



## Supplementary Material

10.1302/2046-3758.109.BJR-2021-0014.R1

**Table i.** Digital droplet polymerase chain reaction primer and probe sequence information.

Gene name and SNV	Forward Primer Sequence	Reverse Primer Sequence	Wild Type Probe	Variant Probe	Amplicon Size (bp)
SMAD4-T1584C	CCAAGACAGAGCATCAAAGA	GCAATCGGCATGGTATGA	ATTCACCTTACACCG	TTCACCTGACACC	103
COL19A1-G1252A	TGGTTTCAAGGGAAGACGAG	CAGACTCTTTAAAGGGACAGTCA	CCCGGAAAACC	CCCAGAAAAACA	96
ADGRG4-T6361C	CAGAACCACAATAACTGCCAA	GGTCAGTTGTAGAAAGGTGACATA	ACTGTGTCTCAT	ACTGTGCTCATC	86
HECW1-C1874A	GGAAGAGGACAGAGAAGAGC	GTGCGTGTGCGCCATC	ACTCCAGGCA	TACTCAAGGCA	115
FOXR2-C130G	CTGGGACATGAGGAATGAGTTA	GCTTCATTACTCCGACTCTGTA	CTGAGCAAATCC	CTGAGGAAATCC	98
DDX3X-A1038C	CCATGTTGATTTCTCCTCAAATTCT	CCCATATCCAACATCCGATCA	CTTCATCTAACACC	TTCATCGAACACCA	101

**Table ii.** Patients with more than one sample.

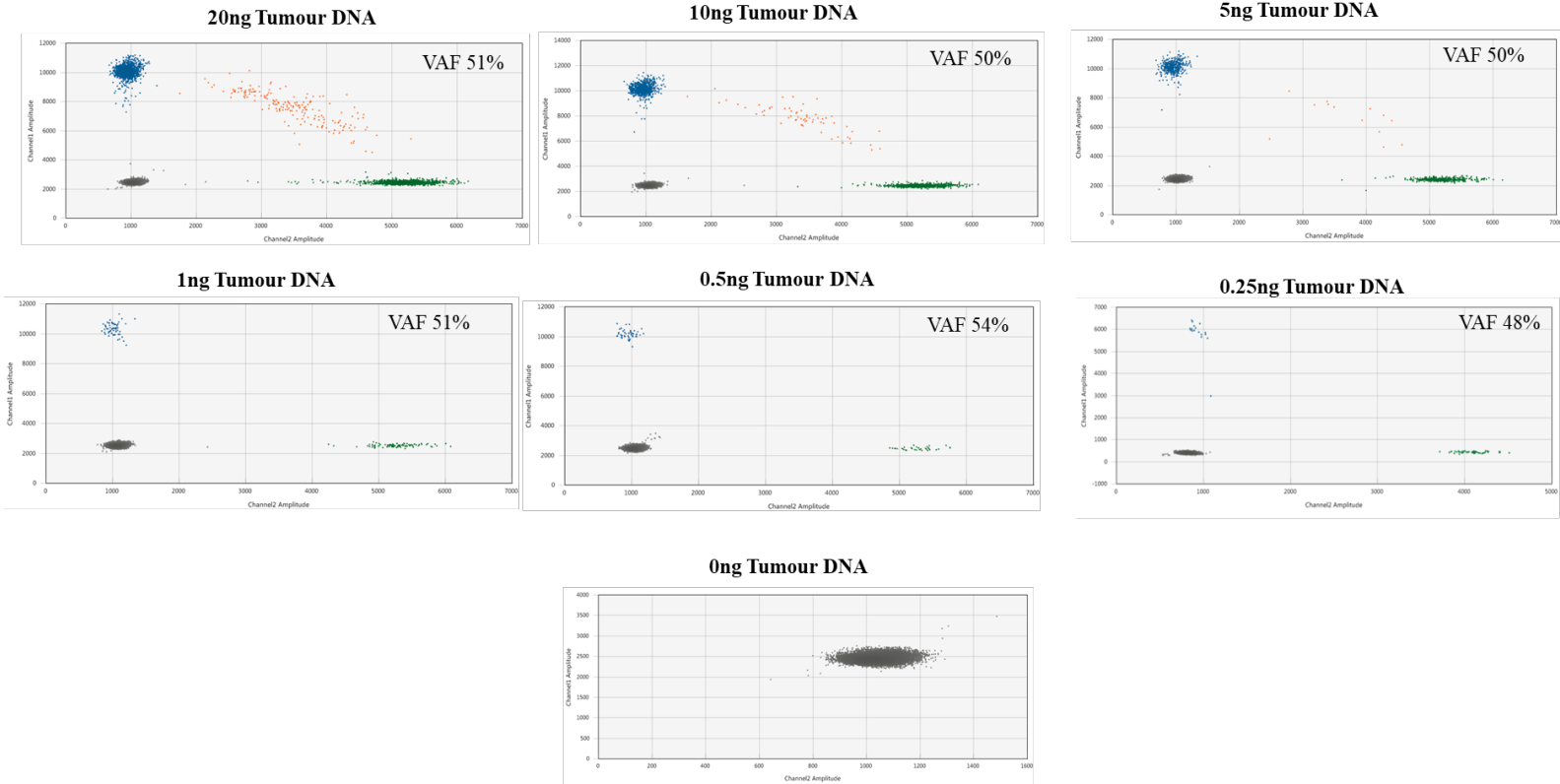
Patient #	Histology	Sample Type	Pre-Treatment	ng cfDNA/mL
1	MFS	Biopsy	None	3.2
		Resection	None	7.5
2	DDLPS	Met	None	5.7
		Met	None	2.1
3	Fibrosarcoma ex-DFSP	Resection	None	24.1
		LR	None	13
4	MFS	Biopsy	None	20.1
		Resection	None	23
5	LPS G1	Biopsy	None	3.1
		Resection	None	3.3
6	LPS G2	Biopsy	None	14
		Resection	Radiation	3.8

cfDNA, cell-free DNA; DDLPS, dedifferentiated liposarcoma; DFSP, Dermatofibrosarcoma protuberans; LPS, liposarcoma; LR, local recurrence; MFS, myxofibrosarcoma.

**Table iii.** Demographic and oncological variables for patients based on amount of cell-free DNA isolated from plasma drawn before sarcoma resection. For the six patients with two samples, the first sample was used. Comparison performed using unpaired *t*-test, chi-squared, or Fisher's exact test, as appropriate. Statistical significance set at  $p < 0.05$ .

			$\leq 6$ ng/mL		$> 6$ ng/mL		p value
			No.	%	No.	%	
<b># of Patients</b>			32		32		
<b>Mean Age (Range)</b>			61 (32-87)		65 (18-90)		0.33
<b>Gender</b>	<b>Male</b>		17	53%	20	63%	0.61
	<b>Female</b>		15	47%	12	38%	
<b>Histology</b>	<b>Soft Tissue Sarcoma</b>	UPS	6	19%	6	19%	0.75
		MFS	7	22%	8	25%	
		LPS	4	13%	5	16%	
		MLS	3	9%	0	0%	
		LMS	1	3%	2	6%	
		Other	7	22%	5	16%	
	<b>Bone Sarcoma</b>	OSA	2	6%	3	9%	
		CSA	2	6%	2	6%	
Other		0	0%	1	3%		
<b>Grade</b>	1		7	22%	1	3%	0.07
	2		10	31%	10	31%	
	3		15	47%	20	63%	
<b>Presenting Status</b>	M0		26	81%	24	75%	0.03
	M1		0	0%	7	22%	
	LR+Mets		6	19%	1	3%	
<b>Location</b>	<b>Deep</b>		28	88%	28	88%	$> 0.99$
	<b>Superficial</b>		4	13%	4	13%	
<b>Maximum Diameter (Range, cm)</b>			11.2 (2.8-34.4)		14.3 (2.0-32.1)		0.095
<b>Treatment at Sample Processing</b>		<b>None</b>	22	69%	25	78%	0.53
		<b>Radiation</b>	8	25%	5	16%	
		<b>Chemotherapy</b>	2	6%	1	3%	
		<b>Both</b>	0	0%	1	3%	
<b>Follow-Up (Range, months)</b>			13.6 (1-39)		13.5 (0-129)		0.97

CSA, chondrosarcoma; LMS, leiomyosarcoma; LPS, liposarcoma; LR ± Mets, presented with a local recurrence and/or metastases from a previously treated sarcoma; M0, no metastases at presentation; M1, metastases at presentation; MFS, myxofibrosarcoma; MLS, myxoid liposarcoma; OSA, osteosarcoma; UPS, undifferentiated pleomorphic sarcoma.



**Fig. a.** Minimum genomic requirement for the COL19A1-G125A variant. Circulating tumour DNA (ctDNA) was serially diluted with water and resultant samples were analyzed with digital droplet polymerase chain reaction (ddPCR). In this representative case, the tumour-specific ctDNA variant was detectable at 0.25ng of DNA. Green = wild-type-positive droplets; blue = variant-positive droplets; orange = variant/wild-type positive droplets; grey = negative droplets. VAF, variant allele frequency.