Cover page

Protocol number: RXC004/0002

Document title: A Multi-arm, Phase II, Open-Label, Multicentre Study to Assess the Preliminary Efficacy of RXC004 in Monotherapy and in Combination with Nivolumab, in Patients with Ring Finger Protein 43 (RNF43) or R-spondin (RSPO) Aberrated, Metastatic, Microsatellite Stable, Colorectal Cancer who have Progressed following Therapy with Current Standard of Care

NCT number: NCT04907539

Version number: 7.0

Date of the document: 19 August 2022

Clinical Study Protocol	
Study Code	RXC004/0002
Version	Final v7.0
Date	19 Aug 2022

A Multi-arm, Phase II, Open-Label, Multicentre Study to Assess the Preliminary Efficacy of RXC004 in Monotherapy and in Combination with Nivolumab, in Patients with Ring Finger Protein 43 (RNF43) or R-spondin (RSPO) Aberrated, Metastatic, Microsatellite Stable, Colorectal Cancer who have Progressed following Therapy with Current Standard of Care

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Regulatory Agency Identifier Number(s)

EudraCT No.: **2020-003132-24**

Protocol Number: **RXC004/0002**

Amendment Number: Final Version 7.0

Study Treatment: RXC004 monotherapy (Arm A) or RXC004 and nivolumab combination (Arm B)

Study Phase: Phase 2

Short Title: Phase II Study to Assess Efficacy of RXC004 +/- Nivolumab in RNF43/RSPO Aberrated mCRC MSS after Progression on SoC.

PORCUPINE STUDY

SIGNATURES

PROTOCOL TITLE: A Multi-arm, Phase II, Open-Label, Multicentre Study to Assess the Preliminary Efficacy of RXC004 in Monotherapy and in Combination with Nivolumab, in Patients with Ring Finger Protein 43 (RNF43) or R-spondin (RSPO) Aberrated, Metastatic, Microsatellite Stable, Colorectal Cancer who have Progressed following Therapy with Current Standard of Care

PROTOCOL NO: RXC004/0002

VERSION NO: 7.0

PPD _____ PPD _____ PPD _____

PPD Signature Date

PPD

PPD

PPD

Medical Monitor Name and Contact Information will be provided separately

International Co-ordinating investigators

PPD

PPD

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Phone: PPD

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INVESTIGATOR SIGNATURES

PROTOCOL TITLE: A Multi-arm, Phase II, Open-Label, Multicentre Study to Assess the Preliminary Efficacy of RXC004 in Monotherapy and in Combination with Nivolumab, in Patients with Ring Finger Protein 43 (RNF43) or R-spondin (RSPO) Aberrated, Metastatic, Microsatellite Stable, Colorectal Cancer who have Progressed following Therapy with Current Standard of Care

PROTOCOL NUMBER: RXC004/0002

VERSION NUMBER: 7.0

This protocol is a confidential communication of Redx Pharma, plc. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with good clinical practices and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from Redx Pharma, plc.

Signature of Investigator: _____ Date: _____

Printed Name: _____

Investigator Title: _____

Name/Address of Centre: _____

_____

_____

Site Number: _____

VERSION HISTORY

Version 7.0 – 19 August 2022
This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union. **Substantial changes:** The RXC004 dose to be used in combination therapy (1.5 mg QD) has been defined and justified by updated and new text, to support combination therapy. Supporting information has been added on the dose regimen and dose reductions. **Sections updated: Section 4.4 and its subsections, Section 6.1.1, Section 6.6** Details of allocation of patients to Arm A or Arm B by randomisation once Arm B has started enrolment have been added, following FDA advice. **Sections updated: Section 1.2, Section 4.1, new Section 6.3** **Non-substantial changes:** Selected Cycle 0 Day 1 pre-dose tests can be performed up to 24 hours prior to Cycle 0 Day 1 treatment administration. **Section updated: Section 1.2** Clarification of timings for baseline biopsy and baseline CCI **Section updated: Section 1.2** Clarification of prophylactic treatment regimens with denosumab and calcium. Sections updated: **Sections updated: Sections 1.2, 2.3.1.2, 6.2.3** At selected sites, digital ECG collection may take place using ambulatory Holter monitoring equipment on Cycle 0 Day 1, and Cycle 1 Day 15. **Sections updated: Section 1.2 and 8.2.4** TSH (reflex T3/T4) sampling at Cycle 0 Day 1 was removed from the Part A schedule of assessment. **Section updated: Section 1.2** Background information has been updated. **Sections updated: Sections 2.2.2, 2.2.3, and 2.3.2** Details of an SAE of ileitis reported in this study were added to the protocol, to provide investigators with details relevant to the safety of RXC004. **Sections updated: Section**

2.3.1.2 and Appendix J Clarifying language was added on the potential assessment of tumour gene and protein expression correlation with treatment response. **Sections updated: Section 3 and 8.6.1** In inclusion criterion 4, the requirement for documented radiological progression has been modified to clarify that the progression does not require having been defined by RECIST 1.1. **Section updated: 5.1** Exclusion criterion 10 and prohibited medication details have been updated to clarify vaccine requirements. **Sections updated: Section 5.2 and 5.3.3.1** Details on central genetic testing for required pre-screening tests have been updated according to the currently used tests and processes, and for consistency with other RXC004 studies. **Section updated: Section 5.5.1** Table 13 (Investigational Products) has been updated to include the RXC004 dosage level for Part B. **Section updated: Section 6.1.1** Update to RXC004 dose modification guidance **Section updated: Section 6.6** Vitamin D and viral tests (HIV, hepatitis B/C) at baseline/screening have been added to the list of laboratory safety variables in Table 16 for consistency with the schedules of assessment. **Section updated: Section 8.2.1** A safety review of the combination data will be conducted after [redacted] patients have received the combination of RXC004 and nivolumab for at least 1 cycle. **Sections updated: Section 4.1 and 9.6** Update to guidance on prohibited CYP3A4 inhibitors and inducers. **Section updated: Appendix E** Guidance on management of events of colitis has been updated. **Section updated: Appendix J** Minor edits have been made throughout the protocol to correct typographical errors and improve cross referencing within the protocol.
Version 6.0 – 13th January 2022
• Inclusion criteria #8, creatinine clearance amended to ≥60mL/min instead of > 60mL/min following FDA advice. **Section updated: Section 5.1** • RXC004 related colitis management guidelines revised to clarify the maximum time

a patient with Grade 1 colitis can continue RXC004 at a lower dose before switching to Grade 2 management and to clarify the maximum time that RXC004 treatment can be held before permanent discontinuation, following FDA advice. **Section updated: Appendix J**

- Dose modification table revised to include interruption of RXC004 if a patient has >5kg weight loss associated with dysgeusia and dysgeusia treatment guidelines modified to clarify management options following FDA advice. **Sections updated: Table 14 and Appendix I**

- Dose modification table revised to include permanent discontinuation of RXC004 in the event of a RXC004 related Grade 4 event following FDA advice. **Sections updated: Table 14 and Table 15**

- The following changes were made to the schedule of Assessments

    - ECG monitoring plan revised to include monitoring around the anticipated maximal RXC400 concentration on Cycle 0 Day 1 (first dose) and Cycle 1 Day 15 (steady state) following FDA advice**.**

    - CCI [redacted] assessments changed from optional to mandatory unless unavailable at site to clarify the original intention that these assessments should be performed if the site has the capability to do so.

    - Footnote added to CCI [redacted] CCI [redacted]

    Sections updated: Table 1 and Table 2

- Minor editorial changes were made throughout the protocol for consistency and clarity

Version 5.0 – 14th September 2021

1. The following updates were made to the eligibility criteria following FDA review:

    a. Exclusion criteria updated to exclude patients with QTcF >470 ms

    b. Creatinine clearance inclusion criteria and monitoring added to enable future population PK analysis of effect of CLcr on PK of RXC004

Sections updated – Section 5.1 and Section 5.2

2. Guidelines for management of colitis events updated after IB updated. Dose modification tables, prohibited medications and safety labs also updated accordingly. Discontinuation of RXC004 and nivolumab (for patients on the combination) for grade 3 colitis events added as per MHRA request. **Sections updated – Appendix J, Table 9, Section 5.3.3.1 and Section 6.6**

3. RXC004 background, risk assessment and dose justification updated to include data from the most recent IB. **Sections updated – Section 2.2.3, Section 2.3.1 and Section 4.4.1**

4. Adverse Events of Potential Interest (AEPI) identified for monitoring: bone toxicities and colitis events. **Sections Updated – Section 8.3.13**

5. Contraception requirements updated to include the definition of sexual abstinence, as per MHRA request. **Section added – Section 5.3.1**

6. Clarification for treating patients after RECIST1.1 progression added. **Sections updated 7.1.1**

7. RXC004 fasting requirements from Section 5.3.2 added to schedule of assessment footnotes and RXC004 handling instructions for clarity. **Sections updated - Table 1, Table 2 and Section 6.2.1**

8. Restrictions on the use of concomitant CYP3A4 inhibitors and inducers updated to include 2 weeks prior to first dose of study treatment, as well as throughout the study treatment following FDA feedback. **Section updated – Section 5.3.3.1**

9. Maximum 14 days RXC004 interruption without Sponsor approval' language removed from protocol as a clarification for situations where it conflicts with advice for management of colitis in Appendix J. **Section updated - Section 6.6**

10. Optional collection of CCI [redacted] in the screening period (if not collected during pre-screening) added to Schedule of Assessments to allow for confirmation of the CCI [redacted] at the central laboratory. Language also amended to allow MSI status of patients to be confirmed at the central laboratory. **Sections updated – Table 1, Table 2, Section 5.5 and Section 8.6**

11. Ability of patients to enrol in concomitant COVID-19 vaccination studies removed as per MHRA request. **Sections updated – Section 5.3.3.2 and 7.1**

12. Dysgeusia dose modifications from Table 10 also added to Table 9 for clarity. **Sections updated – Table 9**

13. Retention of ECG traces added as part of a programme initiative to enable future QT investigations if required. **Section updated – Section 8.2.4**

14. CCI [redacted] CCI [redacted] CCI [redacted] **Sections updated - Table 1, Table 2, Table 3 and Section 8.6.2**

15. Arm A primary endpoint rationale updated for clarity. **Section updated – Section 4.3.1**

Version 4.0 – 18th June 2021

1. Dose of RXC004 in Arm A updated to 2 mg QD, due to recent data from Phase 1 study and Safety Review Committee recommendations. RXC004 background, risk/benefit, dose rationale and dose modification sections also updated with most recent data from the Phase 1 dose escalation study **Sections updated – Section 2.2.3, Section 2.3.1, Section 4.4.1, Section 6.6, Table 1, Table 4, Table 13, and Table 7.**

2. Inclusion criteria for entry into the combination treatment phase in Arm A added, to clarify when crossover to combination treatment can occur. **Sections updated – Figure 1, Section 5.1 and Appendix D**

3. Acceptable methods of contraception in the lifestyle consideration updated to be consistent with the Clinical Trial Facilitation Group (CTFG) recommendations for highly effective methods of contraception. **Sections updated – Section 5.3.1**

4. Management plan for RXC004 related diarrhoea/colitis events added after safety review of data from the Phase 1 study. **Sections added – Appendix J**

5. The following items have been added to the protocol as a result of the COVID-19 vaccination risk assessment;

    a. receipt of live (capable of replication) vaccinations within 4 weeks of starting study treatment eligibility criteria moved from Arm B specific exclusion criteria to core exclusion criteria. **Sections updated – Section 5.2**

    b. Clarification on permitted COVID-19 vaccinations. **Sections updated – Section 5.3.3.1 and 5.3.3.2**

    c. Interruption of RXC004 for COVID-19 infections added to dose interruption

and stopping criteria. **Sections updated – Table 14**

d. Clarification on collection of COVID-19 adverse events added**. Sections updated – Section 8.3.11**

6. The following changes have been made to the schedule of assessments;

   a. Permitted windows added to pharmacokinetic sample timepoints on Cycle 0 Day 1 and Cycle 1 Day 15 for clarity. **Sections updated – Table 1 and Table 2**

   b. HCV, HBV and HIV testing in Arm A moved from pre-first cycle of RXC004+Nivolumab to the screening period following feedback from Ethics Committees. **Sections updated – Table 1**

   c. CCI [redacted] CCI [redacted] CCI [redacted]. **Sections updated – Table 1 and Table 2**

   d. MSI testing removed from pre-screening activities and local MSI test added to screening after feedback from sites on availability of local testing. **Sections updated – Table 1 and Table 2**

   e. Collection of CCI [redacted] after Cycle 2 removed, based on emerging data from Phase 1 study which indicates that collection of samples after Cycle 2 is not required. **Sections updated – Table 1 and Table 2**

7. Assessments to characterise and guidelines to treat RXC004 related dysgeusia added to protocol to better define and clinically manage dysgeusia events. Assessment of dysgeusia also added to Secondary safety objective. **Sections updated - Section 8.3.12, Table 1, Table 2, Table 3 and Appendix I.**

8. CCI [redacted] CCI [redacted] CCI [redacted] CCI [redacted] CCI [redacted] Additional details of the centralised pre-screening assays for RNF43 and RSPO also added to Section 5.5. **Sections updated – Section 5.5, Section 8.6.1, Table 1, Table 2 and Table 3.**

9. Details of a Safety Monitoring Committee for Redx Phase 2 RCX004 studies added to protocol to aid patient safety monitoring. **Sections updated – Section 9.6**

10. Additional language added to sample size determination to ensure that the decision for stopping development is not based purely on patients with RNF43 mutation or RSPO fusions alone. **Sections updated – Section 9.2** 11. Following an update to the Nivolumab SmPC, approval for Nivolumab to treat oesophageal squamous cell carcinoma added to Section 2.2.4 and updates made to the Nivolumab toxicity management guidelines. **Sections updated – Section 2.2.4 and Appendix G** 12. Language about collection of scans preformed before consent moved from Inclusion criteria #4 and added into Section 8.1.1 so that availability of theses scans does not affect patient eligibility for the study. **Sections updated – Section 5.1 and Section 8.1** 13. Changes required for UK local version 3.0 (see below) added into Global protocol. **Sections updated – Section 5.1, 5.3.1, 5.3.4, 8.3.1, 8.3.10, Table 1 and Table 2** 14. Minor editorial changes were made throughout the protocol for consistency and clarity
UK Local Version 3.0 – 18 February 2021
1. Duration of contraception for women of childbearing potential amended to take into account the requirements for patients using nivolumab and denosumab, as per MHRA request. **Sections updated - Section 5.1, 5.3.1 and 8.3.10**. 2. Lifestyle restrictions updated to include avoiding direct sunlight and use of tanning equipment, as per MHRA request, **Section updated - Section 5.3 (new Section 5.3.4 added)** 3. Schedule of assessments and time period for collection of adverse events clarified to be from signature of the main study informed consent, as per MHRA request. **Sections updated - Section 8.3.1, Table 1 and Table 2**
Version 2.0 – 30 November 2020
Initial creation
Version 1.0 – 18 August 2020
Not submitted to Regulatory Authorities

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Multi-arm, Phase II, Open-Label, Multicentre Study to Assess the Preliminary Efficacy of RXC004 in Monotherapy and in Combination with Nivolumab, in Patients with Ring Finger Protein 43 (RNF43) or R-spondin (RSPO) Aberrated, Metastatic, Microsatellite Stable, Colorectal Cancer who have Progressed following Therapy with Current Standard of Care (PORCUPINE study)

Short Title: Phase II Study to Assess Efficacy of RXC004 +/- Nivolumab in RNF43/RSPO Aberrated mCRC MSS after Progression on SoC.

Rationale:

The Wnt signalling pathway plays an important role in tumourigenesis and has been shown to be elevated in both ring finger protein 43 (RNF43) and R-Spondin (RSPO) aberrated colorectal cancer (CRC). There is also evidence that Wnt signalling may have a role in enabling cancers to avoid immunosurveillance and hence resistance to some immunotherapies. RXC004 is a small molecule porcupine (PORCN) inhibitor which reduces Wnt signalling and therefore may offer an opportunity to deliver clinical benefit by both a direct tumour targeting effect in genetically selected patients and by converting the tumour microenvironment from an immune “cold” to an immune “hot” signature making the tumour sensitive to anti-PD1/PD-L1 inhibition.

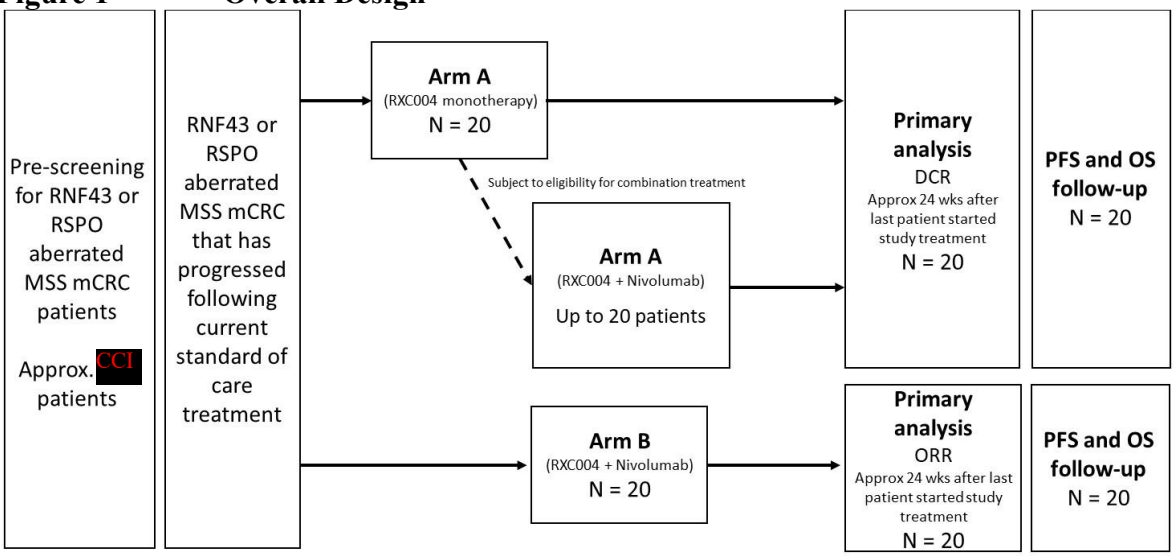
The PORCUPINE study will therefore evaluate RXC004 as monotherapy and in combination with anti-PD1 (nivolumab) in $\geq 2^{nd}$ line metastatic MSS colorectal cancer patients who have either a RNF43 LoF mutation or a RSPO fusion/translocation.

Objectives and Endpoints

Objectives	Endpoints
Primary	
To assess the anti-tumour activity of RXC004 monotherapy and RXC004 + nivolumab	Monotherapy : Disease control rate (DCR) using each patient's Best Overall Response (BOR) according to RECIST 1.1 Combination : Objective response rate (ORR) using each patient's BOR according to RECIST 1.1
Secondary	
To further assess the preliminary efficacy of RXC004 monotherapy and RXC004 + nivolumab	% change in the sum of target lesions, duration of response (DoR), PFS, ORR (monotherapy) and DCR (combination) using investigator assessments according to RECIST 1.1 and OS.
To assess the PK of RXC004 as monotherapy and in combination with nivolumab	Maximum plasma concentration (C_{max}) after Dose 1, C_{max} at steady state, minimum observed plasma concentration (C_{min}) at steady state as well as other relevant parameters (e.g. t_{max}, $t½$, λz, $AUC_{0-\infty}$, CL/F, and Vz/F).
To assess the safety and tolerability profile of RXC004 monotherapy and RXC004 + nivolumab combination	Incidence of AEs, SAEs, dose reductions, interruptions and discontinuations, and assessment of dysgeusia.

A full list of objectives can be found in Table 3.

Figure 1 Overall Design



Patients in Arm A who have undergone dose reduction during monotherapy cannot have the RXC004 dose re-escalated if switching to RXC004 + nivolumab combination therapy. The RXC004 dose must be discussed with the Sponsor prior to starting combination therapy.
DCR disease control rate, ECOG performance status, LoF, mCRC metastatic colorectal cancer; MSS Microsatellite stable; ORR objective response rate; OS Overall survival; PFS progression free survival; RNF43 Ring finger 43; RSPO R-Spondin; RXC004 Porcupine inhibitor

Disclosure Statement: This is an open label study two Arms A & B

Number of Participants:

Approximately CCI patients will be enrolled in total to provide 20 evaluable patients in each arm. Patients who progress on Arm A will have the opportunity to cross over into the combination arm, once this is open to recruitment.

Pre-screened for MSS status, RNF43 loss of function mutations and RSPO fusions	Estimated CCI patients
Screened (Signed main ICF)	Estimated CCI patients
Enrolled (dosed)	Estimated CCI patients
Evaluable patients	Estimated CCI patients

Note: "Enrolled" means patients that receive a dose of RXC004. Potential patients who are screened (sign main ICF) for the purpose of determining eligibility for the study but are not enrolled are considered “screen failures”, unless otherwise specified by the protocol.

1.2 Schedule of Assessments

Table 1 Arm A Schedule of Assessments

	Table 1 – Arm A Schedule of assessments											
Treatment Phase	**Pre-treatment**		**RXC004 monotherapy**				**RXC004 + nivolumab combination** [j]		**Post-treatment**			
	Pre-Screening	**Screening**	**Cycle 0** (3-7 days)	**Cycle 1** (28 day cycle)		**Cycle 2 onwards** (28 day cycles)	**Pre-first Cycle**	**Cycle 1 onwards** (28 day cycles)	**IP Discontinuation**	**30 day Follow-up**[v]	**90 day Follow-up**[v]	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15** +/- 1d	**D1 +/- 3d**		**D1 +/- 3d**	**Within 7 days**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
Pre-Screening informed consent	X											
[redacted]	X											
Genetic screening for RNF43/RSPO aberrations and MSI status [t]	X											
Main study informed consent		X										
Demography & baseline characteristics		X										
Smoking history		X										
Central MSI testing (if not completed in pre-screening)		X										

	Table 1 – Arm A Schedule of assessments											
Treatment Phase	**Pre-treatment**		**RXC004 monotherapy**				**RXC004 + nivolumab combination [j]**		**Post-treatment**			
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**Pre-first Cycle**	**Cycle 1 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[v]**	**90 day Follow-up[v]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**		**D1 +/- 3d**	**Within 7 days**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
CCI CCI CCI CCI		X										
CCI CCI CCI CCI CCI CCI CCI CCI		X										
Medical/ surgical history		X										
Oral and dental assessment[w]		X	Oral assessment at each visit - only if patient reports dysgeusia									
Inclusion/ exclusion criteria		X					X[k]					
HepB, HepC and HIV testing		X										

	Table 1 – Arm A Schedule of assessments											
Treatment Phase	**Pre-treatment**		**RXC004 monotherapy**				**RXC004 + nivolumab combination [j]**		**Post-treatment**			
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**Pre-first Cycle**	**Cycle 1 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[v]**	**90 day Follow-up[v]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**		**D1 +/- 3d**	**Within 7 days**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
CCI CCI CCI CCI CCI		X										
Physical examination		X	X	X		X	X	X	X			
ECOG performance status		X	X	X	X	X	X	X	X			
Pregnancy test (WOCBP only)		(X)	(X)[†]	(X)	(X)	(X)	(X)	(X)				
Baseline and 'on treatment' tumour biopsies (mandatory)[a]		X			X[g]	X[l]		X[l]				
Vital signs (including height and weight)[r]		X	X	X	X	X	X	X	X	X		
Clinical chemistry / Haematology		X	X[†]	X	X	X	X	X	X	X	X	
TSH (reflex T3/T4)[u]		X		X	X	X	X	X	X	X	X	
ECG[c]		X	X	X	X	X[h]	X	X[h]	X			

	Table 1 – Arm A Schedule of assessments											
Treatment Phase	**Pre-treatment**		**RXC004 monotherapy**				**RXC004 + nivolumab combination [j]**		**Post-treatment**			
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**Pre-first Cycle**	**Cycle 1 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[v]**	**90 day Follow-up[v]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**		**D1 +/- 3d**	**Within 7 days**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
DXA scan		X	On Cycle 4 and Cycle 7 of RXC004 treatment and then every 7 RXC004 cycles thereafter whilst on study treatment									
Blood samples for bone turnover biomarkers (β-CTX)		X			X	X		X				
CCI CCI		X		X		X	X	X				
CCI CCI CCI			X†		X	X		X				
CCI CCI			X†		X	X		X				
CCI CCI			X†		X	Cycle 2 only		Cycle 1 and 2 only				
CCI CCI			X†		X	X		X	X			
CCI CCI			X†		X	X		X	X			

	Table 1 – Arm A Schedule of assessments											
Treatment Phase	**Pre-treatment**		**RXC004 monotherapy**				**RXC004 + nivolumab combination [j]**		**Post-treatment**			
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**Pre-first Cycle**	**Cycle 1 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[v]**	**90 day Follow-up[v]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**		**D1 +/- 3d**	**Within 7 days**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
RECIST 1.1 assessments (by CT/MRI)[n]		X		On study tumour assessments should occur q8w +/- 1 week (relative to first dose of IP) for the first 56 weeks, followed by q12w +/- 1 week until RECIST1.1 radiological progression[x]			On study tumour assessments should continue q8w +/- 1 week (relative to first dose of IP) for the first 56 weeks, followed by q12w +/- 1 week until RECIST1.1 radiological progression [q, x].		If a patient discontinues for a reason other than progression then tumour assessments should continue q8w +/- 1 week (relative to first dose of IP) for the first 56 weeks, followed by q12w +/- 1 week until RECIST1.1 radiological progression			
CCI (mandatory unless unavailable at site)[i]		X*		On study CCI assessments should occur at week 8 and week 16 +/- 1 week relative to first dose of IP.			On study CCI assessments should occur at week 8 and week 16 +/- 1 week relative to first dose of combination					
Vitamin D3 and Calcium supplements		Patients should commence 800 IU vitamin D3 (Cholecalciferol) daily and 1000-1500 mg calcium daily supplements from ICF signature until RXC004 discontinuation										
Denosumab dosing[y]			120 mg sc Denosumab once every month from C0D1 until RXC004 discontinuation									
RXC004 dosing[m]			X (2 mg single dose)	Continuous daily dosing (2 mg QD)				Continuous daily dosing (1.5 mg QD)				
RXC004 diary			X	Review at each visit								

	Table 1 – Arm A Schedule of assessments											
Treatment Phase	**Pre-treatment**		**RXC004 monotherapy**				**RXC004 + nivolumab combination [j]**		**Post-treatment**			
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**Pre-first Cycle**	**Cycle 1 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[v]**	**90 day Follow-up[v]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**		**D1 +/- 3d**	**Within 7 days**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
Nivolumab dosing								480 mg q4w +/- 3 days[o]				
RXC004 PK[f]			X		X	X		X	X			
Concomitant medication		X	X	X	X	X	X	X	X	X	X	
Adverse events		X	X	X	X	X	X	X	X	X	X	
Taste assessment[z]		X	At each visit – only if patient reports dysgeusia									
Survival status and post-discontinuation anti-cancer therapy[p]										X	X	X

Once Arm B is opened, patients who are eligible for both Arm A and Arm B will be randomised 2:1 to Arm B: Arm A in an open-label manner on Day 1. Randomisation will be stratified by genetic alteration (RNF43, RSPO).

a A baseline (pre-first dose IP) biopsy is a mandatory requirement, except in exceptional circumstances where biopsy is not technically feasible. All patients without a baseline sample must be approved by Sponsor before starting study treatment. The baseline CCI can be a 'fresh newly acquired' biopsy or an CCI (preferably from the same organ site that 'on treatment' biopsy will be taken) and taken after completion of all prior standard of care treatments. In exceptional circumstances where baseline biopsy cannot be scheduled before C0D1, the procedure may be performed before C1D1 after a minimum of 4 days washout from C0D1. A second 'on treatment' biopsy at Cycle 1 Day 15 (+/- 1 day), is also mandatory providing that investigator judges the second biopsy to be technically and clinically feasible. The Cycle 1 Day 15 biopsy should be collected post-dose and the time of collection should be accurately recorded. RECIST 1.1 target lesions should not be chosen for biopsies.

b CCI

c Triplicate ECGs should be collected as follows;
Cycle 0: CCI
Cycle 1, CCI
Cycle X, CCI
At selected sites, ambulatory Holter monitoring will be used on Cycle 0 Day 1 and Cycle 1 Day 15 for central analysis of digital ECG information.

d CCI

e CCI
CCI

f Blood samples for PK analysis should be collected as follows (the actual time for each blood draw must be accurately recorded);
- Cycle 0: CCI
- Cycle 1, CCI
- Cycle X, CCI

g CCI

h **ECGs are required** at C2D1 and as clinically indicated from C3D1 onwards

i CCI

CCI

CCI

j **RXC004 + nivolumab** combination treatment will be allowed for up to 20 patients that have progressed on RXC004 monotherapy. This treatment phase will only be opened after the RP2D for RXC004 + nivolumab is determined

k Patients that consent to RXC004 + nivolumab combination treatment will have eligibility re-assessed for RXC004 + nivolumab combination specific criteria

l One 'on treatment' biopsy is required during cycle 3 of RXC004 monotherapy **or** RXC004 + nivolumab combination treatment, providing that investigator judges the biopsy to be technically and clinically feasible. The biopsy should be collected post-dose and the time of collection should be accurately recorded.

m Patients must fast (water to drink only) for at least 2 hours prior to taking a dose to at least 1-hour post-dose for all doses

n CT (preferred) or MRI, each preferably with IV contrast. It is strongly recommended to maintain use of the same imaging modality (CT or MRI), acquisition protocol, facility and scanner across all imaging time points per patient.

o 480 mg IV infusion over 60 minutes, q4w +/- 3 days, unless an infusion needs to held due to an adverse event. Results for vital signs (including weight), performance status, chemistry, haematology and thyroid function labs must be available before commencing an infusion (within 3 days) and reviewed by the treating physician or Investigator prior to dosing

p Survival and details of first and subsequent therapies for cancer, after discontinuation of treatment, will be collected.

q Patients treated with RXC004 monotherapy that radiologically progress as per RECIST1.1 at the 8 week scan, may be treated with RXC004 + nivolumab combination, subject to meeting combination treatment eligibility criteria. The most recent scan that shows progression on RXC004 monotherapy treatment will be used as the baseline scan for the combination treatment (see Section 7.1.1 and Appendix D for more details).

r Height is required at screening only

s CCI

CCI

t CCI

CCI

CCI

CCI

u **T3 and T4 will only be measured if TSH is abnormal or if there** is clinical suspicion of an AE related to the endocrine system

v Follow up assessments should be performed 30 days after discontinuation of RXC004 and 90 days after last dose of nivolumab (for patients treated with RXC004 and nivolumab)

w A dental examination with preventive dentistry if appropriate and an individual benefit-risk assessment is mandatory prior to treatment with denosumab. An initial dental examination may be performed by a member of the clinical team who can assess the need for referral to a dentist for further assessment including any preventative dentistry. A visual oral examination (including tongue, palate, uvula, tonsils, buccal mucosa, lips, gums and parotid duct) should also be performed at baseline and at each scheduled visit where dysgeusia is reported as an adverse event.

x Symptomatically stable patients may continue study treatment after RECIST1.1 progression, until either symptomatic progression or second RECIST1.1 progression (relative to the first RECIST1.1 progression on a given study treatment). Patients who continue study treatment after RECIST1.1 progression should continue RECIST1.1 assessments as per protocol until discontinuation of study treatment. See Section 7.1.1 and Appendix D for more details.

y All patients must receive denosumab before first dose of RXC004. 120 mg sc denosumab should be administered approximately once every month from C0D1 until RXC004 discontinuation. Investigators may delay subsequent denosumab doses until the next scheduled visit providing that there is no significant increase in β-CTX compared to baseline, according to Investigator judgement.

z A taste assessment (consisting of a specific set of questions) will be performed at screening and at each study visit when patient reports dysgeusia. Please see Section 8.3.12 for more details

Consent and collection of blood sample during screening is preferred, but patients can consent/sample collected at any time during study

† These tests can be performed up to 24 hours before study treatment is administered on C0D1. They must be performed pre-dose.

* CCI

Table 2 Arm B Schedule of Assessments

	Table 2 – Arm B (RXC004 + nivolumab combination) Schedule of assessments									
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[r]**	**90 day Follow-up[r]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**	**Within 7 days of discontinuation**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
Pre-Screening informed consent	X									
CCI CCI CCI	X									
Genetic screening for RNF43/RSPO aberrations and MSI status[p]	X									
Main study informed consent		X								
Demography & baseline characteristics		X								
Smoking history		X								
Central MSI testing (if not completed in pre-screening)		X								

	Table 2 – Arm B (RXC004 + nivolumab combination) Schedule of assessments									
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[r]**	**90 day Follow-up[r]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**	**Within 7 days of discontinuation**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
CCI CCI CCI CCI CCI CCI CCI		X								
CCI CCI CCI		X								
Medical/surgical history		X								
Oral and dental assessment[s]		X	Oral assessment at each visit - only if patient reports dysgeusia							
Inclusion/exclusion criteria		X								
HepB, HepC and HIV testing		X								
CCI CCI CCI (optional)[w]		X								
Physical examination		X	X	X		X	X			
ECOG performance status		X	X	X	X	X	X			

	Table 2 – Arm B (RXC004 + nivolumab combination) Schedule of assessments									
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[r]**	**90 day Follow-up[r]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**	**Within 7 days of discontinuation**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
Pregnancy test (WOCBP only)		(X)	(X)[x]	(X)	(X)	(X)				
Baseline and 'on treatment' tumour biopsies (mandatory)[a]		X			X	X[g]				
Vital signs (including height and weight)[m]		X	X	X	X	X	X	X		
Clinical chemistry / Haematology		X	X[x]	X	X	X	X	X	X	
TSH (reflex T3/T4)[q]		X		X	X	X	X	X	X	
ECG[v]		X	X	X	X	X[h]	X			
DXA scan		X	On Cycle 4 and Cycle 7 of RXC004 treatment and then every 7 RXC004 cycles thereafter whilst on study treatment							
Blood samples for bone turnover biomarkers (β-CTX)		X			X	X				
CCI CCI		X		X		X				
CCI CCI CCI			X[x]		X	X				
CCI CCI			X[x]		X	X				

	Table 2 – Arm B (RXC004 + nivolumab combination) Schedule of assessments									
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[r]**	**90 day Follow-up[r]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**	**Within 7 days of discontinuation**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
CCI CCI			X[x]		X	Cycle 2 only				
CCI CCI			X[x]		X	X	X			
CCI CCI			X[x]		X	X	X			
RECIST 1.1 assessments (by CT/MRI)[j]		X		On study tumour assessments should occur q8w +/- 1 week (relative to first dose of IP) for the first 56 weeks, followed by q12w +/- 1 week until RECIST1.1 radiological progression [n]						
CCI (mandatory unless unavailable at site)[i]		X[y]	On study CCI assessments should occur at week 8 and week 16 +/- 1 week relative to first dose of IP.							
Vitamin D3 and Calcium supplements		Patients should commence 800 IU vitamin D3 (Cholecalciferol) daily and 1000-1500 mg calcium daily supplements from ICF signature until RXC004 discontinuation								
Denosumab dosing[t]			120 mg sc Denosumab once every month from C0D1 until RXC004 discontinuation							
RXC004 dosing			X (1.5 mg single dose)	Continuous daily dosing (1.5 mg QD)						
RXC004 diary			X	Review at each visit						
Nivolumab dosing				480 mg q4w +/- 3 days[k]						

	Table 2 – Arm B (RXC004 + nivolumab combination) Schedule of assessments									
	Pre-Screening	**Screening**	**Cycle 0 (3-7 days)**	**Cycle 1 (28 day cycle)**		**Cycle 2 onwards (28 day cycles)**	**IP Discontinuation**	**30 day Follow-up[r]**	**90 day Follow-up[r]**	**Survival follow-up**
Window	**n/a**	**-28 to –1**	**D1**	**D1**	**D15 +/- 1d**	**D1 +/- 3d**	**Within 7 days of discontinuation**	**+/- 3d**	**+/- 3d**	**q8w +/- 1wk**
RXC004 PK[f]			X		X	X	X			
Concomitant medication		X	X	X	X	X	X	X	X	
Adverse events		X	X	X	X	X	X	X	X	
Taste assessment[u]		X	At each visit – only if patient reports dysgeusia							
Survival status and post-discontinuation anti-cancer therapy [l]								X	X	X

Once Arm B is opened, patients who are eligible for both Arm A and Arm B will be randomised 2:1 to Arm B: Arm A in an open-label manner on Day 1. Randomisation will be stratified by genetic alteration (RNF43, RSPO).

a A baseline (pre-first dose IP) biopsy is a mandatory requirement, except in exceptional circumstances where biopsy is not technically feasible. All patients without a baseline sample must be approved by Sponsor before starting study treatment. The baseline CCI can be a 'fresh newly acquired' biopsy or an CCI (preferably from the same organ site that 'on treatment' biopsy will be taken) and taken after completion of all prior standard of care treatments. In exceptional circumstances where baseline biopsy cannot be scheduled before C0D1, the procedure may be performed before C1D1 after a minimum of 4 days washout from C0D1. A second 'on treatment' biopsy at Cycle 1 Day 15 (+/- 1 day), is also mandatory providing that investigator judges the second biopsy to be technically and clincally feasible. The Cycle 1 Day 15 biopsy should be collected post-dose and the time of collection should be accurately recorded. RECIST 1.1 target lesions should not be chosen for biopsies.

b CCI

c CCI

d CCI

e CCI
CCI

f Blood samples for PK analysis should be collected as follows (the actual time for each blood draw must be accurately recorded);
Cycle 0: CCI
Cycle 1, CCI
Cycle X, CCI

g One 'on treatment' biopsy is required during Cycle 3 of treatment, providing that investigator judges the biopsy to be technically and clinically feasible. The biopsy should be collected post-dose and the time of collection should be accurately recorded.

h ECGs are required at C2D1 and as clinically indicated from C3D1 onwards

i CCI
CCI
CCI

j CT (preferred) or MRI, each preferably with IV contrast. It is strongly recommended to maintain use of the same imaging modality (CT or MRI), acquisition protocol, facility and scanner across all imaging time points per patient.

k 480 mg IV infusion over 60 minutes, q4w +/- 3 days, unless an infusion needs to held due to an adverse event. Results for vital signs (including weight), performance status, chemistry, haematology, and thyroid function labs must be available before commencing an infusion (within 3 days) and reviewed by the treating physician or Investigator prior to dosing.

l Survival and details of first and subsequent therapies for cancer, after discontinuation of treatment, will be collected

m height is required at screening only

n Symptomatically stable patients may continue RXC004 + nivolumab treatment after the RECIST1.1 progression on combination treatment, until symptomatic progression or second RECIST1.1 progression (relative to the first RECIST1.1 progression). Patients who continue study treatment after RECIST1.1 progression should continue RECIST1.1 assessments as per protocol until discontinuation of study treatment. See Section 7.1.1 and Appendix D for more details.

o CCI

p CCI

q **T3 and T4 will only be measured if TSH is abnormal or if there i**s clinical suspicion of an AE related to the endocrine system

r Follow up assessments should be performed 30 days after discontinuation of IP and 90 days after last dose of Nivolumab

s A dental examination with preventive dentistry if appropriate and an individual benefit-risk assessment is mandatory prior to treatment with denosumab. An initial dental examination may be performed by a member of the clinical team who can assess the need for referral to a dentist for further assessment including any preventative dentistry. A visual oral examination (including tongue, palate, uvula, tonsils, buccal mucosa, lips, gums and parotid duct) should also be performed at baseline and at each scheduled visit were dysgeusia is reported as an adverse event

t All patients must receive first dose of denosumab before first dose of RXC004. 120 mg sc denosumab should be administered approximately once every month from C0D1 until RXC004 discontinuation. Investigators may delay subsequent denosumab doses until the next scheduled visit providing that there is no significant increase in β-CTX compared to baseline, according to Investigator judgement.

u A taste assessment (consisting of a specific set of questions) will be performed at screening and at each study visit when patient reports dysgeusia. Please see Section 8.3.12 for more details. Patients must fast (water to drink only) for at least 2 hours prior to taking a dose to at least 1-hour post-dose for all doses

v Triplicate ECGs should be collected as follows;
Cycle 0: CCI
Cycle 1, CCI
Cycle X, CCI
At selected sites, ambulatory Holter monitoring will be used on Cycle 0 Day 1 and Cycle 1 Day 15 for central analysis of digital ECG information.

w Consent and collection of blood sample during screening is preferred, but patients can consent/sample collected at any time during study

x These tests can be performed up to 24 hours before study treatment is administered. They must be performed pre-dose.

y CCI

2 INTRODUCTION

Colorectal cancer (CRC) accounts for approximately 10% of all cancer related deaths worldwide (Bray et al 2018). Approximately 20-25% of newly diagnosed CRC is metastatic at presentation and approximately 70% will eventually develop metastases (Ansa et al 2018, SEER cancer stats review 2015).

Treatment of metastatic colorectal cancer is based on several factors including patient factors (e.g. performance status, age), treatment choice factors (e.g. QoL, toxicity profile), tumour molecular characteristics CCI and tumour clinical characteristics (tumour burden and biology, related symptoms). Expert treatment guidelines for metastatic colorectal cancer include those by the European society for Medical Oncology (ESMO) (Van Cutsem et al 2016) and the U.S. National Comprehensive Cancer Network (NCCN) (NCCN clinical practice guidelines V3. 2020).

According to the U.S. NCCN guidelines, first line treatment choice is based primarily on the patient's performance status and ability to tolerate intensive versus non-intensive treatment. Typically, 1st line treatment encompasses doublet or triplet chemotherapy with or without targeted therapy with anti-VEGF or anti-EGFR agents. In patients with CCI CCI wild-type disease or with left-sided tumours, oxaliplatin or irinotecan-based doublet chemotherapy treatment (FOLFOX/FOLFIRI) in combination with an anti-EGFR agent is recommended. In patients with CCI mutated tumours, oxaliplatin- or irinotecan-based doublet or triplet chemotherapy (FOLFOX/FOLFIRI/FOLFOXIRI) in combination with anti-VEGF treatment (bevacizumab) is recommended.

In patients with poor performance status or inability to tolerate intensive treatment, 1st line treatment is typically monotherapy chemotherapy with/without targeted treatment (anti-VEGF/EGFR) or targeted monotherapy or immunotherapy treatment. Treatment for patients with CCI wild-type disease includes cetuximab or panitumumab. Patients with CCI wild-type tumours may be offered Trastuzumab in combination with pertuzumab or lapatinib. Patients with MSI-H disease are offered anti-PD1 second line treatment.

Second-line treatment is largely based on the 1st line treatment offered to patients with those who received platinum-based 1st line treatment receiving irinotecan based 2nd line treatment and vice-versa. Recommended third-line treatments include monotherapy treatment with irinotecan, regorafenib (Grothey et al 2013) or TAS-102 (Trifluridine in combination with tipiracil) (Mayer et al 2015).

The median survival of patients with metastatic CRC is 30 months (Van Cutsem et al 2016). One-year PFS and OS rates for patients treated with second line systemic therapy are

approximately 15% and 40% respectively (Cao et al 2020). There is therefore still a significant unmet medical need for additional treatment options for metastatic CRC.

2.1 Study Rationale

The Wnt signalling pathway plays an important role in tumourigenesis and has been shown to be elevated in both ring finger protein 43 (RNF43) and R-Spondin (RSPO) aberrated CRC. There is also evidence that Wnt signalling may have a role in enabling cancers to avoid immunosurveillance and hence resistance to some immunotherapies.

Currently there remains a major unmet medical need in metastatic colorectal cancer. Immunotherapy with anti-PD1 has not demonstrated any efficacy in Microsatellite stable (MSS) colorectal cancer, which represents approximately 85% of colorectal cancer.

RXC004 may offer an opportunity to deliver clinical benefit by both a direct tumour targeting effect (in patients with RNF43 LoF mutations or RSPO fusion/translocation) and an immune-oncology effect, by converting the tumour microenvironment from an immune "cold" to an immune "hot" signature, making the tumour sensitive to anti-PD1/PD-L1 inhibition. This immune effect may be seen in genetically unselected patients as the target cells are the immune cells and not the tumour cells themselves.

RXC004 will therefore be evaluated in $\geq 2^{nd}$ line metastatic MSS colorectal cancer patients, who have either a RNF43 LoF mutation or a RSPO fusion/translocation.

2.2 Background

2.2.1 Wnt signalling in colorectal cancer

The Wnt signaling pathway plays an important role in tumourigenesis (Zhan et al 2016) and in immune evasion (Goldsberry et al 2019) in many common cancers. The Wnt pathway is initiated by the binding of Wnt glycoproteins to Frizzled (Fz) and low-density lipoprotein receptor-related protein (LRP) 5 or 6 co-receptors, resulting in the phosphorylation of Dishevelled (DSH) which in turn recruits a destruction complex comprising axin, adenomatous polyposis coli (APC) and glycogen synthase kinase 3β (GSK3β) to the cell membrane. The movement of these proteins inhibits the phosphorylation of β-catenin allowing β-catenin to accumulate near the cell nucleus and induces the transcription of target genes.

RNF43 is an integral membrane E3 ubiquitin ligase that promotes degradation of Fz receptors. Loss of function (LoF) RNF43 mutations have been described in 3-13% of all colorectal cancers (cBioPortal for cancer genomics, Matsumoto et al 2020, Cerami et al 2012). These LoF RNF43 mutations lead to increased Fz receptors at the cell surface and hence elevated Wnt signaling.

RSPOs are secreted proteins that act as ligands for the LRP 4/5/6 receptors. RSPO binding to

LRPs potentiates Wnt signaling by sequestering RNF43, resulting in the accumulation of Fz/LRP-receptor complexes at the cell membrane (De Lau et al 2014 and Seshagiri et al 2012). Chromosome rearrangements of RSPO2 and RSPO3 have been described in approximately 5% of CRC (Seshagiri et al 2012, Shinmura et al 2014, Kleeman et al 2019). The rearrangements result in fusion transcripts that drive marked overexpression of the RSPO gene.

PORCN is a membrane-bound protein-serine O-palmitoleoyltransferase which is required for the acetylation, activation and secretion of Wnt ligands. RXC004 inhibits PORCN leading to reduced canonical and non-canonical Wnt signalling. Blocking Wnt signalling with PORCN inhibitors has been shown to suppress the growth of RNF43 and RSPO aberrated CRC cancers in preclinical models (Madan et al 2016 and Koo et al 2015).

2.2.2 Role of Wnt signalling in the immune response to cancer

Anti-PD-1 monoclonal antibodies such as nivolumab have been shown to improve survival in metastatic mis-match repair deficient (dMMR) and MSI-H CRC, but patients with MSS CRC are largely unresponsive to immune checkpoint inhibitor treatment (Overman et al 2017, Le et al 2015).

There is strong scientific rationale, from both preclinical and clinical studies, that Wnt pathway activity drives the immune evasion of tumours (Spranger and Gajewski 2018, Wang et al 2018 and Luke et al 2019). In order for immune cells to recognize cancer cells, they must first infiltrate the tumour and then remain activated. Wnt pathway activated tumours have been shown to be poorly infiltrated by immune cells (Grasso et al 2018) and pharmacological inhibition of Wnt signaling promotes cytotoxic T cell infiltration sensitizing cancer cells to PD-1 inhibitors (Feng et al 2019, Xiao et al 2018).

There are currently a number of ongoing clinical trials investigating Porcupine inhibitors combined with PD-1 checkpoint inhibitors, in genetically unselected patients with advanced tumours (e.g. NCT02521844, NCT01351103 and NCT02675946).

The results from an ongoing Phase 1 study (NCT01351103) of the small molecule PORCN inhibitor WNT974, indicated WNT974 monotherapy had a manageable safety profile and the potential for antitumor activity in a molecularly selected population (Janku et al 2015, Rodon et al 2021). Preliminary data from the dose escalation part of the study, in which patients with advanced solid tumours received WNT974 in combination with the anti-PD-1 monoclonal antibody, spartalizumab have been reported (Janku et al 2020). Of 27 enrolled patients (as of 02 September 2019), 63% had received prior anti-PD-1 therapy. One patient (4%) had a partial response (PR), 11 (41%) had stable disease (SD), 13 (48%) had progressive disease, and response was unknown in 2 patients. The combination of WNT974 + spartalizumab was reported as being well tolerated. Dose-limiting toxicities were reported in 2 patients: Grade 2

spinal compression fracture that occurred in the setting of trauma and Grade 3 arthralgia. The most common treatment-related AE was hypothyroidism (19%). Four patients (15%) had 7 suspected-related Grade 3/4 AEs (arthralgia, atrial fibrillation, diabetes mellitus, diabetic ketoacidosis, hyperglycemia, hyponatremia, and maculopapular rash)..

2.2.3 RXC004

RXC004 is a potent and selective small molecule inhibitor of Porcupine (PORCN) that is required for the post-translational modification of all Wnt ligands. Inhibition of PORCN reduces both Wnt secretion and downstream canonical and non-canonical Wnt signaling. RXC004 has demonstrated in vitro and in vivo efficacy in preclinical models with RNF43 loss of function mutations and RSPO fusions. This data supports the genetic selection of patients with MSS CRC with upstream Wnt pathway aberrations such as RNF43 mutation and RSPO fusions. RXC004 was not effective in 3 human cancer cell lines with downstream Wnt pathway mutations such as CCI mutations, however there is evidence to suggest that patients with CCI mutated tumours may also benefit from a reduction in ligand drive because of PORCN inhibition. It has been reported that many colorectal cancer cell lines with mutations in CCI remain dependent on Wnt ligands (Voloshenenko et al 2013) and in these Wnt dependent cell lines, PORCN inhibition with a small molecule in resulted in decreased pathway activation, anti-proliferative effects and reduced cell viability. Taken together these data suggest patients with genetically selected cancers bearing downstream mutations in the Wnt pathway, may also receive some therapeutic benefit from treatment with RXC004

RXC004 has demonstrated an immune stimulant effect and efficacy in monotherapy and in combination with anti-PD1 in mouse syngeneic models, supporting testing of combination of RXC004 with nivolumab in MSS CRC patients.

RXC004 is being assessed in the Phase 1 RXC004/0001 trial (NCT03447470) as monotherapy and also in combination with the anti-PD-1 monoclonal antibody nivolumab, for safety and tolerability in advanced malignancies. RXC004 monotherapy at doses of 0.5, 1.0, 1.5, 2.0 mg, 3.0 mg and 10 mg have been evaluated to date. RXC004 at doses of 1.0 and 1.5 mg has been evaluated in combination with nivolumab.

In addition, RXC004 is being tested as monotherapy in patients with RNF43 mutated pancreatic cancer and biliary tract cancers, and is planned to be tested in combination with pembrolizumab in biliary tract cancers, following SOC therapies, in an ongoing Phase 2 study RXC004/0003.

A detailed description of the chemistry, pharmacology, and pre-clinical efficacy, and safety of RXC004 is provided in the RXC004 Investigator's Brochure

As of the 30 July 2021 a total of 25 patients have been dosed with RXC004 monotherapy in the phase 1 study at doses between 0.5 mg and 10 mg QD. The most frequently occurring

treatment emergent adverse events (TEAEs) were fatigue (64%), nausea (56%), anorexia (48%), vomiting (40%), dysgeusia (36%), diarrhoea (32%), aspartate aminotransferase increased (28%) anaemia (20%) and constipation (20%). The most frequently occurring treatment related AEs (TRAEs) of any grade were fatigue (52%), nausea (44%), decreased appetite (40%), dysgeusia (40%) and vomiting (24%). Doses up to 2 mg QD were considered safe and tolerable. Events of intestinal inflammation (colitis, enteritis or enterocolitis) were observed in 4 patients who started treatment with RXC004 at doses of 3 mg QD or higher and 3 of these were dose-limiting toxicities.

A detailed description of the Phase 1 study results is provided in Section 4.4.1.

2.2.4 Nivolumab

Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody that binds to the programmed death receptor-1 (PD-1) receptor. Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumours and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumours. Nivolumab blocks the interaction of PD-L1 and PD-L2 with the PD-1 receptor, releasing the inhibition of the immune response and hence generating an anti-tumour immune response.

Nivolumab has been approved in the European Union (EU) for the treatment of multiple cancers including metastatic melanoma, metastatic non-small cell lung cancer, advanced renal cell carcinoma, metastatic or recurrent squamous cell head and neck cancer, metastatic urothelial cancer, unresectable, recurrent or metastatic oesophageal squamous cell carcinoma and classical Hodgkin lymphomas that have progressed or recurred after an autologous stem cell transplant. Nivolumab has also been approved by the US Food and Drug Administration (FDA) for the treatment of chemoresistant microsatellite instability-high (MSI-H) or mismatch repair deficient metastatic colorectal cancer (CRC).

A detailed description of the chemistry, pharmacology, efficacy, and safety of nivolumab is provided in the nivolumab prescribing information.

2.3 Benefit/Risk Assessment

2.3.1 RXC004 risks

2.3.1.1 Risks associated with targeting the Wnt pathway

The principal on-target adverse events of significant interest known to occur with porcupine inhibition include decreases in bone density (including bone fragility fractures) and loss of taste (dysgeusia). Inhibiting the Wnt pathway may also predispose to inflammation in the gastrointestinal tract because of the role of Wnt in the immune micro-environment.

The safety profile of RXC004 is similar to that reported for two other Porcupine inhibitors in

clinical trials: ETC-159 and WNT974. Preliminary data from 16 patients in the ongoing Phase 1a/b, first in human (FIH), dose escalation study of PORCN inhibitor, ETC-159, in advanced or metastatic solid tumours [NCT02521844] were first reported in 2017. At that time, the maximum tolerated dose (MTD) was 30 mg every other day, with dose limiting toxicities of compression fractures and hyperbilirubinemia. Adverse events (>20%) included vomiting (32%), anorexia and fatigue (31%) dysgeusia and constipation (25%) Elevations of serum β-CTX, a marker of bone turnover, were observed (Ng et al 2017). Subsequently, prophylactic denosumab was introduced during the first 2 cycles. Data from the completed Phase 1, open-label trial of WNT974, administered in monotherapy have recently been published [NCT01351103] (Rodon et al 2021). In this study, 94 patients received oral WNT974 at doses of 5–30 mg once-daily, plus intermittent dosing schedules. AEs suspected to be related to study treatment were reported for 75 patients (80%), with the most common (≥20%) being dysgeusia (44 patients; 47%), decreased appetite (27 patients; 29%), and nausea (23 patients; 24%). Six patients (6.4%) experienced seven bone related disorders, five of which were suspected to be related to study treatment: osteoporosis, pathological fracture, osteopenia, and 2 Grade 3 spinal fractures.

2.3.1.2 Risks of RXC004 associated adverse events

As described in Section 4.4.1, RXC004 has been evaluated as a monotherapy in 25 patients in a phase 1 monotherapy dose-escalation trial at doses of 0.5–10 mg QD, in which doses of up to 2 mg QD were found to be tolerable and safe.

At doses up to and including 2 mg QD, the most common treatment related AEs of any grade were fatigue (10/20 patients), nausea (7/20), anorexia (6/20), dysgeusia (6/20), and vomiting (4/20).

Dysgeusia Events

In the Phase 1 study, RXC004-related dysgeusia was observed in 5 out of 20 patients who received doses of up to 2 mg QD, 3 out of 4 patients receiving 3 mg QD, and by the 1 patient who received 10 mg QD RXC004. This study protocol includes an assessment for the further characterisation of this event and include guidance for the management of dysgeusia (Appendix I) Guidance for dose modifications of RXC004 and nivolumab for all toxicities are provided in Section 6.6.

Colitis Events

In the Phase 1 study, events of intestinal inflammation (colitis, enteritis or enterocolitis) were observed in 4 patients who started treatment with RXC004 at doses of 3 mg QD or higher and 3 were dose-limiting toxicities. CTCAE Grade 3 RXC004 related colitis occurred in the one patient who received 10 mg and CTCAE Grade 3 enteritis occurred in one patient who received RXC004 3 mg QD. CTCAE Grade 2 colitis also developed in two more patients who

started treatment with 3 mg RXC004, both Grade 2 events presented during Cycle 2, Symptoms included abdominal pain, intermittent constipation, diarrhoea, nausea and/or vomiting. Blood and mucus in the stool were not apparent in any of the cases. Symptoms were usually accompanied by, and preceded by, a raised CRP. In two patients, the event recurred after initially responding to steroids, but responded again to further treatment.

Colitis events were not observed in any patients who commenced treatment with doses of RXC004 2 mg QD and lower, and the 3 mg QD dose gave a disproportionately high exposure compared to 2 mg QD. However, at the time of writing this protocol, one SAE of ileitis had been reported in this Phase 2 study and, considering the known risk of colitis for patients receiving nivolumab, there is a potential risk for colitis in patients receiving RXC004 as a monotherapy in Arm A and as an overlapping toxicity in patients who receive RXC004 in combination with nivolumab in Arm B.

Guidelines for the investigation and management of diarrhoea/colitis for RXC004 both as a monotherapy and in combination with nivolumab are provided in Appendix J. Guidance for dose modifications of RXC004 and nivolumab for all toxicities are provided in Section 6.6.

Colitis events are also defined as an Adverse Event of Potential Interest (AEPI) for RXC004, requiring additional details to be recorded on routine safety reporting.

Bone Events

Since Wnt inhibition both reduces bone formation and increases bone resorption, the concomitant use of the anti-resorbing agent, denosumab is expected to prevent drug-induced bone loss, and this has been a successful approach in the Phase 1 study, along with patient selection, and monitoring. Patients with high risk of bone fractures are excluded study protocols and participating patients will be monitored for bone turnover biomarkers and bone mineral density while on study.

In this study, patients will receive prophylactic denosumab (120 mg s.c. once every month) along with Vitamin D3 and Calcium supplements (800 IU vitamin D3 daily [Cholecalciferol] and 1000-1500 mg calcium daily supplements) from the time of signing the ICF and throughout the study until discontinuation of RXC004.

Guidance for dose modifications of RXC004 and nivolumab for all toxicities are provided in Section 6.6. Bone toxicity events are defined as an AEPI for RXC004, requiring additional details to be recorded on routine safety reporting.

Assessment of potential for other risks

Potential for Drug-Drug Interactions with CYP3A4 inhibitors

RXC004 has not shown the potential to induce or inhibit CYP enzymes or transporters at the dose to be investigated in this study but it is exclusively metabolised by CYP3A4 and as such has the potential to be a victim for drug-drug interactions with potent CYP inhibitors (which could increase levels of RXC004, leading to a higher risk of adverse events). As such, strong and moderate CYP3A4 inhibitors are contra indicated in this protocol (see Appendix E). Patients with colorectal cancers commonly require co-medication with analgesics, antibiotics, anti-emetics and anti-depressant or anxiolytic drugs, some of which are CYP3A4 inhibitors. The table in Appendix E highlights the CYP3A4 inhibitors which are also likely to be commonly used in colorectal cancer patients. These drugs should be avoided, and alternatives used wherever possible. If it is not possible to use an alternative (e.g. if a specific antibiotic or anti-fungal treatment is needed), RXC004 should be interrupted.

Potential for QTc prolongation

Based on preclinical data [hERG assay, ECG monitoring in 28-day GLP toxicology studies], RXC004 is not considered to have a significant potential to prolong the QTc interval. RXC004 did not lead to cardiac adverse events including arrhythmias, or syncope in the Phase 1 study.

Triplicate ECGs will be collected in this study and other studies of RXC004 so that the potential for QTc prolongation can be explored in future as part of a population PK analysis.

Potential for an effect of food on RXC004 exposure

It is not yet known whether food may increase or decrease the exposure of RXC004. Until such data are generated, RXC004 must be given in a fasted state, as described in Section 5.3.2, and this is explained to patients in the Information Sheet.

Potential for risks in specific patient populations

A preliminary analysis of data from the Phase 1 study showed no effect of age, sex, BMI or race on RXC004 pharmacokinetics.

Pre-clinical development showed that RXC004 is exclusively metabolised by CYP3A4 and there was no renal clearance of RXC004. However, there is currently no information on the clearance of potential human metabolites of RXC004. While data are limited, the study inclusion criteria will restrict the use of RXC004 to patients with adequate renal and hepatic function

More detailed information about the known and potential risks of RXC004 may be found in

the most recent Investigator's Brochure and Development Safety Update Report.

In summary the pre-clinical information and emerging PK and safety profile has not identified any risks that would preclude investigation of RXC004 at a dose of 2 mg QD in the advanced cancer setting.

2.3.2 Nivolumab Risks

Checkpoint inhibitors including anti PD-1, as well as antibodies directed against PDL-1 and CTLA-4, boost the endogenous immune responses directed against tumours. Adverse events seen with checkpoint inhibitors are frequently due to immune mediated mechanisms and can occur in any organ system. The adverse events known to occur with nivolumab treatment (as monotherapy and in combination with various agents) are well documented (Haanen et al 2017) and include gastrointestinal effects (e.g. diarrhoea or colitis), pneumonitis, hepatic events, skin rashes myocarditis and endocrinopathies (including hypo and hyper-thyroidism).

The safety of nivolumab administered as a single agent or in combination with ipilimumab in patients with mCRC was evaluated in the CHECKMATE-142 study. (NCT02060188). The most frequent serious adverse reactions reported in at least 2% of patients treated with nivolumab monotherapy were colitis/diarrhoea, hepatic events, abdominal pain, acute kidney injury, pyrexia, and dehydration. The most common adverse reactions for single agent nivolumab (reported in at least 20% of patients) were fatigue, diarrhea, pyrexia, musculoskeletal pain, abdominal pain, pruritus, nausea, rash, decreased appetite, and vomiting (Overman et al 2017).

Immune mediated adverse events that can occur during treatment with nivolumab can be seen weeks or months after discontinuation of treatment. Symptoms of these events will be monitored throughout treatment (e.g. diarrhoea, rash, and neuropathy). Laboratory assessments will check for elevated liver enzymes, kidney and thyroid function, electrolytes, glucose and blood counts.

More detailed information about the known and expected benefits and potential risks of nivolumab may be found in the nivolumab Package Insert.

The safety profile of RXC004 in combination with nivolumab has not been fully characterized in patients. The toxicity profiles of RXC004 and nivolumab as monotherapy are largely non-overlapping apart from gastrointestinal effects (diarrhoea/colitis), which were observed at the 3 mg dose of RXC004 but not at doses up to and including doses of 2 mg. The porcupine inhibitor WNT974 has been combined with an anti-PD1 in a phase 1 trial (NCT01351103) in 27 patients with metastatic solid tumours, most of whom (63%) had received prior anti-PD1 treatment. DLTs of Grade 2 spinal compression fracture (in the setting of trauma) and Grade 3 arthralgia were reported in 2 patients. The authors concluded that WNT974 + spartalizumab was well tolerated (Janku et al 2020). The safety profile of RXC004 in combination with

nivolumab, while unknown, is expected to be similar to that of WNT974 in combination with spartalizumab.

2.3.3 Benefit assessment

This trial will evaluate RXC004 monotherapy and RXC004 in combination with nivolumab in Microsatellite Stable (MSS) metastatic colorectal cancer (CRC) patients with upstream WNT pathway aberrations (RNF43 LoF mutations and RSPO fusions). RXC004 monotherapy may offer clinical benefit by both a direct tumour targeting mechanism and by an immune-oncology mechanism.

Wnt pathway activated tumours have been shown to be poorly infiltrated by immune cells (Grasso et al 2018) and pharmacological inhibition of Wnt signaling promotes cytotoxic T cell infiltration, sensitizing cancer cells to PD-1 inhibitors (Feng et al 2019, Xiao et al 2018). Thus, there is clear rationale for the combination of RXC004 with checkpoint inhibitors.

The Phase 1 study of RXC004 enrolled unselected patients with advanced cancers with a median of 3 prior lines of treatment. Out of 18 RECIST-evaluable patients, 5 had stable disease, in one case lasting for up to 26 weeks. All five of these patients with stable disease had Wnt-ligand-dependent tumours, supporting the hypothesis that RXC004 will have clinical activity in selected tumours that are dependent on Wnt ligand signaling pathways.

In the expansion cohort of the WNT 974 study (Rodon et al 2021), stable disease was observed in 10 out of 28 patients, which was enriched in patients with RNF43 mutations.

WNT974 has also been combined with anti-PD1 (spartalizumab) in an ongoing Phase 1 trial in 27 patients (NCT01351103). The combination was well tolerated with efficacy being observed in some patients (DCR of 41%) (Janku et al 2020).

In addition, in house data with RXC004 in mouse syngeneic models have demonstrated that RXC004 combined with anti-PD1 results in a more immunogenic tumour microenvironment.

Currently the prognosis of previously treated MSS metastatic colorectal cancer patients is poor with current 3rd line standard of care agents (regorafenib and TAS-102) with a response rate of <5%, median PFS and OS of approximately 2 and 6 months respectively and a DCR of approximately 40% (Grothey et al 2013; Mayer et al 2015). MSS colorectal cancer represents approximately 85% of all colorectal cancer. Immunotherapy has, to-date, proved ineffective in MSS CRC with no efficacy observed with anti-PD1 treatment- in contrast to MSI-H CRC where anti-PD1 treatment is approved for use as a single agent.

There is insufficient clinical data to reach a definitive conclusion about the efficacy of PORCN inhibitors or other treatments in genetically selected MSS CRC with RNF43 mutations and RSPO fusions. However, there is some preliminary evidence to suggest that

patients with RNF43 mutations have a poor prognosis compared to the general patient population with MSS CRC, (Cerami et al 2012, Gao et al 2013) suggesting that alternative treatments are needed for this subset of patients.

RXC004, both as a single agent and in combination with nivolumab in this genetically selected MSS metastatic colorectal cancer population has therefore the potential to demonstrate clinical benefit in this major patient population with a large unmet medical need.

2.3.4 Overall Benefit: Risk Conclusion

Considering the measures taken to minimise risk to participants participating in this study, the potential risks identified in association with study treatment seem justified by the anticipated benefits that may be afforded to participants with RNF43 or RSPO aberrated MSS mCRC.

3 OBJECTIVES AND ENDPOINTS

Table 3 Objectives and Endpoints

Objectives	Endpoints
Primary	
To assess the anti-tumour activity of RXC004 monotherapy and RXC004 +nivolumab	Monotherapy : Disease control rate (DCR) using each patients Best Overall Response (BOR) according to RECIST 1.1 Combination : Objective response rate (ORR) using each patients BOR according to RECIST 1.1
Secondary	
To further assess the preliminary efficacy of RXC004 monotherapy and RXC004 + nivolumab	% change in the sum of target lesions, duration of response (DoR), PFS, ORR (monotherapy) and DCR (combination) using investigator assessments according to RECIST 1.1 and OS.
To assess the PK of RXC004 in monotherapy and in combination with nivolumab	Maximum plasma concentration (C_{max}) after Dose 1, C_{max} at steady state, minimum observed plasma concentration (C_{min}) at steady state as well as other relevant parameters (e.g. t_{max}, $t½$, λz, $AUC_{0-\infty}$, CL/F, and Vz/F).
To assess the safety and tolerability profile of RXC004 monotherapy and RXC004 + nivolumab combination	Incidence of AEs SAEs, dose reductions, interruptions and discontinuations, and assessment of dysgeusia.
Exploratory	
CCI CCI CCI CCI	CCI CCI CCI CCI CCI CCI CCI CCI
CCI CCI CCI	CCI CCI CCI

Objectives	Endpoints
CCI CCI	
CCI CCI CCI	CCI CCI CCI CCI CCI
CCI CCI	CCI CCI CCI

4 STUDY DESIGN

4.1 Overall Design

This is Phase II, open label, multicentre, multi-arm, study to evaluate the preliminary efficacy and safety of RXC004 as monotherapy and in combination with nivolumab in patients with Ring finger protein 43 (RNF43) or R-spondin (RSPO) aberrated, microsatellite stable (MSS), colorectal cancer (CRC), that have progressed following current standard of care treatment.

The study is composed of two arms, RXC004 monotherapy (Arm A) and RXC004 in combination with nivolumab (Arm B). 20 evaluable patients will be enrolled in Arm A and 20 evaluable patients in Arm B.

The study initially opened with Arm A; Arm B will be opened once a recommended Phase II dose (RP2D) for RXC004 in combination with nivolumab is established in the Phase I dose escalation study (NCI 03447470). A safety review of the combination data will be conducted after [redacted] patients have received the combination of RXC004 and nivolumab for at least 1 cycle.

Once Arm B is opened, patients who are eligible for both Arm A and Arm B will be randomised 2:1 to Arm B: Arm A in an open-label manner. Randomisation will be stratified by genetic alteration (RNF43 vs RSPO).

Patients in Arm A may be treated with RXC004 + nivolumab if they have progressive disease on the 8-week scan, as long as they are eligible for Arm B and have Sponsor approval. Patients who have undergone dose reduction during monotherapy cannot have the RXC004 dose re-escalated if switching to RXC004 + nivolumab combination therapy. The RXC004 dose must be discussed with the Sponsor prior to starting combination therapy.

The primary objectives of the study are to assess the preliminary efficacy of each treatment arm; RXC004 monotherapy and RXC004+nivolumab based on an individual patients best overall response.) Tumour assessment will be performed by Investigators every 8 weeks ± 1 week (relative to the date of initiation of study treatment) for the first 56 weeks, followed by q12w weeks ± 1 until radiological disease progression (as defined by Response Evaluation Criteria in Solid Tumours, version 1.1 [RECIST1.1]). The primary efficacy endpoint for RXC004 monotherapy is disease control rate (i.e. proportion of patients who achieve CR, PR or SD) and the primary efficacy endpoint for RXC004 + nivolumab is objective response rate. Following radiological progression, patients will be followed-up for safety and survival.

The general study design is summarized in Figure 1.

4.2 Scientific Rationale for Study Design

The Wnt signaling pathway plays an important role in tumourigenesis (Zhan et al 2016) and in immune evasion (Goldsberry et al 2019) in many common cancers. RNF43 is an integral

membrane E3 ubiquitin ligase that promotes degradation of Fz receptors. Loss of function (LoF) RNF43 mutations have been described in 3-13% of all colorectal cancers (cBioPortal for cancer genomics, Matsumoto et al 2020, and Cerami et al 2012). These LoF RNF43 mutations lead to increased Fz receptors at the cell surface and hence elevated Wnt signaling.

RSPOs are secreted proteins that act as ligands for the LRP 4/5/6 receptors. RSPO binding to LRPs potentiates Wnt signaling by sequestering RNF43, resulting in the accumulation of Fz/LRP-receptor complexes at the cell membrane (De Lau et al 2014 and Seshagiri et al 2012). Chromosome rearrangements of RSPO2 and RSPO3 have been described in approximately 5% of CRC (Seshagiri et al 2012, Shinmura et al 2014, Kleeman et al 2019). The rearrangements result in fusion transcripts that drive marked overexpression of the RSPO gene.

PORCN is a membrane-bound protein-serine O-palmitoleoyltransferase which is required for the acetylation, activation and secretion of Wnt ligands. RXC004 inhibits PORCN leading to reduced canonical and non-canonical Wnt signalling. Blocking Wnt signalling with PORCN inhibitors has been shown to suppress the growth of RNF43 and RSPO aberrated CRC cancers in preclinical models (Madan et al 2016 and Koo et al 2015).

There is strong scientific rationale, from both preclinical and clinical studies, that Wnt pathway activity drives the immune evasion of tumours (Spranger and Gajewski 2018, Wang et al 2018 and Luke et al 2019). In order for immune cells to recognize cancer cells, they must first infiltrate the tumour and then remain activated. Wnt pathway activated tumours have been shown to be poorly infiltrated by immune cells (Grasso et al 2018) and pharmacological inhibition of Wnt signaling promotes cytotoxic T cell infiltration sensitizing cancer cells to PD-1 inhibitors (Feng et al 2019, Xiao et al 2018).

There are currently a number of ongoing clinical trials investigating combining Porcupine inhibitors with PD-1 checkpoint inhibitors, in patients with advanced tumours. This trial will therefore evaluate both the direct tumour targeting potential of RXC004 monotherapy and the dual potential of direct tumour targeting and reversal of immune evasion/restoration of sensitivity to anti-PD1 treatment in MicroSatellite Stable (MSS) metastatic colorectal cancer patients with upstream WNT pathway aberrations (RNF43 LoF and RSPO fusion).

4.3 Rationale for Study Endpoints

4.3.1 Arm A Primary Endpoint

RXC004 has shown efficacy in both RNF43 LoF and RSPO fusion human xenograft models. As part of these preclinical studies, it was observed that RXC004 did not show a clear dose response relationship when a calliper measurement was utilised for study endpoint. The callipers measure the size of the tumour in the mouse similar to a RECIST scan in a cancer

patient. In addition to the calliper measurement Redx conducted immunohistochemistry (IHC) analysis and CCI scanning on these RSPO fusion tumours. By IHC a clear dose response relationship was shown on reduction of Ki67 staining, a marker of proliferation in tumour cells, and a dose dependent increase in Alcian Blue/Periodic acid-Schiff staining, markers of mucins in the tumour. Therefore, in addition to the dramatic dose dependent reduction in tumour cell proliferation, RXC004 also resulted in a striking change in cell morphology. As indicated by Alcian blue/Period acid-Shiff staining, RXC004 treatment was shown to increase the presence of mucins in the extracellular space in a dose dependent manner. This increase in mucin in the tumour microenvironment we believe is responsible for the lack of apparent dose response in the calliper tumour volume measurement in this model. Furthermore, CCI scanning in the same RSPO fusion tumour model showed a reduction in metabolic activity in the tumours, and when tumours were removed and implanted into secondary host animals, they had a significantly reduced rate of growth compared to tumours pre-treated with vehicle alone. This suggests potential continued benefit of RXC004 induced cell changes even post drug treatment.

Based on these pre-clinical observations, and also taking into consideration the low published response rates CCI %) with single agents TAS-102 and regorafenib in MSS CRC, it is considered unlikely that RXC004 as a monotherapy will deliver RECIST-confirmed clinical responses. The RECIST measurement on tumour size may be confounded if this cell differentiation and increase in mucous production in the tumour microenvironment that was seen in pre-clinical models is also observed in human tumours as this would result in a lack of tumour area shrinkage. For these reasons, the exploratory objectives of the study include an CCI for IHC and an CCI scan.

Disease Control Rate (DCR; defined as CR+ PR+ durable stable disease lasting for at least 16 weeks) was selected as the primary endpoint for the RXC004 monotherapy arm because it considers both tumour shrinkage and durable disease stabilisation. It is possible and more likely that RXC004 efficacy could result in prolonged stable disease rather than tumour shrinkage. As complete ablation of tumour cell proliferation has been observed in preclinical models, this mechanism of action could still lead to patient benefit, increased progression free survival and ultimately an overall survival benefit for the patient.

For this endpoint, a value of CCI% DCR will be used as a reference, as this was consistently reported in the studies of TAS-102 and regorafenib in a similar but unselected patient population (Grothey et al 2013 and Mayer et al 2015) and an improvement over this value would be considered relevant, firstly because the DCR rate in the reported studies was based on SD at [redacted] weeks, whereas in this study at least 16 weeks of SD is required, and secondly because of the preliminary evidence that RNF43 mutated MSS CRC may have a poorer prognosis than the general MSS CRC population.

However, acknowledging that Disease Control Rate is difficult to interpret in a single arm study, the secondary endpoints of the study include the best % change in the sum of target lesions from baseline, the ORR (monotherapy) and duration of response (DoR) according to

RECIST 1.1, as well as PFS and OS. All of these endpoints will be taken into account when determining whether RXC004 has demonstrated sufficient clinical activity to support further clinical development as a monotherapy. Further details are provided in sections 9.2 and 9.4.

4.3.2 Arm B Primary Endpoint

The mechanism of action of RXC004 on the tumour epithelial cell could lead to prolonged stable disease and hence DCR is considered an appropriate primary endpoint for RXC004 monotherapy. In contrast, combination of RXC004 with nivolumab would be expected to have both a RXC004 effect on the growth of the tumour cells and a synergistic effect with nivolumab on the immune system. RXC004 should reverse Wnt pathway induced immune evasion and nivolumab will allow activated CD8 T cells to function without the immune suppression resulting from tumour PD-L1 expression. Therefore, it is believed that in combination, the active CD8 T cells will have the ability to destroy tumour cells and thus shrink the size of the tumour; hence ORR is thought to be a suitable endpoint for the combination arm.

4.4 Justification for Doses of RXC004 and Nivolumab

Patients who enrol in Arm A RXC004 monotherapy will receive 2.0 mg RXC004 orally once a day in a fasted state. This dose was chosen using the predicted efficacious dose ranges from pre-clinical pharmacology models, preclinical and clinical target engagement assays, information from toxicology and toxicokinetic studies, and safety, efficacy and pharmacokinetics data from the Phase 1 study of RXC004 in patients with advanced solid tumours (NCT03447470).

Patients who receive combination therapy with RXC004 and nivolumab in Arm A or B will receive RXC004 1.5 mg orally once a day in combination with nivolumab 480 mg q4w. The RXC004 1.5 mg dose is supported by the available safety and PK data for this dose given in combination with nivolumab in the ongoing Phase 1 study RXC004/0001 (NCT03447470; see Sections 4.4.1.2 and 4.4.1.3). At a meeting held on 07 July 2022, the Phase 1 Safety Review Committee (SRC) determined that RXC004 1.5 mg in combination with nivolumab is a tolerated dose, based on safety and PK data (see Sections 4.4.1.2 and 4.4.1.3).

4.4.1 RXC004

4.4.1.1 RXC004 Clinical Safety Data (NCT03447470) – Monotherapy Module 1

As of 30 July 2021, 25 patients had received treatment with RXC004 monotherapy, which completed the Module. This study opened in 2018 with a RXC004 dose of 10 mg once daily, but the dose was not tolerated, in the first patient who developed grade 3 diarrhoea, colitis and asymptomatic bone fragility fractures in a thoracic vertebra and clavicle, which are on-target toxicities of Wnt pathway inhibitors. Recruitment was halted to allow for the manufacture of lower doses of compound and to amend the protocol with a revised dose schedule and additional safety measures. The study restarted the dose escalation on 18th March, 2019 with

0.5 mg RXC004.

As of the 30 July 2021, 24 additional patients had been dosed with RXC004 monotherapy in Module 1 of the study at doses of 0.5 mg, 1 mg, 1.5 mg, 2 mg and 3 mg, a six-fold dose range. Six patients received treatment at highest declared tolerated dose (2 mg), and all were evaluable for dose limiting toxicity (DLT) analysis.

Table 4 Grade 2 or higher RXC004 related adverse events, including DLTs observed in DLT evaluable patients as of 30 July 2021 (Phase 1 study RXC004/0001, monotherapy Module 1)

Dose	Patients dosed	Patients evaluable for DLT	Grade 2 or higher RXC004 related AEs in DLT evaluable[a] patients
0.5 mg QD	4	3	**Tolerated dose** RXC004 related AEs ≥ CTCAE G2 (2/3 patients); G2 fatigue (1/3 patients) G2 increase in β-CTX (1/3 patients)
1 mg QD	3	3	**Tolerated dose** RXC004 related AEs ≥ CTCAE G2 (1/3 patients); G2 fatigue (1/3 patients) G2 increase in β-CTX (1/3 patients) G2 loss of appetite (1/3 patients) G2 Nausea (1/3 patients)
1.5 mg QD	7	6	**Tolerated dose** RXC004 related AEs ≥ CTCAE G2 (4/6 patients); G2 fatigue (4/6 patients)[b] G2 loss of appetite/anorexia (2/6 patients)[b] G2 Nausea (1/6 patients), G3 Nausea (1/6 patients)[b] G2 Dysgeusia (1/6 patients)[b] G2 increased AST (1/6 patients)[b] G2 decreased sodium (1/6 patients)[b] G3 diarrhoea (1/6 patients)[b] G3 vomiting (1/6 patients)[b] G3 weight loss (1/6 patients)[b]
2 mg QD	6	6	**Tolerated dose** RXC004 related AEs ≥ CTCAE G2 (4/6 patients); G3 Pancreatitis (1/6 patients) [DLT]

Dose	Patients dosed	Patients evaluable for DLT	Grade 2 or higher RXC004 related AEs in DLT evaluable[a] patients
			G2 Diarrhoea (2/6 patients) G2 Dysgeusia (1/6 patients)[b]
3 mg QD	4	4	**Non-tolerated dose** RXC004 related AEs ≥ CTCAE G2 (4/4 patients); G2 Fatigue (1/4 patients)[b] G2 Nausea (1/4 patients)[b] G2 Constipation (1/4 patients)[b] G2 Anorexia (2/4 patients)[b] G2 Colitis (2/4 patients)[b] [DLT] G2 Dysgeusia (1/4 patients)[b] G3 Enteritis (1/4 patients) [DLT] G2 hyponatremia (1/4 patients) G2 Humerus fracture (1/4 patients) G5 Subdural Hematoma (1/4 patients)
10 mg QD	1	1	**Non-tolerated dose** One DLT – G3 Diarrhoea. G3 proctitis, G2 enterocolitis, dysgeusia and fragility bone fractures also reported by this patient

a The DLT assessment period runs from the start of dosing with RXC004 (Cycle 0 Day 1) up to Cycle 1 Day 21. To be evaluable for DLT assessment patient must had received at least 66% of the intended dose of RXC004 during Cycle 1 or have experienced a DLT event in the assessment period

b The start date of one or more events was outside of the DLT period. However, AEs occurring in Cycle 2 and beyond which meet the definition of a DLT or are considered clinically significant may also be considered by the Safety Review Committee, when making dose escalation decisions.

No DLTs were observed in the patients dosed in the first three cohorts since the study was restarted (RXC004 doses from 0.5 mg to 1.5 mg). One DLT of CTCAE grade 3 pancreatitis was observed at the 2 mg dose level. At the 3 mg dose level, two DLTs of colitis and enteritis were observed in 2 out of 4 evaluable patients, which led to the SRC declaring 3 mg QD RXC004 as non-tolerated.

Denosumab (XGEVA™ - 120 mg s.c. once every month) was administered prophylactically from Cycle 0 to all patients from cohort 3 (1.5 mg RXC004) onwards, to maximize bone safety.

A summary of the treatment emergent adverse events (TEAEs) regardless of causality, reported for RXC004 monotherapy in the phase 1 RXC004/0001 study (NCT03447470) up to 30 July 2021 is included in Table 5.

Table 5 Summary of Treatment-Emergent Adverse Events Phase 1 study RXC004/0001, monotherapy Module 1)

Number of patients with:	0.5 mg (N=4) N (%)	1.0 mg (N=3) N (%)	1.5 mg (N=7) N (%)	2.0 mg (N=6) N (%)	3.0 mg (N=4) N (%)	10.0 mg (N=1) N (%)	Overall (N=25) N (%)
Any TEAE	4 (100)	3 (100)	7 (100)	6 (100)	4 (100)	1 (100)	25 (100)
Any Treatment-Related TEAE	4 (100)	3 (100)	5 (71)	5 (83)	4 (100)	1 (100)	22 (88)
TEAE >= Grade 3	0 (0)	1 (33)	6 (86)	1 (17)	3 (75)	1 (100)	12 (48)
Treatment-Related TEAE >= Grade 3	0 (0)	0 (0)	2 (29)	1 (17)	1 (25)	1 (100)	5 (20)
Serious TEAE	0 (0)	1 (33)	5 (71)	117)	3 (75)	1 (100)	11 (44)
Serious Treatment-Related TEAE	0 (0)	0 (0)	1 (14)	0 (0)	2 (50)	1 (100)	4 (16)
TEAE Leading to Discontinuation of Study Treatment	1 (25)	0 (0)	2 (29)	0 (0)	2 (50)	1 (100)	6 (24)
Treatment-Related TEAE Leading to Discontinuation of Study Treatment	1 (25)	0 (0)	2 (29)	0 (0)	2 (50)	1 (100)	6 (24)
TEAE Leading to Dose Modification	0 (0)	1 (33)	3 (43)	2 (33)	2 (50)	1 (100)	9 (36)
TEAE Leading to Death	0 (0)	0 (0)	1 (14)	0 (0)	1 (25)	0 (0)	2 (8)

TEAE = treatment-emergent adverse event

The most common TEAEs and TRAEs (occurring in at least 20% patients) are summarised in Table 6 and Table 7. The most frequently occurring TEAEs were fatigue (64%), nausea (56%), decreased appetite (48%), vomiting (40%), dysgeusia (36%), diarrhoea (32%), aspartate aminotransferase increased (28%), anaemia (20%), and constipation (20%).

Table 6 Most common (occurring in at least 20% patients) treatment emergent AEs in Phase 1 study RXC004/0001 (monotherapy Module 1)

Preferred Term	0.5 mg (N=4)	1.0 mg (N=3)	1.5 mg (N=7)	2.0 mg (N=6)	3.0 mg (N=4)	10.0 mg (N=1)	Overall (N=25)
Patients with Any TEAEs	4 (100)	3 (100)	7 (100)	6 (100)	4 (100)	1 (100)	25 (100)
Fatigue	2 (50)	2 (67)	6 (86)	3 (50)	2 (50)	1 (100)	16 (64)
Nausea	2 (50)	2 (67)	5 (71)	1 (17)	3 (75)	1 (100)	14 (56)
Decreased appetite	1 (25)	2 (67)	3 (43)	2 (33)	3 (75)	1 (100)	12 (48)
Vomiting	0 (0)	2 (67)	4 (57)	2 (33)	1 (25)	1 (100)	10 (40)
Dysgeusia	0 (0)	0 (0)	2 (29)	3 (50)	3 (75)	1 (100)	9 (36)
Diarrhoea	1 (25)	2 (67)	3 (43)	1 (17)	0 (0)	1 (100)	8 (32)
AST increased	1 (25)	0 (0)	3 (43)	1 (17)	1 (25)	1 (100)	7 (28)
Anaemia	1 (25)	2 (67)	2 (29)	0 (0)	0 (0)	0 (0)	5 (20)
Constipation	0 (0)	0 (0)	1 (14)	1 (17)	2 (50)	1 (100)	5 (20)

The most common RXC004-related AEs reported were: fatigue (52%), nausea (44%), decreased appetite (40%), dysgeusia (40%) and vomiting (24%). At doses up to and including 2 mg QD, the most common treatment related AEs were fatigue (10/20 patients), nausea (7/20), anorexia (6/20), dysgeusia (6/20), and vomiting (4/20), No grade 4/5 treatment related AEs or bone fragility events were reported at any dose levels up to and including 2 mg QD. Only dysgeusia appeared to be dose-related.

Table 7 Most common (occurring in at least 20% patients) treatment related AEs in Phase 1 study RXC004/0001 (monotherapy Module 1)

Preferred Term	0.5 mg (N=4)	1.0 mg (N=3)	1.5 mg (N=7)	2.0 mg (N=6)	3.0 mg (N=4)	10.0 mg (N=1)	Overall (N=25)
Patients with Any TRAEs	4 (100)	3 (100)	5 (71)	5 (83)	4 (100)	1 (100)	22 (88)
Fatigue	2 (50)	1 (33)	4 (57)	3 (50)	2 (55)	1 (100)	13 (52)
Nausea	1 (25)	2 (67)	3 (43)	1 (17)	3 (75)	1 (100)	11 (44)
Decreased appetite	1 (25)	1 (33)	2 (29)	2 (33)	3 (75)	1 (100)	10 (40)
Dysgeusia	0 (0)	0 (0)	2 (29)	4 (66)	3 (75)	1 (100)	10 (40)
Vomiting	0 (0)	1 (33)	2 (29)	1 (17)	1 (25)	1 (100)	6 (24)

Five patients reported nine CTCAE Grade 3 events that were assessed as possibly or probably related to RXC004 by the investigator, and these patients all received doses of 1.5 mg or higher. These events were: weight loss (1 patient, 1.5 mg); nausea, vomiting and diarrhoea (all in one patient, 1.5 mg); pancreatitis (1 patient, 2 mg); enteritis (1 patient, 3 mg) and diarrhoea, proctitis and hyponatraemia (all in 1 patient, 10 mg). No Grade 4 events were reported. Two Grade 5 events were reported – COVID-19 infection (1 patient, 1.5 mg, not-related to RXC004) and subdural hematoma following a fall (1 patient, 3 mg QD, assessed as possibly related to RXC004). See Table 4.

A total of 16 Serious Adverse Events (SAEs) were reported in 11 patients. Nine of the 16 SAEs reported were assessed as unrelated to the RXC004 by the investigator and the sponsor. These were: gastroenteritis viral (Grade 3, 1 patient, 1 mg), Escherichia urinary tract infection (Grade 3, 1 patient, 1.5 mg), pulmonary haemorrhage (Grade 1, 1 patient, 1.5 mg), melaena (2 SAEs in 1 patient at 1.5 mg – one Grade 1 then other Grade 3 – both due to a new primary malignancy), small intestinal obstruction (Grade 3, 1 patient 1.5 mg), COVID-19 (Grade 5, 1 patient, 1.5 mg), Stoma site infection (Grade 2, 1 patient, 2 mg) and biliary track infection (Grade 3, 1 patient, 3 mg). 7 SAEs (which occurred in 4 patients) were assessed as possibly or probably related to RXC004 by the investigator and are described below:

2. 2 SAEs of NCI CTCAE grade 3/2 diarrhoea that occurred in 1 patient at 10 mg QD RXC004 (before the study was re-started) were both assessed as probably related to RXC004 and led to discontinuation of study drug. The events subsequently resolved/recovered and 10 mg QD RXC004 was declared not tolerated by the Safety Review Committee (SRC).
3. 1 SAE of NCI CTCAE grade 3 diarrhoea that occurred at 1.5 mg RXC004 was assessed as probably related to RXC004 and led to discontinuation of study drug. The event was also associated with a *clostridium difficile* infection and subsequently resolved/recovered.

4. 1 SAE of NCI CTCAE grade 2 colitis that occurred at 3 mg QD RXC004 was assessed as probably related to RXC004. The event subsequently resolved, and the patient continued study treatment at a lower dose (1.5 mg RXC004). The 3 mg QD dose of RXC004 was subsequently declared not tolerated by the SRC.
5. 1 SAE of NCI CTCAE grade 3 enteritis that occurred at 3 mg QD RXC004 was assessed as probably related to RXC004. This patient also had a SAE of NCI CTCAE grade 2 humerus fracture (following a fall whilst hospitalised for enteritis) which was assessed as probably related to RXC004. The enteritis and fracture events were both unresolved at the time that the patient subsequently died due to a CTCAE grade 5 subdural hematoma (following a fall). The subdural hematoma was assessed as possibly related to RXC004 by investigator due to unknown mechanism as an association between the study drug and potential factors that could have contributed to this event, such as coagulation, had not been ruled out at the time of death. The 3 mg QD dose of RXC004 was subsequently declared not tolerated by the SRC.

4.4.1.2 RXC004 Clinical Safety Data (NCT03447470) – in Combination with Nivolumab Module 2)

As of 01 July 2022, 13 patients have received RXC004 in combination with nivolumab 480 mg q4w at doses of RXC004 1 mg and 1.5 mg in Module 2 of the ongoing Phase 1 study RXC004/0001. Eight of these patients received treatment at the highest declared tolerated dose (RXC004 1.5 mg). Treatment-related AEs of CTCAE ≥ Grade 2 reported in DLT-evaluable patients are summarised in Table 8.

Overall, the AE profile of the RXC004 1.5 mg dose in combination with nivolumab is similar to the RXC004 2 mg monotherapy dose. More patients who received the 1.5 mg dose had treatment related AEs of Grade 2 or higher (4/6) than patients who received the 1 mg dose (2/5). One patient who received 1.5 mg in combination had drug-induced liver injury (DILI), which was considered drug-related and a DLT.

The SRC determined at a meeting held on 07 July 2022, that both RXC004 1 mg and 1.5 mg QD in combination with nivolumab are tolerated doses. A decision was made not to further escalate the dose in the study, based on the safety and PK profiles of the 1.5 mg dose, as the combination of RXC004 2 mg with nivolumab was anticipated to lead to slightly higher exposure and potentially more AEs than the 2 mg monotherapy dose.

Table 8 Treatment-related AEs Grade 2 or higher including DLTs, observed in DLT evaluable patients as of 01 July 2022, Phase 1 study RXC004/0001 (RXC004 + nivolumab Module 2)

Dose	Number of patients dosed	Patients evaluable for DLT	Study treatment-related AEs, CTCAE ≥ Grade 2 in DLT-evaluable [a] patients
1.0 mg QD RXC004 + nivolumab 480 mg q4w	5	5	**Tolerated dose** Events ≥ Grade 2 in DLT-evaluable patients (2/5 patients): G2 back pain (1/5 patients) [b] G2 nausea (1/5 patients) [b] G2 fatigue (1/5 patients) [c] G2 poor appetite (1/5 patients) [c] G3 fatigue (1/5 patients) [b]
1.5 mg QD RXC004 + nivolumab 480 mg q4w	8	6 [e]	**Tolerated dose** Events ≥ Grade 2 in DLT evaluable patients (4/6 patients): G2 dysgeusia (2/6 patients) [b] G2 diarrhoea (1/6 patients) [b] G2 diarrhoea (1/6 patients) [c] G2 reduced appetite (1/6 patients) [b] G2 nausea (1/6 patients) [b] G2 constipation (1/6 patients) [d] G2 anorexia (1/6 patients) [b] G2 increased total bilirubin (1/6 patients) [b] G2 High TBL (1/6 patients) [b] G3 DILI (1/6 patients) **[DLT]** [c]

[a] The DLT assessment period runs from the start of dosing with RXC004 (Cycle 0 Day 1) up to Cycle 1 Day 28. To be evaluable for DLT assessment patient must had received ≥ 66% of the intended dose of RXC004 and all of the nivolumab infusion during Cycle 1, or have experienced a DLT event in the assessment period.

[b] Related to RXC004.

[c] Related to RXC004 and nivolumab.

[d] Related to nivolumab.

[e] Two of the 8 patients who received RXC004 1.5 mg in combination with nivolumab discontinued prior to the end of Cycle 1 without meeting DLT criteria, and were replaced.

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events; DLT, dose-limiting toxicity; q4w, every 4 weeks; QD, once daily.

A summary of the treatment-emergent adverse events (TEAEs) reported in Module 2 (RXC004 + nivolumab combination therapy) up to 01 July 2022 is presented in Table 9.

Table 9 Summary of Treatment-Emergent Adverse Events as of 01 July 2022, Phase 1 study RXC004/0001 (RXC004 + Nivolumab Module 2)

Number of patients with:	RXC004 1.0 mg + nivolumab (N=5)	RXC004 1.5 mg + nivolumab (N=8)	Overall (N=13)
TEAE	5 (100)	8 (100)	13 (100)
Treatment-Related TEAE	5 (100)	8 (100)	13 (100)
RXC004-Related TEAE	5 (100)	8 (100)	13 (100)
Nivolumab-Related TEAE	3 (60)	6 (75)	9 (69)
TEAE ≥ Grade 3	3 (60)	4 (50)	7 (54)
Treatment-Related TEAE ≥ Grade 3	1 (20)	3 (38)	4 (31)
RXC004-Related TEAE ≥ Grade 3	1 (20)	3 (38)	4 (31)
Nivolumab-Related TEAE ≥ Grade 3	0 (0)	2 (25)	2 (15)
TEAE ≥ Grade 4	0 (0)	0 (0)	0 (0)
Treatment-Related TEAE ≥ Grade 4	0 (0)	0 (0)	0 (0)
Serious TEAE	3 (60)	3 (38)	6 (46)
Serious Treatment-Related TEAE	0 (0)	1 (13)	1 (8)
Serious RXC004-Related TEAE	0 (0)	1 (13)	1 (8)
Serious nivolumab-Related TEAE	0 (0)	1 (13)	1 (8)
TEAE Leading to Discontinuation of Study Treatment (RXC004)	0 (0)	2 (25)	2 (15)
Treatment-Related TEAE Leading to Discontinuation of Study Treatment	0 (0)	2 (25)	2 (15)
TEAE Leading to RXC004 Dose Modification	2 (40)	5 (63)	7 (54)
Dose Reduced	0 (0)	1 (13)	1 (8)
Dose Interrupted	2 (40)	5 (63)	7 (54)
TEAE Leading to Death	0 (0)	0 (0)	0 (0)
TEAE of Dose Limiting Toxicity	0 (0)	1 (13)	1 (8)

Adverse events were coded using MedDRA Version 24.1. A TEAE was an AE with onset on or after date of first dose of study treatment and within 30 days of last dose.
Treatment-related adverse event was an adverse event that had relationship to study drug designated by the investigator as Possibly Related or Probably Related.
Treatment-Related: adverse events related to RXC004 and/or nivolumab. If the relationship to RXC004 or nivolumab was missing, the event was conservatively treated as related to the corresponding study treatment.
AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event.

The most common TEAEs (that were reported by ≥ 20% of patients) are summarised by RXC004 dose level and overall in Table 10. The most common TEAEs were decreased appetite, fatigue, and nausea which were all reported for 6 patients each (46%).

Table 10 Most common (occurring in at least 20% patients) TEAEs, as of 01 July 2022, Phase 1 study RXC004/0001 (RXC004 + nivolumab Module 2)

Preferred term	Number (%) of patients		
	RXC004 1.0 mg QD + nivolumab (N = 5)	RXC004 1.5 mg QD + nivolumab (N = 8)	Overall (N = 13)
Patients with any TEAEs	**5 (100)**	**8 (100)**	**13 (100)**
Decreased appetite	2 (40)	4 (50)	6 (46)
Fatigue	3 (60)	3 (38)	6 (46)
Nausea	2 (40)	4 (50)	6 (46)
Dysgeusia	1 (20)	4 (50)	5 (38)
Constipation	2 (40)	2 (25)	4 (31)
Headache	2 (40)	2 (25)	4 (31)
Weight decreased	1 (20)	3 (38)	4 (31)
Abdominal distension	1 (20)	2 (25)	3 (23)
Blood bilirubin increased	0 (0)	3 (38)	3 (23)
Pruritus	1 (20)	2 (25)	3 (23)
Urinary tract infection	0 (0)	3 (38)	3 (23)

QD, once daily; TEAE, treatment-emergent adverse event.

The most common AEs (that were reported by ≥ 20% of patients) related to study treatment (RXC004 or nivolumab) are summarised in Table 11. All 13 patients had at least one AE related to study treatment; all 13 patients had events related to RXC004, 9 patients (69%) had events related to nivolumab. The most common treatment-related AEs were nausea (46%), dysgeusia (38%), and fatigue (38%). With the exception of fatigue, all of these events were more frequently observed at the 1.5 mg dose than the 1 mg dose level. Dysgeusia is considered to be an on-target effect of Wnt inhibition.

Table 11 Most common (occurring in at least 20% patients) AEs considered related to RXC004 or nivolumab, as of 01 July 2022, Phase 1 study RXC004/0001 (RXC004 + nivolumab Module 2)

Preferred term	Number (%) of patients		
	RXC004 1.0 mg QD + nivolumab (N = 5)	RXC004 1.5 mg QD + nivolumab (N = 8)	Overall (N = 13)
Patients with any TRAEs	**5 (100)**	**8 (100)**	**13 (100)**
Nausea	2 (40)	4 (50)	6 (46)
Dysgeusia	1 (20)	4 (50)	5 (38)
Fatigue	2 (40)	3 (38)	5 (38)
Decreased appetite	1 (20)	3 (38)	4 (31)
Weight decreased	1 (20)	3 (38)	4 (31)
Blood bilirubin increased	0 (0)	3 (38)	3 (23)

QD, once daily; TRAE, treatment-related adverse event.

Three patients had CTCAE Grade 3 events that were assessed by the Investigator as possibly or probably related to RXC004: Grade 3 fatigue was reported by one patient who received RXC004 1 mg + nivolumab and one patient who received RXC004 1.5 mg. One patient who received RXC004 1.5 mg had Grade 3 events of aspartate aminotransferase increased, blood alkaline phosphatase increased, and DILI, which were considered to be related to both RXC004 and nivolumab. No other events of DILI were reported in either Module 1 or Module 2. A fourth patient who received RXC004 1.5 mg had a Grade 3 event of anaemia with missing relationship details.

A total of 11 treatment-emergent SAEs were reported in 6 patients. Ten of the 11 SAEs reported were assessed by the Investigator and the Sponsor as unrelated to RXC004. These were as follows: infection (NOS), left leg weakness, and infection (all reported in one patient at 1 mg); kidney infection and liver pain (in one patient at 1 mg), and 2 events of ascites (in one patient at 1 mg); diarrhoea (in one patient at 1.5 mg); and chest infection and pneumonia (in one patient at 1.5 mg).

One SAE was assessed as being related to RXC004 and nivolumab: this was Grade 3 DILI in a patient who had colorectal cancer with liver metastases, and was reported on Cycle 1 Day 17. The event was considered to be a DLT, and study treatment was permanently discontinued. The liver function tests subsequently returned to baseline. The patient subsequently died due to disease progression. No other events of DILI were reported in either Module 1 or Module 2.

4.4.1.3 RXC004 pharmacokinetic profile

The data from the ongoing Phase 1 study (NCT03447470) have characterised the PK of RXC004 monotherapy in patients following single and multiple oral doses ranging from 0.5 mg to 10 mg, and in combination with standard dose nivolumab at doses of RXC004 1 mg and 1.5 mg.

RXC004 was rapidly absorbed following oral dosing in fasting conditions, with a peak plasma concentration observed typically after 1-2 hours post-dose and eliminated with a half-life of approximately CCI hours. The exposures achieved in patients dosed with 0.5–2 mg were broadly dose proportional and predictable from the exposure observed in the patient dosed at 10 mg in terms of half-life, C_{max}, C_{min} and AUC. There was limited accumulation to steady state and it is in line with expectations from the measured half-life. The 3 mg dose C_{max}, C_{min} and AUC were all disproportionately high compared to the 2 mg dose, giving an estimated CCI in exposure for the 50% rise in dose.

There was little difference in RXC004 plasma exposure between monotherapy and combination therapy on Cycle 0 Day 1. On Cycle 1 Day 15, the steady state RXC004 plasma exposure appeared to be slightly greater on combination therapy compared with monotherapy, although patient numbers are small. The mechanism for a potential small increase in exposure when RXC004 is given with nivolumab is unclear since a PK interaction with a monoclonal antibody is unlikely, but an increase in bioavailability cannot be ruled out.

The plasma exposure of RXC004 when given at 1.5 mg in combination with nivolumab was no greater than that observed at 2 mg monotherapy and was around CCI than that observed at 3 mg monotherapy.

The steady state PK parameters of RXC004 from Study RXC004/0001, for RXC004 monotherapy and in combination with nivolumab are summarised in Table 12.

Table 12 Steady state pharmacokinetic parameters, as of 01 July 2022, Phase 1 study RXC004/0001 RXC004 monotherapy and combination with nivolumab (Module 1 and Module 2)

Parameter (units)		RXC004 monotherapy			CCI	RXC004 in combination with nivolumab	
		1 mg	1.5 mg	2 mg		1 mg	1.5 mg
C_{min} (ng/mL)	N	3	6	4		4	4
	Geometric mean (%CV)	7.15 (120)	12.6 (142)	26.8 (134)		8.62 (163)	13.6 (48.4)
T_{max} (h)	N	3	6	5		5	6
	Median (min – max)	1.00 (1–2.00)	2.00 (1.00–4.00)	1.00 (0.5 – 2.00)		2.00 (0.5-6.0)	1.50 (0.5-2.00)
C_{max} (ng/mL)	N	3	6	5		5	5
	Geometric mean (%CV)	42.4 (89.1)	56.6 (53.7)	68.5 (61.6)		58.2 (46.3)	76.4 (57.6)
AUC_{0-24} (h*ng/mL)	N	3	6	4		5	5
	Geometric mean (%CV)	392 (42)	630 (76)	931 (61.1)		544 (65.5)	706 (40.1)

AUC_{0-24}, area under the plasma concentration-time curve from zero to 24 hours; C_{max}, maximum observed plasma concentration; C_{min}, minimum observed plasma concentration across the dosing interval; T_{max}, time to maximum plasma concentration.

4.4.1.4 RXC004 Pharmacodynamic and Efficacy Data

In pre-clinical pharmacology models, RXC004 demonstrated anti-tumour activity at steady state C_{min} exposures of between CCI and CCI fold IC_{50}, with evidence of improved efficacy at higher doses. The target human exposure to achieve monotherapy efficacy is a C_{min} value of CCI fold IC_{50}.

Pharmacodynamic effects (>50% reduction in AXIN2 expression in skin) were observed in patients who achieved C_{min} exposure of CCI fold IC_{50}.

As of 30 July 2021, 5/18 evaluable patients in the monotherapy module, with at least 2 scheduled RECIST1.1 scans had achieved durable SD of 10 weeks or longer – 1 patient dosed at 1.0 mg QD (RNF43 LoF mCRC), 2 patients dosed at 1.5 mg (CCA and RSPO fusion mCRC) and 2 patients dosed at 3 mg (CCA and thymus cancer. The CCA patient had a dose reduction to 1.5 mg RXC004 after experiencing colitis). Of note all 5 patients who achieved SD had a type of cancer in which RXC004 is most likely to demonstrate clinical benefit based on its mechanism of action. These selected tumour types are CRC or PDAC with a RNF43 LoF mutation or RSPO fusion, or biliary tract cancer or thymus cancer, both of which are reported to have high Wnt ligand activity (Boulter et al 2015, Loilome et al 2014, Vodicka et al 2020). All patients with RECIST SD also achieved C_{min} exposures of CCI-fold IC_{50}.

Efficacy data for the combination of RXC004 + nivolumab in Module 2 are not yet available.

4.4.1.5 RXC004 monotherapy dose

Based on all of the available information, patients who enrol in Arm A RXC004 monotherapy will receive 2.0 mg RXC004 orally once a day.

The 2 mg QD dose was both tolerable and able to consistently deliver Cmin values of CCI fold IC50 in all patients, whereas the 1.5 mg QD dose did not consistently achieve the target Cmin values of CCI fold IC50 in all patients.

The 2 mg dose has been selected for Phase 2 development as the optimal dose to demonstrate an efficacy signal in patients with genetically selected tumours and to allow for potential dose reductions to manage adverse events.

4.4.1.6 RXC004 dose in combination with nivolumab

Patients who receive combination therapy with RXC004 and nivolumab in Arm A or B will receive RXC004 1.5 mg orally once a day. Justification for the choice of doses and regimens is provided in Sections 4.4.1 and 4.4.2.

4.4.2 Nivolumab

The nivolumab dose and regimen selected for this study are based on the FDA approved prescribing information for nivolumab. Patients with MSI-H or dMMR metastatic colorectal cancer can receive 240 mg q2w or 480 mg q4w. 480 mg q4w was selected to complement a 28-day cycle of RXC004 and reduce the number of patient visits required.

4.5 End of Study Definition

A participant is considered to have completed the study if he/she has completed all phases of the study (including the last visit and the last scheduled assessment shown in the Schedule of Assessments) or withdraws consent whichever occurs first.

The end of the study is defined as the date of the last visit of the last participant in the study or the last scheduled assessment shown in the Schedule of Assessments for the last participant in the study before the data cut off (DCO).

Sponsor may terminate this study at any time for reasons that include but are not limited to;

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study
- Inability to complete the trial in a meaningful timeframe due to a lack of recruitment.
- A decision on the part of the Sponsor to modify/suspend or discontinue development of the drug

5 STUDY POPULATION

The study will recruit male or female patients aged 18 years or older with histologically documented, RNF43 or RSPO genetically aberrated, MSS mCRC (Stage IV), who have progressed after standard of care treatment(s), have a minimum life expectancy of 16 weeks and an Eastern Cooperative Oncology Group performance status of 0 or 1.

Prospective approval of protocol deviations to recruitment and enrolment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1 Participant must be ≥18 years of age at the time of signing the informed consent.

2 Ability to give written informed consent and capable of understanding the protocol requirements listed in the informed consent form (ICF). Written informed consent must be obtained prior to performing any protocol-related procedures, including screening and pre-screening evaluations Participants must be willing and able to comply with the study protocol procedures and restrictions.

3 Histological documentation of metastatic (Stage IV) CRC and;

   (a) documented tumour tissue aberration in RNF43 and/or RSPO from central pre-screening or from a recognised panel approved by the sponsor. Please see Section 5.5 and Appendix F for more details

   (b) documented confirmation of MSS status, performed as a standard of care procedure at site. Please see Section 5.5 for more details

4 Patients must have had documented radiological progression following a minimum of 1 prior standard of care treatment regimen for metastatic disease.

5 ECOG performance status 0 or 1 with no deterioration over the previous 2 weeks and an estimated life expectancy of greater than 16 weeks

6 At least one lesion that is measurable by RECIST 1.1 at baseline (within 28 days prior to start of study treatment). The measurable lesion must not be chosen for the mandatory paired biopsies.

7 Mandatory paired biopsies*; Patients must have at least one lesion suitable for biopsy at screening (which must not be a target lesion for RECIST 1.1) and be willing to provide mandatory tumour biopsy samples as follows:

   Arm A: Baseline*; C1D15 (+ up to 7 days) and during Cycle 3 of monotherapy treatment (3 biopsies)

   Arm A combination treatment phase patients: Baseline*, C1D15 (+ up to 7 days) and during Cycle 3 of combination treatment (3 biopsies)

Arm B: Baseline**, C1D15 (+ up to 7 days) and during Cycle 3 of combination treatment (3 biopsies)

*Baseline biopsy is mandatory except in exceptional circumstances where biopsy is not technically feasible. All patients without a baseline sample must be approved by Sponsor before starting study treatment. On treatment biopsies are also mandatory unless deemed not to be technically or clinically feasible by Investigator.

**CCI , ideally from the same site of disease (e.g. liver, lymph node etc) that the 'on treatment' biopsy will be taken from, and taken after completion of all prior standard of care treatments will be accepted as a baseline biopsy.

8 Patients with adequate organ functions as described below;

- AST/ALT ≤ 2.5X ULN (upper limit of normal) [with no underlying Liver Metastasis]
- AST/ALT ≤ 5 X ULN [with underlying Liver Metastasis]
- Total Bilirubin ≤ 1.5 X ULN
- Serum Creatinine ≤ 1.5 X ULN
- ANC ≥ 1.5 x 10^9/L
- Platelets > 100 x 10^9/L
- Hb >8.5g/dL (with or without transfusional support)
- Calculated creatinine clearance (CrCL) ≥60 mL/min as determined by Cockcroft Gault (using actual body weight).

  Males:

$$\text{CrCL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}}$$

  Females:

$$\text{CrCL (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{72 \times \text{serum creatinine (mg/dL)}} \times 0.85$$

-

9 Female patients of childbearing potential must have a negative pregnancy test prior to start of dosing

10 Female patients of childbearing potential and male patients with female partners of childbearing potential must agree to use a highly effective method of contraception during the study and for at least 5 months after the last dose of study drug. See Section 5.3.1 for details of acceptable methods of contraception

For patients on RXC004 monotherapy treatment (Arm A) the following inclusion criteria will also apply to enter the RXC004 + nivolumab treatment phase;

11. Patients must have had documented RECIST1.1 defined radiological progression on RXC004 monotherapy treatment on the first scheduled scan (week 8 +/- 1 week)
12. Patients must receive Cycle 1 Day 1 of combination study treatment within 28 days of the first scheduled scan (week 8 +/- 1 week) showing RECIST1.1 defined radiological progression, or for clinically stable patients that remain on RXC004 after RECIST1.1 defined radiological progression, within 28 days of the most recent scan showing further radiological progression or confirmation of the week 8 RECIST1.1 progression (see Section 7.1.1 and Appendix D for more details)

5.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1 Prior therapy with a compound of the same mechanism of action as RXC004.

2 Patients at higher risk of bone fractures, including;

(a) Patients with Vitamin D [25(OH)D_3] deficiency defined as < 30 nmol/L (<12 ng/mL). [Note - Patients who fail on this criteria can be retested within the screening window]

(b) Patients with a corrected total serum calcium level of <2 mmol/L and serum magnesium level of < 0.60 mmol/L

(c) Patients with osteoporosis (as defined by a T-score of < -2.5 at any of the 3 following sites, all of which must be assessed: L/R total hip, L/R femoral neck, lumbar spine (L1-4) by DEXA scan) or history of fragility fractures (any fracture occurring with low-level trauma or as a result of falling < standing height)

(d) Patients with ongoing or a history of clinically significant hyperparathyroidism, Pagets disease or Osteomalacia. Patients with a prior diagnosis of hyperparathyroidism, Pagets disease or Osteomalacia, considered to have no increased bone fragility risk, may be included only after consultation with the Sponsor's Medical Monitor.

(e) Patients who have received treatment for type 2 Diabetes Mellitus with a Thiazolidinedione peroxisome proliferator-activated receptor gamma agonist (e.g. pioglitazone or rosiglitazone) within 4 weeks prior to study drug dosing.

3 Any known uncontrolled inter-current illness or persistent clinically significant toxicity related to prior anti-cancer treatment (as assessed by the Investigator) which in the investigator's opinion makes it undesirable for the patient to participate in the study

4 Patients who have any history of an active (requiring treatment) other malignancy (except any in-situ carcinoma, non-melanoma skin carcinoma and early prostate cancer with a normal PSA) within 2 years of study entry.

5 Patients with known or suspected brain metastases

6 Use of anti-neoplastic agents (including immunotherapy), immunosuppressants and other investigational drugs within 3 weeks prior to the first dose of study treatment, or any residual AEs from prior anti-cancer therapies that have not resolved to Grade ≤1. Note: COVID-19 vaccinations that are authorised but not approved should not be considered as investigational agents for the purposes of study eligibility assessment.

7 Patients with a known hypersensitivity to any RXC004 excipients.

8 Patients with a contra-indication for denosumab treatment including:

- Known hypersensitivity to Denosumab or any of the excipients
- Severe untreated hypocalcaemia
- Unhealed lesions from dental or oral surgery

9 Patients who are pregnant or breast-feeding

10 Use of any live or live-attenuated vaccines against infectious diseases (e.g., influenza nasal spray, varicella) within 4 weeks (28 days) of initiation of study treatment. Note: Patients who require COVID-19 vaccination within 4 weeks of initiation of study treatment should receive a non-live vaccine (e.g. one based on mRNA or fully inactivated/genetically modified viruses incapable of replication)

11 Judgment by the investigator that the participant should not participate in the study if the participant is unlikely to comply with study procedures, restrictions and requirements.

12 Patients with a mean resting corrected QTcF >470 ms, obtained from triplicate ECGs performed at screening

For patients on RXC004 + nivolumab combination treatment (Arm B or Arm A RXC004 + nivolumab treatment phase) the following exclusion criteria will also apply;

13 Patients with any contraindication to the use of nivolumab as per approved label (Summary of Product Characteristics or equivalent)

14 Patients with active or prior documented autoimmune or inflammatory disorders within the past 5 years, including inflammatory bowel disease, rheumatoid arthritis, autoimmune hepatitis, Grave's disease, lupus and celiac disease. The following are exceptions to this criterion;

(a) Patients with vitiligo or alopecia
(b) Patients with type I diabetes mellitus
(c) Patients with residual hypothyroidism due to autoimmune condition only requiring hormone replacement
(d) Patients with psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger.
(e) Patients with celiac disease controlled by diet alone

15 Patients with active infections, including tuberculosis, hepatitis B, hepatitis C or human immunodeficiency virus. Patients with a resolved HBV infection (defined as presence of

hepatitis B core antibody [HBcAb] and absence of HBV surface antigen [HBsAg] or negative on polymerase chain reaction [PCR] for HBV DNA) are eligible. Patients positive for HCV antibody are eligible only if PCR is negative for HCV RNA.

16 Patients with body weight <40kg

17 Patients with a history of allogeneic organ transplant or active primary immunodeficiency

18 Patients with a known hypersensitivity to nivolumab or any of the excipients of the product

5.3 Lifestyle Considerations

5.3.1 Contraception

1 Women must be 1 year post-menopausal, surgically sterile or must agree to using enhanced contraceptive measures for the duration of the study and for 5 months after the last dose of study drug, with all male sexual partners. Highly effective methods of contraception must be used, which include having a vasectomised partner, sexual abstinence (defined as refraining from heterosexual intercourse during the study and for 5 months after the last dose of study drug. The reliability of sexual abstinence needs to be evaluated by the Investigator, in relation to the duration of the study and the preferred/usual lifestyle of the subject), or one of the following: hormonal contraceptives which inhibit ovulation (oral, injectable, transdermal, intravaginal or implants), Intrauterine Device (IUD), Intrauterine Hormone-releasing System (IUS) (e.g., Mirena), or bilateral tubal occlusion.

2 Men must use a condom (with spermicide) during the study, and for 5 months after the last dose of study drug, with all sexual partners.

3 Men must not donate sperm for 5 months after the last dose of study drug.

5.3.2 Meals and Dietary Restrictions

Patients must fast (water to drink only) from at least 2 hours prior to taking a dose to at least 1-hour post-dose for all doses. Patients should abstain from eating large amounts of grapefruit and Seville oranges (and other products containing these fruits e.g. grapefruit juice or marmalade) during the study **(**e.g., no more than a small glass of grapefruit juice (120 mL) or half a grapefruit or 1-2 teaspoons (15g) of Seville orange marmalade daily).

5.3.3 Concomitant treatments

5.3.3.1 Prohibited medications

The following concomitant medication is prohibited without approval from the Sponsor Physician;

1 The use of any natural/herbal products or other ‘folk remedies’.

2 No other chemotherapy, hormonal therapy (contraception and hormone replacement therapy is acceptable), or other investigational product is permitted other than the study treatments.

3 Live or live-attenuated virus and bacterial vaccines should not be administered, e.g. yellow fever, measles, influenza nasal spray, COVID-19, rubella, mumps, typhoid, mycobacterium tuberculosis (BCG), Yersinia pestis (EV) within 4 weeks of starting study treatment, whilst the patient is receiving study medication and for 30 days following discontinuation of study treatment. Patients who require COVID-19 vaccination whilst on study should receive a non-live vaccine (e.g. one based on mRNA or fully inactivated/genetically modified viruses incapable of replication).

4 The use of some CYP3A4 inhibitors and inducers should be restricted prior to first dose of study treatment and throughout the study treatment, as RXC004 is observed to be exclusively metabolised by CYP3A4. For a list of prohibited CYP3A4 inhibitors and inducers and the required washout periods, please refer to Appendix E.

5 Drugs that are immunosuppressive or may cause secondary osteoporosis should not be administered (e.g. corticosteroids, pioglitazone or rosiglitazone).

The following exceptions are allowed;

- Use of immunosuppressive medication for the management of nivolumab-related AEs (as per Appendix G) or colitis events (as per Appendix J). Steroids required for the management of RXC004 or nivolumab related AEs, should be tapered to doses ≤ 10 mg/day prednisone equivalent, before re-starting study treatment.
- Use of topical, inhaled or intranasal corticosteroids
- Systemic steroids at doses ≤ 10 mg/day prednisone equivalent

5.3.3.2 COVID-19 Vaccinations

COVID-19 vaccination is permitted before or during study treatment, with the exception of live-attenuated vaccines and replication-competent vector vaccines, which are prohibited within 4 weeks of starting study treatment and whilst on study treatment.

As COVID-19 vaccines are often prioritised for cancer patients, vaccination prior to the first dose of study treatment is advisable. Where possible, COVID-19 vaccinations should be given at least 72 hours before starting study treatment and study treatment should not commence until any acute adverse effects from vaccination have resolved to at least Grade 1. If this is not possible, investigators should follow their local prescribing information and policies when considering whether a patient on study treatment should receive a COVID-19 vaccination. If COVID-19 vaccination is given during the study (including the screening period), then please ensure that all the relevant information is recorded in the concomitant medication eCRF (i.e vaccine name, manufacturer, date(s) given and dose).

5.3.4 Other Lifestyle Considerations

Patients taking RXC004 are advised to avoid exposure to direct sunlight and the use of tanning equipment.

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently treated in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the Calcium and/or Vitamin D3 laboratory criteria for participation in this study may be retested after starting Calcium/Vitamin D3 supplementation. Repeating laboratory tests within the screening period will not be considered as a rescreen.

5.5 Pre-Screening Genetic Testing

All enrolled patients' tumours will be required to have a genetic mutation which is predicted to be loss of function in RNF43, or an RSPO fusion/translocation detectable in CCI [redacted]. Eligible patients also need to have evidence of MSS status. Eligible subjects will be identified by either a central genetics screening approach or local laboratory assessments.

5.5.1 Central Testing

CCI [redacted]

CCI

CCI

CCI

CCI

CCI

Data from central genetic screening will be shared with investigators to aid future treatment decisions.

CCI

CCI

CCI

CCI

5.5.2 Local Testing

Patients with predicted loss of function RNF43 mutations or RSPO fusions from other local testing will also be eligible to enter the study, provided that the assay used for testing has adequate analytical validation for this purpose (i.e. fully CCI for the purpose of investigating RNF43 mutations or RSPO fusions; partially CCI for their analytical performance or having achieved a minimum validation specification of >90% for Analytical Sensitivity / PPA and >95% for Analytical Specificity / NPA for the detection of the aberrations in the genes of interest and performed in an ISO accredited, CLIA or CAP accredited lab). If aberrations are detected by local tests that are not included in the list in Appendix F, these may be included in the study on discussion with the Sponsor. CCI

CCI

CCI

CCI

5.5.3 MSS/MSI Testing

Tumour samples that test positive for RNF43 mutations or RSPO fusions will also be tested for MSI status at CCI. The MSI status from CCI

CCI is not required for eligibility (which will be based on the local standard of care IHC, NGS or PCR test that has been taken at diagnosis to identify patients with Lynch Syndrome or patients with MSI CRC who may be eligible for treatment with a PD-1 inhibitor), but will be used to retrospectively evaluate the concordance between local and central MSI assays.

6 STUDY TREATMENT

6.1 Study Treatment(s) Administered

6.1.1 Investigational Products

Table 13 Investigational Products

Treatment	RXC004	Nivolumab	Denosumab
ARM	Arm A and B	Arm B and Arm A combination treatment phase	Arm A and B
Type	Drug	Biologic	Biologic
Dose Formulation	0.5 mg or 1 mg capsules	Ampule	Ampule
Dosage Level(s)	Monotherapy: 2 mg QD Combination therapy: 1.5 mg QD	480 mg q4w	120 mg once every month
Route of Administration	oral	IV infusion	SC injection
Use	Experimental	Experimental	Prophylactic
IMP and NIMP	IMP	IMP	IMP
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor	Provided centrally by the Sponsor
Packaging and Labelling	RXC004 will be provided in white 50 mL HDPE Duma bottles. Each bottle will be labelled as required per country requirement	Study Intervention will be provided in packets of 240 mg/24 mL vials. Each packet will be labelled as required per country requirement	Study Intervention will be provided in a vial containing 120 mg in 1.7 mL solution Each vial will be labelled as required per country requirement

HDPE, high-density polyethylene; IMP, investigational medicinal product; IV, intravenous; NIMP, non-investigational medicinal product; QD, once daily; q4w, once every 4 weeks; SC, subcutaneous.

6.2 Preparation/Handling/Storage/Accountability of Study Treatments

6.2.1 RXC004

RXC004 will be supplied by Redx as 0.5 and 1.0 mg capsules. A complete description of the chemistry and formulation may be found in the Investigational Medicinal Product Dossier.

The Quality Control Standards and requirements for RXC004 study medication are described in separate release protocols/ Certificate of Analysis.

RXC004 is formulated as two dosage strengths capsules (0.5 mg and 1.0 mg), manufactured by Quay Pharma, Deeside Industrial Park, CH5 2NS, United Kingdom. The RXC004 capsules strengths are 0.5, 1.0 mg for oral administration. Stability testing of RXC004 is ongoing. Please refer to the current IMP label for the Expiry Date associated with the current shelf-life of the product.

Patients must fast (water to drink only) for at least 2 hours prior to taking a dose to at least 1-hour post-dose for all doses.

6.2.2 Nivolumab

Nivolumab will be supplied by 240 mg/24 mL vial solutions for infusion after dilution. Two (2) vials will be needed for the 480 mg dose.

Storage;

- Unopened vials must be stored in a refrigerator (2°C to 8°C). The vials must be kept in the original package in order to protect from light. Vials should not be frozen. The unopened vial can be stored at controlled room temperature up to 25°C with room light for up to 48 hours.

- Infusion must be completed within 24 hours of preparation. If not used immediately, the solution may be stored under refrigeration conditions (2°C-8°C) and protected from light for up to 24 hours [a maximum of 8 hours of the total 24 hours can be at room temperature (20°C-25°C) and room light].

- Do not use after the expiry date which is stated on the carton and on the vial label after EXP. The expiry date refers to the last day of that month.

- Do not store any unused portion of the infusion solution for reuse. Any unused medicine or waste material should be disposed of in accordance with local requirements.

Preparation and administration of nivolumab;

- Visually inspect drug product solution for particulate matter and discoloration prior to administration. Nivolumab is a clear to opalescent, colourless to pale-yellow solution. Discard the vial if the solution is cloudy, discoloured, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do

not shake the vial.

- Withdraw the required volume of nivolumab and transfer into an intravenous container. Dilute nivolumab with either 0.9% Sodium Chloride Injection, USP or 5% Dextrose/Glucose Injection, USP to prepare an infusion with a final concentration ranging from 1 mg/mL to 10 mg/mL. The total volume of infusion must not exceed 160 mL. For patients with body weights less than 40 kg, the total volume of infusion must not exceed 4 mL/kg of body weight.

- Mix diluted solution by gentle inversion. Do not shake

- The dose of nivolumab for administration must be prepared by the Investigator's or site's designated IP manager using aseptic technique. Total time from needle puncture of the vial to the end of administration should not exceed;

    - 8 hours at room temperature

    - 24 hours under refrigeration at 2°C to 8°C (36°F to 46°F)

- The infusion should be administered over 60 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micrometre to 1.2 micrometre).

- Do not co-administer other drugs through the same intravenous line

- The intravenous line should be flushed at the end of the infusion.

6.2.3 Denosumab

Denosumab is a human monoclonal antibody (IgG2) that targets and binds with high affinity specifically to RANKL, preventing RANKL/RANK interaction from occurring and resulting in reduced osteoclast numbers and function, thereby decreasing bone resorption and cancer-induced bone destruction.

Denosumab is approved to be administered at 120 mg S.C. once monthly for use in the prevention of skeletal-related events in patients with advanced cancers with bone metastases (XGEVA®).

The use of denosumab in this trial is outside labelled use as patients may not have bone metastases. The rationale for its prophylactic use in all patients is as a bone protection measure based on the increased risk of bone fragility with RXC004 treatment.

6.3 Randomisation

Once Arm B is opened, patients who are eligible for both Arm A and Arm B will be randomised 2:1 to Arm B: Arm A to ensure there is no conscious or unconscious selection bias. A 2:1 randomisation was selected to ensure that the combination and monotherapy arms will complete at approximately the same time and to maximise the number of patients recruited under randomisation conditions. Patients will be centrally assigned to a Study Arm using IRT.

Randomisation will be open-label, and will be stratified by genetic alteration (RNF43 vs RSPO).

6.4 Study Treatment Compliance

6.4.1 RXC004

The first dose of RXC004 will be taken at the site under medical supervision. The date and time of dose administered at site will be recorded in the source documents and recorded in the eCRF. When patients self-administer RXC004 at home, compliance with study treatment will be assessed at each visit. Compliance will be assessed by collection of a patient diary cards and counting of returned capsules, during the site visits and documented in the source documents and eCRF. Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF.

A record of the number of RXC004 capsules dispensed to and taken by each participant must be maintained and reconciled with study treatment and compliance records. Treatment start and stop dates, including dates for treatment delays and/or dose reductions will also be recorded in the eCRF.

6.4.2 Nivolumab

Nivolumab will be dosed at the site, directly from the investigator or designee, under medical supervision. The date, and time of dose administered at site will be recorded in the source documents and recorded in the eCRF. The dose of nivolumab and the study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention.

6.5 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrolment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates

- Dosage information including dose and frequency

Concomitant medication may be given as medically indicated with the exception of prohibited concomitant medication (Section 5.3.3 and Appendix E). The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.5.1 Rescue Medicine

As a result of immune mediated AEs that could potentially be experienced by patients on nivolumab, steroids and other immunosuppressant rescue medication must be available to these patients. The two products that fall into the category of other immunosuppressants are infliximab (e.g., for colitis) and mycophenolate (e.g. for hepatitis). Rescue medications for immune mediated adverse events will be obtained locally.

The use of infliximab and mycophenolate are considered to be used as off-label for management of immunotherapy related toxicities. These rescue medications must be receipted, controlled, and administered by the pharmacist and stored according to the labelled storage conditions.

6.6 Dose Modification

RXC004 dose reduction and interruption are permitted. Nivolumab dose reduction is not permitted but dose interruption/delay is permitted as per Appendix G.

RXC004 may be dose interrupted (withheld) until a clinically significant (any grade) and/or a grade $\geq$ 3 related adverse event resolves to baseline or $\leq$ grade 2. RXC004 may also be withheld if it is necessary to give a concomitant medication that is a moderate or strong CYP3A4 inhibitor. RXC004 related colitis events should be managed as per Appendix J.

Adverse events that are assessed as being causally related nivolumab should be managed as per the Nivolumab Toxicity Management and Dose Modification Guidelines (Appendix G). If either agent is interrupted or discontinued for a related-AE the other agent (nivolumab or RXC004) may be continued if the patient is judged (by the investigator) to be receiving clinical benefit.

RXC004 and nivolumab dose interruption and stopping criteria are detailed in Table 14.

Table 14 Dose Modification and Stopping Criteria

Event	Action
Colitis events (e,g. Colitis, ileitis, enterocolitis etc)	
Grade 1	If receiving RXC004, either as monotherapy or in combination with nivolumab; Reduce RXC004 to a lower dose (see Table 15 for dose reduction scheme) and manage as per Appendix J (and Appendix G if applicable)
Grade 2	If receiving RXC004, either as monotherapy or in combination with nivolumab Interrupt RXC004. Manage as per Appendix J and Appendix G if applicable. When event has resolved to Grade 1[a] and steroids tapered to ≤10 mg of prednisone per day (or equivalent), resume RXC004 at a lower dose (see Table 15 for dose reduction scheme) and nivolumab (if applicable) at 480 mg q4w. If event re-occurs after re-starting study treatment, then RXC004 and nivolumab (if applicable) should be permanently discontinued.
Grade 3	If receiving RXC004 monotherapy; Interrupt RXC004. Manage as per Appendix J. When event has resolved to Grade 1[a] and steroids tapered to ≤10 mg of prednisone per day (or equivalent), resume RXC004 at a lower dose (see Table 15 for dose reduction scheme). If event re-occurs after re-starting study treatment, then RXC004 should be permanently discontinued. If receiving RXC004 in combination with nivolumab; **permanently discontinue both agents** and manage as per Appendix J and Appendix G.
Grade 4	If receiving RXC004, either as monotherapy or in combination with nivolumab; **Permanently discontinue study treatment (both agents if applicable)**. Manage as per Appendix J and Appendix G

Event	Action
Dysgeusia events	
Grade 1/2	RXC004 related; Consider dose reduction, depending on clinical symptoms Manage as per Appendix I Interrupt RXC004 treatment if dysgeusia is associated with >5kg weight loss from baseline
Grade 2 associated with a 10-20% decrease in body weight	RXC004 related; Manage as per Appendix I Reduce RXC004 to a lower dose (see Table 15 for dose reduction scheme).
Grade 2 associated with a >20% decrease in body weight	RXC004 related; Permanently discontinue RXC004. Nivolumab can continue if clinical benefit is judged by investigator.
Other adverse events	
Any event that meets discontinuation criteria for nivolumab (see Appendix G)	Permanently discontinue nivolumab. RXC004 can continue if clinical benefit is judged by investigator.
Any event that meets nivolumab treatment hold (see Appendix G)	Interrupt nivolumab and manage as per Appendix G. Nivolumab can be re-started when event is resolved to ≤Grade 1 and any steroid taper completed (to ≤10 mg prednisone equivalent). RXC004 can continue whilst nivolumab is held if the patient is judged (by the investigator) to be receiving clinical benefit.
Grade 3 toxicity (1st event)	RXC004 related; Interrupt RXC004 and provide supportive care, resume at a lower dose level when resolved (grade ≤ 2 or returns to baseline). See Table 15 for dose reduction scheme. Nivolumab related; See guidelines in Appendix G.
Grade 3 toxicity (subsequent recurrence of a previously experienced event)	RXC004 and/or nivolumab related; Permanently discontinue the related study treatment.

Event	Action
Grade 4 toxicity	RXC004 and/or nivolumab related; Permanently discontinue RXC004 and nivolumab (if applicable)
All Grade events	Study treatment can be interrupted or discontinued for any clinically significant AEs that in the investigator's opinion warrants treatment interrupted or discontinuation.
Bone events	
Confirmed (by imaging) RXC004 related fragility bone fracture (excluding grade 1 vertebral deformities)	Permanently discontinue RXC004. Nivolumab can continue if the patient is receiving clinical benefit as judged by the investigator.
Patients with a DXA scan showing ≥ 7% worsening in BMD at lumbar spine or total hip compared to baseline	Permanently discontinue RXC004. Nivolumab can continue if the patient is receiving clinical benefit as judged by the investigator.
An individual increase in β-CTX of CCI pg/ml from the baseline (screening) value **or** an individual measurement of CCI pg/ml	Permanently discontinue study treatment unless the patient has received clinical benefit (investigator assessment) and continued treatment is warranted.
COVID-19 infection Positive COVID-19 test	Interrupt RXC004, until acute symptoms have resolved.
Major surgery	Interrupt RXC004, resume at full dose. Nivolumab does not need to be interrupted. No stoppage is required for biopsy procedures.
Vomiting	If vomiting occurs shortly after RXC004 is swallowed, the dose may be replaced if all of the intact capsules can be counted. Resume with the following scheduled dose.
Missed RXC004 dose	Allowed to take the scheduled dose up to 4 hours after the scheduled dose time. If greater than 4 hours, the missed dose should not be taken, and patient should continue with next dose at allotted time.

[a] Attempts should be made to confirm that the colitis has resolved to Grade ≤ 1 (e.g. normalised CRP or fecal calprotectin if raised during event; GI appearance normalised on X-ray/CT or endoscopy) before restarting RXC004.

AE, adverse event; BMD, bone mineral density; COVID-19, Coronavirus disease 2019; QD, once daily.

Toxicity will be assessed utilizing the NCI Common Terminology Criteria for Adverse Event (CTCAE) version 5.0, unless otherwise specified. Every effort should be made to administer trial treatment at the planned dose and schedule. However, patients experiencing RXC004 related toxicities may have their RXC004 dose modified as outlined below.

RXC004 related toxicities observed during the course of the study will be managed by interruption of RXC004 and initiation of appropriate treatment as judged by the Investigator. Upon re-starting treatment the guidance for dose reduction in RXC004 contained in Table 15 should be followed.

Patients in Arm A who have undergone dose reduction during monotherapy cannot have the RXC004 dose re-escalated if switching to RXC004 + nivolumab combination therapy. The RXC004 dose must be discussed with the Sponsor prior to starting combination therapy.

Table 15 Guidance for dose reductions for RXC004-related adverse events

Dose Level	**RXC004 (monotherapy)**	**RXC004 (in combination therapy)**
Initial dose level	2 mg QD	1.5 mg QD
1st dose reduction • Grade 3 toxicity (1st event) • Grade 2 dysgeusia associated with 10-20% weight loss[a]	Hold RXC004[b]. Upon resolution to ≤ grade 2, restart at 1.5 mg QD Dose reduce to 1.5 mg QD	Hold RXC004b. Upon resolution to ≤ Grade 2, restart at 1.0 mg QD Dose reduce to 1.0 mg QD
2nd dose reduction • Grade 3 toxicity (1st unique event) • Grade 3 toxicity (recurrence of a previously experienced Grade 3-4 event) • Grade 2 dysgeusia associated with >20%	Hold RXC004[b]. Upon resolution to ≤ grade 2, restart at 1 mg QD Permanently discontinue RXC004 Permanently discontinue RXC004	Hold RXC004[b]. Upon resolution to ≤ Grade 2, restart at 0.5 mg QD Permanently discontinue RXC004 Permanently discontinue RXC004

Dose Level	RXC004 (monotherapy)	RXC004 (in combination therapy)
weight loss[a]		
3rd dose reduction	Not permitted. Permanently discontinue RXC004	Not permitted. Permanently discontinue RXC004

a excluding other possible causes and despite optimal treatment
b RXC004 can be resumed after an interruption, as long as no other treatment discontinuation criteria have been met. Patients who experience disease progression whilst RXC004 is held, will not be eligible to be treated beyond progression.

For patients treated with RXC004 and nivolumab, dose reductions in nivolumab are not allowed. Patients experiencing nivolumab related toxicities may delay nivolumab infusions or permanently discontinue nivolumab as described in Appendix G and Table 14.

6.7 Access to Study Treatment after the End of the Study

Any patients still receiving investigational product at the time of the data cut off will be able to continue to receive investigational products within the current study, if in the Investigator's opinion, they are deriving clinical benefits and not meeting any of the discontinuation criteria. Assessments will revert to standard of care for each individual site. Patients will continue to be monitored for all SAEs up to 30 days after the last dose of RXC004 (for patients treated with RXC004 monotherapy only) or 90 days after the last dose of nivolumab (for patients treated with RXC004 and nivolumab). A paper form process will be used for SAE reporting.

All SAEs, overdoses and pregnancies will be reported until 30 days after the last dose of RXC004 (for patients treated with RXC004 monotherapy only) or 90 days after the last dose of nivolumab (for patients treated with RXC004 and nivolumab). The investigational product will be supplied to sites manually. The investigational product dispensation and reconciliation will be handled by the study at each patient's visit. The investigational product accountability information must still be collected until all patients have completed treatment.

7 DISCONTINUATION OF STUDY TREATMENT AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Treatment

An individual patient will not receive any further IP (RXC004 or nivolumab) if any of the following occur;

- Patient decision to withdraw from further treatment with IP. The patient is, at any time,

free to discontinue treatment, without prejudice to further treatment

- Clinical or RECIST 1.1-defined radiological progression (symptomatically/clinically stable patients may continue treatment until symptomatic/clinical progression or second RECIST 1.1 progression [relative to the first 'on study treatment' RECIST 1.1 progression]). See Section 7.1.1 for details for continuing study treatment after RECIST1.1 defined progression
- An AE that meets discontinuation criteria in Appendix G or Table 14
- Pregnancy or intent to become pregnant
- Initiation of alternative anticancer therapy including another investigational agent
- Non-compliance with the study protocol that, in the opinion of the Investigator or Sponsor, warrants withdrawal from treatment with IP

Discontinuation from study treatment is NOT the same thing as a withdrawal from the study. If study treatment is permanently discontinued, the participant will remain in the study to be evaluated for safety, progression (if the patient discontinues for a reason other than progression) and survival. See the SoA for data to be collected at the time of discontinuation of study treatment and during follow-up.

7.1.1 Treatment of Symptomatically Stable Patients after RECIST 1.1 Defined Progression

Patients with symptomatically stable disease may continue treatment after the initial RECIST 1.1 – defined progression, until symptomatic/clinical progression or a second RECIST 1.1 PD assessment (relative to the initial RECIST1.1 progression, for a given study treatment).

Patients who wish to continue study treatment after RECIST1.1 defined progression, should be informed of all other alternative treatment options available to them by the treating physician and sign an additional consent that they wish to continue study treatment. A decision to continue study treatment after initial progression must also be discussed and agreed by the treating physician and the Sponsor.

The criteria for continuing treatment after initial RECIST 1.1-defined progression are as follows:

- Signed patient consent for treatment after progression
- The patient does not have any significant, unacceptable, or irreversible toxicities indicating that continuing treatment will not further harm the patient.

- There is absence of clinical symptoms or signs indicating clinically significant disease progression accompanied by a decline in ECOG performance status>1.

- There is absence of rapid disease progression or threat to vital organs or critical anatomical sites (e.g. central nervous system metastasis, respiratory failure due to tumour compression, or spinal cord compression) requiring urgent alternative medical intervention.

- Study treatment was not on hold/interrupted at time of progression

Patients continuing study treatment after initial RECIST 1.1 defined progression should continue RECIST scans as per the Schedule of Assessments (the next tumour assessment should be at least 4 weeks after the first RECIST 1.1 defined progression and no later than the next regularly scheduled imaging timepoint).

7.2 Participant Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance, or administrative reasons. This is expected to be uncommon.
- A participant who considers withdrawing from the study must be informed by the investigator about modified follow-up options (e.g., telephone contact, a contact with a relative or treating physician, or information from medical records).
- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, it should be confirmed if he/she is still agrees for existing samples to be used in line with the original consent. If he/she requests withdrawal of consent for use of samples, destruction of any samples taken and not tested should be carried in line with what was stated in the informed consent and local regulation. The investigator must document the decision on use of existing samples in the site study records and inform the Global Study Team.

7.3 Lost to Follow up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit

schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.

8 STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarised in the SoA. Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed by Investigator to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g., blood count) and obtained before signing of the ICF may be utilised for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within 28 days of the first dose of study treatment.
- The maximum amount of blood collected from each participant over the duration of the study, is expected to not exceed 500 mL.

8.1 Efficacy Assessments

The primary endpoint for the RXC004 monotherapy arm, will be the DCR using a patients best overall response, with secondary endpoints of % change in the sum of target lesions, ORR, DoR, PFS and OS. For the RXC004 + nivolumab arm, the primary endpoint will be ORR using a patients BOR with secondary endpoints of % change in the sum of target lesions, DCR, DoR, PFS and OS.

Efficacy assessments of DCR, ORR, % change in the sum of target lesions, DoR and PFS will be derived using investigator RECIST 1.1 assessments. In addition, OS will also be evaluated.

8.1.1 Tumour assessments

Tumour assessments utilize images from CT or MRI, collected during screening/baseline and at regular (follow-up) intervals during the study. The same imaging method should be used throughout the study. It is important to follow the tumour assessment schedule as per the SoA. Tumour assessments will continue until RECIST1.1-defined radiological progression. Patients treated with RXC004+nivolumab that continue treatment after RECIST1.1 progression, will continue tumour assessments until second RECIST1.1 defined radiological progression (relative to the initial RXC004+nivolumab progression event).

Sites will be required to store electronic copies of all scans, and the Sponsor may arrange for possible centralised storage of all imaging data. Centralised storage of imaging data would be possible to support an independent centralized review of disease assessments.

In addition, if available, the last 2 imaging scans taken prior to the screening scan for this study and/or their reports should be kept for possible central/sponsor review.

8.1.2 Survival assessments

Assessments for survival must be made following treatment discontinuation, as indicated in the SoA. Survival information may be obtained via telephone contact with the patient or the patient's family, or by contact with the patient's current physician. The details of first and subsequent therapies for cancer, after discontinuation of treatment, will be collected. In addition, patients on treatment or in survival follow-up will be contacted within 7 days of the DCO for any survival analyses to provide complete survival data.

8.2 Safety Assessments

8.2.1 Clinical Safety Laboratory Assessments

Blood samples for determination of clinical chemistry and haematology will be taken at the visits indicated in the SoA. Additional safety samples may be collected if clinically indicated at the discretion of the investigator. The date, time of collection and results (values, units and reference ranges) will be recorded on the appropriate eCRF.

The clinical chemistry and haematology will be performed at a local laboratory at or near to the investigator site. Sample tubes and sample sizes may vary depending on laboratory method used and routine practice at the site.

The following laboratory variables will be measured.

Table 16 Laboratory safety variables

Haematology/Haemostasis (whole blood)	Clinical Chemistry (serum or plasma)
Haemoglobin (Hb)	Creatinine and creatinine clearance[a]
Total white cell count	Bilirubin, total
Neutrophils (absolute count or %)	Alkaline phosphatise (ALP)
Platelet count	Aspartate transaminase (AST)
Lymphocytes (absolute count or %)	Alanine transaminase (ALT)
	Albumin
	Potassium
Other tests:	Calcium, total
HIV antibodies[c]	Phosphate
Hepatitis B surface antigen[c]	Sodium
Hepatitis C antibodies[c]	Glucose
	Thyroid-stimulating hormone (TSH)
	Lipase
	Lactate dehydrogenase (LDH)
	Amylase
	T3 free (reflex)[b]
	T4 free (reflex)[b]
	C-reactive protein (CRP)
	Vitamin D[c]

[a] Creatinine clearance at baseline only
[b] Free T3 or T4 only need to be measured if TSH is abnormal or if Investigator judges that there may be an AE related to the endocrine system
[c] Required at baseline/screening only.

8.2.2 Physical Examinations

A physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen). A neurological exam should also be conducted at screening/baseline and thereafter as clinically indicated.

Physical examinations will be performed at timelines as specified in the SoA,

8.2.3 Vital Signs

The following vital signs will be performed at timelines as specified in the SoA;

- Blood pressure

- Pulse rate
- Temperature
- Respiration rate
- Weight
- Height (recorded at baseline only)

8.2.4 Electrocardiograms

Triplicate 12-lead ECGs will be performed at timepoints as specified in the SoA. ECGs should be obtained after the patient has been in a supine position for 5 minutes and recorded while the patient remains in that position. Mean QT intervals corrected for heart rate should be calculated using Fridericia's formula (QTcF).

All ECG traces should be kept by site for possible central/sponsor review.

At selected sites, digital ECG collection may take place using ambulatory Holter monitoring equipment for approximately 24 hours on Cycle 0 Day 1 and Cycle 1 Day 15. The information will be stored and analysed centrally.

8.2.5 ECOG performance status

ECOG performance status will be assessed at the times specified in the assessment schedules based on the following:

0. Fully active; able to carry out all usual activities without restrictions

1. Restricted in strenuous activity, but ambulatory and able to carry out light work or work of a sedentary nature (e.g. light housework or office work)

2. Ambulatory and capable of self-care, but unable to carry out any work activities; up and about more than 50% of waking hours

3. Capable of only limited self-care; confined to bed or chair more than 50% of waking hours

4. Completely disabled; unable to carry out any self-care and totally confined to bed or chair

5 Dead

8.2.6 Other Safety Assessments

Pregnancy tests

All women of childbearing potential will have pregnancy tests (urine or serum) performed at timepoints as specified in the SoA

Screening safety tests

Tests for hepatitis B surface antigen, hepatitis C antibodies, and HIV antibodies will be performed for all patients at screening.

8.3 Adverse Events and Serious Adverse Events

The Principal investigator (PI) is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

The definitions of an AE or SAE can be found in Appendix B.

AEs will be reported by the patient (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorised representative).

The investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE.

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

Adverse Events will be collected from time of signature of the main study informed consent form throughout the treatment period and until 30 days after the last dose of RXC004 (for patients treated with RXC004 monotherapy only) or 90 days after the last dose of nivolumab (for patients treated with RXC004 and nivolumab).

SAEs will be recorded from the time of signing of the main study informed consent form until the follow-up period is completed (30 days after the last dose of RXC004 [for patients treated with RXC004 monotherapy only] or 90 days after the last dose of nivolumab [for patients treated with RXC004 and nivolumab]). If an event starts after the safety follow-up period defined above is considered to be a late-onset toxicity related to study treatment, then it should be reported as an AE or SAE, as applicable.

If the investigator becomes aware of a serious adverse event with a suspected causal relationship to the investigational medicinal product that occurs after the end of the clinical study in a participant treated by him or her, the investigator shall, without undue delay, report the serious adverse event to the sponsor.

8.3.2 Follow-up of AEs and SAEs

Any AEs that are unresolved at 90 days after last dose of IP should be followed up by the

investigator for as long as medically indicated, but without further recording in the eCRF. Redx retains the right to request additional information for any participant with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Adverse event variables

'The following variables will be collected for each AE;

- AE (verbatim)
- The date when the AE started and stopped
- CTCAE grade changes
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product(s) (yes or no)
- Action taken with regard to Investigational Product(s)
- Outcome
- Whether treatment was required

In addition, the following variables will be collected for SAEs:

- Date AE met criteria for serious AE
- Date investigator became aware of serious AE
- Serious criteria
- Date of hospitalisation
- Date of discharge
- Probable cause of death
- Date of death
- Autopsy performed
- Causality assessment in relation to Study procedure(s)
- Causality assessment to other medication

8.3.3 Causality Collection

The investigator should assess causal relationship between Investigational Product and each Adverse Event, and answer ‘yes’ or ‘no’ to the question ‘Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?’

8.3.4 Adverse Events Based on Signs and Symptoms

All AEs spontaneously reported by the patient or reported in response to the open question

from the study site staff: 'Have you had any health problems since the previous visit/you were last asked?', or revealed by observation will be collected and recorded in the eCRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

8.3.5 Adverse Events Based on Examinations and Tests

The results from the Clinical Study Protocol mandated laboratory tests and vital signs will be summarised in the Clinical Study Report (CSR).

Deterioration as compared to baseline in protocol-mandated, laboratory values, vital signs, bone turnover biomarkers and DXA scans, should therefore only be reported as AEs if they fulfil any of the SAE criteria, are the reason for discontinuation of treatment with the investigational product or are considered to be clinically relevant as judged by the investigator (which may include but not limited to consideration as to whether treatment or non-planned visits were required or other action was taken with the study treatment, e.g. dose adjustment or drug interruption).

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (e.g. anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Deterioration of a laboratory value, which is unequivocally due to disease progression, should not be reported as an AE/SAE.

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE unless unequivocally related to the disease under study.

8.3.6 Hy's Law

If a patient demonstrates an AST or ALT ≥3x ULN together with total bilirubin ≥2x ULN, it must be reported to the Sponsor and/or designated CRO within 24 hours. Prompt reporting of cases meeting Hy's Law criteria (via the SAE expedited reporting system) is required for compliance with regulatory guidelines. The Investigator is responsible for immediately determining whether a patient meets potential Hy's Law criteria. The Investigator needs to perform additional examinations for this and confirm the results with the medical monitor.

8.3.7 Disease Progression

Disease progression can be considered as a worsening of a patient's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the disease under study and/or increases in the symptoms of the disease. The development of new, or progression of existing metastasis from the primary cancer under study should be considered as disease progression and not an AE. Events, which are unequivocally due to disease progression, should not be reported as an AE during the study.

8.3.8 Disease Under study

Events which are unequivocally due to disease under study should not be reported as an AE during the study unless they meet SAE criteria or lead to discontinuation of the investigational product.

8.3.9 Reporting of Serious Adverse Events

All SAEs must be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the eCRF.

If any SAE occurs in the course of the study, then investigators or other site personnel must inform the appropriate Sponsor representatives within one day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative works with the investigator to ensure that all the necessary information is provided to Patient Safety **within 1 calendar day** of initial receipt for fatal and life-threatening events **and within 5 calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform Sponsor representatives of any follow-up information on a previously reported SAE within one calendar day i.e., immediately but **no later than 24 hours** of when he or she becomes aware of it.

For further guidance on the definition of a SAE, see Appendix B of the Clinical Study Protocol.

The reference document for definition of expectedness is the IB for RXC004 and the EU/FDA/Local label for nivolumab.

8.3.10 Pregnancy

All pregnancies and outcomes of pregnancy should be reported to Sponsor representative except for if the pregnancy is discovered before the study participant has received any study

drug.

8.3.10.1 Maternal Exposure

The Sponsor and Sponsor designated Safety Services must be notified **within 24 hours** of the initial report and any follow-up reports of a female patient or a male patient's female partner becoming pregnant during the course of the study and for 5 months after the last dose of the study drug(s) via the Pregnancy Report Form. Pregnancy itself is not regarded as an adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication or a female patient or male patient's female partner experiences signs or symptoms of pregnancy complications; congenital abnormalities/birth defects and spontaneous miscarriages should be reported as SAEs. The contact information for pregnancy reporting is the same as for SAE reporting. Female patient(s) who become pregnant will be followed until the outcome of the pregnancy is known. In order for the Sponsor or designee to collect pregnancy surveillance information, the pregnant patient or partner must sign an ICF. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects in the offspring.

If a female patient becomes pregnant, administration of the study drug(s) must be discontinued immediately.

8.3.10.2 Paternal Exposure

Male participants should refrain from fathering a child or donating sperm during the study and for at least 5 months following the last dose.

Pregnancy of the participant's partners is not considered to be an adverse event. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality), occurring from the date of the first dose until 5 months after the last dose should, if possible, be followed up and documented in the Pregnancy Report Form. Consent from the partner must be obtained before the Pregnancy Report Form is completed.

8.3.11 COVID-19 infections

COVID-19 infections occurring from the time of signature of the main study ICF until 30 days after discontinuation of study treatment should be reported as adverse events. Patients who test positive for COVID-19, but do not have any symptoms should be reported as 'asymptomatic COVID-19'. If COVID-19 is suspected but not confirmed by a diagnostic test, then 'suspected COVID-19' should be reported. The AE term for the 'suspected COVID-19' should be updated when the results of the diagnostic test are known to reflect the confirmed COVID-19 or alternative diagnosis (e.g. common cold, influenza etc).

8.3.12 Dysgeusia

Loss of taste (dysgeusia) is an on-target adverse event known to occur with porcupine inhibition (Ng et al 2017, Janku et al 2015). According to CTCAEv5, there are only two grades of dysgeusia;

Grade 1 – Altered taste but no change in diet

Grade 2 – Altered taste with change in diet (e.g., oral supplements); noxious or unpleasant taste; loss of taste.

As dysgeusia is poorly characterized by CTCAE grade alone, site research personnel will complete a taste assessment (consisting of a specific set of questions and an oral examination) at screening for each patient enrolled, and for any patient who reports dysgeusia in response to the open question from the study site staff; 'Have you had any health problems since the previous visit/you were last asked?. In such cases, the assessment will be performed at each visit until the adverse event has resolved. The information will be recorded and analysed as part of the overall safety objective of the study.

8.3.13 Adverse Events of Potential Interest (AEPI)

As discussed in Section 2.3.1, Colitis events and Bone Toxicity events are classified as AEPIs for RXC004. This means that additional information will be captured on the case report forms for any CTCAE preferred terms that fall into these categories. These terms will be defined in the CRF Guidelines and reviewed at any update of the CTCAE definitions.

8.4 Overdose

For this study, any dose of RXC004 greater than the daily assigned dose will be considered an overdose.

Investigators should be advised that any patient who receives an overdose should be monitored closely, managed with appropriate supportive care and followed up expectantly. There are no data regarding RXC004 overdose in humans. An overdose and AEs should be treated as per standard medical practice.

Dosing details should be captured in the eCRF. If the subject receives a dose of a study drug that exceeds protocol specifications and the subject is symptomatic, then the symptom(s) should be documented as AEs in the eCRF and, if serious, submitted to the Sponsor's designated safety contact on an SAE Report Form. Do not record the overdose as an AE if the subject is not symptomatic.

Please refer to the local label for management of nivolumab overdoses.

8.5 Human Biological Samples

Instructions for the collection and handling of biological samples will be provided in the study specific laboratory manual. Samples should be stored in a secure storage space with adequate measures to protect confidentiality. For further details on Handling of Human Biological Sample see Appendix C.

Samples will be stored for a maximum of 15 years from the date of the issue of the CSR in line with consent and local requirements, after which they will be destroyed/repatriated.

- Pharmacokinetic (PK) samples will be disposed of after the Bioanalytical Report finalization or six months after issuance of the draft Bioanalytical Report (whichever is earlier), unless consented for future analyses.
  - Pharmacokinetic samples may be disposed of or anonymised by pooling. Additional analyses may be conducted on the anonymised, pooled pharmacokinetic samples to further evaluate and validate the analytical method. Any results from such analyses may be reported separately from the Clinical Study Report (CSR).

8.5.1 Pharmacokinetics

RXC004 PK data will be summarised using descriptive statistics (N, mean, SD, median, min, max for all parameters, also geometric mean for C_{max} and AUC) to determine the pharmacokinetic behaviour of RXC004 on multiple dosing and to confirm relevant exposure to RXC004. This may also permit investigation of any significant metabolites. Venous blood samples (2 mL) for determination of concentrations of RXC004 in plasma will be taken at the times described in the schedule of assessments (Table 1 and Table 2). The date and time that the last dose of RXC004 was administered before the PK assessment day, the date and time of the dose of RXC004 administered on the PK assessment day and the date and time of collection of each sample will be recorded.

The date and time of collection of each sample will be recorded.

The timing of the PK samples may be adjusted during the study, dependent on emerging data, in order to ensure appropriate characterisation of the plasma concentration-time profiles. The total number of samples and the total volume of blood taken from each patient will not exceed that outlined in the Lab Manual.

If a patient misses any doses of RXC004 within 3 days of PK sampling, please contact the Sponsor representative as to any effect on the changes required on the timing of the PK assessments. All other assessments, including laboratory safety assessments, vital signs and RECIST should continue to be performed as per study plan, relative to baseline assessments.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

8.5.1.1 Determination of Drug Concentration

Samples for determination of RXC004 concentrations in plasma will be analysed using an appropriate bioanalytical method. Full details of the analytical method used will be described in a separate bioanalytical report.

All samples still within the known stability of the analytes of interest at the time of receipt by the bioanalytical laboratory will be analysed.

In addition, the pharmacokinetic samples may be subjected to further analyses in order to investigate the presence and/or identity of drug metabolites. Any results from such analyses will be reported separately from the Clinical Study Report.

PK parameters will be estimated for each patient using WinNonlin® (Phoenix 64 V8 or higher) or suitable alternative. The following parameters will be derived, where appropriate, from the individual plasma concentration versus time profiles of RXC004.

Table 17 Definition of PK Parameters

PARAMETER	DEFINITION
C_{max}	The maximum observed concentration.
t_{max}	The time at which C_{max} was apparent.
AUC_{0-t}	The area under the concentration versus time curve from time zero to the sampling time at the last quantifiable concentration (C_t) at t_{last} (the time of the last quantifiable concentration) calculated by the linear trapezoidal rule.
λ_z	The apparent terminal rate constant, estimated using the negative slope of the least square regression analysis of the log concentration versus time data for the terminal linear portion of the curve.
$t_{½}$	The apparent terminal half-life, calculated from $Log_e 2 / \lambda_z$.
$AUC_{0-\infty}$	The area under the concentration-time curve estimated from time zero to infinity as the sum of the two areas: AUC_{0-t} and AUC_{extrap}, where AUC_{extrap} is calculated as C_t / λ_z.
CL	The systemic clearance calculated as: Dose/ $AUC_{0-\infty}$.
V_{ss}	The apparent volume of distribution at steady state calculated as: Dose/AUC x (AUMC/ $AUC_{0-\infty}$ - T/2) where T is the duration of intravenous injection.

Where appropriate the following RXC004 plasm pharmacokinetic parameters will be estimated. include

• **C_{max}**	Maximum observed plasma concentration
• **t_{max}**	Time to C_{max}

• **C_{min}**	Minimum observed concentration across the dosing interval
• **λz**	Terminal rate constant
• **t½**	Terminal half-life
• **AUC0-24 and from zero to infinity**	Area under the plasma concentration-time curve from zero to 24 hours
• **AUC_{0-t}**	Area under the plasma concentration-time curve from zero to the time of the last quantifiable concentration
• **$AUC_{0-\infty}$**	Area under the plasma concentration-time curve from zero to infinity
• **AUC_{τ}**	Area under the plasma concentration-time curve across the dosing interval
• **CL/F**	Total plasma clearance after oral administration
• **Vz/F**	Apparent volume of distribution after oral administration
• **$V_{ss/F}$**	The apparent volume of distribution at steady state
• **MRT**	Mean residence time
• **R_{ac}**	Accumulation ratio based on AUC_{τ} and C_{min} after the first and the last dose
• **Swing**	(Cmax-Cmin)/Cmin

Dose proportionality of exposure parameters will be explored. In addition to dose normalisation of Cmax and AUC values.

Additional PK parameters may be calculated as appropriate.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples. The results from the evaluation, if performed, will be reported in a separate Bioanalytical Report.

8.5.2 Pharmacodynamics

8.5.2.1 Collection of Samples

CCI [redacted]

CCI [redacted]

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CCI

8.6 Human Biological Sample Biomarkers

8.6.1 Collection of mandatory samples for biomarker analysis

By consenting to participate in the study the subject consents to participate in the mandatory research components of the study.

Samples for biomarker research are required and will be collected from all participants in this study as specified in the SoA. CCI
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Details for collection, volumes, storage, and shipment of biologic samples will be detailed in a separate Laboratory Manual

CCI

All enrolled patient's tumours will be required to have a genetic mutation which is predicted to be loss of function in RNF43, or an RSPO fusion/translocation detectable in CCI
CCI Eligible patients also need to have documented MSS status. Eligible subjects will be identified by either a central genetics screening approach or local laboratory assessments. Please see Section 5.5 for more details

If patients are enrolled in the study based on local laboratory assessments, then an CCI
CCI will be collected at screening and sent for central genetic screening as described in Section 5.5. If an CCI is not available or not consented to, then a sample of the study baseline tumour biopsy may be sent for the central genetic screening.

Exploratory biomarkers include;

CCI

The study mandates that CCI are collected at baseline and 'on treatment' at time points specified in the SoA. CCI that may be assessed include (but are not limited to), CCI
CCI. The CCI of these CCI may be correlated to clinical response.

CCI

CCI may be prepared from whole blood samples obtained from patients at timepoints specified in the SoA. Quantification of CCI and/or CCI

CCI

CCI Analysis may include (but is not limited to), the CCI of CCI. Correlations with outcome data may be performed on CCI with the aim of identifying useful CCI thresholds for identifying patients likely to receive benefit.

CCI

Blood samples for analysis of CCI or CCI will be obtained from all patients as specified in the SoA. Overall CCI or CCI and/or CCI in CCI may be assessed using CCI CCI

CCI

Blood samples for analysis of CCI will be obtained from patients at timepoints specified in the SoA. The concentrations of a panel of relevant CCI CCI CCI Such measurements may be correlated with response.

CCI

CCI will be taken from a subset of patients recruited by sites within close proximity to Redx Pharma. CCI CCI will be used for analysis of CCI using CCI Such measurements may be correlated with response.

CCI

CCI frequently increased in CCI. Blood samples for CCI will be obtained from patients at timepoints specified in the SoA and analysed locally. CCI may be correlated with response.

8.6.2 CCI

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8.6.3 CCI

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9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The statistical hypotheses for testing the activity of monotherapy and the combination independently are:

$H_0 : p \leq p_0$

$H_1 : p \geq p_1$

Where H_0 is the null hypothesis, p is the observed response rate, p_0 is the response rate for available treatment options – CCI, H_1 is the alternative hypothesis and p_1 is the target response rate – CCI. For RXC004 monotherapy the primary summary measure is the disease control rate (DCR) and the corresponding p_0 and p_1 are CCI% and CCI%. For the combination of RXC004 and nivolumab the primary summary measure is objective response rate (ORR) and the corresponding p_0 and p_1 are CCI% and CCI%.

9.2 Sample Size Determination

The method by Frewer et al (Frewer et al 2016), together with the totality of the data collected, will be used to guide go/no-go decision making:

For the combination RXC004+nivolumab arm, assuming a tCCI rate of CCI% response rate which is considered to be clinically significant in the target population, against a reference value of CCI% response rate (Eng et al 2019) as a CCI, then a sample size of 20 achieves greater than CCI% power, at the CCI
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CCI
CCI These hurdles will be used to guide the decision for onward development. The totality of the data, including the population enrolled and the results from the secondary endpoints will be used for the final decision.

For the monotherapy RXC004 arm, assuming a CCI% disease control rate which is considered to be clinically significant improvement over standard of care in the target population, against a reference value of CCI% DCR (Grothey et al 2013 and Mayer et al 2015) as a CCI, then a sample size of 20 achieves greater than CCI% power, at the CCI
CCI
CCI
CCI These hurdles will be used to guide the decision for onward development. The totality of the data, including the population enrolled and the results from the secondary endpoints will be used for the final decision.

Approximately CCI patients are expected to be enrolled in order for approximately 20 patients to be in the evaluable dataset in each treatment arm.

Note: "Enrolled" means the subject has signed the main study ICF, completed screening and been dosed.

The intention of the trial is to recruit patients with tumours with either RNF43 LoF mutation or RSPO fusion/translocation tumours to ensure the optimum decision is made at the end of the trial. If, after approximately half the patients have been recruited it is found that patients with tumours harbouring RNF43 LoF mutation or RSPO fusions dominate recruitment and at that time the results are such there is a CCI % probability of the CCI being met in patients with one of the aberrations then recruitment may be focussed on patients with the alternative aberration. For example, if all 10 patients in both arms have RNF43 LoF mutation tumours and no patients respond on the combination arm and/or 3 or less have disease control on the monotherapy arm then recruitment in one or both arms may be focussed on patients with RSPO fusion/translocation tumours and recruitment expanded to allow 20 patients with RSPO fusion/translocation tumours to be recruited.

9.3 Populations for Analyses

The following populations are defined:

Table 18 Populations for Analysis

Population/Analysis set	Description
Full analysis set / Safety analysis set	All subjects who enrolled and received at least one dose of study drug (RXC004 in the case of the monotherapy arm and at least one of RXC004 and nivolumab in the combination arm)
CCI	CCI CCI CCI CCI CCI CCI CCI
PK analysis set	All subjects in the safety analysis set who have had at least 1 blood sample

9.4 Statistical Analyses

A detailed description of the statistical summaries and analyses will be presented in the Statistical Analysis Plan (SAP) which will be finalised prior to DBL.

9.4.1 General Considerations

The study data is planned to be analysed and reported based on all subject data at the primary completion data cut-off date (approximately 24 weeks after cycle 1 day 1 for the last subject in), or at the End of Study, whichever comes first.

All analyses, summaries and listings will be performed using SAS software (version 9.3 or higher). For categorical variables the frequency and percentage will be presented. For continuous variables, the sample size (n), mean, median, and standard deviation, as well as the minimum and maximum values will be presented. Missing data will not be imputed unless otherwise stated.

A baseline assessment will be defined as the last assessment performed prior to the first dose of study treatment. While many of these assessments will be performed on the day of the first dose, others will be performed during screening. If a patient is missing an assessment typically performed on the day of the first dose, screening values may be substituted as baseline

Data will be presented separately for the monotherapy arm and the combination arm. There will be no formal comparisons of the results in the two arms. ORR, DCR and PFS will be presented separately for the patients who received RXC004+nivolumab as combination therapy as sequential therapy after progression on RXC004 monotherapy at first RECIST progression scan.

9.4.1.1 Patient Disposition

Patient disposition will be presented together with withdrawals from treatments, withdrawals from the study, and the reason for withdrawal.

9.4.1.2 Protocol Deviations

Protocol deviations will be collected and reviewed during the study. The final classification and effect of each protocol deviation as well as the composite effect of a set of protocol deviations on analysis populations will be assessed prior to the database lock.

9.4.1.3 Demographic and other baseline characteristics

Demographic and baseline characteristics will be summarised. For continuous demographic variables, results will be summarized and presented as n, mean, standard deviations, median and minimum and maximum values. For categorical variables the frequency and percentage of subjects will be used.

9.4.1.4 Medical History

Medical History will be coded according to the latest version of Medical Dictionary for Regulatory Activities (MedDRA). The number (percent) of patients reporting a history of any disease related medical condition, as recorded on the CRF, will be summarized by system

organ class (SOC) and preferred term (PT) for the FAS.

9.4.1.5 Prior and concomitant therapy

All investigator terms for medications recorded in the eCRF will be coded to standard names using the World Health Organization Drug Dictionary and further coded to the appropriate Anatomical-Therapeutic-Chemical code.

Prior medications will be defined as medications that stopped before the first dose of study drug and will be summarised and listed.

Concomitant medications will be defined as medications that (1) started before the first dose of study drug and were continuing at the time of the first dose of study drug or (2) started on or after the date of the first dose of study drug. Concomitant medications will be summarised and listed.

9.4.2 Efficacy

9.4.2.1 Primary Endpoint(s)

The primary endpoint for the monotherapy arm is disease control rate and the primary endpoint for the combination arm is the objective response rate, both based on a individuals best overall response using investigator RECIST 1.1 assessments.

Objective response rate is defined as the proportion of subjects with a best overall response of complete response or partial response, based on local investigator assessment, as defined in RECIST 1.1. Confirmation of response is required for declaring PR or CR as the BOR. A confirmed response is defined as a CR or PR followed by a CR or PR a least 4 weeks later. The ORR will be summarised with accompanying 90% confidence interval calculated using the Clopper Pearson method. This will be calculated on the full analysis set and the evaluable analysis set with the evaluable analysis set calculation being considered primary. In the summary tables the incidence of unconfirmed responses will be presented as well as the patients with confirmed responses.

Disease control rate (DCR) is defined as the proportion of patients with a BOR of either CR, PR or stable disease (SD) for at least 16 weeks post baseline (corresponding to SD for 2 scheduled scans post baseline). A time window of 1 week around the week 16 visit will be applied. The DCR and associated 90% confidence interval, calculated using the Clopper Pearson method will be presented. This will be calculated on the full analysis set and the evaluable analysis set with the evaluable analysis set calculation being considered primary.

9.4.2.2 Secondary Endpoint(s)

Progression free survival is defined as the time from first dose of study treatment until the date of disease progression or death (by any cause in the absence of progression), regardless whether the subject withdraws from the assigned study treatment or receives another

anticancer prior to progression. Patients who have not progressed or died at the time of analysis will be censored at the time of the latest date of RECIST v1.1 assessment of their last evaluable scan, however if the subject progresses or dies after 2 or more missed scheduled scanning visits, the subject will be censored at the time of their last evaluable scan prior to the missing scan visits. If the subject has no evaluable visits or does not have baseline data they will be censored at 0 days unless they die within 2 scan visits of baseline. Progression free survival will be listed by subject and will be presented graphically using Kaplan-Meier plots. Median PFS, together with 3 month, 6 month and 12 months PFS rates will be estimated.

Percentage change in tumour size will be derived at each visit by the percentage change from baseline in the sum of diameters of target lesions. The best percentage change in tumour size will be the subjects value representing the largest decrease (or smallest increase) from baseline in tumour size. Best percentage change in tumour size will be presented in waterfall plots and percentage change in tumour size at each visit will be presented in a spider plot.

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Overall survival is defined as the time from first day of study treatment until death due to any cause. Any subject not known to have died at the time of analysis will be censored based on the last recorded date on which the subject was known to be alive. Overall survival will be listed by subject and presented graphically using Kaplan-Meier plots. Median, 6 month and 12 month OS rates will be estimated.

9.4.2.3 Tertiary/Exploratory Endpoint(s)

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9.4.3 Safety

In general, safety data will be reported according to treatment initially received – i.e. RXC004

monotherapy or RXC004 + nivolumab combination. In order to explore any difference in the subjects who just received monotherapy compared to those who receive combination after progressing at the 8-week scan some safety data presentations will split the RXC004 into two groups – the monotherapy only subjects and the monotherapy followed by combination subjects as well as presenting them together. The tables that will be produced this way will be detailed in the SAP.

9.4.3.1 Adverse events

Treatment emergent AEs (TEAEs) are defined as those AEs with an onset after dosing and those pre-existing AEs that worsen after the start of dosing and within 30 days of stopping RXC004 monotherapy or 90 days after the last actual nivolumab dose (for patients on RXC004 and nivolumab combination). For AEs, verbatim terms in the eCRF will be mapped to preferred terms and system organ class using the Medical Dictionary for Regulatory activities (version 22.0 or later) CTCAE criteria v5.0 will be used to grade the severity of AEs. The incidence of treatment emergent AEs will be summarised by preferred term and system organ class as well as severity. Treatment related TEAEs will be summarised in the same way. Subject incidence of SAEs will also be produced.

By subject listings of all AEs, deaths, SAEs and TEAEs leading to discontinuation will also be produced.

9.4.3.2 Laboratory parameters

Shift tables to characterize changes from baseline to on treatment will be presented for haematology, clinical chemistry and coagulation parameters. Laboratory tests with numerical grading criteria in the NCI CTCAE v5.0 will be graded accordingly. Grade 0 will be assigned for all non-missing values not graded as 1 or higher. For laboratory tests where grades are not defined by CTCAE v5.0 results will be classified as low, normal or high based on laboratory normal ranges,

9.4.3.3 Vital signs

Vital signs data will be presented in listings. Abnormal vital signs values will be identified as those outside (above or below) the reference range. Vital signs data will be summarized using descriptive statistics.

9.4.4 Other Analyses

Other analyses that may be deemed necessary will be outlined and presented in the SAP.

9.5 Interim Analyses

There are no formal interim analyses planned.

9.6 Safety Monitoring Committee

A safety monitoring committee (SMC) will be appointed to review safety data from patients in this study and a second study of RXC004 in patients with pancreatic and biliary tract cancer (RXC004/0003).

The SMC will comprise experts in the disease areas under investigation, who may be study investigators, a biostatistician and an independent bone metabolism expert. The SMC will be convened to review the safety and tolerability of RXC004 at regular intervals.

The safety reviews will start when approximately [redacted] patients across the phase 2 clinical trial programme have been dosed for at least 1 cycle of study treatment and will continue every 6 months until the phase 2 studies close or last patient discontinues study treatment (whichever occurs first). A safety review of the combination data will be conducted after [redacted] patients have received the combination of RXC004 and nivolumab for at least 1 cycle. Additional safety reviews may be performed at other times as deemed appropriate by the Sponsor.

Since the studies are open label, there are no blinding issues associated with performing interim safety reviews. Full details of the SMC procedures, processes, and analyses can be found in the SMC Charter.

10 APPENDICES

Appendix A Regulatory, Ethical, and Study Oversight Considerations

A 1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC and applicable Regulatory Authority approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- Redx will be responsible for obtaining the required authorisations to conduct the study from the concerned Regulatory Authority. This responsibility may be delegated to a CRO but the accountability remains with Redx.

Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- For all studies except those utilizing medical devices investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

A 2 Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial

information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests, this includes any changes to financial interests throughout the study and for 1 year after the final CSR.

A 3 Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the patient or his/her legally authorised representative and answer all questions regarding the study.
- Patients must be informed that their participation is voluntary and they are free to refuse to participate and may withdraw their consent at any time and for any reason during the study. Patients or their legally authorised representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study centre.
- The medical record must include a statement that written informed consent was obtained before the patient was enrolled in the study and the date the written consent was obtained. The authorised person obtaining the informed consent must also sign the ICF.
- Patients must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorised representative.

The ICF will contain a separate section that addresses and documents the collection and use of any mandatory and/or optional human biological samples. The investigator or authorised designee will explain to each participant the objectives of the analysis to be done on the samples and any potential future use. Participants will be told that they are free to refuse to participate in any optional samples or the future use and may withdraw their consent at any time and for any reason during the retention period.

A 4 Data Protection

- Patients will be assigned a unique identifier by the sponsor. Any patient records or datasets that are transferred to the sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred.
- The patient must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure and use of their data must also be explained to the patient in the informed consent

- The patient must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorised personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

A 5 Dissemination of Clinical Study Data

A description of this clinical study will be available on http://www.clinicaltrials.gov, as will the summary of the main study results when they are available. The clinical study and/or summary of main study results may also be available on other websites according to the regulations of the countries in which the study is conducted.

A 6 Data Quality Assurance

- All patient data relating to the study will be recorded on eCRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorised site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 2 years after the last marketing application unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

A 7 Source Documents

- Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the investigator’s site.

- Data reported on the eCRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

A 8 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of patients.

The first act of recruitment is the first site open and will be the study start date.

The sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

A 9 Publication Policy

- The data generated by this study are confidential information of the Sponsor. The Sponsor will make the results of the study publicly available. The publication policy with respect to the Investigator and study centre will be set forth in the Clinical Trial Agreement.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicentre studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Appendix B Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

B 1 Definition of adverse events

An adverse event is the development of any untoward medical occurrence in a patient or clinical study participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (e.g., an abnormal laboratory finding), symptom (for example nausea, chest pain), or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

The term AE is used to include both serious and non-serious AEs and can include a deterioration of a pre-existing medical occurrence. An AE may occur at any time, including run-in or washout periods, even if no Study treatment has been administered.

B 2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-participant hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardise the participant or may require medical treatment to prevent one of the outcomes listed above.

Adverse Events (AEs) for **malignant tumours** reported during a study should generally be assessed as **Serious** AEs. If no other seriousness criteria apply, the 'Important Medical Event' criterion should be used. In certain situations, however, medical judgement on an individual event basis should be applied to clarify that the malignant tumour event should be assessed and reported as a **Non-Serious** AE. For example, if the tumour is included as medical history and progression occurs during the study, but the progression does not change treatment and/or prognosis of the malignant tumour, the AE may not fulfil the attributes for being assessed as Serious, although reporting of the progression of the malignant tumour as an AE is valid and should occur. Also, some types of malignant tumours, which do not spread remotely after a routine treatment that does not require hospitalization, may be assessed as Non-Serious; examples in adults include Stage 1 basal cell carcinoma and Stage 1A1 cervical cancer removed via cone biopsy.

The above instruction applies only when the malignant tumour event in question is a new malignant tumour (i.e., it is *not* the tumour for which entry into the study is a criterion and that is being treated by the IP under study and is not the development of new or progression of existing metastasis to the tumour under study). Malignant tumours that – as part of normal, if rare, progression – undergo transformation (e.g., Richter's transformation of B cell chronic lymphocytic leukaemia into diffuse large B cell lymphoma) should not be considered a new malignant tumour.

Life threatening

'Life-threatening' means that the participant was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the participant's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (e.g., hepatitis that resolved without hepatic failure).

Hospitalisation

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (e.g., bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the participant was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical treatment

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life threatening or result in death, hospitalisation, disability or incapacity but may jeopardise the participant or may require medical treatment to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (e.g., neutropenia or anaemia requiring blood transfusion, etc.) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse

Intensity rating scale:

The grading scales found in the revised National Cancer Institute CTCAE latest version will be utilised for all events with an assigned CTCAE grading. For those events without assigned CTCAE grades, the recommendation in the CTCAE criteria that converts mild, moderate and severe events into CTCAE grades should be used. A copy of the CTCAE can be downloaded from the Cancer Therapy Evaluation Program website (http://ctep.cancer.gov). The applicable version of CTCAE should be described clearly.

B 3 A Guide to Interpreting the Causality Question

When making an assessment of causality consider the following factors when deciding if there is a 'reasonable possibility' that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the participant actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?
- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? Or could the AE be anticipated from its pharmacological properties?
- De-challenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.
- Re-challenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped?
- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?
- Is there a known mechanism?

Causality of 'related' is made if following a review of the relevant data, there is evidence for a 'reasonable possibility' of a causal relationship for the individual case. The expression 'reasonable possibility' of a causal relationship is meant to convey, in general, that there are facts (evidence) or arguments to suggest a causal relationship.

The causality assessment is performed based on the available data including enough

information to make an informed judgment. With no available facts or arguments to suggest a causal relationship, the event(s) will be assessed as 'not related'.

Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

B 4 Medication Error

For the purposes of this clinical study a medication error is an unintended failure or mistake in the treatment process for a Redx study drug that either causes harm to the participant or has the potential to cause harm to the participant.

A medication error is not lack of efficacy of the drug, but rather a human or process related failure while the drug is in control of the study site staff or participant.

Medication error includes situations where an error.

- occurred
- was identified and intercepted before the participant received the drug
- did not occur, but circumstances were recognised that could have led to an error

Examples of events to be reported in clinical studies as medication errors:

- Drug name confusion
- Dispensing error e.g. medication prepared incorrectly, even if it was not actually given to the participant
- Drug not administered as indicated, for example, wrong route or wrong site of administration
- Drug not taken as indicated e.g. tablet dissolved in water when it should be taken as a solid tablet, tablet taken with food when it should be taken fasted.
- Drug not stored as instructed e.g. kept in the fridge when it should be at room temperature
- Wrong participant received the medication
- Wrong drug administered to participant

Examples of events that **do not** require reporting as medication errors in clinical studies:

- Participant accidentally missed drug dose(s) e.g. forgot to take medication
- Accidental overdose (will be captured as an overdose)
- Participant failed to return unused medication or empty packaging

- Errors related to background and rescue medication, or standard of care medication in open label studies

Medication errors are not regarded as AEs but AEs may occur as a consequence of the medication error.

Appendix C Handling of Human Biological Samples

C 1 Chain of custody

A full chain of custody is maintained for all samples throughout their lifecycle.

The investigator at each centre keeps full traceability of collected biological samples from the patients while in storage at the centre until shipment or disposal (where appropriate) and records relevant processing information related to the samples whilst at site.

The sample receiver keeps full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps record of receipt of arrival and onward shipment or disposal.

Redx or delegated representatives will keep oversight of the entire life cycle through internal procedures, monitoring of study sites, auditing or process checks, and contractual requirements of external laboratory providers.

Samples retained for further use will be stored in the Redx-assigned biobanks or other sample archive facilities and will be tracked by the appropriate Redx Team for the remainder of the sample life cycle.

C 2 Withdrawal of Informed Consent for donated biological samples

Redx ensures that biological samples are returned to the source or destroyed at the end of a specified period as described in the informed consent.

If a participant withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed/repatriated, and the action documented. If samples are already analysed, Redx is not obliged to destroy the results of this research.

Following withdrawal of consent for biological samples, further study participation should be considered in relation to the withdrawal processes outlined in the informed consent.

The investigator:

- Ensures participant's[2] withdrawal of informed consent to the use of donated samples is highlighted immediately to Redx or delegate.
- Ensures that relevant human biological samples from that participant, if stored at the study site, are immediately identified, disposed of as appropriate, and the action documented.
- Ensures that the participant and Redx are informed about the sample disposal.

Redx ensures the organization(s) holding the samples is/are informed about the withdrawn

consent as soon as reasonably possible and that samples are disposed of or repatriated as appropriate, and the action documented and study site notified.

C 3 International Airline Transportation Association (IATA) 6.2 Guidance Document

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) (**https://www.iata.org/whatwedo/cargo/dgr/Pages/download.aspx**) classifies infectious substances into 3 categories: Category A, Category B or Exempt

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.
Category A pathogens are e.g., Ebola, Lassa fever virus. Infectious substances meeting these criteria which cause disease in humans or both in humans and animals must be assigned to UN 2814. Infectious substances which cause disease only in animals must be assigned to UN 2900:

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are e.g., Hepatitis A, C, D, and E viruses. They are assigned the following UN number and proper shipping name

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - Substances which do not contain infectious substances or substances which are unlikely to cause disease in humans or animals are not subject to these Regulations unless they meet the criteria for inclusion in another class.

- Clinical study samples will fall into Category B or exempt under IATA regulations
- Clinical study samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging (https://www.iata.org/whatwedo/cargo/dgr/Documents/DGR-60-EN-PI650.pdf).
- Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content
- files would remain physically separate.

Appendix D RECIST 1.1 (Response Evaluation Criteria in Solid Tumours) Responses

Term	Definition
Complete response (CR)	Disappearance of all target lesions. Any pathological lymph nodes must have reduction in short axis to <10 mm.
Partial response (PR)	A ≥ 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
Progressive disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 0.5 cm. The appearance of one or more new lesions is also considered progression.
Stable disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
New measureable lesions[1]	Always represents progressive disease (PD)
New non-measureable lesions	Always represents progressive disease (PD)
Non-index lesions	Changes contribute to defining best overall response of CR, PR, SD, and PD

From RECIST v1.1

1) Measureable lesion must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10 mm by CT scan (CT slice thickness no greater than 5 mm)

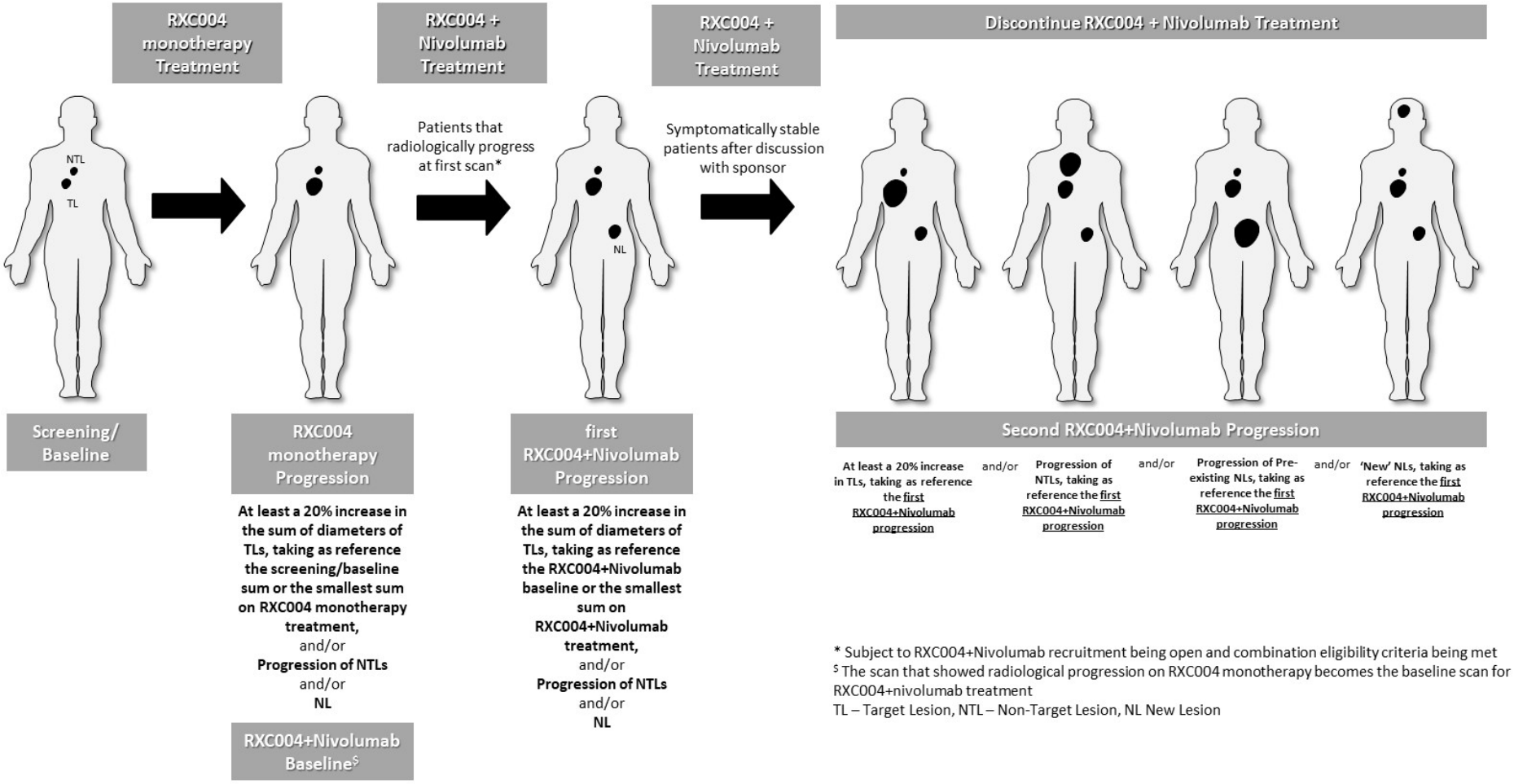
Patients with symptomatically stable disease may continue study treatment after the initial RECIST 1.1 - defined progression until symptomatic progression or second RECIST 1.1 PD assessment (relative to the initial RECIST1.1 progression for a given study treatment). A decision to continue study treatment after initial progression must be discussed and agreed by the treating physician and the Sponsor.

The criteria for continuing treatment after initial RECIST 1.1-defined progression are as follows:

- The patient does not have any significant, unacceptable, or irreversible toxicities indicating that continuing treatment will not further benefit the patient.
- There is absence of clinical symptoms or signs indicating clinically significant disease progression accompanied by a decline in ECOG performance status>1.

- There is absence of rapid disease progression or threat to vital organs or critical anatomical sites (e.g. central nervous system metastasis, respiratory failure due to tumour compression, or spinal cord compression) requiring urgent alternative medical intervention.

Figure 2 Treatment after first progression (Arm A)



* Subject to RXC004+Nivolumab recruitment being open and combination eligibility criteria being met
$ The scan that showed radiological progression on RXC004 monotherapy becomes the baseline scan for RXC004+nivolumab treatment
TL – Target Lesion, NTL – Non-Target Lesion, NL New Lesion

Figure 3 Treatment after first progression (Arm B)

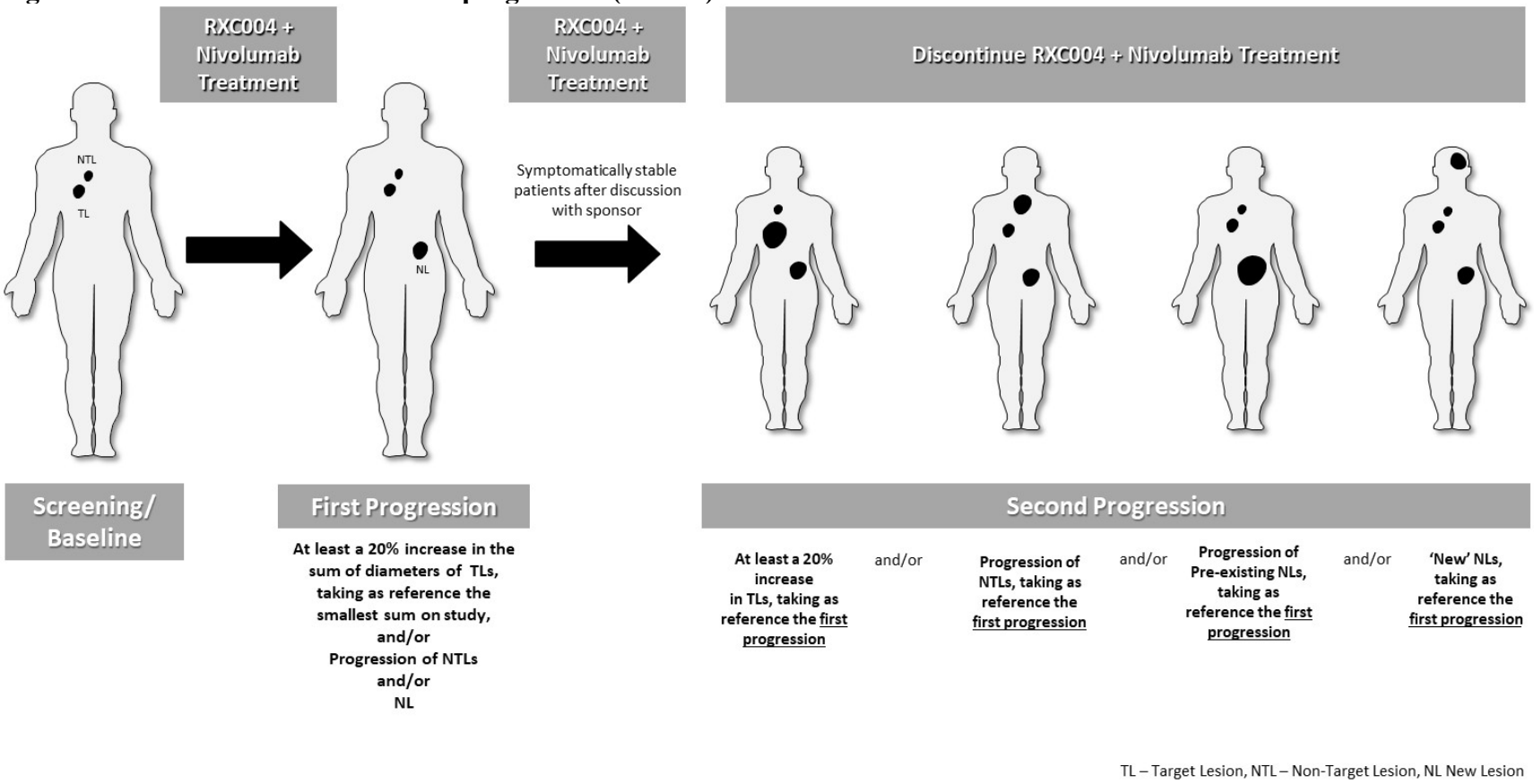
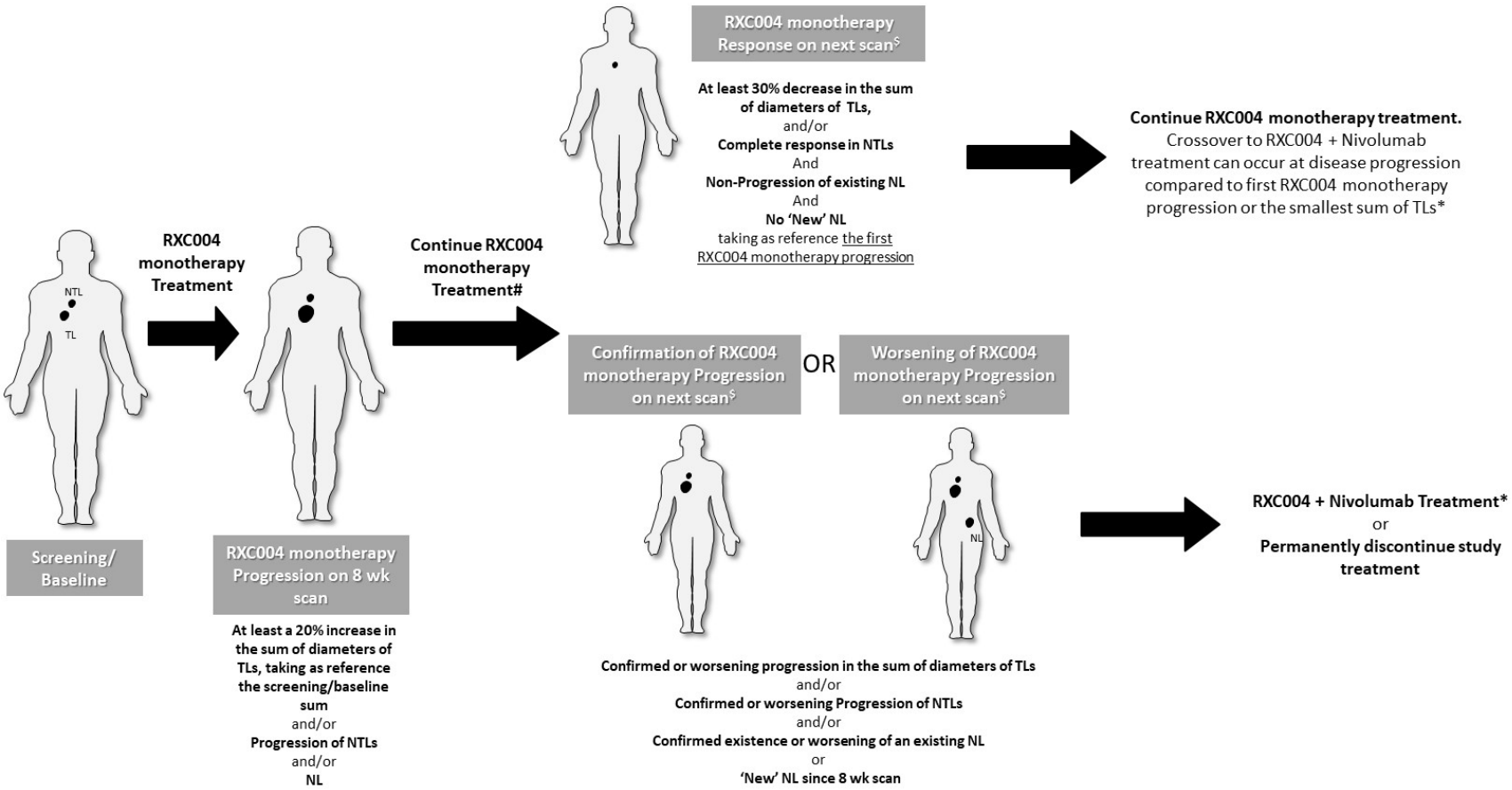


Figure 4 Treatment after first progression for patients that remain on RXC004 monotherapy (Arm A)



* Subject to RXC004+Nivolumab recruitment being open and combination eligibility criteria being met. The most recent progression scan will become the RXC004 + Nivolumab baseline scan
# Symptomatically stable patients after discussion with sponsor
$ at least 4 weeks after initial RECIST1.1 progression 8 wk (+/- 1 week) scan or scheduled 16 wk (+/- 1 week) scan, whichever occurs first
TL – Target Lesion, NTL – Non-Target Lesion, NL New Lesion

Appendix E Prohibited CYP3A4 Inhibitors, and Inducers

CYP3A4 Inhibitors **[Bold text denotes commonly used co-medications in patients with colorectal cancer]**	**CYP3A4 Inducers** **[None of these drugs are commonly used co-medications in patients with colorectal cancer]**
Strong CYP3A4 Inhibitors *Concomitant use of these drugs has the potential to increase the exposure of RXC004 over 5-fold* *These agents must not be given within 14 days of first dose of study treatment* boceprevir, cobicistat, danoprevir and ritonavir, elvitegravir and ritonavir, grapefruit juice, indinavir and ritonavir, **itraconazole**, **ketoconazole**, lopinavir and ritonavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), **posaconazole,** ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, **troleandomycin, voriconazole, clarithromycin**, idelalisib, **nefazodone,** nelfinavir,	**Strong CYP3A4 Inducers** *Concomitant use of these drugs has the potential to decrease the exposure of RXC004 by over 80%* aptalutimide, carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, St. John's wort *These agents must not be given within 14 days of first dose of study treatment*
Moderate CYP3A4 Inhibitors *Concomitant use of these drugs has the potential to increase the exposure of RXC004 by 2-5-fold* *A washout period of 14 days is recommended and a minimum washout period of 5 half-lives (See SPC or USPI) is required before first dose of study treatment.* **aprepitant, ciprofloxacin,** conivaptan, crizotinib, cyclosporine, diltiazem, dronedarone, **erythromycin, fluconazole**, **fluvoxamine,** imatinib, **tofisopam,** verapamil **lansoprazole**	**Moderate CYP3A4 Inducers** *Concomitant use of these drugs has the potential to decrease the exposure of RXC004 by 60-80%* *A washout period of 14 days is recommended and a minimum washout period of 5 half-lives (See SPC or USPI) is required before first dose of study treatment* bosentan, efavirenz, etravirine, phenobarbital, primidone
Weak CYP3A4 Inhibitors *These are not prohibited but should be used with caution as they may increase the exposure of RXC004 by 1.25 -2- fold* **chlorzoxazone,** cilostazol, **cimetidine**, **clotrimazole, fosaprepitant**, istradefylline, ivacaftor, lomitapide, **ranitidine,** ranolazine, ticagrelor, **omeprazole**	**Weak CYP3A4 Inducers** *These drugs are not prohibited- they have the potential to decrease the exposure of RXC004 by 20-50% which is within the scope of the dose reduction allowances*

	armodafinil, modafinil, rufinamide

For updated information, please refer to https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers

Appendix F RNF43/RSPO aberrations

The study will recruit patients with documented RNF43 or RSPO genetically aberrated, MSS mCRC.

Patients with the following RNF43 mutations are eligible for the study;

CCI

[redacted]

Patients with documented loss of function RNF43 mutations other than those listed above may be allowed after consultation with Sponsor.

Patients with the following RSPO fusions are eligible for the study:

CCI

[redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]

CCI [redacted]
CCI [redacted]

Patients with documented RSPO fusions other than those listed above may be allowed after consultation with Sponsor.

Appendix G Nivolumab Toxicity Management and Dose Modification Guidelines

Recommendations for nivolumab modifications are provided in the dose modification section of the nivolumab Prescribing Information and summarized here.

Table 19 Nivolumab toxicity management and dose modifications

Adverse Event	Severity	Investigations, Treatment and Dose modification
Colitis	Grade 1 diarrhoea or colitis	Investigations: FBC, UEC, LFTs, CRP, TFTs. Stool microscopy for leucocytes/ova/parasites, culture, viral PCR, C. diff. toxin and cryptosporidia Treat with oral fluids and loperamide. Avoid high fibre/lactose diet. If Grade 1 symptoms persist for >14 days treat as per Grade 2 Continue treatment
	Grade 2 diarrhoea or colitis	Investigations as per Grade 1. Exclude steatorrhea Consider abdominal X-ray and sigmoidoscopy/colonoscopy for signs of Colitis. Start prednisolone 0.5-1 mg/kg or consider oral budesonide 9 mg od if no bloody diarrhoea. Once recovered to Grade 1, wean steroids over 2-4 weeks. If no improvement in 72hrs, treat as per Grade 3. Withhold dose[a]
	Grade 3 diarrhoea or colitis	Investigations as per Grade 2 with daily FBC, UEC, LFTs and CRP Conduct Sigmoidoscopy/colonoscopy. Early surgical review if bleeding, pain or distension. Review diet (e.g. nil by mouth, clear fluids, TPN) Treat with IV (methyl)prednisolone 1-2 mg/kg. Wean steroids over 4-8 weeks. If no improvement in 72 hours start Infliximab 5 mg/kg (if no perforation/sepsis/TB/hepatitis/congestive heart failure – Must have had flexsigmoidoscopy/colonoscopy prior)[b] Withhold dose[a]
	Grade 4 diarrhoea or colitis	Investigations and treatment as per Grade 3 Permanently discontinue
Pneumonitis	Grade 1	Bloods (FBC/UEC/LFTs/Ca/ESR/CRP). Consider sputum sample screening for viral/bacterial infection.

Adverse Event	Severity	Investigations, Treatment and Dose modification
		Chest X-ray Continue treatment
	Grade 2	Investigations as per Grade 1. Monitor symptoms daily Start antibiotics if suspicion of infection. If no evidence of infection or no improvement with antibiotics after 48hrs add in prednisolone 1 mg/kg/day orally. Once improved to baseline, wean steroids over at least 6 weeks. If no improvement after 48hrs, manage as per Grade 3 Consider Pneumocystis prophylaxis depending on the clinical context High resolution CT +/- bronchoscopy and BAL pending appearances Withhold dose[a]
	Grade 3 or 4	Admit patient and perform investigations as per Grade 2. Treat with (methyl)prednisolone IV 2-4 mg/kg/day and cover with empiric antibiotics. Once improved to baseline wean steroids over at least 8 weeks. If no improvement after 48hrs add infliximab 5 mg/kg (or MMF if concurrent hepatic toxicity). High resolution CT +/- bronchoscopy and BAL pending appearances Permanently discontinue
Hepatitis	Grade 1: ALT or AST >ULN-3XULN	If > ULN-3XULN repeat in 1 week Continue treatment
	Grade 2: ALT or AST 3-5XULN	Re-check LFTs/INR/albumin every 3 days. Review medications, e.g. statins, antibiotics and alcohol history. Perform liver screen: Hepatitis A/B/C serology, Hepatitis E PCR, anti-ANA/SMA/LKM/SLA/LP/LCI, iron studies If rising ALT/AST when re-checked start oral prednisolone 1 mg/kg. Once resolved to Grade 1, wean steroids over 2 weeks. Re-escalate if worsening; nivolumab treatment may be resumed once prednisolone ≤ 10 mg

Adverse Event	Severity	Investigations, Treatment and Dose modification
		Consider imaging for metastases/clot Withhold dose[a]
	Grade 3:AST or ALT 5-20XULN	Re-check LFTs/INR/albumin every day. Other investigation as per Grade 2. If ALT/AST < 400 and normal bilirubin/INR/albumin treat with oral prednisolone 1 mg/kg. If ALT/AST > 400 or raised bilirubin/INR/low albumin treat with i.v. (methyl)prednisolone 2 mg/kg. Once improved to G2, can change to oral prednisolone and wean over 4 weeks Perform US with Doppler. Low threshold to admit if clinical concern Permanently discontinue
	Grade 4: ALT or AST >20xULN	Investigations as per Grade 3 Treat with i.v. (methyl)prednisolone 2 mg/kg. If condition worsens despite steroids, add mycophenolate mofetil (MMF) 500-1000 mg bd. If worse on MMF, consider addition of tacrolimus. Hepatology consult and consider liver biopsy Permanently discontinue
Hypophysitis	Grade 2	Pituitary axis assessment, MRI pituitary protocol (also exclude brain metastases), visual field assessment. Monitor TFTs. Start oral prednisolone 0.5-1 mg/kg od after pituitary axis assessment. If no improvement in 48h, treat as severe with i.v. (methyl)prednisolone. Wean steroids based on symptoms over 2-4 weeks to 5 mg prednisolone. Do not stop steroids Refer to or consult endocrinologist Withhold dose[c]
	Grade 3	Investigations as per Grade 2 Initiate i.v. (methyl)prednisolone 1 mg/kg after sending bloods for pituitary axis assessment. Analgesia as needed for headache. Aim convert to prednisolone and wean as symptoms allow over 4 weeks to 5 mg

Adverse Event	Severity	Investigations, Treatment and Dose modification
		Refer to or consult endocrinologist Withhold dose[c]
	Grade 4	Investigations as per Grade 1 Treatment as per Grade 3 Refer to or consult endocrinologist Permanently discontinue
Adrenal Insufficiency	Grade 2	Consider management with corticosteroids (if needed for symptoms of acute inflammation) Withhold dose[c]
	Grade 3 or 4	Permanently discontinue
Type 1 Diabetes Mellitus	Grade 3 hyperglycaemia/diabetes	Admit to hospital immediately and start treatment of newly onset type I DM. Consider management with corticosteroids (if needed for symptoms of acute inflammation) Withhold dose[c]
	Grade 4 hyperglycaemia/diabetes	Treat as per Grade 3 Permanently discontinue
Nephritis and Renal Dysfunction	Grade 1 (creatinine 1.5 x baseline or > ULN-1.5x ULN)	Review hydration status, medications, urine test/culture if urinary tract infection symptoms Dipstick urine and send for protein assessment UPCR. Repeat creatinine weekly. If worsens, manage as per Grade 2 If obstruction suspected: renal ultrasound +/- doppler to exclude obstruction/clot Continue treatment
	Grade 2: creatinine > 1.5-3x baseline or > 1.5-3x ULN	Investigation as per Grade 1 and review creatinine/K+ in 48h-72h; if not improving discuss with nephrologist and need for biopsy and if attributed to irAE, If proteinuria: for 24 h collection or UPCR If blood: phase contrast microscopy and GN screen* if nephrologist recommends Initiate steroids (oral prednisolone 0.5-1 mg/kg)

Adverse Event	Severity	Investigations, Treatment and Dose modification
		Renal ultrasound +/- doppler to exclude obstruction/clot Withhold dose[a] - Repeat creatinine/K+ every 48h If returns to G1/baseline – recommence treatment (if on steroids, only once < 10 mg prednisolone) If not attributed to irAE - may continue treatment
	Grade 3: creatinine > 3x baseline or > 3-6x ULN Serum creatinine more than 1.5 and up to 6 times the ULN	Investigation as per Grade 2, plus admit patient for monitoring and fluid balance; repeat creatinine every 24h; early discussion with nephrologist and need for biopsy; if worsening, initiate i.v. (methyl)prednisolone 1-2 mg/kg Withhold dose[a]
	Serum creatinine >6 x ULN	Investigations as per G3, plus patient should be managed in hospital where renal replacement therapy is available initiate i.v. (methyl)prednisolone 1-2 mg/kg Permanently discontinue
Skin	Grade 1 (skin rash with or without symptoms, <10% BSA)	Physical examination. Exclude other causes e.g. viral illness, infection, other drug rash etc Topical steroids (mild strength) +/- oral or topical anti-histamines may be administered Continue treatment
	Grade 2 (10-30% BSA)	Investigations as per Grade 1 Topical steroids (moderate strength) +/- oral or topical anti-histamines may be administered Consider dermatology referral and skin biopsy Continue treatment
	Grade 3 rash (>30% BSA, or 10-30% BSA with substantial symptoms) or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)	Investigations as per Grade 1 Topical treatments as above (potent). Initiate steroids: if mild to moderate 0.5-1 mg/kg prednisolone od for 3 days then wean over 1-2 weeks; or if severe i.v. (methyl)prednisolone 0.5-1 mg/kg and convert to oral steroids on response, wean over 2-4 weeks Dermatology referral and skin biopsy

Adverse Event	Severity	Investigations, Treatment and Dose modification
		Withhold dose[a]
	Grade 4 rash or confirmed SJS or TEN	Investigations as per Grade 1 i.v. (methyl)prednisolone 1-2 mg/kg Seek urgent dermatology review Permanently discontinue
Peripheral neurological toxicity (For specific recommendations for Myasthenia Gravis and Guillain-Barré syndrome, see 'other')	Grade 1	Comprehensive neurological examination Diabetic screen, B12/folate, HIV, TSH, consider vasculitic & autoimmune screen, review alcohol history & other medications Consider need for MRI/MRA brain or spine (exclude CVA, structural cause) Continue treatment
	Grade 2	Investigations as per Grade 1, plus consider NCS/EMG for lower motor neurone motor and/or sensory change Consider pulmonary function/sniff/diaphragmatic function tests and neurological consult Initial observation reasonable or initiate prednisolone 0.5-1 mg/kg (if progressing, e.g. from mild) and/or pregabalin or duloxetine for pain. Taper steroids over 4-8 weeks. If worsening, treat as per Grade 3 Withhold dose[a]
	Grade 3	Admit patient. Involve neurologist in care Daily neurological review +/- daily vital capacity MRI brain/spine advised NCS/EMG Lumbar puncture Pulmonary function assessment Initiate (methyl)prednisolone 2 mg/kg i.v. Taper steroids over 4-8 weeks. Withhold dose[a]
Encephalitis	New-onset moderate or severe neurologic signs or symptoms	Lumbar puncture, PCR for HSV, cytology and consider viral culture Oral prednisolone 0.5-1 mg/kg or i.v. (methyl)prednisolone 1-2 mg/kg if very unwell (Exclude bacterial and ideally viral infections prior

Adverse Event	Severity	Investigations, Treatment and Dose modification
		to high-dose steroids). Consider concurrent empiric antiviral (i.v. acyclovir) and antibacterial therapy CNS imaging and consider neurological consult Withhold dose[a]
	Immune-mediated encephalitis	Lumbar puncture, PCR for HSV, cytology and consider viral culture Oral prednisolone 0.5-1 mg/kg or i.v. (methyl)prednisolone 1-2 mg/kg if very unwell (Exclude bacterial and ideally viral infections prior to high-dose steroids). Consider concurrent empiric antiviral (i.v. acyclovir) and antibacterial therapy CNS imaging Permanently discontinue
Thyroid function	Hypothyroidism: Low FT4 with elevated TSH or TSH > 10 with normal FT4	Thyroxine 0.5-1.5 µg/kg (start low in elderly, if cardiac history) Continue treatment
	Thyrotoxicosis (DDx thyroiditis, Grave's disease)	Investigations: Anti-TSH Receptor Ab, anti-TPO Ab, nuclear medicine thyroid uptake scan Propranolol or atenolol for symptoms; consider carbimazole if anti-TSH Receptor Ab positive Painful thyroiditis – consider prednisolone 0.5 mg/kg and taper if unwell, Withhold dose[c]
	Grade 4 hypothyroidism or hyperthyroidism	Investigations and treatments as above. Permanently discontinue
Infusion reactions	Grade 1 or 2	Interrupt or slow rate of infusion
	Grade 3 or 4	Permanently discontinue
Other	Other Grade 3AEs - First occurrence - Recurrence of same Grade 3 adverse events	Withhold dose[a] Permanently discontinue
	Life-threatening or Grade 4 adverse reaction	Permanently discontinue
	Grade 3 myocarditis	If myocarditis is suspected, admit the patient and immediately start high-dose (methyl)prednisone (1-2 mg/kg). In the case of deterioration, consider

Adverse Event	Severity	Investigations, Treatment and Dose modification
		adding another immunosuppressive drug (MMF or tacrolimus) Permanently discontinue
	Guillain-Barré syndrome (GBS)	Nerve conduction studies (acute polyneuropathy) Lumbar puncture (elevated protein with normal WBC count). Pulmonary function tests with vital capacity and maximum inspiratory/expiratory pressures. Antibody testing for GBS variants, e.g. GQ1b in Miller Fisher variant Use of steroids not recommended in idiopathic GBS If no improvement or worsening, plasmapheresis or intravenous immunoglobulin indicated. Neurological consult. Consider location of care where ventilatory support available (required in 15%-30% idiopathic cases) Permanently discontinue
	Myasthenia Gravis	Check for ocular muscle and proximal muscle fatigability. AChR and anti-MuSK antibodies Bedside tests, e.g. Tensilon test or ice pack test with neurological input. Repetitive nerve stimulation and single fibre EMG Steroids indicated (oral or i.v. depending on symptoms). Pyridostigmine initial dose 30 mg tds. If no improvement or worsening, plasmapheresis or IVIG may be considered Additional immunosuppressants azathioprine, cyclosporine, mycophenolate Avoid certain medications, e.g. ciprofloxacin, beta-blockers, that may precipitate cholinergic crisis Neurological consult Permanently discontinue
	Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks	Permanently discontinue
	Persistent Grade 2 or 3 adverse event lasting 12 weeks or longer	Permanently discontinue

a Resume treatment when adverse reaction improves to Grade 0 or 1
b Other immunosuppressive treatment options – MMF 500-1000 mg bid or tacrolimus
c Withhold dose until symptoms resolve and any steroid treatment is complete. Treatment should be continued in the presence of hormone replacement therapy as long as no symptoms are present

Appendix H Abbreviations

Abbreviation or special term	Explanation
AE	Adverse Event
AUC	Area under the concentration-time curve
BOR	Best Overall Response
CCA	Cholangiocarcinoma
C_{max}	Maximum plasma concentration
C_{min}	Minimum observed plasma concentration
CR	Complete response
CRC	Colorectal cancer
CRO	Contract Research Organisation
CSR	Clinical Study Report
CT	Clinical Trial
CTCAE	Common Terminology Criteria for Adverse Events (National Cancer Institute)
DBL	Data Base Lock
DCO	Data Cut Off
DILI	Drug-induced liver injury
DLT	Dose-limiting toxicity
DNA	deoxyribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
eCRF	electronic Case Report Form
ESMO	European Society for Medical Oncology
EU	European Union
FDA	U.S. Food and Drug Administration
CCI [redacted]	CCI [redacted]
FSI	First Subject In
Fz	Frizzled
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC_{50}	Half maximal inhibitory concentration
ICF	Informed Consent Form
IRT	Interactive response technology
LoF	Loss of function
LRP	low-density lipoprotein receptor-related protein

Abbreviation or special term	Explanation
mCRC	Metastatic colorectal cancer
MSI-H	Microsatellite instability high
MSS	Microsatellite stable
NCCN	National Comprehensive Cancer Network
PD-1	Programmed death receptor 1
PDAC	Pancreatic ductal adenocarcinoma
PI	Principal Investigator
PORCN	Porcupines
PR	Partial response
QoL	Quality of life
RECIST	Response Evaluation Criteria In Solid Tumours
RNF43	Ring finger protein 43
RSPO	R-Spondin
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable disease
SmPC	Summary of Product Characteristics
SoA	Schedule of Assessments
SOC	Standard of Care
SRC	Safety Review Committee
TEAE	Treatment-emergent adverse event
TRAE	Treatment-related adverse event
TSH	Thyroid-stimulating hormone

Appendix I Dysgeusia treatment guidelines

As there are no recognised standard international guidelines for the management of this multifactorial event, investigators are advised to consult their local practice guidelines for the management of dysgeusia. Please refer to Table 14 and Section 6.6 for instructions on dose reductions and interruptions for dysgeusia.

Some interventions may be effective in treating dysgeusia:

• Treatment with oral pilocarpine and artificial saliva may help in cases where there is also dry mouth
• Zinc supplements may help in cases where there is evidence of zinc deficiency
• Alpha lipoic acid is an antioxidant capsule supplement which may improve flavour sensation
• Good oral hygiene and chlorhexidine (or similar) mouthwashes

Patients may be advised of some ways that they can manage dysgeusia, for example:

• Cooking or eating food in non-metallic cookware
• Avoiding foods that taste bitter or metallic
• Flavouring foods with seasonings and spices
• Eating food cold may help to reduce unpleasant flavour sensations
• Frequently brushing teeth
• Rinsing with mouth wash regularly
• Using chewing gum. Lozenges or mints to stimulate saliva production

A dysgeusia treatment algorithm has been developed by Sevryugin et al 2021 based on their review of published studies of dysgeusia interventions and is included to assist investigators with the management of dysgeusia that occurs during treatment with RXC004. However, it is acknowledged that not all of the management suggestions may be readily available or applicable to patients in this study.

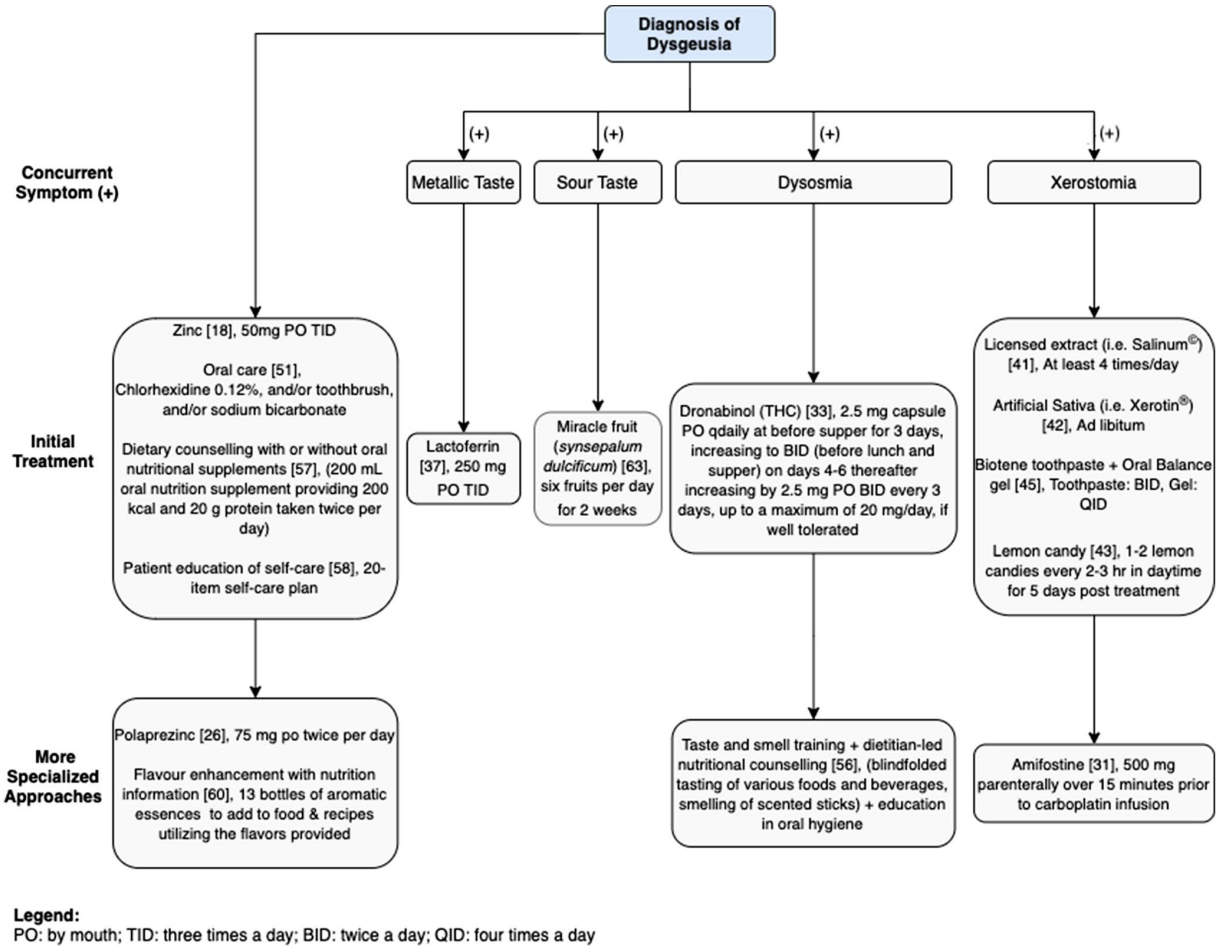


Figure from Sevryugin et al 2021

Appendix J Management of Colitis Events

In the Phase 1 study of RXC004, events of colitis or enteritis were reported in 4 patients who commenced treatment at doses of 3 mg QD or 10 mg QD as a monotherapy. One event of ileitis has also been reported to date in this study.

RXC004-related diarrhoea/colitis may present as abdominal pain with other symptoms including intermittent constipation, diarrhoea, nausea and/or vomiting. In some cases, symptoms may be minimal. Blood and mucus in the stool may not be apparent.

Symptoms may be preceded by or accompanied by a raised CRP and neutrophil count.

If lower GI tract inflammation is suspected, infectious and other causes should be excluded, including CMV and *C .difficile*. Attempts should be made to definitively diagnose the condition with a CT scan, and colonoscopy/sigmoidoscopy with biopsy, if clinically safe to do so.

Nivolumab is known to cause Colitis (See Appendix G) so patients receiving the combination of RXC004 and nivolumab may be at higher risk of this event. These guidelines are consistent with guidance for nivolumab-related colitis. However, for patients who are receiving RXC004 plus nivolumab, please also refer to the most current version of the nivolumab SPC/USPI.

Management:

Monitoring of CRP or fecal calprotectin levels may help to monitor the response to treatment.

Table 20 Management of colitis events

Grade	Management
Grade 1 colitis [asymptomatic but evidence on CT scan]	Continue RXC004 at a lower dose and repeat the CT scan within 4 weeks. No action is needed for nivolumab. No additional treatment is indicated. If the Grade 1 colitis is evident on the second scan despite the lower dose of RXC004, it should be managed as Grade 2 colitis and the scan should be repeated within 4 weeks.
Grade 2 colitis [abdominal pain, mucus or blood in stool]	Pause RXC004 (and nivolumab if applicable) Manage with corticosteroids at a dose of 0.5 mg/kg methylprednisolone equivalent. When symptoms have resolved, taper over 2-4 weeks.

Grade	Management
	If symptoms recur during tapering, the steroid dose may need to be increased again. If no improvement in 72 hrs despite treatment, treat as Grade 3 RXC004 can be restarted at a lower dose after colitis has resolved to Grade ≤ 1* and steroids tapered to physiological levels (<10 mg prednisone per day equivalent dose) over at least 1 month. If steroids cannot be tapered to physiological levels within 12 weeks of commencing steroid treatment, then RXC004 (and nivolumab if applicable) should be permanently discontinued.
Grade 3 colitis [severe or persistent abdominal pain, fever, ileus; peritoneal signs]	Pause RXC004 (and nivolumab, if applicable) Seek specialist advice Manage with corticosteroids at a dose of 1 mg/kg methylprednisolone equivalent. When symptoms improve, taper steroids to physiological levels over 4-8 weeks and consider use of prophylactic antibiotics and anti-fungals If the patient is receiving RXC004 as a monotherapy, RXC004 can be restarted at a lower dose after colitis has resolved to Grade ≤ 1* and steroids tapered to physiological levels (<10 mg prednisolone equivalent dose)**. If the patient is receiving RXC004 in combination with nivolumab, both agents should be permanently discontinued** If steroids cannot be tapered to physiological levels within 12 weeks of commencing steroid treatment, then RXC004 (and nivolumab, if applicable) should be permanently discontinued. Additional anti-inflammatory medications (e.g infliximab) may be indicated if no improvement in 48-72hrs. **It is**

Grade	Management
	important to rule out bowel perforation and refer to infliximab SMPC/USPI for general guidance before using infliximab If anti-inflammatories are required, RXC004 (and nivolumab, if applicable) should be permanently discontinued.
Grade 4 colitis [life threatening consequences]	Manage as per Grade 3 Colitis. **RXC004 and nivolumab (if applicable) should be permanently discontinued.**

*Attempts should be made to confirm that the colitis has resolved to Grade ≤ 1 (e.g normalised CRP or fecal calprotectin if raised during event; GI appearance normalised on X ray/ CT or endoscopy) before restarting RXC004.

If there is any recurrence of colitis ≥ Grade 2 after recommencing RXC004, **RXC004 and nivolumab, if applicable should be permanently discontinued.**

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