#4399 RXC005 (REDX08608), a Novel, Potent and Selective, Reversible BTK Inhibitor with Efficacy and Equivalent Potency Against Wild-Type and Mutant C481S BTK

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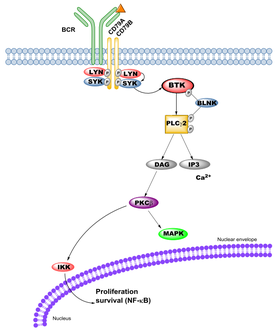
BACKGROUND

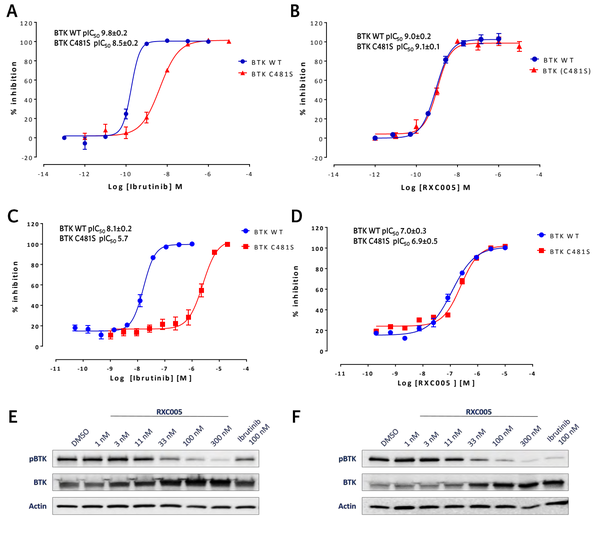
Bruton's tyrosine kinase (BTK) is a member of the src-related Tec family of cytoplasmic tyrosine kinases and plays a key role in the BCR signaling pathway, which is required for the development, activation and survival of B-cells. BTK is a clinically validated target to treat B-cell malignancies that 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 (Byrd2016, Inhye2016, Maddocks2015, Woaych2014). Redx's reversible BTK inhibitor, RXC005 (REDX08608), aims to overcome this resistance mechanism by targeting both wild type and C481-mutated BTK. Redx has recently presented REDX06961 our BTK probe (Guisot2016, AACR#4795) and, following lead optimization, we are now disclosing RXC005, our development candidate, a potent, reversible and selective BTK inhibitor, which displays an improved profile including superior pharmacokinetics.

RESULTS

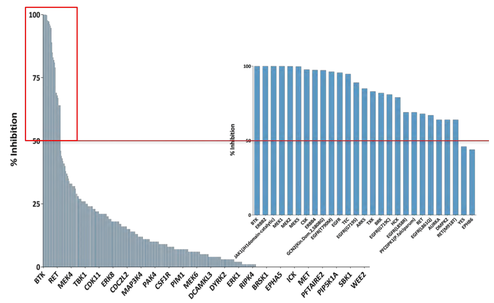
RXC005 displays nanomolar affinity and potency in both WT and mutant BTK cells



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

RESULTS

RXC005 is highly selective when tested against a panel of 468 kinases

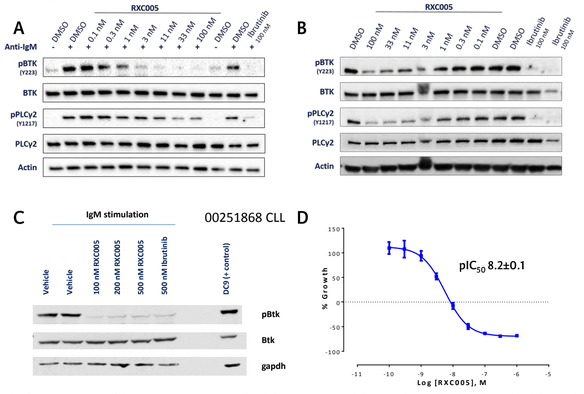


At 1 µM, only 24 off-target kinases were inhibited with more than 50% inhibition in the kinase panel. Follow-up analysis revealed exquisite selectivity with ARK5, AURKA, AURKB, DMPK2, EGFR, GCN2, JAK1, MEK1, MEK5, RET, BMX, ITK, TEC, TXK, BLK, FYN, FRK, HCK, LCK, LYN A, LYN B, SRC displaying > 200-fold selectivity over BTK.

Kinase	pIC_{50}	Selectivity to BTK
BTK WT	8.7	-
CSK	6.6	129
FGR	6.5	186
YES1	7.2	34
BRK	6.9	64
EGFR(T790M)	6.6	170
ERBB4	7.2	33
MEK2	6.5	170

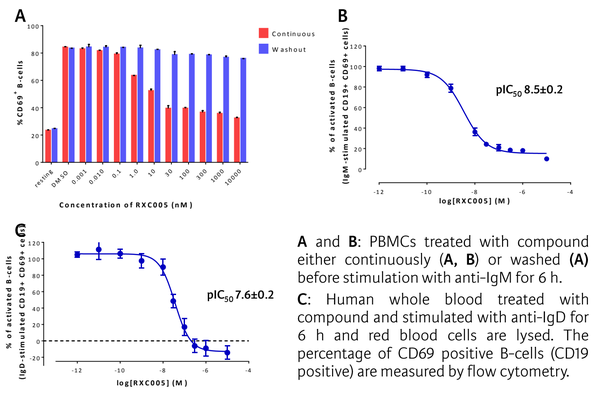
Selectivity to BTK:
Fold off-target/BTK WT IC_{50}
Thermo Fisher Scientific Z'-Lyte™ – Biochemical activity

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



A: Serum starved Ramos cells treated with compound for 1.5 h and stimulated with anti-IgM for 10 min. **B:** OCI-Ly10 cells treated with compound for 2 h. **C:** cells treated with compound for 1 h and stimulated with anti-IgM for 15 min. **D:** Proliferation measured in the CD79A mutated OCI-Ly10 cell line of the activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL) subtype. Metabolically active cells are measured using resazurin.

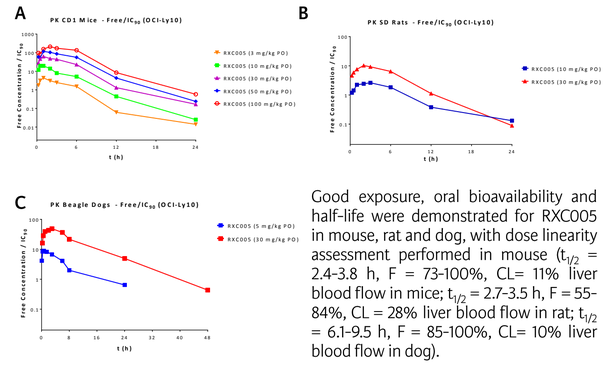
RXC005 reversibly inhibits BTK in isolated human PBMCs and in human whole blood



A and **B**: PBMCs treated with compound either continuously (**A, B**) or washed (**A**) before stimulation with anti-IgM for 6 h.

C: 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 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).

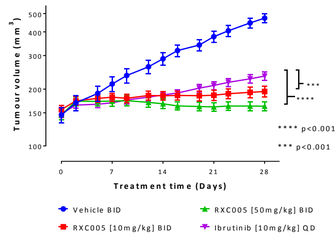
RXC005 displays a good *in vitro* safety profile

- The pIC_{50}s of voltage-gated ion channels hERG, hNav1.5, hCav1.2 of RXC005 were respectively 4.8, < 4.5, < 4.5.
- 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 is also non-cytotoxic in HepG2 and HCT-116 cell cytotoxicity assays.

	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4 (Midazolam)	CYP3A4 (Testosterone)
CYP inhibition (IC_{50} µM)	> 30	16	17	> 30	22	> 30
CYP450 TDI at 30 µM (% TDI)	< 20	< 20	< 20	< 20	< 20	-

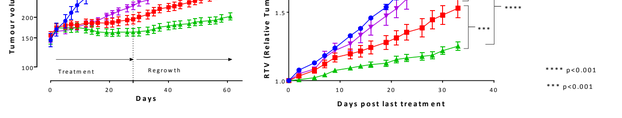
RXC005 is efficacious in an OCI-Ly10 xenograft mouse model

NOD/SCID mice bearing OCI-Ly10 tumors subcutaneously were orally administered daily with RXC005 at 10 or 50 mg/kg BID, ibrutinib 10 mg/kg QD or vehicle control. Tumor volume (mm^3) was monitored.



- RXC005 ([10 mg/kg], [50 mg/kg]) and ibrutinib [10 mg/kg] showed significant efficacy versus Vehicle (P <0.001).
- RXC005 [10 mg/kg or 50 mg/kg) showed significantly greater efficacy than ibrutinib after 28 days treatment (P=0.001/ <P=0.001 respectively) using One way Anova (multiple comparisons with uncorrected Fischer's LSD).

Dosing was stopped at day 28 and tumour growth delay was monitored.



CONCLUSION

RXC005, our reversible BTK inhibitor development candidate, is showing:

- Nanomolar affinity and cellular activity for BTK WT and BTK C481S
- Nanomolar anti-proliferative activity in an ABC-DLBCL cell line
- 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 OCI-Ly10 xenograft in NOD/SCID mouse model

Further studies are currently ongoing with an anticipated entry to clinic by the end of 2017.

DISCLOSURES

NESG, SAB, VW, AT, VA, DC, PC, JE, KH, JR, JKT, KL, MM, JR, LS, FT, MB, CP, RA were employed by Redx Pharma Plc at the time of abstract submission.

JW: Research funding from Morphosys, Acerta, Karyopharm

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