

Liquid biopsy assay for testing *RNF43* mutated gastric and pancreatic cancers

Research findings presented at the European Society for Molecular Oncology (ESMO) 2017 meeting by Dr. Matilda Bingham, Head of Research and Operations at Redx Pharma Plc., Manchester, UK. The study identified a sub-group of cancer patients whose tumours are dependent upon the Wnt signalling pathway, and developed a circulating tumour DNA (ctDNA) screening assay to identify such patients for recruitment to clinical trials of the Wnt pathway inhibitor, RXC004.

“Redx Pharma are developing RXC004, a potent inhibitor of the protein Porcupine, an important component of the Wnt signalling pathway. Tumours containing RNF43 mutations are likely to be Wnt-dependent, and we hope patients with this type of cancer will therefore benefit from treatment with RXC004. Identifying these patients for recruitment to clinical trials requires an effective Clinical Trial Assay (CTA), which combines high sensitivity and specificity for mutations of interest with rapid turn-around-times, analysis of readily obtained sample material and competitive per-sample cost. A CTA which analyses circulating tumour DNA (ctDNA) isolated from stabilized whole peripheral blood can satisfy all these requirements.”

– Dr. Matilda Bingham

In collaboration with Redx Pharma, NewGene Ltd. has developed a clinical trial assay (CTA) to detect specific *RNF43* mutations in ctDNA samples. The assay was developed using the highly-sensitive UltraSEEK™ chemistry on the MassARRAY® System from Agena Bioscience, which has a limit of detection as low as 0.1% variant allele frequency.



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CASE STUDY

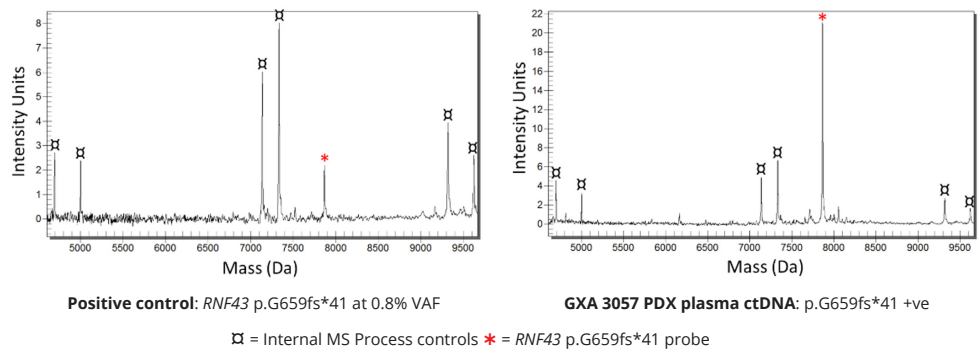
THE METHOD

The MassARRAY System utilises highly robust and specific oligonucleotide primer extension and termination chemistry to probe for base pair alterations at the targets of interest. Variants are identified using mass spectrometry. The technology is sensitive, label-free, rapid and cost effective. It also enables simultaneous analysis of multiple targets, which maximises the use of sparse sample material and greatly increases laboratory operating efficiency.

RESULTS

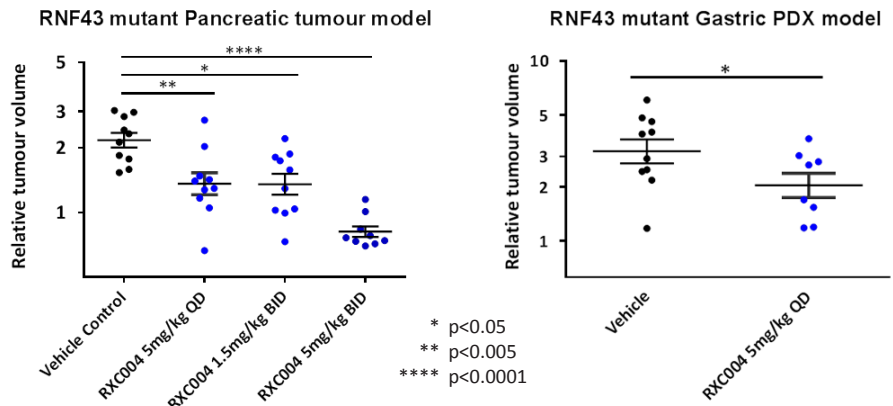
The assay was first developed using samples from cell lines known to harbour specific *RNF43* mutations. Testing tumour samples from patient-derived xenograft (PDX) models then confirmed that the probe oligonucleotides do not have reactivity towards murine *RNF43*. This provides reassurance that mutations detected in the ctDNA purified from PDX animal plasma are exclusively representative of the tumour graft.

Technical validation of the assay was carried out on ctDNA purified from blood plasma obtained from PDX animals that were part of pre-clinical RXC004 efficacy studies. Analysis of this sample material indicated successful detection of the p.G659fs*41 *RNF43* mutation, known to be present in this model.



Furthermore, results of the efficacy studies indicated that *RNF43*-mutant pancreatic and gastric cancer xenograft models with established tumours are sensitive to treatment with RXC004, with tumour growth significantly inhibited in both cases.

In summary, this study demonstrates that a Porcupine inhibitor such as RXC004 has potential beneficial therapeutic effects upon tumours harbouring *RNF43* mutations, which are known to comprise a noteworthy proportion of gastric and pancreatic cancer cases. Patients with such tumours can potentially be identified for recruitment to clinical trials by detection of mutations of interest in ctDNA samples.



The MassARRAY UltraSEEK assay described here is well-suited for this application as it combines high sensitivity and specificity for mutations of interest with rapid turn-around-times, analysis of readily obtained sample material and a competitive per-sample cost.

The MassARRAY System is For Research Use Only. Not for use in diagnostic procedures. The MassARRAY Dx is CE-IVD; not for sale in the United States.