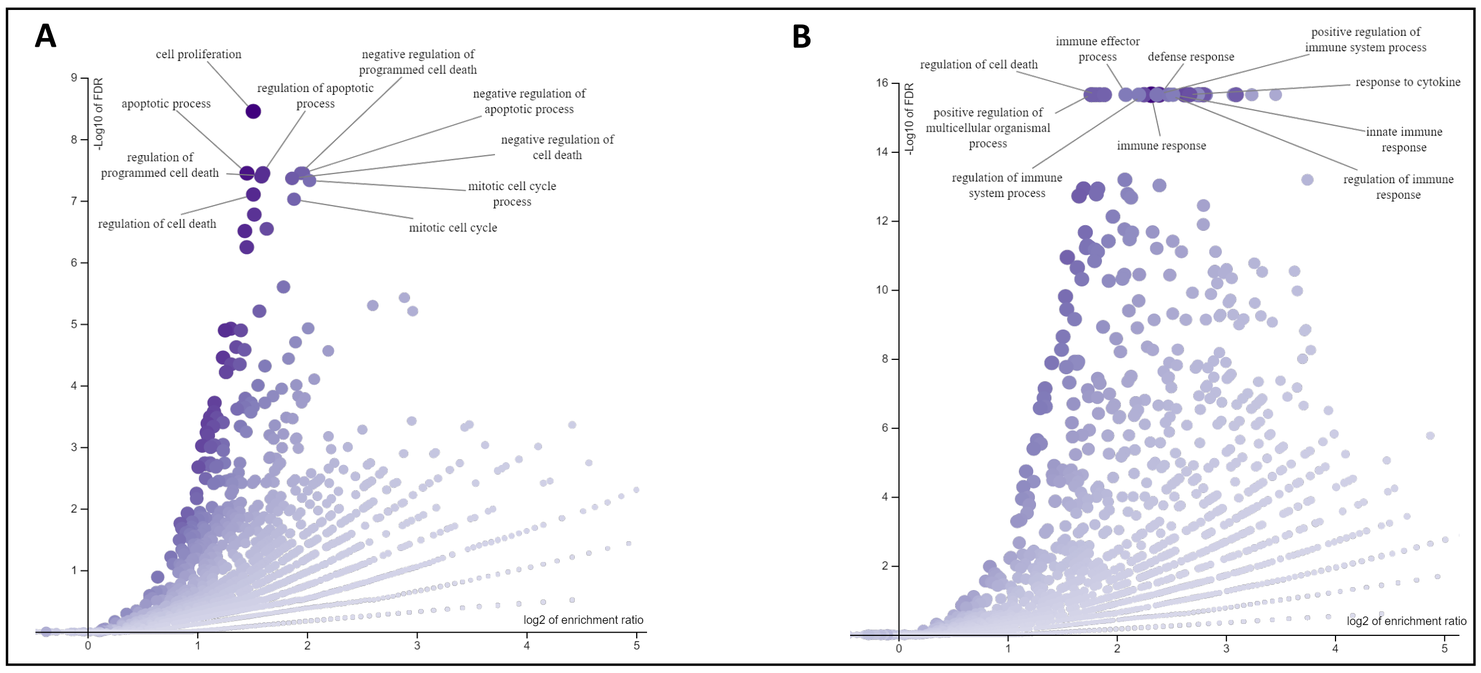

Figure 3. Gene ontology analysis for genes significantly up-regulated in A) Responders and B) Non-responders. Analysis performed using WebGestalt – www.webgestalt.org

Predicting Response to Neoadjuvant Therapy:

Using the 26 differentially expressed genes from the biomarker panel, we created a neuronal artificial network to predict response to NAT (Figure 5). This model had an overall accuracy of 73% with sensitivity of 80% and specificity of 70%.

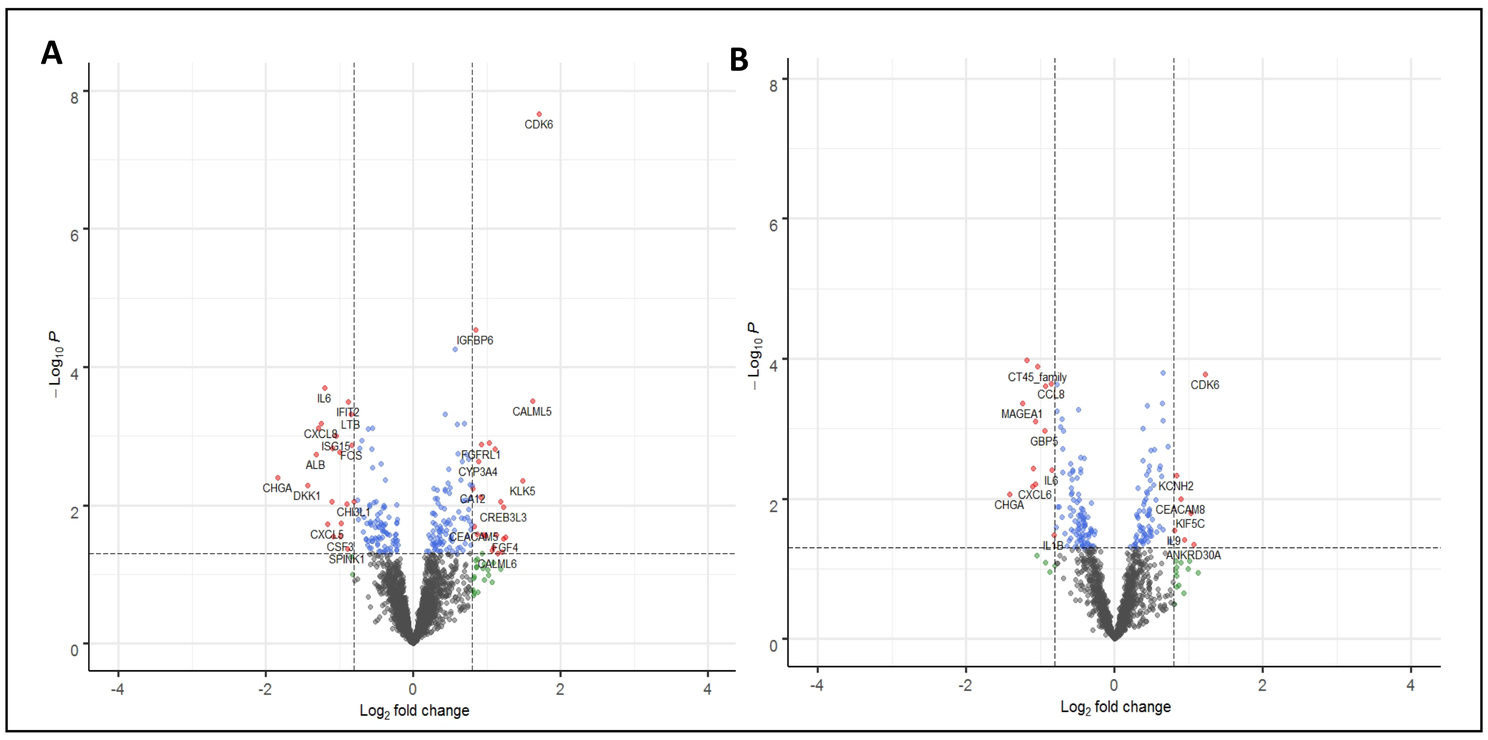

Figure 4. Differential Gene Expression between responders and non-responders A) Oncology Biomarker Panel and B) Precision Immuno-Oncology Panel

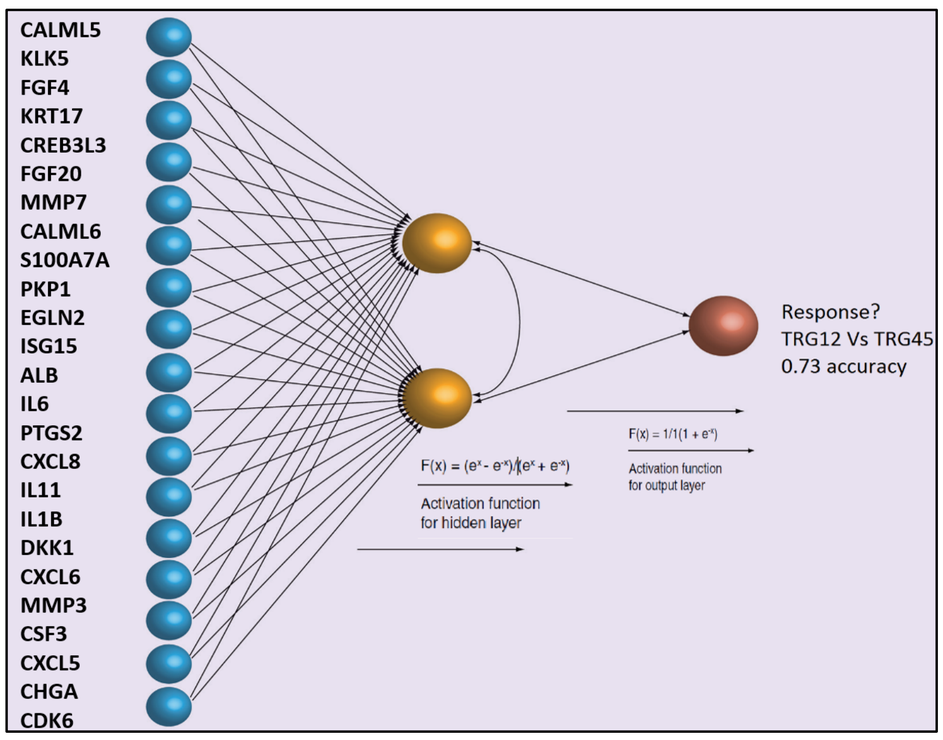

Figure 5. Model Predicting Response To Neoadjuvant Therapy

Conclusion:

Accurate prediction of response to NAT would allow non-responders to be diverted straight to surgery or to receive an alternative treatment, which will reduce morbidity and may increase survival. This pilot study informs a biologically sound hypothesis for predicting response to NAT. Further study using widely available stored tissue may allow us to improve the performance of our model to predict response to NAT in the clinic.

1. Noble et al. Multicentre cohort study to define and validate pathological assessment of response to neoadjuvant therapy in oesophagogastric adenocarcinoma. *British Journal of Surgery. 2017;* Doi: 10.1002/bjs.10627
2. Mandard et al. Pathologic assessment of tumour regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. *Cancer*. 1994; 73: 2680-6

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