RXC005, a Potent and Selective, Reversible BTK Inhibitor Targeting both Wild-type and Mutant C481S BTK with Potent Efficacy in ABC-DLBCL Xenograft Mouse Models

Abstract #219
Poster #210

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INTRODUCTION

Bruton's tyrosine kinase (BTK) is a member of the Src-related Tec family of cytoplasmic tyrosine kinases and plays a key role in the BCR signaling pathway, which is required for the development, activation and survival of B-cells.

BTK is a clinically validated target to treat B-cell malignancies that are dependent on BCR signaling *i.e.* CLL and NHL with ibrutinib approved for the treatment of CLL, MCL and WM.

Irreversible and covalent reversible BTK inhibitors such as ibrutinib, acalabrutinib and GS-4059 specifically target a cysteine residue C481 within BTK and mutations at this site clearly interfere with covalent drug binding. C481S, C481Y, C481R, C481F mutations have been reported and linked to cases of resistance that have emerged in patients with CLL progression following treatment with ibrutinib.

Redx development candidate, RXC005, aims to overcome this resistance mechanism by targeting both wild type and C481-mutated BTK.

METHODS

Kinase binding assays (LanthaScreen™)
Kinase binding affinity was determined using TR-FRET assay.

Kinase biochemical activity assays (Z-Lyte™)
Biochemical activity determined using a synthetic FRET peptide to measure phosphorylation of the desired kinases.

Cellular kinase assay (ClariCELL™)
Tyrosine phosphorylation of BTK in HEK293 cells transfected with either BTK WT or BTK C481S vectors measured by ELISA.

Inhibition of BTK WT and BTK C481S by immunoblot
DT40 LV125 BTK C481S cells (10^7 cells/condition) reconstituted in 1 mL fresh RPMI, 15% FBS, 1% pen/strep/glut media. The cells were drugged for 2 h at 37 °C in a six well plate. The 1 µM ibrutinib condition was washed out by centrifugation and the cells were reconstituted in fresh media. Soluble chicken IgM stimulation at 10 µg/mL for 15 min at 37 °C. The 1 µM ibrutinib condition rested for 1 h prior to lysing.

Anti-proliferative activity
Proliferation measured in the CD79A mutated OCI-Ly10 and TMD8 cell lines of the activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL) subtype. Metabolically active cells are measured using resazurin.

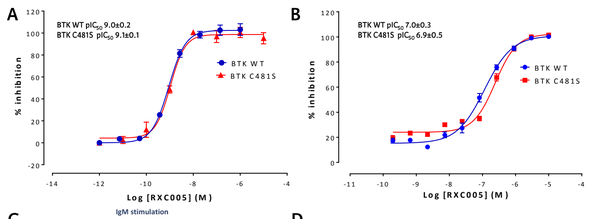
Inhibition of B-cell activation and BTK inhibition
PBMCs treated continuously before stimulation with anti-IgM for 6 h. Human whole blood treated with compound and stimulated with anti-IgD for 6 h and red blood cells are lysed. The percentage of CD69 positive B-cells (CD19 positive) are measured by flow cytometry.

Inhibition of BCR signaling and BTK inhibition
OCI-Ly10 cells treated with compound for 2 h and stimulated with anti-IgM for 10 min. Cells are lysed and western blots used to determine the levels of pBTK(Y223), total BTK, pPLCγ2 (Y1217), total PLCγ2, pAKT (S473), Total AKT and Vinculin. Primary CLL B-cells were isolated from whole blood of consented patients by Ficoll density centrifugation and Rosette-sep negative selection. The cells were treated with compound for 1 h and stimulated for 15 min with α-IgM.

ABC-DLBCL xenograft mouse models
Female NOD/SCID mice were subcutaneously implanted with OCI-Ly10 or TMD8 cells in 50% Matrigel. Treatment was initiated when tumors reached an average size of 150 mm^3 (OCI-Ly10) or 200 mm^3 (TMD8). The groups were then dosed BID, with vehicle or RXC005. Tumor volume (mm^3) was monitored.

RESULTS

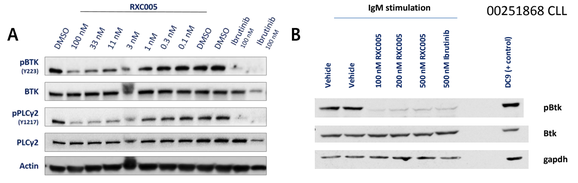
RXC005 displays nanomolar affinity and potency in both WT and mutant BTK cells



A: Binding affinity (LanthaScreen™); **B:** Cellular activity in HEK293 cells expressing BTK WT and C481S (ClariCELL™); **C:** Western blotting in DT40 cells expressing BTK C481S.

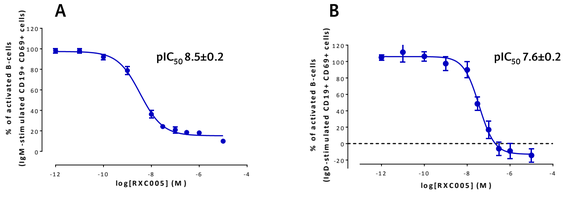
- RXC005 inhibits both BTK WT and C481S activation.
- RXC005 displays exquisite selectivity towards Tec family kinases Tec, ITK, BMX, TXK/ RLK. When tested against 468 kinases, only YES1 and ERBB2 displayed a pIC_{50} greater than 7 (pIC_{50} 7.2 for both kinases) in a biochemical activity assay.

RXC005 inhibits BCR signalling in ABC-DLBCL cell lines and in CLL patient cells



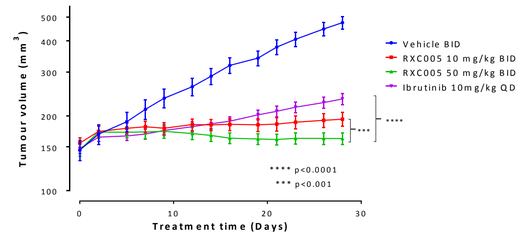
A: OCI-Ly10 cells treated with compound for 2 h. **B:** CLL cells treated with compound for 1 h and stimulated with anti-IgM for 15 min.

RXC005 inhibits BTK in isolated human PBMCs and in human whole blood

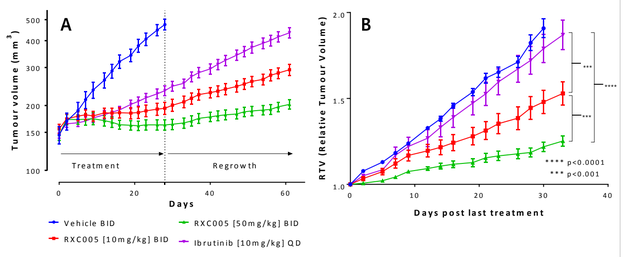


A: PBMCs treated with compound continuously before stimulation with anti-IgM for 6 h.
B: Human whole blood treated with compound and stimulated with anti-IgD for 6 h and red blood cells are lysed. The percentage of CD69 positive B-cells (CD19 positive) are measured by flow cytometry.

RXC005 is efficacious in an OCI-Ly10 xenograft mouse model

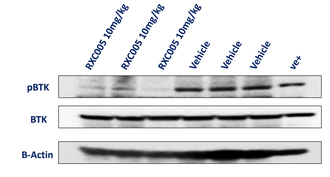


- RXC005 (10 mg/kg and 50 mg/kg) and ibrutinib (10 mg/kg) showed significant efficacy versus Vehicle (P <0.0001).
- RXC005 (10 mg/kg or 50 mg/kg) showed significantly greater efficacy than ibrutinib after 28 days treatment (P=0.001/ <P=0.0001 respectively) using One way Anova (multiple comparisons with uncorrected Fischer's LSD).
- Dosing was stopped at day 28 and tumour growth delay was monitored.



A: Tumor growth inhibition and regrowth rates following cessation of treatment. **B:** Rate of regrowth after day 28 measured by relative tumor volume (RTV)

RXC005 inhibits pBTK in an OCI-Ly10 xenograft

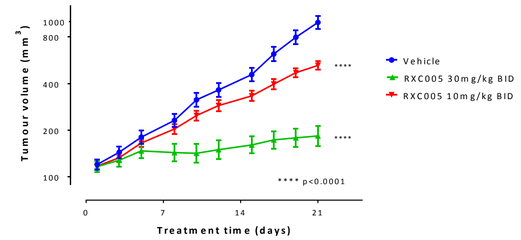


OCI-ly10 xenograft model (NOD-SCID) dosed with RXC005 10 mg/kg for 3 dosing occasions prior to samples being taken 2 h post final dose.

RXC005 inhibits proliferation in ABC-DLBCL cell lines

Cell line	RXC005	Ibrutinib	Acalabrutinib	ONO-4059
OCI-Ly10 (pIC_{50})	8.2±0.1	9.3±0.2	8.3±0.1	8.4±0.2
TMD8 (pIC_{50})	8.3±0.2	9.1±0.2	8.3 (N=2)	8.2 (N=2)

RXC005 is efficacious in a TMD8 xenograft mouse model



- RXC005 at 10 and 30 mg/kg BID showed significant efficacy versus Vehicle (P <0.0001).

RXC005 has a suitable ADME profile and excellent bioavailability

- Good exposure, oral bioavailability and half-life were demonstrated for RXC005 in mouse, rat and dog, with dose linearity assessment performed in mouse ($t_{1/2}$ = 2.4-3.8 h, F = 73-100%, CL= 11% liver blood flow in mice; $t_{1/2}$ = 2.7-3.5 h, F = 55-84%, CL = 28% liver blood flow in rat; $t_{1/2}$ = 6.1-9.5 h, F = 85-100%, CL= 10% liver blood flow in dog).
- The pIC_{50} values of voltage-gated ion channels hERG, hNav1.5, hCav1.2 of RXC005 were respectively 4.8, < 4.5, < 4.5.
- In a dog CV safety study, RXC005 displayed no effects at the dose levels tested.
- RCX005 is non-mutagenic in mini-Ames and micronucleus assays.
- RXC005 displays good selectivity in a CEREP safety panel with no counter-activities observed.
- RXC005 shows no inhibition of major CYP isoforms or activation of PXR *in vitro*.

CONCLUSION

RXC005 aims to overcome ibrutinib-resistance by targeting both wild type and C481-mutated BTK.

Our reversible BTK inhibitor development candidate, is showing:

- Nanomolar affinity and cellular activity for BTK WT and BTK C481S.
- Nanomolar anti-proliferative activity in ABC-DLBCL cell lines.
- High selectivity against 468 kinases and >100-fold selectivity for BTK-dependent vs. EGFR-, ITK-, Tec- and or Lck- signaling pathways.
- No safety issues observed in preliminary studies (hERG, cytotoxicity, genotoxicity, CYP inhibition).
- Preclinical ADME and predicted human PK properties suitable for BID administration.
- *In vivo* efficacy in ABC-DLBCL xenograft models.

Further studies are currently ongoing in advance of anticipated IND and CTA filings around the end of 2017.

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