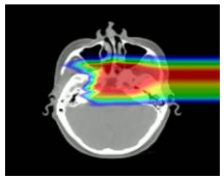








Tissue Biobanking in the UK VORTEX trial

Catharine West
University of Manchester
9th SPAEN Annual Conference
1-3 February 2019, Athens

MCRC Driving Cancer Team Science
MANCHESTER 1824 The University of Manchester
CANCER RESEARCH UK
NHS The Christie NHS Foundation Trust
Sarcoma Patients EuroNet

THE VORTEX TRIAL

RTTQA
Radiotherapy Trials Quality Assurance

VorteX

Cancer Research UK Clinical Trials Unit
@CRCTU

CRCTU Protocol Number: SA3002
ISRCTN76456502

VorteX trial: A randomised controlled multi-centre phase III trial of Volume of post-operative radiotherapy given to adult patients with eXtremity soft tissue sarcoma

Martin H Robinson, University of Sheffield

P Gaunt, R Grimer, B Seddon, J Wylie, A Davis, D Hughes, D Peake, A Cassoni, D Spooner, A Miah, A Hughes, C West,

K Venables and L Billingham

CANCER RESEARCH UK

The University Of Sheffield.

UNIVERSITY OF BIRMINGHAM

Trial Rationale

To assess whether using a reduced volume of post-operative radiotherapy improves limb function without compromising local control

Randomisation

Stratified by: tumour grade, adequacy of surgical clearance and treatment centre

Control Arm

Phase 1: 50 Gy in 25 fractions to CTV1
(GTV + 5cm cranio-caudally and 2cm axially).

Phase 2: 16 Gy in 8 fractions to CTV2
(GTV + 2cm c-c and axially).

Research Arm

66 Gy in 33 fractions to CTV2 alone

Inclusion Criteria

- Soft tissue sarcoma of extremity in adults
- Post-operative radiotherapy indicated

Primary Outcome Measures

- Limb function measured by the Toronto Extremity Salvage Score (TESS) – 210 patients required
- Time to local recurrence – 400 patients required

Timeline

- Funding confirmed April 2004

PLANNED STARTING AND CLOSING DATES

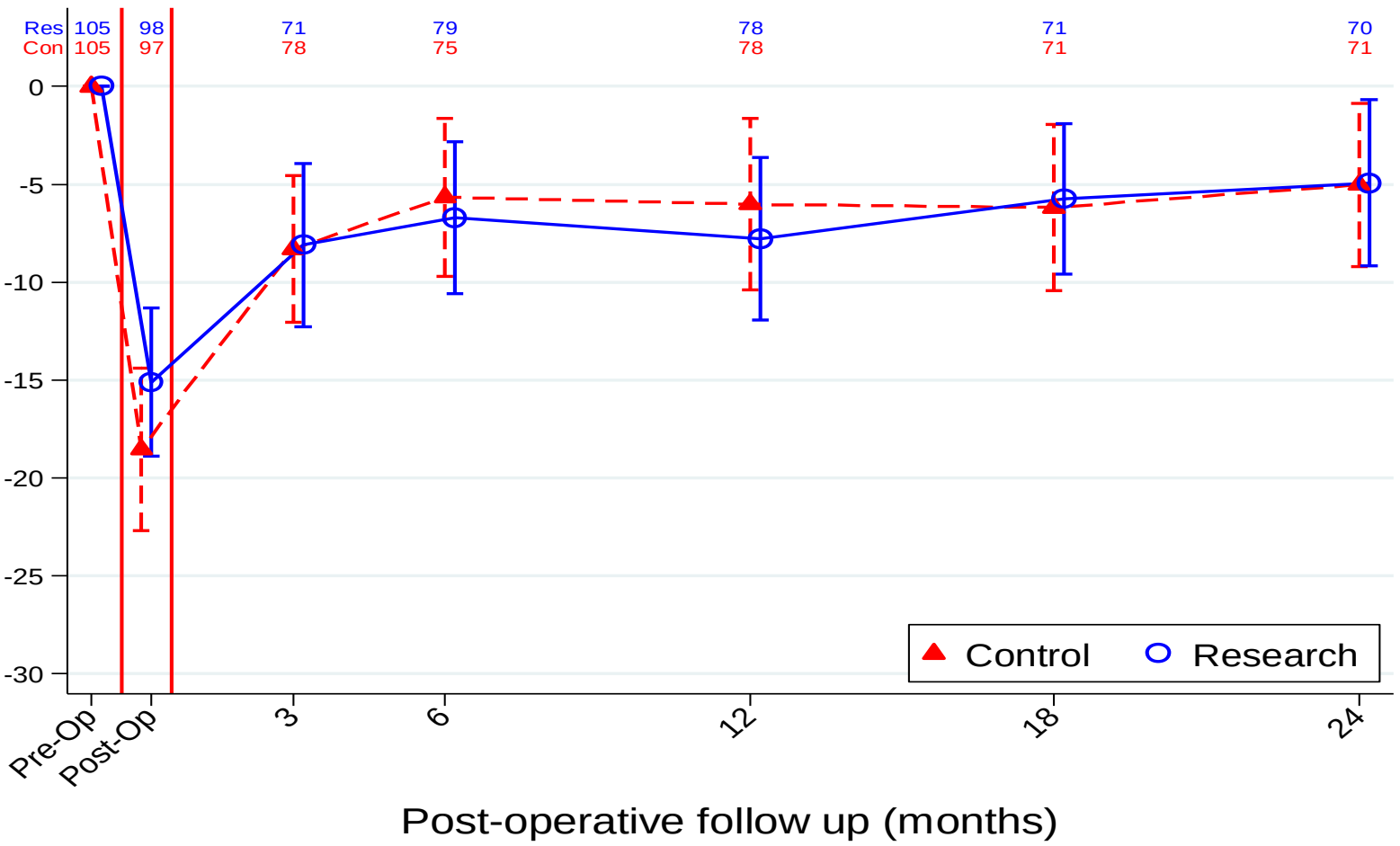
- October 2004 Open trial to recruitment
- October 2008 400 patients recruited
- October 2010 Final analysis

ACTUAL CLOSING DATE

- September 2013 Closed to recruitment
- September 2018 End data collection

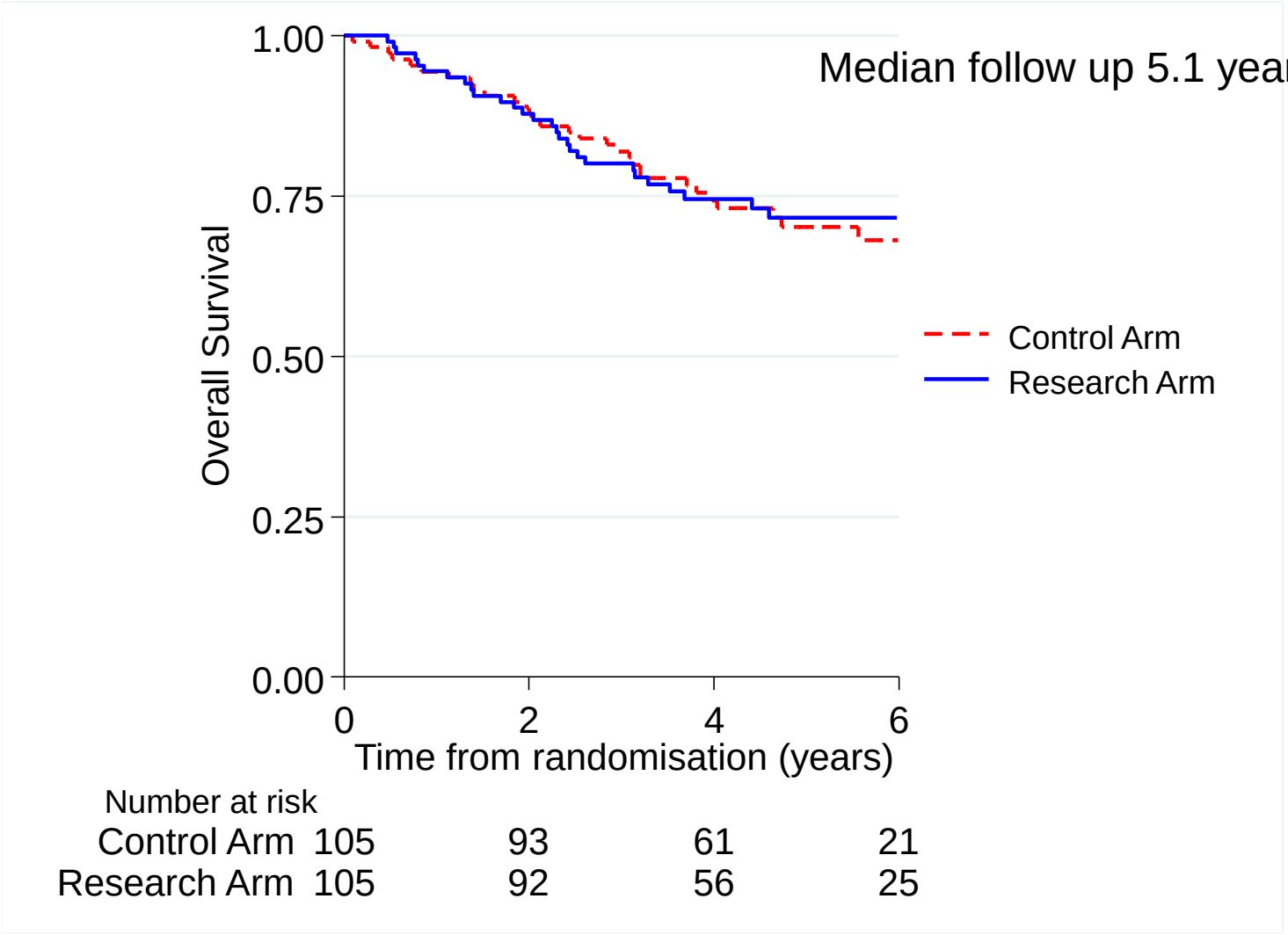
A reduced radiotherapy volume did not improve limb function

n = 210



A reduced radiotherapy volume did not compromise overall survival

n = 210



VORTEX BIOBANK

My role as a scientist

- Martin Robinson asked me to lead biology research in patients enrolled in VORTEX
- Obtained £195,940 from Cancer Research UK in 2005 for a prospective sample collection
- Money for a half-time technician in Manchester and sample re-imbursement costs

Why tissue was collected

TRANSLATIONAL HYPOTHESES

- A pre-treatment tumour molecular profile will identify patients with a poor prognosis
- The profile would identify patients likely to benefit from adjuvant systemic therapy with a hypoxia targeted agent
- Common genetic variants - single nucleotide polymorphisms (SNPs) – will identify patients likely to suffer with toxicity

What we collected

- Translational study collection
  - Fresh tissue during surgery — *RNA microarrays*
  - Diagnostic paraffin-embedded biopsies — *Tissue microarrays*
  - Blood samples just before radiotherapy — *SNP genotyping*

TISSUE COLLECTION WAS CENTRALISED
Samples sent to Manchester
Sent in the post

SNP = single nucleotide polymorphism

Quality Control: Protocol & SOPs

Patient signs consent form to agree to participate in the VORTEX-BIOBANK translational research.
Copy is faxed to VORTEX-BIOBANK office.

↓

Research team at the registration hospital notify the surgeon and pathologist of the patient's participation in the translational research

↓

At the time of surgery tumour and normal tissue samples will be taken and placed in RNAlater for future microarray analyses. Tumour samples will be taken in triplicate.

↓

Samples labelled and pseudo-anonymised in theatre

↓

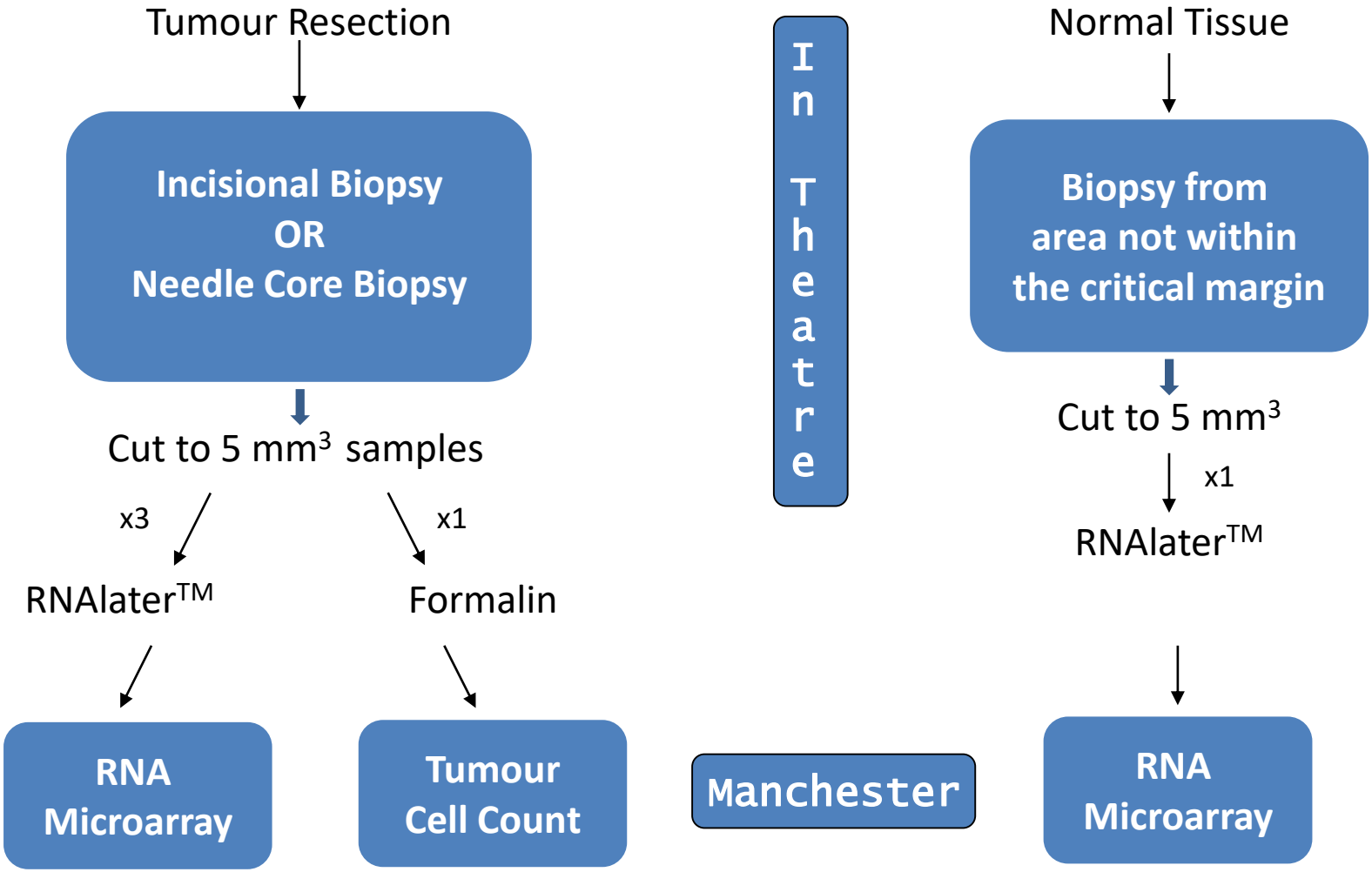
Fresh samples left overnight at room temperature ensuring they are submerged in RNAlater.

↓

Tubes placed in specimen transport tubes, which will be placed in pre-labelled, franked envelopes and placed in mail for delivery to the VORTEX- BIOBANK office.

Quality Control: Protocol & SOPs

Fresh Tissue Collection



Quality Control: Protocol & SOPs

Tissue Sample Identify Card

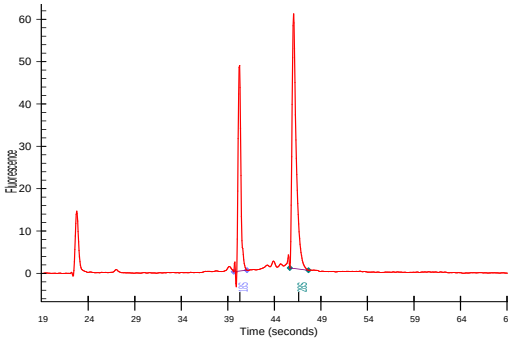
VORTEX-BIOBANK Tissue Sample ID Card	
VORTEX Trial Number	
Patient Date of Birth	
Date Sample/s Taken	
Time between resection & biopsy	
Hospital / Site	
Name of Researcher	
Signature of Researcher	
Contact Number	

Please return with tissue samples in the jiffy bag provided

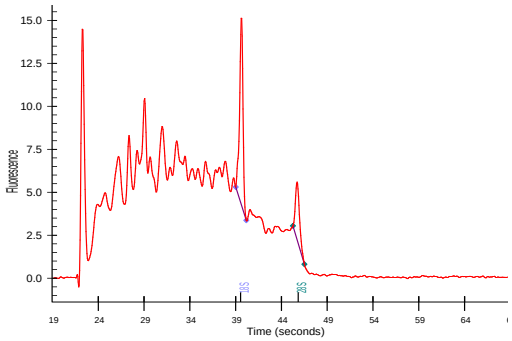
Link to trial ID and patient data

Quality Control: Monitoring

Quality Control: Prospective Monitoring of Fresh Tissue Quality



Good RNA



Bad RNA

Site	Sample ID	Extraction method	Conc. RNA	Total RNA yield	RIN (RNA integrity number)	260/230 ratio. (contaminants)	260/280 ratio.	RNA Later	Weight
ROH Birm	R003	Phen/chlo.	290	8.7	8.7	0.57	2.09	5	
ROH Birm	R004	Phen/chlo.	2492.3	74.0	3.3	1.95	2.1	7	
Stan.ULCH	R005	Phen/chlo.	1328.3	39.0	9.4	1.53	2.07	15	
ROH Birm	R006	Phen/chlo.	1741	52.0	9.4	2.13	2.08	2	
Stan.ULCH	R008	Phen/chlo.	75.1	2.2	9.8	2.12	1.74	14	
ROH Birm	R021	TRIZOL+	289.6	8.6	8.5	2.04	2.14	5	15
ROH Birm	R022	TRIZOL+	212.4	6.3	9.7	1.59	1.95	0	18.7
Sheffield	R024	TRIZOL+	487.3	14.6	9.4	2.22	2	20	37
ROH Birm	R026	TRIZOL+	392.9	11.8	7.7	1.96	2.04	10	63
ROH Birm	R027	TRIZOL+	1990.7	59.7	6.9	2.2	2.03	0	61
Sheffield	R057	mi RNEASY	205	10.2	5.8	0.45	2.05	90	87
Stan.ULCH	R059	mi RNEASY	291.1	14.5		0.59	2.04		46
Stan.ULCH	R060	mi RNEASY	41	2.0	1.8	0.15	1.72	10	24
Stan.ULCH	R065	mi RNEASY+Trizol	19.7	0.9	1.4	0.34	1.92	20	9
ROH Birm	R066	mi RNEASY	243.7	12.0	7	0.73	2.05	2	29
MRI	R067	mi RNEASY	208.1	10.4	2.4	0.93	2.04	30	60
ROH Birm	R068	TRIZOL+	12.1	0.6		0.88	2.16		
ROH Birm	R070	mi RNEASY+Trizol	516.1	25.8		1.5	2	5	60
ROH Birm	R072	TRIZOL+	3.28	0.1		0.03	3.02		
UCL	R073	TRIZOL+	433	21.6	8.8	1.61	2.07		
Sheffield	R074	mi RNEASY+Trizol	157	7.8	7.5	1.84	2.17	15	31
Stan.ULCH	R084	TRIZOL+	3.6	0.2	3.1	1.84	0.41	10	31
Stan.ULCH	R086	TRIZOL+	14.5	0.7	6.1	1.7	0.95	10	20
Stan.ULCH	R087	TRIZOL+	126	6.3	10	2.1	2.1	10	15
MRI	R088	TRIZOL+	873	43.0	9.2	2.09	2.31	30	30
MRI	R089	TRIZOL+	3731.1	186.0	6.5	1.84	1.77	30	70
ostwestry	R091	TRIZOL+	204.5	20.4	8.4	1.72	2.05		51
Sheffield	R101	TRIZOL+	247.4	12.3	8.6	1.78	2.1	20	80
Sheffield	R102	TRIZOL+	595.8	29.7	8.5	1.91	1.96	15	70
Stan.ULCH	R103	TRIZOL+	18.4	0.9	2.5	0.57	0.59	5	18
MRI	R105	TRIZOL +	321.9	16.1	6.8	2.2	2.05		75
Stan.ULCH	R107	TRIZOL +	11.4	0.5	1	1.78	1.88	10	20
ROH Birm	R108	TRIZOL +	973.5	48.6	4	2.27	2.06	2	80
Stan.ULCH	R109	TRIZOL +	20.1			1.7	0.88		7

Monitoring Tissue Quality

- Sample size and weight varies but at least one dimension should be cut to <5 mm to avoid RNA degradation
- RNA quality values suggest good quality RNA is still obtained where sample size exceeds recommendations
- Most RNA extracted is good enough for future use
- S2 (R033) RNA quality was poor despite being stabilised within advised time (20 min) but a tissue section revealed low cellular content and necrosis
- Stan6 (R036) was very small and difficult to extract

Any issues with the collection?

- Very slow
- Organised regular phone conference
- Presented updates at UK sarcoma meetings
  - 2008: 31 tumour samples
  - 2011: 155 tumour samples
  - 2012: 184 tumour samples
  - 2014: 234 tumour samples
- Obtained no-cost extensions from Cancer Research UK

Final Figures

	Registered	Randomised	%
RNA later ('fresh' tumour)	211	169	80%
RNA later (normal)	218	158	75%
FFPE (tumour)	295	**203**	**97%**
Peripheral blood (normal)	202	169	80%

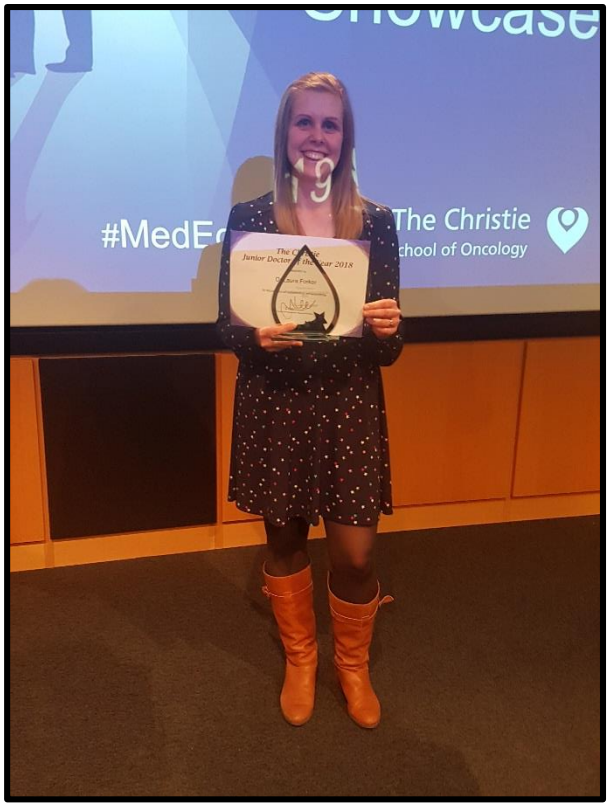
FFPE = formalin-fixed paraffin-embedded

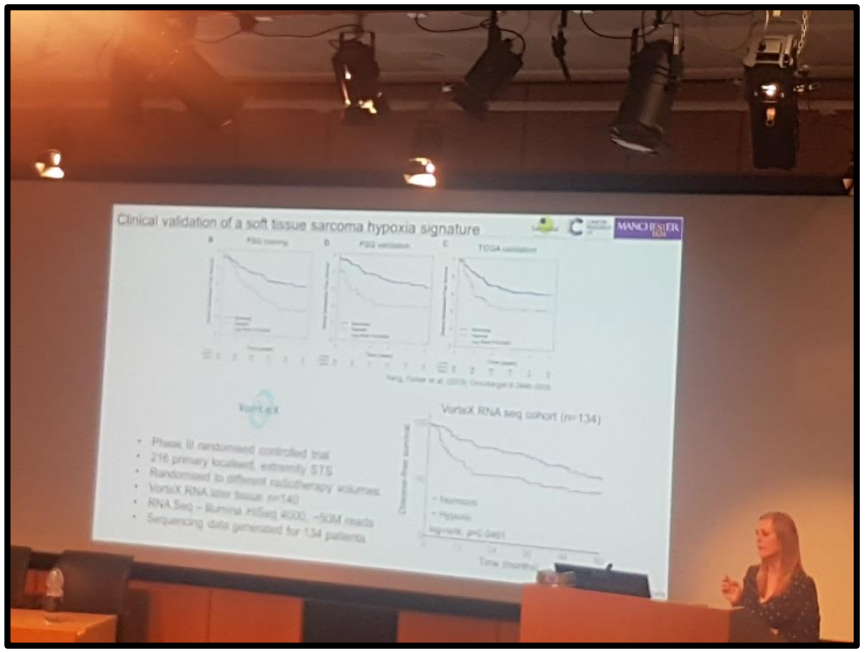
CANCER RESEARCH UK

VorteX BIOBANK

STUDIES UNDERTAKEN

Laura Forker



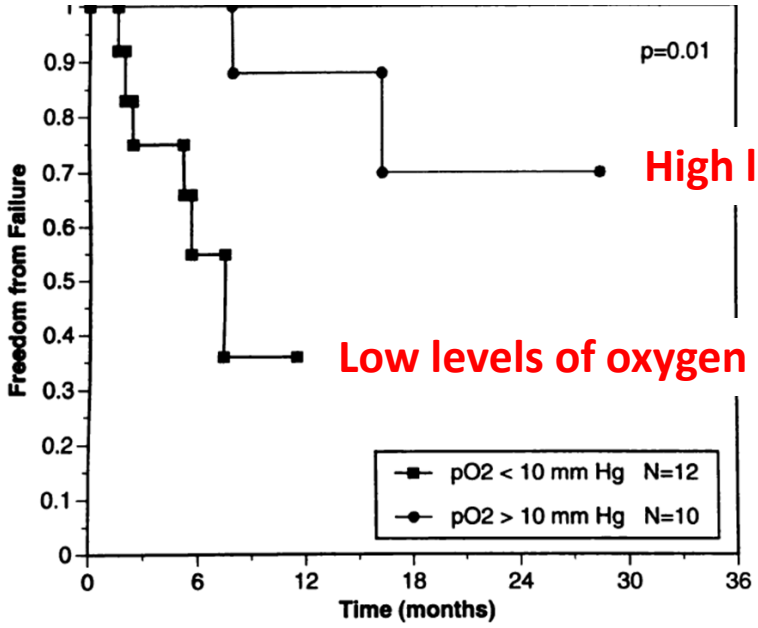


Manchester Cancer Research Centre funded PhD student

Scientific Purpose

- Radiotherapy + surgery can cure patients
- Patients tend to die with metastases
- Sarcomas are heterogeneous
- Limits use of new agents targeting genetic changes
- Hypoxia (low oxygen) is a common feature
- Hypoxia can be targeted
- But only in patients with hypoxic tumours

Patients with hypoxic tumours are more likely to die



Brizel *et al.* (1996) Cancer Research 56:941-43.

Using the collection for research

FULL PAPER

BJC

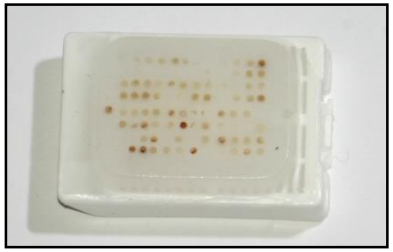
British Journal of Cancer (2017), 1–7 | doi: 10.1038/bjc.2017.430

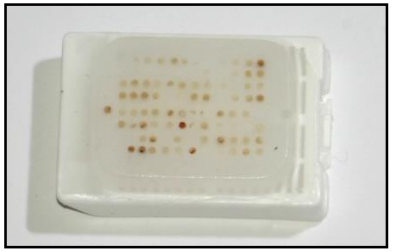
BJC OPEN

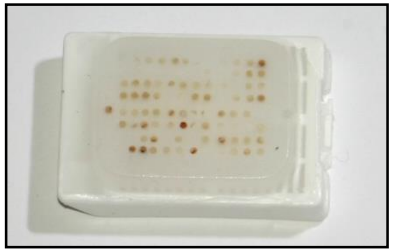
Keywords: sarcoma; hypoxia; biomarker; CAIX; HIF-1α; GLUT1

The hypoxia marker CAIX is prognostic in the UK phase III VorteX-Biobank cohort: an important resource for translational research in soft tissue sarcoma

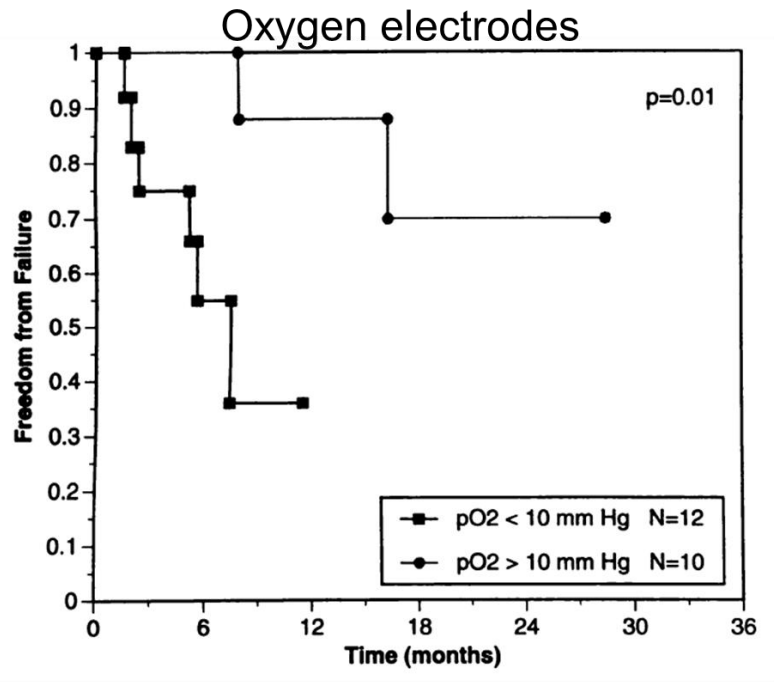
Laura Forker[1], Piers Gaunt[2], Stefano Sioletic[3], Patrick Shenjere[4], Robert Potter[1], Darren Roberts[1], Joely Irlam[1], Helen Valentine[1], David Hughes[5], Ana Hughes[2], Lucinda Billingham[2], Rob Grimer[6], Beatrice Seddon[7], Ananya Choudhury[1], Martin Robinson[8] and Catharine M L West*[,1]



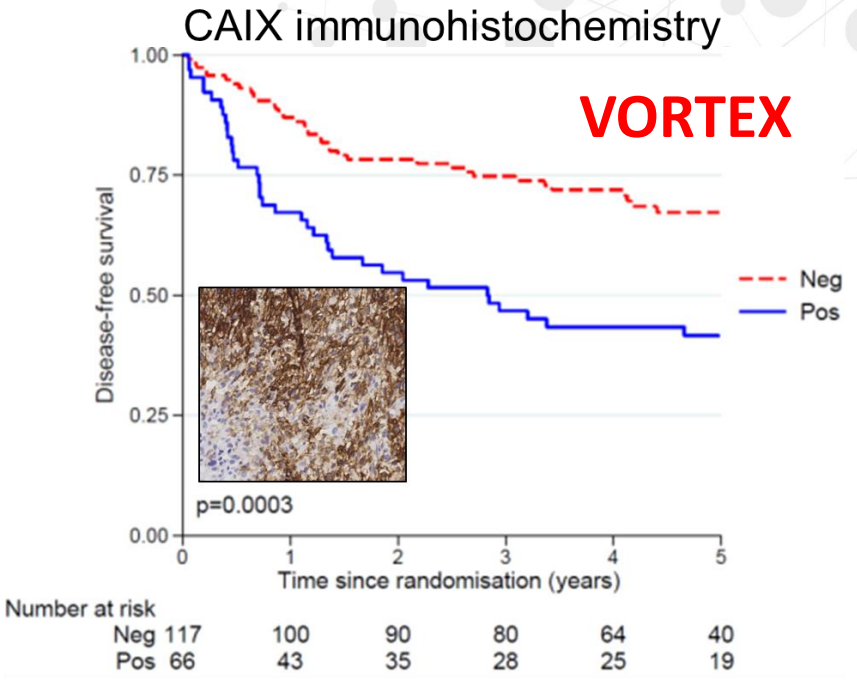




Hypoxia = poor prognosis



Brizel *et al.* (1996) Cancer Research 56:941-43.



Forker *et al.* (2018) British Journal of Cancer 118:698-704.

Hypoxia targeted drugs can reduce lung metastases in pre-clinical models in the adjuvant/neo-adjuvant setting (Liapis et al, 2015; Lunt et al, 2010)

MANCHESTER CANCER RESEARCH CENTRE
MANCHESTER 1824 The University of Manchester
CANCER RESEARCH UK
NHS The Christie NHS Foundation Trust

Deriving a gene signature to measure sarcoma hypoxia

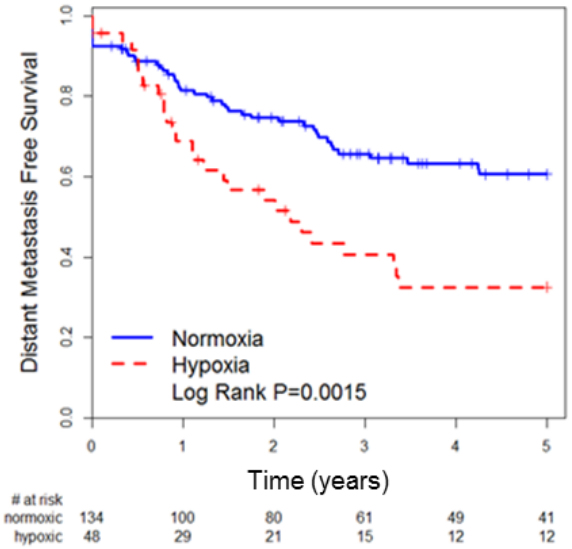
www.impactjournals.com/oncotarget/ Oncotarget, 2018, Vol. 9, (No. 3), pp: 3946-3955

Research Paper

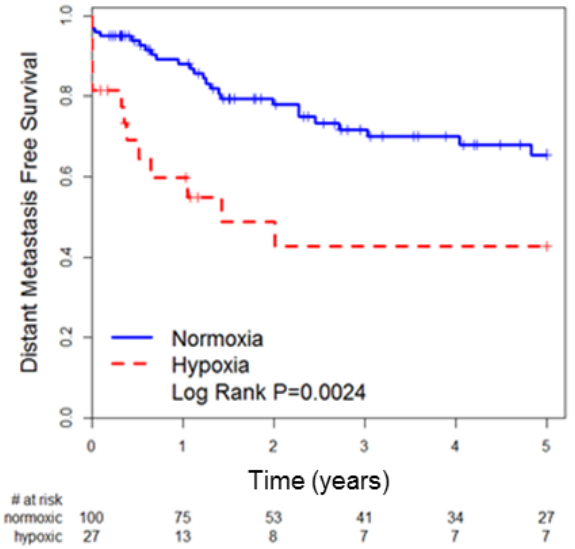
Validation of a hypoxia related gene signature in multiple soft tissue sarcoma cohorts

Lingjian Yang[1], Laura Forker[1], Joely J. Irlam[1], Nischalan Pillay[2,3], Ananya Choudhury[1] and Catharine M. L. West[1,4]

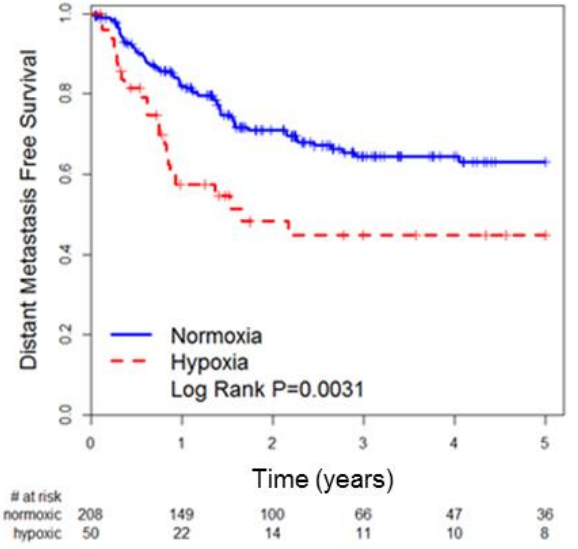
FSG training



FSG validation



TCGA validation



April 2017


northern cranes

Generating a resource for future research: RNAseq

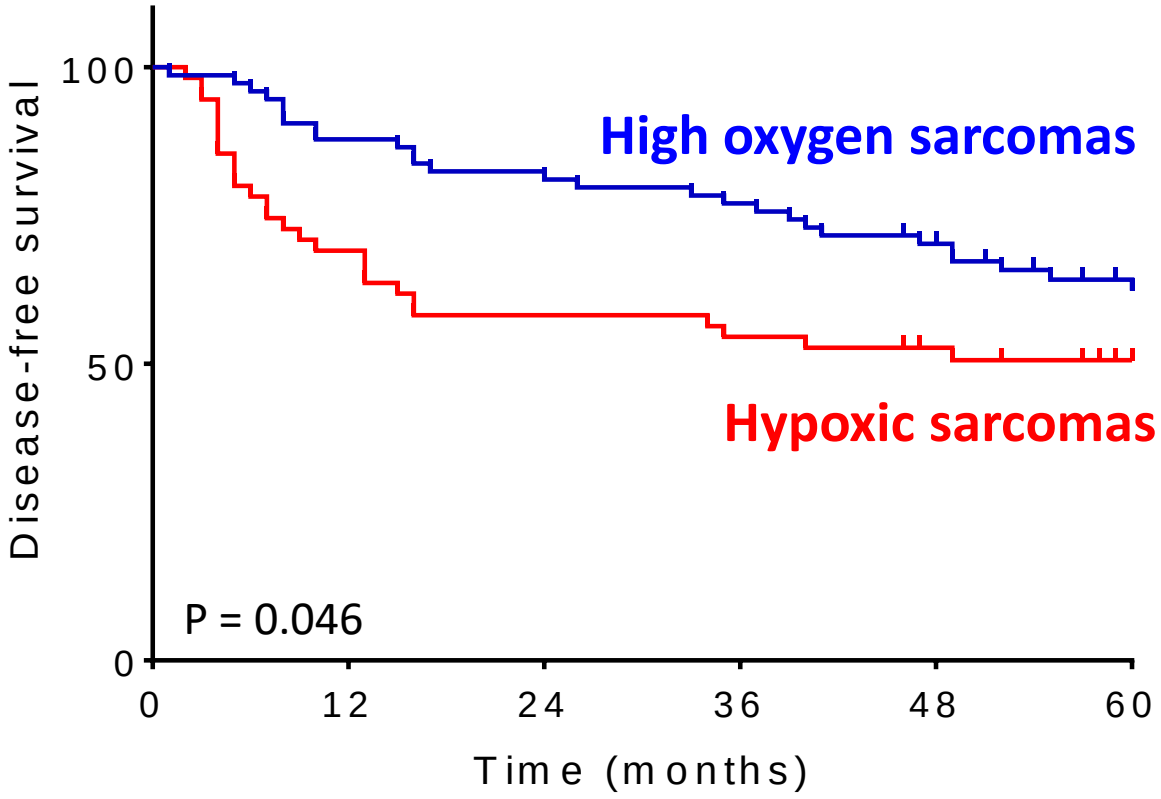
- VorteX RNA later tissue
- 86 extracted pre-fire (median RIN 7.0)
- 64 extracted post-fire (median RIN 2.5)
- RNA Seq – Illumina HiSeq 4000, ~50M reads

RIN = RNA quality measure

Sarcoma UK
CANCER RESEARCH UK
MANCHESTER 1824

RNAseq Data

VorteX RNAseq cohort (n=134)



What next?

Assay development

FFPE

Prospective samples	Retrospective samples
Local Biobank <3 years (n=34)	VorteX Biobank, 5-12 years (n~200) Christie Cohort, 3-15 years (n~200)
TLDA, Nanostring	TLDA, Nanostring

Biomarker driven trial

FFPE = formalin-fixed paraffin-embedded

Generating a resource for future research

- DNA extracted from blood samples
- Genome-wide SNP genotyping completed
- Waiting to be analysed
- Being analysed with >20,000 samples

A resource built by team work for sharing

University of Manchester, UK
Laura Forker
Lingjian Yang
Becky Bibby
Joely Irlam
Helen Valentine
Kaye Williams
Victoria Tessyman
Rebecca Elliott
Holly Summersgill
Keren Dawson
Andy Hayes
Leo Zeef
Ananya Choudhury
Catharine West

University of Sheffield, UK
Martin Robinson
David Hughes
Will English

Birmingham CRUK Clinical Trials Unit, UK
Ana Hughes
Piers Gaunt
Lucinda Billingham

University Hospital of Udine, Italy
Stefano Sioletic

Christie NHS Foundation Trust, UK
Patrick Shenjere
James Wylie
Michael Leahy

University College London, UK
Nischalan Pillay
Sandra Strauss – link with GEDDIS

New research

CANCER RESEARCH UK

Sarcoma UK
The bone & soft tissue cancer charity

Final Thoughts

- Would I do anything differently – probably not
- Lessons learned – don't give up
- Trial recruitment could be better
- No opt out of sample donation? Mandatory sample collection?
- Small cohorts are valuable (essential)
- Standardise to enable sharing
- Collaboration is important (essential)
- Collaborative tissue banks should be underpinned by scientific research questions that are relevant for patients