Abstract 846

Inhibition of Focal Adhesion Kinase in Combination With Bevacizumab Reduces the Rate of Tumor Revascularization and Increases Survival in a Pre-clinical Model of Basal Breast Cancer

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Background - Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that provides a critical hub for signaling from at least four different classes of cellular sensing mechanisms including growth factor receptors, GPCRs, integrins and mechanical stress forces. By temporal and spatial integration of signals from these sources, FAK plays a central role in cell migration, invasion and proliferation; processes vital for tumorigenesis. The significance of FAK to the function of signal transduction pathways provides a strong rationale for the combination of FAK inhibitors with other targeted agents to achieve improved efficacy against a range of cancers. Others have demonstrated the importance of FAK in angiogenesis and therefore combining a FAKi with anti-VEGF agents is attractive as it employs two complementary mechanisms of suppressing the formation of tumor vasculature.

Vascular Endothelial Growth Factor Receptor 3 (VEGFR3, also known as Flt4) is activated by VEGF-C and -D and under normal physiological conditions in adults is restricted to lymphatic and some fenestrated vascular endothelium. Expression levels of VEGFR3 are significantly increased in sprouting endothelial tip cells of angiogenic blood vessels in tumours and wounds and blocking VEGFR3 signalling has been demonstrated to reduce the number of vessel branches and endothelial sprouts both during development and in tumours. Furthermore, VEGFR3 signalling mediates lymphangiogenesis in tumours and appears to have a significant role in tumour metastasis though the lymphatics. Consequently inhibition of VEGFR3 could have therapeutic potential in treating lymph node metastatic diseases and possibly a subset of primary cancers. Furthermore, acquired resistance to anti-VEGF treatments such as bevacizumab (bev) is a frequent occurrence. Studies have shown that development of resistance often correlates with a significant increase in the levels of the VEGFR3 ligand, VEGF-C. Therefore, treatment with VEGFR3 inhibitors could be of clinical benefit to patients with acquired resistance to bev.

Aim - The aim of this study was to investigate the effect of inhibition of FAK (CTx-0294945) or FAK+VEGFR3 (CTx-0294886) on tumour response to anti-VEGF treatment in an aggressive model of basal breast cancer. Here we present results from the co-administration of, bev, with either CTx-0294945, a highly selective FAKi, or CTx-0294886, a potent inhibitor of FAK and VEGFR3 in an orthotopic model of human breast cancer.

CTx-0294945 (selective FAK) and CTx-0294886 (FAK +VEGFR3) kinase inhibitors suitable for combination therapy

Compound Activity Profile

Compound	FAK Biacore K_D (nM)	VEGFR3 Kinase IC_{50} (nM)	MDA-MB-231-LNA cells IC_{50} (nM)			HMVEC IC_{50} (nM)	HUVEC IC_{50} (nM)
			p397Y-FAK	3DoT proliferation	2D Proliferation	pY-VEGFR3	pY-VEGFR2
CTx-0294945	0.21	559	7	214	6,600	> 1000	NT
CTx-0294886	1.3	12	36	-	1,700	36	2,700

Kinase Selectivity Profile

% Inhibition	Number of Kinases			
	CTx-0294945 (100 nM)	CTx-0294945 (1 µM)	CTx-0294886 (100 nM)	CTx-0294886 (1 µM)
≤ 50	120	116	111	90
51 - 90	1	8	9	31
> 90	0	1*	1#	4

* IC_{50} = 209 nM
# Flt3 IC_{50} = 9 nM

Pharmacokinetic Properties (Rat)

		Measured dose (mg/kg)	Apparent $t_{½}$ (h)	Plasma Cl_{total} (ml/min/kg)	V_{ss} (L/kg)	Cmax (µM)	Tmax (min)	BA(%)
CTx-0294945	IV	4.5	4.8	31.0	12.1	-	-	-
	Oral	20.1	5.1	-	-	1.1	240	58.4
CTx-0294886	IV	5.8	3.4	38.7	7.5	-	-	-
	Oral	21.7	3.6	-	-	0.8	240	42

CYP Inhibition and Reactive Metabolite Profile

Compound	1A2 IC_{50} (µM)	2C9 IC_{50} (µM)	2C19 IC_{50} (µM)	2D6 IC_{50} (µM)	3A4/5 (Midazolam) IC_{50} (µM)	3A4/5 (Testosterone) IC_{50} (µM)	Glutathione Trapping
CTx-0294945	>20 (nmi)	>20 (25%)	>20 (nmi)	> 20 (nmi)	>20 (nmi)	> 20 (nmi)	Negative
CTx-0294886	>20 (nmi)	>20 (25%)	>20 (nmi)	> 20 (nmi)	>10 (nmi)	> 20 (nmi)	Negative

nmi = no measurable inhibition

CTx-0294945 reduces rate of tumour regrowth after cessation of bevacizumab therapy in the MDA-MB-231-LNA breast cancer model

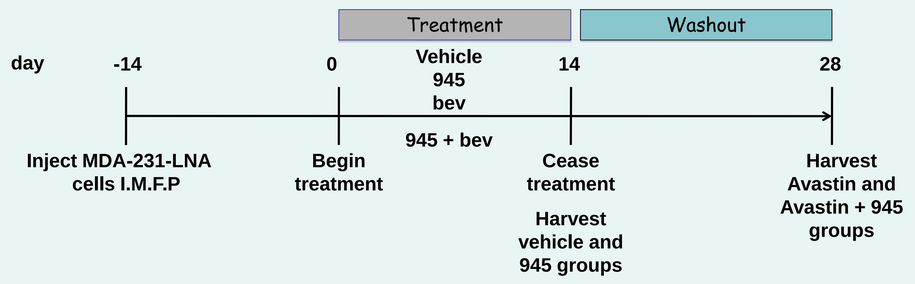

Figure 1: CTx-0294945 augments MDA-MB-231-LNA tumor growth inhibition in combination with bev. 1 x 10^6 MDA-231-LNA cells were implanted into the right mammary fat pad of Balb/c SCID mice. Once tumors were palpable (day 14), the mice were randomised into groups of 8 to receive drug vehicle alone (vehicle; black), 80 mg/kg CTx-0294945 po once daily (red), 12.5 mg/kg bev ip twice weekly (blue) or the two drugs in combination (purple) for a period of 14 days (day 14 – 28). Bars represent the mean ± S.E.M of 8 mice per treatment group. * $p < 0.05$ by t-test compared to bev alone.

CTx-0294886 in combination with bevacizumab significantly increases survival in the MDA-MB-231-LNA breast cancer model

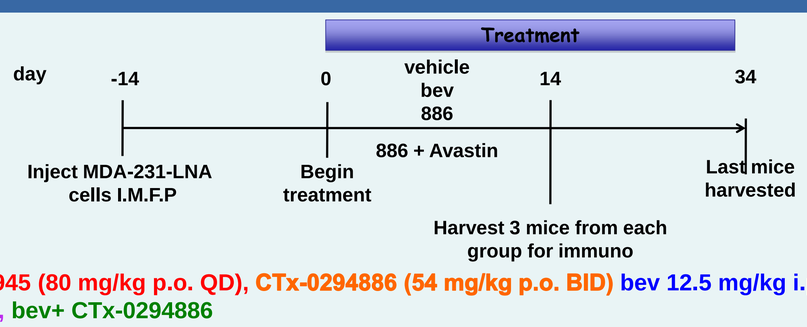

Figure 2: CTx-0294886 in combination with bevacizumab (bev) increases survival time MDA-MB-231-LNA model compared to bev alone. 1 x 10^6 MDA-231-LNA cells were implanted into the right mammary fat pad of Balb/c SCID mice. Once tumors were palpable (day 0), the mice were randomised 8 to receive drug vehicle alone (vehicle; black), 54 mg/kg CTX-0294886 po twice daily (yellow), 12.5 mg/kg bev ip twice weekly (blue) or the two drugs in combination (green). Individual mice were culled when tumours reached 1500 mm^3. Bars represent the mean ± S.E.M of 8 mice per treatment group. * $p = 0.02$ by Log-Rank (Mandel-Cox) test compared to bev alone.

CTx-0294945 may inhibit tumour revascularization and macrophage infiltration in MDA-MB-231-LNA primary tumours following cessation of bevacizumab therapy

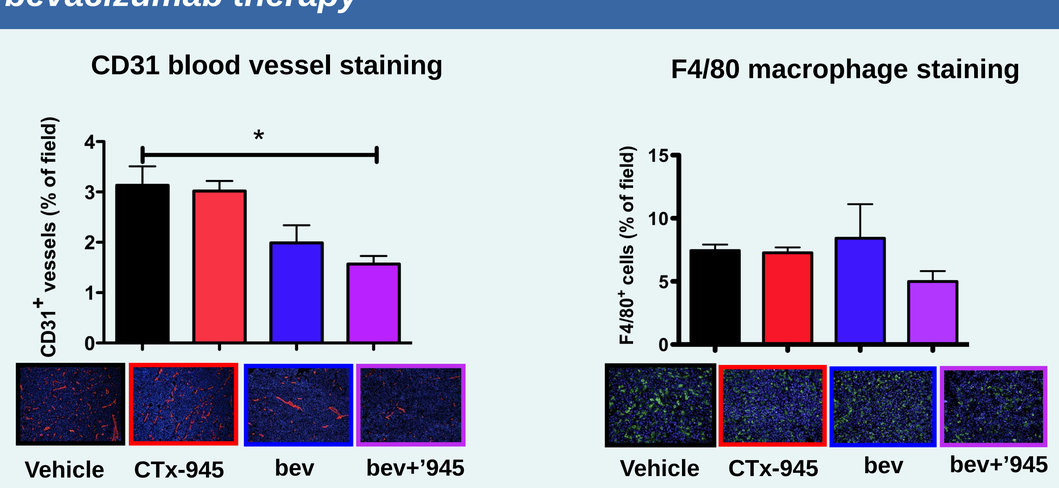

Figure 3a $CD31^+$ blood vessels (red) and cell nuclei (DAPI; blue) in MDA-231-LNA primary tumors at day 14 (vehicle and CTx-0294945 groups) or day 28 (bev and combination groups). Bars represent the mean ± SEM of 4-8 tumours per group and 3 random quantitated fields per tumour.

Figure 3b $F480^+$ macrophages (green) and cell nuclei (DAPI; blue) in MDA-231-LNA primary tumors at day 14 (vehicle and CTx-0294945 groups) or day 28 (bev and combination groups). Bars represent the mean ± SEM of 4-8 tumours per group and 3 random quantitated fields per tumour.

CTx-0294886 inhibits angiogenesis in MDA-MB-231-LNA primary tumours

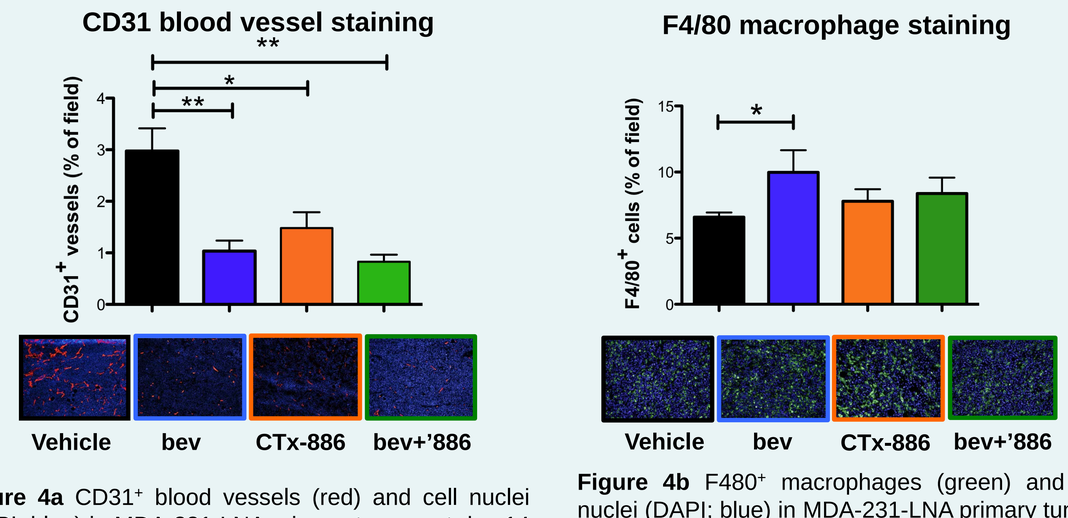

Figure 4a $CD31^+$ blood vessels (red) and cell nuclei (DAPI; blue) in MDA-231-LNA primary tumors at day 14 Bars represent the mean ± SEM of 3 tumours per group and 3 random quantitated fields per tumour.

Figure 4b $F480^+$ macrophages (green) and cell nuclei (DAPI; blue) in MDA-231-LNA primary tumors at day 14. Bars represent the mean ± SEM of 3 tumours per group and 3 random quantitated fields per tumour.

Summary

- **CTx-0294945 is a potent and selective inhibitor of FAK with excellent development potential**
- **CTx-0294886 is a potent inhibitor of FAK and VEGFR3 with excellent development potential**
- **Our data support the potential clinical utility of combining CTx-0294886 with bevacizumab to enhance anti-tumor effects and increase the durability of response**

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