Identification of an RNF43 mutated gastric cancer patient population with potential sensitivity to porcupine inhibitor RXC004 and development of a complimentary ctDNA liquid biopsy assay for patient screening

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Introduction

RXC004 is a potent small molecule inhibitor of the membrane bound *O*-acyl transferase (MBOAT) Porcupine (PORCN). PORCN is required for and dedicated to post-translational modification of all known Wnt ligand isoforms, a necessary step in the initiation of canonical Wnt signalling (figure 1).[1]

Aberrant Wnt signalling as a result of mutations in or abnormal expression of pathway components initiates key oncogenic pathways in cancer. Wnt signalling pathways are implicated in tumour initiation, growth, cell senescence, cell death, differentiation and metastasis in multiple cancer types.[2]

A growing body of literature also suggests that Wnt signalling plays a role in the host immune response to tumours and that activation of the pathway may result in poor response and indeed resistance to checkpoint inhibitors.[3]

A PORCN inhibitor has the potential to benefit patients with cancers in which Wnt signalling is implicated. RXC004 demonstrates potent Wnt inhibitory activity in a cellular reporter gene assay and antiproliferative activity in Wnt pathway dependant pancreatic cancer cell lines bearing loss of function mutations in RNF43, a negative regulator of canonical Wnt signalling.[4]

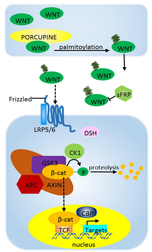


Figure 1: Canonical WNT pathway

RXC004 displays anti-proliferative activity in pre-clinical *in vivo* models, has a favourable ADMET profile *in vitro* and *in vivo* in pre-clinical species[4] leading to an achievable predicted human efficacious dose following oral administration. Pre-clinical safety studies have been completed and MHRA approval gained for first-in-human clinical studies (CT 2017-000720-98).

RNF43 mutation incidence in gastric and pancreatic cancer

- Gastric cancer harbours the highest percentage of RNF43 mutations (15.6%), the majority (86.7%) of which are potentially deactivating.
- Similarly, the majority (73.7%) of pancreatic cancer RNF43 mutations are potentially deactivating (RNF43 mutation rate: 4.53%).

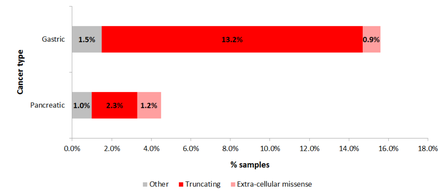


Figure 2a: RNF43 mutation frequency in gastric and pancreatic cancer based on 674 and 1677 samples respectively. A deactivating mutation is classed as either a frameshift or nonsense (truncating) mutation anywhere in RNF43 sequence, or missense mutation in the protease associated (residues 87-186) or extracellular (residues 34-198) domains. Source: COSMIC v77.

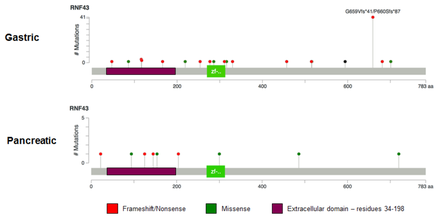
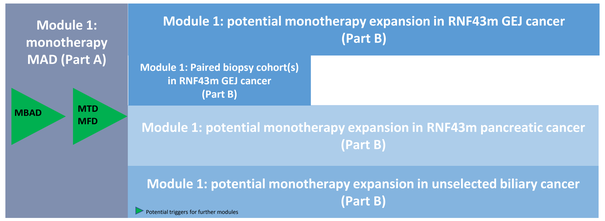


Figure 2b: Predicted hotspot RNF43 deactivating mutations. Source: TCGA, plots generated using cBioPortal (http://www.cbioportal.org/).

CT 2017-000720-98: A Modular, Multi-arm, Multi-part, Phase 1/2a, Adaptive Design Study to Evaluate the Safety and Tolerability of RXC004, Alone and in Combination with Anti-cancer Treatments, in Patients with Advanced Malignancies



Study Duration

Figure 3: Outline of RXC004 modular clinical phase 1/2a study. The minimally biologically active dose (MBAD) of RXC004 is defined as: Biologically relevant PK exposure (i.e. dose predicted to sustain free plasma concentration >IC50) And/ or Biomarker (AXIN2) or clinical evidence of target engagement in either normal or tumour tissue.

Objectives for study module 1:

Primary objectives:

- Part A: Assessment of safety and tolerability of RXC004 when given orally as a single agent to patients with advanced malignancies, and to define the doses and schedules for further clinical evaluation.
- Part B: To assess preliminary signs of monotherapy anti-tumour activity of RXC004 in patients with selected advanced solid malignancies, by evaluation of tumour response using RECIST v1.1.; objective response rate (ORR), duration of response (DRR) and disease control rate (DCR), to provide a monotherapy 'proof of concept' (PoC).

Secondary objectives:

- Characterisation of the pharmacokinetics (PK) of monotherapy RXC004, following a single dose and at steady state after multiple dosing
- Preliminary assessment of the activity of RXC004 by evaluation of tumour pharmacodynamic (PDc) biomarkers
- Preliminary assessment of overall survival (OS) and, progression free survival (PFS)

Further study modules may include an assessment of the safety, tolerability and efficacy of RXC004 in combination with anti-cancer treatments, including anti-PD1 checkpoint inhibitors

Eligibility criteria

Study inclusion criteria:

- Histological or cytological confirmation of advanced malignancy not considered to be appropriate for further conventional treatment
- Aged at least 18 years
- ECOG or WHO performance status 0 or 1
- Adequate organ function
- Evaluable disease, either measurable on imaging, or with informative tumour marker(s), as assessed by Response Evaluation Criteria in Solid Tumours [RECIST 1.1] or other relevant response assessment criteria for tumour type

Module 1, Part B specific inclusion criteria:

- Gastric/pancreatic tumour expansion group: patients should have a solid tumour with genetic alterations upstream in the Wnt signaling pathway.

Module 1, Part B, paired biopsy cohort(s) specific inclusion criteria

- Gastric or gastroesophageal junction (GEJ) cancer
- Provide a mandatory tumour biopsy sample at baseline (pre-dose first dose of RXC004) and up to 4 weeks after the first dose.

Study exclusion criteria:

- Prior therapy with a compound of the same mechanism of action as RXC004
- No other anti-cancer therapy and/or hormonal therapy or other investigational product is permitted other than the combination agent(s) described in the relevant study module
- Radiotherapy with a limited field of radiation for palliation within 1 week of the first dose of study treatment
- QTc prolongation (> 470 msec or 60 msec above baseline)
- Any known uncontrolled inter-current illness or psychiatric illness/social situations that would limit compliance with study requirements
- Subjects with any of the following medications within 4 weeks prior to enrolment: anti-neoplastic agents, immunotherapy, immunosuppressants, another investigational drug
- Patients with pleural effusions and/or ascites, due to malignancy, requiring paracentesis every 2 weeks or more frequently
- Patients with bladder inflammation and urinary outflow obstruction

Module 1 Part B specific exclusion criteria

- Subjects who have any history of other malignancy (except non-melanoma skin carcinoma and carcinoma-in-situ of the uterine cervix) within 5 years of study entry
- Subjects with metastasis limited to the bone only; however, patients with current metastasis limited to the bone only and with a history of locally advanced disease or distant metastasis are eligible.

Development of an RNF43 ctDNA liquid biopsy

Identifying patients with tumours containing *RNF43* mutations for recruitment to clinical trials requires an effective Clinical Trial Assay (CTA), combining high sensitivity and specificity for mutations of interest with rapid turn-around-times, analysis of readily obtained sample material and low per-sample cost. A CTA which analyses circulating tumour DNA (ctDNA) isolated from stabilised whole peripheral blood can satisfy all these requirements.

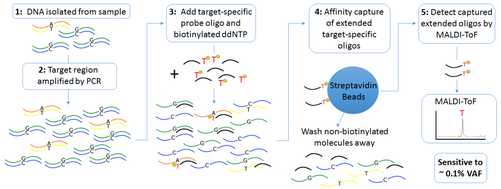


Figure 4a: The MassARRAY® UltraSEEK™ analytical process

Fig 4b: The benchtop MassARRAY® instrument

MassARRAY® Analysis

To support the RXC004 trial Redx Pharma Plc. and NewGene Ltd. have developed a CTA using the Agena Bioscience MassARRAY® instrument, particularly highly-sensitive UltraSEEK™ detection chemistry. This enables targeted detection of specific mutations to an allelic frequency as low as 0.1% Variant Allele Frequency (VAF) against a background of Wild Type sequence.

The MassARRAY® system utilises highly robust and specific oligonucleotide primer extension and termination technology to probe for base pair alterations which are characteristic of the mutations of interest. This is combined with a biotin-streptavidin capture / clean-up step to selectively enrich the pool of reaction products for those which are positive for the assay target. Read-out is via determination of reaction product masses, achieved using MALDI-ToF mass spectrometry. The system is label-free, rapid, cost-effective and allows many targets to be analysed in parallel, preserving laboratory efficiency.

Fig 4c: Close-up of MALDI chips and carrier

Mutations targeted

Six *RNF43* mutations were selected for inclusion in the CTA on the basis of their COSMIC variant count (http://cancer.sanger.ac.uk/cosmic), reported frequency of occurrence in gastric and pancreatic cancer cohorts recorded in the cBioPortal resource (http://www.cbioportal.org/) and reported frequency of occurrence in the literature.

Table 1: Mutations targeted for detection by the RNF43 ctDNA liquid biopsy Clinical Trial Assay

Mutation	Mutation type	COSMIC ID	COSMIC variant count	Source material	Assay multiplex
c.1970 del G, p.G659fs*41	Deletion – Frameshift	COSM6190365	122	Cell line: 23132-87 PDX: GXA 3057	1
c.349 del C, p.R117fs*41	Deletion – Frameshift	COSM1384762	20	Cell line: HCT-116	2
c.1977 del T, p.S661fs*39	Deletion – Frameshift	COSM1734865	9	Synthetic plasmid	2
c.394 C>T, p.R132*	Substitution - Nonsense	COSM981870	9	Synthetic plasmid	3
c.433 C>T, p.R145*	Substitution - Nonsense	COSM248786	7	Synthetic plasmid	3
c.988 C>T, p.R330*	Substitution - Nonsense	COSM3388049	4	Synthetic plasmid	3

Initial assay development

Source DNA for assay development was obtained from either cell lines known to harbour the mutations of interest, or if this was not available, synthesised as a plasmid construct. Normal genomic Wild Type control DNA was isolated from healthy donor whole blood samples.

Initial development was carried out using iPLEX® analytical chemistry. This identifies all variants present at a given genetic locus, rather than specifically detecting a given mutation of interest and can therefore be used to determine the zygosity and allelic frequency of a sample.

Results indicated that the 23132-87 cell line is heterozygous for the p.G659fs*41 mutation, whereas the GXA 3057 PDX line is homozygous for this mutation. Furthermore, in PDX FFPE tumour samples there is no evidence of probe reactivity towards mouse *RNF43*, providing reassurance that ctDNA detected in PDX plasma is representative of the tumour graft.

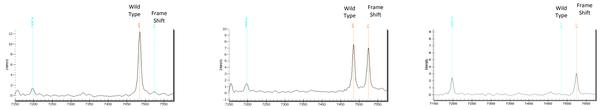


Figure 5: Example MassARRAY® iPLEX® RNF43 p.G659fs*41 assay results

Assay transfer to UltraSEEK® chemistry and technical validation

Following development of the iPLEX® assays, transfer to UltraSEEK® chemistry was carried out. As illustrated in Figure 3a, this method incorporates a biotin-streptavidin capture and clean-up step, enhancing assay sensitivity to well below 1% VAF, making it particularly suitable for detection for rare molecular species such as ctDNA.

Spike recovery of the RNF43 p.G659fs*41 mutation to a VAF of <0.8% was achieved, as was detection of the mutation in ctDNA purified from PDX plasma samples.

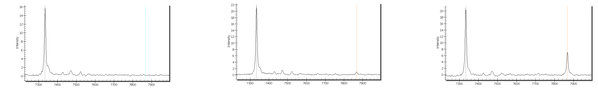


Figure 6: Example MassARRAY® UltraSEEK™ RNF43 p.G659fs*41 assay results.
NB: *Due to the target-specific capture step only the target sequence is detected using this method, rather than both target and WT.*

RXC004 is efficacious in RNF43 mutant tumours

RNF43 mutant pancreatic and gastric xenograft models with established tumours are sensitive to treatment with RXC004.

- RXC004 demonstrates tumour growth inhibition in Capan-2 human pancreatic tumour xenograft. Figure 7a and 7b; RXC004 dosed at 5mg QD in this model caused significant tumour growth inhibition (TGI) at day 28 (TGI = 67.6%, ** p <0.005). RXC004 dosed at 5mg BID in this model caused significant tumour regression at day 29 (TGI = 116.9%, **** p <0.0001). RXC004 dosed at 1.5mg BID in this model caused significant TGI at day 29 (TGI = 65.6%, ** p <0.005).
- RXC004 demonstrates tumour growth inhibition in a human gastric cancer PDX model. Figure 6c and 7d; RXC004 dosed at 5mg QD in this model caused significant TGI at day 11 (TGI = 49%, p <0.05)

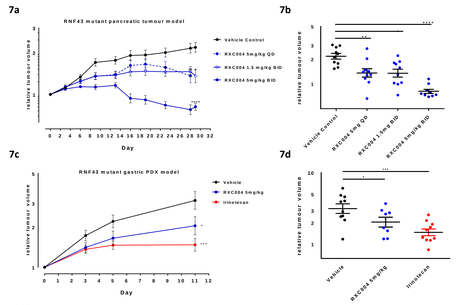


Figure 7a and 7b: Capan-2 cells ($3x10^6$) were implanted subcutaneously into the flanks of Scid Beige mice. Animals were randomized in to groups of 10 animals when tumours reached an average size of 200mm³. RXC004 was dosed orally either BID for 29 days (0.5mg/kg, 1.5mg/kg and 5mg/kg) or QD (5mg/kg) for 28 days.
Figure 7c and 7d: nu/nu Mice were implanted with tumour cells from Champions TumorGraft® model CTG-0147. After the tumours reached 1-1.5 cm³, they were harvested and the tumour fragments were implanted SC in the left flank of the female study mice. Each animal was implanted with a specific passage lot (passage 5) and documented. Tumour growth was monitored twice a week using digital calipers and the tumour volume (TV) was calculated. Tumours were selected at between 150-300mm³, and animals were randomized into groups for treatment. Dosing was initiated on Day 0.

Conclusions

RXC004 is entering first-in-human trials with a modular phase I/IIa clinical protocol design which allows for phase IIa expansion arms in molecularly selected patient segments including gastric cancer.

We demonstrate here that there is an RNF43 mutated patient segment which may benefit from therapy with a porcupine inhibitor such as RXC004, and that these patients have the potential to be identified by a ctDNA screening approach.

References

1. Biechele s, Cox BJ, Rossant J; *Dev Biol,* 2011, 355 (2): 275-285; 2. Nusse R, Varmus H; *EMBO J,* 2012, 31 (12) : 2670-2684; 3. Gopalkrishna Pai S *et al*; J Haematol Oncol, 2017, 10:101; 4. Bhamra I *et al*; *Eur J Cancer*, 2016, 69, S100-S101.

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