

# Sunitinib treatment enhances metastasis of innately drug resistant breast tumors

Wragg, Joseph; Heath, Victoria; Bicknell, Roy

DOI:

[10.1158/0008-5472.CAN-16-1982](https://doi.org/10.1158/0008-5472.CAN-16-1982)

License:

None: All rights reserved

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Wragg, J, Heath, V & Bicknell, R 2017, 'Sunitinib treatment enhances metastasis of innately drug resistant breast tumors', *Cancer Research*, vol. 77, no. 4, pp. 1008-1020. <https://doi.org/10.1158/0008-5472.CAN-16-1982>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**

<http://cancerres.aacrjournals.org/content/77/4/1008>

**General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

**Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

**Supplementary table 1.** Murine RTqPCR primers used for the research detailed in this paper.

Target name	Primer sequences	Probe number
CD11b	Fwd: gcacctgggtatcagcatatt	9
	Rev: cccaggtaccgaaattctcc	
CD68	Fwd: ttctcttgcaaccgtgacc	34
	Rev: gaggaggaccaggccaat	
EPCAM	Fwd: ggtagcgcttccgaggta	53
	Rev: tggtagtagtcaaggccagt	
PDGFRA	Fwd: aacggggctagaagtcaacc	4
	Rev: tgacatgaagccaagaacttaaac	
PECAM	Fwd: gctgggtctctatgcaagc	30
	Rev: atggatgctgttgatgggtga	
$\beta$ -ACTIN	Fwd: ggagggggttgaggtgtt	71
	Rev: gtgtgcactttattggctcaa	
LEPR	Fwd: cctccatctaactgaaaagcaga	47
	Rev: tggctttccaagatacttc	
PRLR	Fwd: gccttctgctctgtctcac	55
	Rev: cctgagccccgtgtaaaat	
ESAM	Fwd: tgattcttcaggctggaacc	54
	Rev: tcagtcagggaacaaaacc	
PTN	Fwd: tgtcactttgctctccttgg	55
	Rev: agtgggcttctctggcttc	
AQP1	Fwd: atcaactcagcaccctcactc	25
	Rev: caggtgggtccctcacttt	
ANGPT2	Fwd: aagagcgtggacagcacag	8
	Rev: gtagctgcagggtcgttc	
DARC	Fwd: cttcaccttgggactcagtggt	32
	Rev: gactggcagccctaagagg	
ECSCR	Fwd: gctagacacttggcctgctc	18
	Rev: ttgactcctcgttctctgagttt	
TSPAN7	Fwd: ttggatgctttgctacatgc	89
	Rev: gggacaggaacatggcatac	
STC2	Fwd: catgccctgcgtcataaat	18
	Rev: catttcctaattgctggaca	
RET	Fwd: cacagcccagcaacttacg	2
	Rev: ggccttgagaattctgtct	
MMRN2	Fwd: agcccctaccatgatcc	3
	Rev: agtccccagctcaggtacac	
VEGFR2	Fwd: accagagaccctcgtttca	22
	Rev: catttgcttcaggagggtt	
EDN1	Fwd: cagcatccttgatccaaca	30
	Rev: gacgcagacaggctaggg	

**Supplementary table 2.** Expression change of genes on which sunitinib has a known or predicted effect, in tumour bulk harvested at each time-point. Log2 fold change in gene expression shown.

Gene ID	Gene symbol	GeneBank accession no.	Predicted sunitinib effect	Responsive vs Untreated 9 days	Responsive vs Untreated 600 mm <sup>3</sup>	Responsive vs Untreated 1300 mm <sup>3</sup>
kit oncogene	KIT	NM_001122733	Downregulated	-2.33	-0.78	0.27
platelet derived growth factor receptor, beta	PDGFRB	NM_001146268	Downregulated	-2.11	-0.41	-0.60
CD69 antigen	CD69	NM_001033122	Downregulated	-2.00	0.47	0.47
Vascular endothelial growth factor receptor 2	VEGFR2	NM_010612	Downregulated	-1.63	-0.18	-0.95
FMS-like tyrosine kinase 3	Flt3	NM_010229	Downregulated	-1.53	-0.21	-0.82
colony stimulating factor 1 receptor	Csf1r	NM_001037859	Downregulated	-1.35	-0.91	-0.82
interleukin 2 receptor, alpha	IL2RA	NM_008367	Downregulated	-1.28	-0.24	0.99

**Supplementary table 3.** Expression change of genes on which sunitinib has a predicted effect, in EC isolates from tumours harvested at 1300 mm<sup>3</sup>. Log2 fold change in gene expression shown.

Gene ID	Gene symbol	GeneBank accession no.	Predicted sunitinib effect	Non-responsive vs. Untreated EC	Responsive vs. Untreated EC	Non-responsive vs. Responsive EC
chemokine (C-X-C motif) ligand 3	CXCL3	NM_203320	Downregulated	-1.97	-0.35	-1.62
FMS-like tyrosine kinase 1	FLT1	AK005502	Downregulated	-0.95	0.89	-1.84
kinase insert domain protein receptor	KDR	NM_010612	Downregulated	-0.14	1.04	-1.18
FMS-like tyrosine kinase 3	FLT3	AK045865	Downregulated	-1.04	-0.01	-1.04
ret proto-oncogene	RET	NM_001080780	Downregulated	0.60	1.26	-0.41
baculoviral IAP repeat-containing 5	BIRC5	NM_001012273	Downregulated	1.15	0.20	0.97
BCL2-like 11 (apoptosis facilitator)	BCL2L11	NM_207680	Upregulated	1.30	-0.33	1.64

**Supplementary table 4.** Expression change of genes that enhance metastasis in tumour bulk harvested at each time-point. Log2 fold change in gene expression shown.

Gene ID	Gene symbol	GeneBank accession no.	Effect on metastasis	Responsive vs. Untreated 9 days	Responsive vs. Untreated 600 mm <sup>3</sup>	Responsive vs. Untreated 1300 mm <sup>3</sup>
bone morphogenetic protein 2	BMP2	NM_007553	Increased	-3.30	-0.45	-1.05
snail homolog 2 (Drosophila)	SNAI2	NM_011415	Increased	-2.91	0.08	0.46
ubiquitin D	UBD	NM_023137	Increased	-2.06	0.72	0.04
thymus cell antigen 1, theta	THY1	NM_009382	Increased	-1.98	-0.67	0.19
collagen triple helix repeat containing 1	CTHRC1	NM_026778	Increased	-1.37	-0.71	-0.70
nitric oxide synthase 3, endothelial cell	NOS3	NM_008713	Increased	-1.34	-0.32	-0.24
angiopoietin-like 4	ANGPTL4	NM_020581	Increased	-1.32	-1.04	0.22
heparanase	HPSE	NM_152803	Increased	-1.30	-0.18	-0.21
O-6-methylguanine-DNA methyltransferase	MGMT	NM_008598	Increased	-1.28	-0.52	-0.08
tumor necrosis factor superfamily, 11	TNFSF11	NM_011613	Increased	-1.27	0.29	0.36
zeta-chain (TCR) associated protein kinase	ZAP70	NM_009539	Increased	-1.27	0.19	0.89
CD274 antigen	CD274	NM_021893	Increased	-1.25	-0.21	0.00
Fyn proto-oncogene	FYN	NM_001122893	Increased	-1.13	-0.35	-0.08
tumor necrosis factor	TNF	NM_013693	Increased	-1.11	-0.30	0.96
chemokine (C-X-C motif) receptor 4	CXCR4	NM_009911	Increased	-1.07	-0.73	0.00
matrix metalloproteinase 2	MMP2	NM_008610	Increased	-0.29	-1.14	-0.40
chemokine (C-X-C motif) ligand 1	CXCL1	NM_008176	Increased	0.25	1.08	0.77
TOX high mobility group box 4	TOX4	NM_023434	Increased	0.31	-0.02	1.95
chemokine (C-X-C motif) ligand 3	CXCL3	NM_203320	Increased	0.40	1.41	0.07
lysyl oxidase	LOX	NM_010728	Increased	0.41	-1.05	-0.29
c-fos induced growth factor	FIGF	NM_010216	Increased	0.42	-1.21	-0.36
syndecan binding protein	SDCBP	AK014678	Increased	0.60	-0.06	1.11
leukotriene B4 receptor 2	LTB4R2	NM_020490	Increased	0.74	-0.69	1.29
CD151 antigen	CD151	NM_009842	Increased	1.03	0.13	0.21
inositol hexaphosphate kinase 2	IP6K2	NM_029634	Increased	1.05	-0.15	0.04
chemokine (C-X-C motif) ligand 5	CXCL5	NM_009141	Increased	1.07	0.32	0.04
S100 calcium binding protein A4	S100A4	NM_011311	Increased	1.13	0.38	0.33
bone morphogenetic protein 7	BMP7	NM_007557	Increased	2.34	-0.14	0.54

**Supplementary table 5.** Expression change of genes that enhance metastasis in tumour bulk harvested at 1300 mm<sup>3</sup>. Log2 fold change in gene expression shown.

Gene ID	Gene symbol	GeneBank accession no.	Effect of metastasis	Non-responsive vs. Untreated W	Responsive vs. Untreated W	Non-responsive vs. Responsive W
TOX high mobility group box family member 4	TOX4	NM_023434	Increased	-1.96	1.95	-3.94
insulin-like growth factor 1	IGF1	NM_010512	Increased	-1.52	0.10	-1.62
cadherin 2	CDH2	NM_007664	Increased	-1.12	-0.11	-1.01
neurotrophic tyrosine kinase, receptor, type 2	NTRK2	NM_001025074	Increased	-1.12	0.64	-1.74
leukotriene B4 receptor 2	LTB4R2	NM_020490	Increased	-1.01	1.29	-2.04
bone morphogenetic protein 2	BMP2	NM_007553	Increased	-0.02	-1.05	0.91
syndecan binding protein	SDCBP	AK014678	Increased	0.77	1.11	-0.51
netrin 1	NTN1	NM_008744	Increased	1.01	0.30	0.72
CD44 antigen	CD44	NM_009851	Increased	1.02	0.17	0.85
Notch gene homolog 1 (Drosophila)	NOTCH1	NM_008714	Increased	1.16	0.75	0.40
ubiquitin D	UBD	NM_023137	Increased	1.23	0.04	1.17
melanoma cell adhesion molecule	MCAM	NM_023061	Increased	1.27	-0.05	1.35
chemokine (C-X-C motif) ligand 5	CXCL5	NM_009141	Increased	1.55	0.04	1.52

**Supplementary table 6.** Expression change of genes that enhance endothelial migration in tumour bulk harvested at each time-point. Log2 fold change in gene expression shown.

Gene ID	Gene symbol	GeneBank accession no.	Effect on endothelial migration	Responsive vs. Untreated 9 days	Responsive vs. Untreated 600 mm <sup>3</sup>	Responsive vs. Untreated 1300 mm <sup>3</sup>
tenascin N	TNN	NM_177839	Increased	-3.45	-0.42	-0.14
bone morphogenetic protein 2	BMP2	NM_007553	Increased	-3.30	-0.45	-1.05
matrix metalloproteinase 13	MMP13	NM_008607	Increased	-2.90	1.24	-0.75
wingless-related MMTV integration site 5A	WNT5A	NM_009524	Increased	-2.86	-0.36	0.17
endothelial cell-specific adhesion molecule	ESAM	NM_027102	Increased	-2.62	-0.29	-1.08
integrin alpha 9	ITGA9	NM_133721	Increased	-2.33	0.20	-0.47
EGF-like domain 7	EGFL7	NM_178444	Increased	-2.00	-0.38	-0.42
elastin	ELN	NM_007925	Increased	-1.65	-1.04	-0.15
growth arrest specific 6	GAS6	NM_019521	Increased	-1.64	-0.75	0.12
phosphoinositide-3-kinase, catalytic, gamma	PIK3CG	NM_020272	Increased	-1.63	-0.46	-0.34
tumor necrosis factor (ligand) superfamily, 10	TNFSF10	NM_009425	Increased	-1.62	1.01	-0.03
tachykinin 1	TAC1	NM_009311	Increased	-1.60	0.35	0.01
insulin-like growth factor binding protein 3	IGFBP3	NM_008343	Increased	-1.59	-0.79	-0.43
Rac/Cdc42 guanine nucleotide exchange factor 6	ARHGEF6	NM_152801	Increased	-1.52	-0.08	0.18
sphingosine-1-phosphate receptor 3	S1PR3	NM_010101	Increased	-1.50	-1.03	0.16
sphingosine-1-phosphate receptor 1	S1PR1	NM_007901	Increased	-1.44	-0.70	-0.96
endothelial-specific receptor tyrosine kinase	TEK	NM_013690	Increased	-1.42	-0.45	-0.38
nitric oxide synthase 3, endothelial cell	NOS3	NM_008713	Increased	-1.34	-0.32	-0.24
heparanase	HPSE	NM_152803	Increased	-1.30	-0.18	-0.21
chemokine (C-C motif) ligand 5	CCL5	NM_013653	Increased	-1.25	-0.15	0.43
phosphodiesterase 2A, cGMP-stimulated	PDE2A	NM_001143848	Increased	-1.21	-0.77	-0.62
protein kinase D1	PRKD1	NM_008858	Increased	-1.13	-0.07	-0.51
GATA binding protein 1	GATA1	NM_008089	Increased	-0.82	-1.45	-0.60
pleiotrophin	PTN	NM_008973	Increased	-0.34	-0.15	3.23
matrix metalloproteinase 2	MMP2	NM_008610	Increased	-0.29	-1.14	-0.40
chemokine (C-X-C motif) ligand 1	CXCL1	NM_008176	Increased	0.25	1.08	0.77
teratocarcinoma-derived growth factor 1	TDGF1	NM_011562	Increased	0.30	0.56	1.03
lysyl oxidase	LOX	NM_010728	Increased	0.41	-1.05	-0.29
c-fos induced growth factor	FIGF	NM_010216	Increased	0.42	-1.21	-0.36
gastrin releasing peptide	GRP	NM_175012	Increased	0.49	0.47	-2.22
syndecan 4	SDC4	NM_011521	Increased	0.73	-0.55	1.01
adrenomedullin	ADM	NM_009627	Increased	0.78	-1.31	0.10
CD151 antigen	CD151	NM_009842	Increased	1.03	0.13	0.21
angiopoietin 1	ANGPT1	NM_009640	Increased	1.77	0.15	-0.19
colony stimulating factor 2	CSF2	NM_009969	Increased	1.80	0.28	0.06
kininogen 1	KNG1	NM_001102411	Increased	2.58	-0.08	-0.09
calcitonin/calcitonin-related polypeptide, alpha	CALCA	NM_001033954	Increased	2.73	-0.08	0.67