Supplementary Appendix

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Table S1. Antibody panel optimised for use in Trans-CHHiP
PTM: DAKO PT module, PC: pressure cooker

Marker	Function	Antibody clone	Antigen retrieval	Dilution	Positive control
CK5/6	Basal cell marker	Monoclonal (D5/16)	PTM 9.0	1:75	normal breast
Ki-67	Proliferation	Monoclonal (MIB1)	PC 6.0	1:300	appendix
Geminin	Cell cycle phase	Polyclonal	PTM 9.0	1:2000 plus protein block	tonsil
p53	G1/S checkpoint and apoptosis	Monoclonal (DO-7)	PC 6.0	1:200	p53 mutant PDX
p16	G1/S + senesence checkpoint	Monoclonal (E6H4)	Automated Ventana system	VENTANA machine	osteosarcoma
MDM2	p53 degradation	Monoclonal (IF2)	PC 6.0	1:400 + linker	clear cell ovary
ATM	DNA damage response	Monoclonal (Y170)	PTM pH9.0	1:150	normal breast
p-chk1	G2/M checkpoint	Polyclonal	PC 6.0	1:300	skin +/- RT
MRE11	DNA damage response	Monoclonal (12D7)	PTM pH9.0	1:3000	MRE11 TMA
EGFR	Growth signalling	Monoclonal (E30)	5 mins Dako proteinase K	1:60	skin
PTEN	Growth signalling	Monoclonal (6H2.1)	PTM 9.0	1:100	breast multiblock
HIF1-alpha	Hypoxia	BD Biosciences (clone 54)	PTM 9.0	1:200	hypoxic xenograft
VEGF	Hypoxia	Polyclonal	PTM pH9.0	1:200-300	placenta
Bcl-2	Apoptosis	Monoclonal (124)	PC 6.0	1:100	appendix
COX2	Pro-inflammatory/metastatic	Monoclonal (CX-294)	PTM 9.0	1:200	stomach
osteopontin	Pro-metastatic	Polyclonal	No antigen retrieval	1:2000	prostate tumour

Table S2. Distribution of the matching variables as categorical variables by fractionation schedules in Ki67 data set (PSA: prostate specific antigen).

	Ki67 data set							
	74Gy/37f Total N = 116		60Gy/20f Total N = 98		57Gy/19f Total N = 122		Total Total N = 336	
	N	%	N	%	N	%	N	%
PSA (ng/ml)								
<10	32	27.6	38	38.8	62	50.8	132	39.3
10- & <20	76	65.5	50	51.0	50	41.0	176	52.4
20-	8	6.9	10	10.2	10	8.2	28	8.3
Tumour stage								
T1	22	19.0	16	16.3	38	31.1	76	22.6
T2	86	74.1	74	75.5	74	60.7	234	69.6
T3	8	6.9	8	8.2	10	8.2	26	7.7
Gleason Score								
6	12	10.3	10	10.2	16	13.1	38	11.3
3+4	62	53.4	58	59.2	62	50.8	182	54.2
4+3	32	27.6	18	18.4	26	21.3	76	22.6
8+	10	8.6	12	12.2	18	14.8	40	11.9

Table S3. Distribution of the matching variables as categorical variables by fractionation schedules in P53 data set (PSA: prostate specific antigen).

	P53 data set							
	74Gy/37f Total N = 114		60Gy/20f Total N = 96		57Gy/19f Total N = 118		Total Total N = 328	
	N	%	N	%	N	%	N	%
PSA (ng/ml)								
<10	30	26.3	40	41.7	56	47.5	126	38.4
10- & <20	76	66.7	48	50	52	44.1	176	53.7
20-	8	7	8	8.3	10	8.5	26	7.9
Tumour stage								
T1	22	19.3	16	16.7	34	28.8	72	22
T2	84	73.7	74	77.1	72	61	230	70.1
T3	8	7	6	6.3	12	10.2	26	7.9
Gleason Score								
6	12	10.5	10	10.4	16	13.6	38	11.6
3+4	60	52.6	58	60.4	64	54.2	182	55.5
4+3	32	28.1	18	18.8	22	18.6	72	22
8+	10	8.8	10	10.4	16	13.6	36	11

Table S4. Distribution of the matching variables as categorical variables by fractionation schedules in P16 data set (PSA: prostate specific antigen).

	P16 data set							
	74Gy/37f Total N = 94		60Gy/20f Total N = 78		57Gy/19f Total N = 104		Total Total N = 276	
	N	%	N	%	N	%	N	%
PSA (ng/ml)								
<10	26	27.7	30	38.5	46	44.2	102	37
10- & <20	64	68.1	42	53.8	50	48.1	156	56.5
20-	4	4.3	6	7.7	8	7.7	18	6.5
Tumour stage								
T1	16	17	14	17.9	32	30.8	62	22.5
T2	70	74.5	60	76.9	64	61.5	194	70.3
T3	8	8.5	4	5.1	8	7.7	20	7.2
Gleason Score								
6	6	6.4	6	7.7	18	17.3	30	10.9
3+4	50	53.2	50	64.1	52	50	152	55.1
4+3	28	29.8	14	17.9	20	19.2	62	22.5
8+	10	10.6	8	10.3	14	13.5	32	11.6

Table S5. Distribution of the matching variables as categorical variables by fractionation schedules in PTEN data set (PSA: prostate specific antigen).

	PTEN data set							
	74Gy/37f Total N = 96		60Gy/20f Total N = 92		57Gy/19f Total N = 112		Total Total N =300	
	N	%	N	%	N	%	N	%
PSA (ng/ml)								
<10	26	27.1	40	43.5	54	48.2	120	40
10- & <20	62	64.6	44	47.8	48	42.9	154	51.3
20-	8	8.3	8	8.7	10	8.9	26	8.7
Tumour stage								
T1	14	14.6	16	17.4	36	32.1	66	22
T2	74	77.1	72	78.3	68	60.7	214	71.3
T3	8	8.3	4	4.3	8	7.1	20	6.7
Gleason Score								
6	10	10.4	10	10.9	16	14.3	36	12
3+4	48	50	52	56.5	58	51.8	158	52.7
4+3	28	29.2	18	19.6	24	21.4	70	23.3
8+	10	10.4	12	13	14	12.5	36	12

Table S6. Distribution of the matching variables as categorical variables by fractionation schedules in Bcl2 data set (PSA: prostate specific antigen).

	74Gy/37f Total N = 106		60Gy/20f Total N = 96		57Gy/19f Total N = 108		Total Total N = 310	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
PSA (ng/ml)								
<10	28	26.4	38	39.6	52	48.1	118	38.1
10- & <20	72	67.9	50	52.1	48	44.4	170	54.8
20-	6	5.7	8	8.3	8	7.4	22	7.1
Tumour stage								
T1	20	18.9	16	16.7	34	31.5	70	22.6
T2	78	73.6	72	75	64	59.3	214	69
T3	8	7.5	8	8.3	10	9.3	26	8.4
Gleason Score								
6	12	11.3	10	10.4	14	13	36	11.6
3+4	54	50.9	56	58.3	56	51.9	166	53.5
4+3	30	28.3	18	18.8	22	20.4	70	22.6
8+	10	9.4	12	12.5	16	14.8	38	12.3

Table S7. Distribution of the matching variables as categorical variables by fractionation schedules in Geminin data set (PSA: prostate specific antigen).

	74Gy/37f Total N = 104		60Gy/20f Total N = 94		57Gy/19f Total N = 110		Total Total N = 308	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
PSA (ng/ml)								
<10	28	26.9	36	38.3	54	49.1	118	38.3
10- & <20	70	67.3	48	51.1	46	41.8	164	53.2
20-	6	5.8	10	10.6	10	9.1	26	8.4
Tumour Stage								
T1	18	17.3	14	14.9	36	32.7	68	22.1
T2	78	75	74	78.7	64	58.2	216	70.1
T3	8	7.7	6	6.4	10	9.1	24	7.8
Gleason Score								
6	10	9.6	10	10.6	16	14.5	36	11.7
3+4	54	51.9	56	59.6	58	52.7	168	54.5
4+3	30	28.8	16	17	22	20	68	22.1
8+	10	9.6	12	12.8	14	12.7	36	11.7

Table S8. Distribution of the matching variables as categorical variables by fractionation schedules in HIF1α data set (PSA: prostate specific antigen).

	HIF1α data set							
	74Gy/37f Total N = 110		60Gy/20f Total N = 92		57Gy/19 Total N = 108		Total Total N = 310	
	N	%	N	%	N	%	N	%
PSA (ng/ml)								
<10	30	27.3	40	43.5	52	48.1	122	39.4
10- & <20	74	67.3	44	47.8	48	44.4	166	53.5
20-	6	5.5	8	8.7	8	7.4	22	7.1
Tumour Stage								
T1	24	21.8	16	17.4	32	29.6	72	23.2
T2	78	70.9	70	76.1	66	61.1	214	69
T3	8	7.3	6	6.5	10	9.3	24	7.7
Gleason Score								
6	12	10.9	8	8.7	12	11.1	32	10.3
3+4	58	52.7	56	60.9	60	55.6	174	56.1
4+3	32	29.1	16	17.4	22	20.4	70	22.6
8+	8	7.3	12	13	14	13	34	11

Table S9. Distribution of the matching variables as categorical variables by fractionation schedules in P-CHK1 data set (PSA: prostate specific antigen).

	P-CHK1 data set							
	74Gy/37f Total N = 102		60Gy/20f Total N = 86		57Gy/19 Total N = 110		Total Total N = 298	
	N	%	N	%	N	%	N	%
PSA (ng/ml)								
<10	28	27.5	38	44.2	54	49.1	120	40.3
10- & <20	68	66.7	40	46.5	48	43.6	156	52.3
20-	6	5.9	8	9.3	8	7.3	22	7.4
Tumour Stage								
T1	18	17.6	16	18.6	38	34.5	72	24.2
T2	76	74.5	64	74.4	64	58.2	204	68.5
T3	8	7.8	6	7	8	7.3	22	7.4
Gleason Score								
6	10	9.8	8	9.3	16	14.5	34	11.4
3+4	54	52.9	50	58.1	58	52.7	162	54.4
4+3	28	27.5	18	20.9	22	20	68	22.8
8+	10	9.8	10	11.6	14	12.7	34	11.4

Figure S1: Different staining patterns for p53 IHC. A: clonal mutant, B: total mutant, C and D: wild type. A-C 10X, D:20X
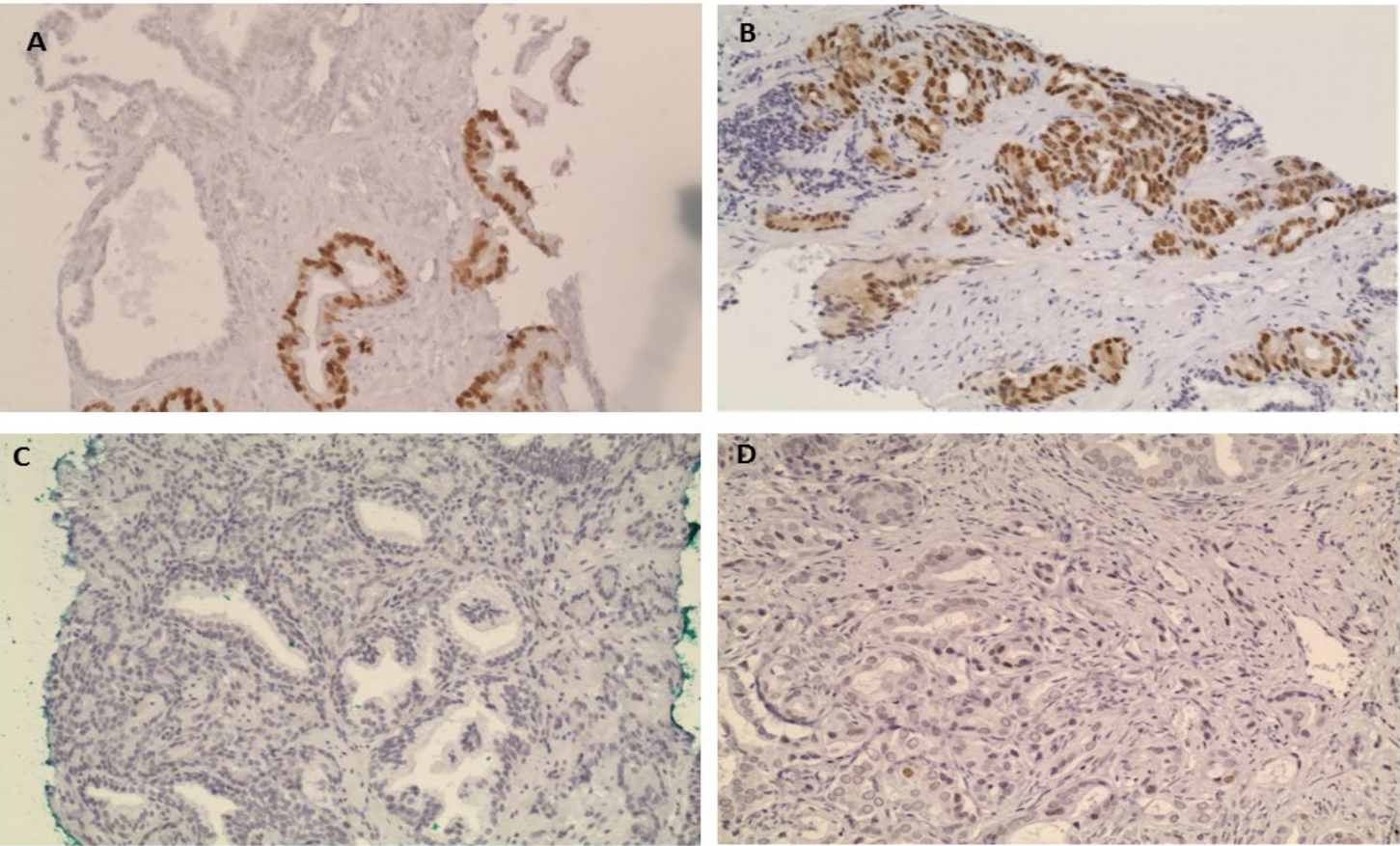
Table S10: Agreement in IHC markers in Part 1 between scoring investigators 1 and 2 for p53, Bcl2, HIF1α, ATM, MRE11 and VEGF.
*less than 110 slides were stained due to lack of tissue

p53	Investigator 1					ATM	Investigator 1			
Investigator 2	Wild-type	Mutant	Focal mutant	Insufficient	Total	**Investigator 2**	No loss of staining	Staining loss	Insufficient	Total
Wild-type	94	0	1	2	97	No loss of staining	101	1	1	103
Mutant	0	3	0	0	3	Straining loss	0	0	0	0
Focal mutant	0	0	4	0	4	Insufficient	0	0	7	7
Insufficient	0	0	0	6	6					
Total	94	3	5	8	110	Total	101	1	8	110
Cohen's kappa for agreement: 0.89 (SE: 0.07)						Cohen's kappa for agreement: 0.87 (SE: 0.09)				

Bcl2*	Investigator 1					MRE11	Investigator 1			
Investigator 2	No expression	Any expression	Insufficient	Total		**Investigator 2**	No loss of staining	Staining loss	Insufficient	Total
No expression	83	0	0	83		No loss of staining	103	0	0	103
Any expression	0	13	0	13		Staining loss	0	0	0	0
Insufficient	1	1	8	10		Insufficient	1	0	6	7
Total	84	14	8	106		Total	104	0	6	110
Cohen's kappa for agreement: 0.95 (SE: 0.07)						Cohen's kappa for agreement: 0.92 (SE: 0.10)				

HIF1α*	Investigator 1					VEGF*	Investigator 1			
Investigator 2	No staining	Any staining	Insufficient	Total		**Investigator 2**	Normal expression	Increased expression	Insufficient	Total
No staining	68	0	0	68		Normal expression	85	0	0	85
Any staining	0	21	0	21		Increased expression	3	1	0	4
Insufficient	0	0	12	12		Insufficient	0	0	12	12
Total	68	21	12	101		Total	88	1	12	101
Cohen's kappa for agreement: 1.00 (SE: 0.08)						Cohen's kappa for agreement: 0.88 (SE: 0.09)				

Table S11: Agreement in IHC markers in Part 1 between scoring investigators 1 and 2 for PTEN, EGFR, Geminin and p16.
*less than 110 slides were stained due to lack of tissue
$Mixed score for p16 included at least 100 cells ≤25% and at least 100 cells >25%

PTEN	Investigator 1					
Investigator 2	No loss	Loss	Focal loss	H-score ≤10	Insufficient	Total
No loss	52	0	1	0	1	54
Loss	0	17		0	0	17
Focal loss	0	6	25	0	0	31
H-score ≤10	0	0	0	1	0	1
Insufficient	0	0	0	0	7	7
Total	52	23	26	1	8	110

Cohen's kappa for agreement: 0.89 (SE: 0.06)

Geminin	Investigator 1						
Investigator 2	0-5%	5.1-10%	10.1-15%	15.1-20%	>20%	Insufficient	Total
0-5%	60	0	0	0	0	0	60
5.1-10%	6	27	0	0	0	0	33
10.1-15%	0	0	5	1	0	0	6
15.1-20%	0	0	0	0	2	0	2
>20%	0	0	0	0	2	0	2
Insufficient	0	0	0	0	0	7	7
Total	66	27	5	1	4	7	110

Cohen's kappa for agreement: 0.86 (SE: 0.06)

EGFR	Investigator 1			
Investigator 2	Normal expression	Increased expression	Insufficient	Total
Normal expression	98	0	0	98
Increased expression	1	4	0	5
Insufficient	0	0	7	7
Total	99	4	7	110

Cohen's kappa for agreement: 0.95 (SE: 0.08)

p16*	Investigator 1						
Investigator 2	1-25%	25.1-50%	50.1-75%	>75%	Mixed$	Insufficient	Total
1-25%	33	0	0	0	0	0	33
25.1-50%	1	27	2	0	0	0	30
50.1-75%	0	0	18	2	0	0	20
>75%	0	0	0	5	0	0	5
Mixed$	0	0	0	2	5	0	7
Insufficient	0	0	0	0	0	11	11
Total	34	27	20	9	5	11	106

Cohen's kappa for agreement: 0.91 (SE: 0.05)

Figure S2: IHC markers excluded after Part 1. Homogenous staining in normal prostate and tumour is shown for ATM in nuclei (A) and VEGF in cytoplasm (B). Heterogeneous cytoplasmic staining within cases is shown for OPN (C) and COX2 (D). A:10X, B-D:20X
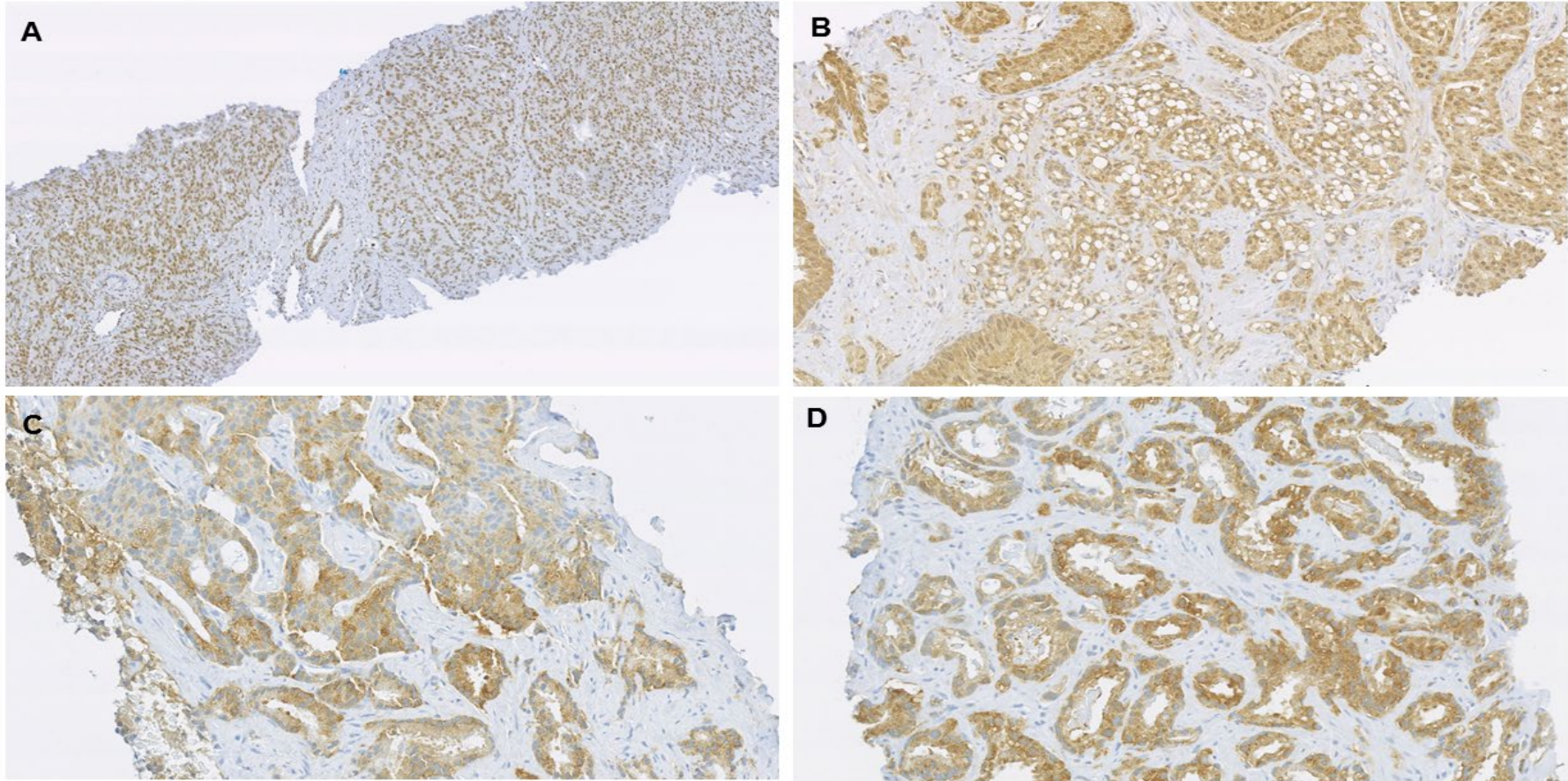
Table S12: Distribution of IHC scores between cases and controls and according to fractionation schedule for p53, p16 and Bcl2

p53	74Gy/37f (n=114)				60Gy/20f (n=96)				57Gy/19f (n=118)				Total (n=300)			
	Controls (n=57)		Cases (n=57)		Controls (n=48)		Cases (n=48)		Controls (n=59)		Cases (n=59)		Controls (n=164)		Cases (n=164)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Wild type	55	96.5	55	96.5	46	95.8	46	95.8	57	96.6	56	94.9	158	96.3	157	95.7
Focal/total gain	2	3.5	2	3.5	2	4.2	2	4.2	2	3.4	3	5.1	6	3.7	7	2.1
	>0.99				>0.99				>0.99				>0.99			
p16	74Gy/37f (n=94)				60Gy/20f (n=78)				57Gy/19f (n=104)				Total (n=276			
	Controls (n=47)		Cases (n=47)		Controls (n=39)		Cases (n=39)		Controls (n=52)		Cases (n=52)		Controls (n=138)		Cases (n=138)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Wild type	20	42.6	28	59.6	15	58.5	19	48.7	28	53.9	31	59.6	63	45.7	78	56.5
Focal/total loss	27	57.5	19	40.4	24	61.5	20	51.3	24	46.2	21	40.4	75	54.3	60	43.5
	0.099				0.361				0.553				0.071			
Bcl2	74Gy/37f (n=106)				60Gy/20f (n=96)				57Gy/19f (n=108)				Total (n=310)			
	Controls (n=53)		Cases (n=53)		Controls (n=48)		Cases (n=48)		Controls (n=54)		Cases (n=54)		Controls (n=155)		Cases (n=155)	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Not increased	48	90.6	47	88.7	39	81.3	42	87.5	51	94.4	49	90.7	138	89	138	89
Increased	5	9.4	6	11.3	9	18.8	6	12.5	3	5.6	5	9.3	17	11	17	11
	0.99				0.399				0.716				>0.99			

Table S13: Distribution of IHC scores between cases and controls and according to fractionation schedule for HIF1α and pCHK1

Biomarkers	Fractionation	Events	N	Median score	IQR (score) Q1	Q3	Mean score	SD (Score)
HIF1α	74Gy/37f	Controls	55	0.00	0.00	0.00	1.47	6.08
	(n=82)	Cases	55	0.00	0.00	0.00	0.95	4.26
	60Gy/20f	Controls	46	0.00	0.00	0.00	0.17	0.76
	(n=78)	Cases	46	0.00	0.00	0.00	0.34	0.77
	57Gy/19f	Controls	54	0.00	0.00	0.00	0.20	0.70
	(n=88)	Cases	54	0.00	0.00	0.50	1.72	5.45
pCHK1	74Gy/37f	Controls	51	0.36	0.18	0.56	0.52	0.52
	(n=102)	Cases	51	0.42	0.26	0.78	0.64	0.58
	60Gy/20f	Controls	43	0.40	0.20	1.08	0.76	0.77
	(n=86)	Cases	43	0.44	0.20	0.90	0.61	0.53
	57Gy/19f	Controls	55	0.42	0.24	0.84	0.59	0.50
	(n=110)	Cases	55	0.28	0.14	0.52	0.39	0.34

Table S14: Odds ratios for BCR estimated from univariate conditional logistic regression models without and with interaction terms between continuous variables (mean and maximum Ki67, Geminin, HIF1α and p-chk1) and fractionation schedules.

Schedules	OR	95 % CI (OR)	P value (OR)	P value for interaction*
mean Ki67				
74 Gy & 60 Gy	1.09	1.02 – 1.17	0.007	0.26
74 Gy & 57 Gy	1.07	1.01 – 1.14	0.03	0.59
60 Gy & 57 Gy	1.11	1.04 – 1.19	0.001	0.59
max Ki67				
74 Gy & 60 Gy	1.06	1.02 – 1.11	0.008	0.34
74 Gy & 57 Gy	1.04	1.00 – 1.08	0.08	0.82
60 Gy & 57 Gy	1.06	1.01 – 1.11	0.02	0.27
Geminin				
74 Gy & 60 Gy	1.14	1.05 - 1.25	0.003	0.56
74 Gy & 57 Gy	1.05	0.98 - 1.13	0.2	0.06
60 Gy & 57 Gy	1.04	0.98 - 1.12	0.21	0.1
ln(HIF1α)				
74 Gy & 60 Gy	0.57	0.13 - 2.46	0.45	n/a
74 Gy & 57 Gy	1.13	0.56 - 2.3	0.74	0.27
60 Gy & 57 Gy	1.6	0.54 - 4.75	0.4	n/a
P-chk1				
74 Gy & 60 Gy	1.24	0.8 - 1.91	0.34	0.27
74 Gy & 57 Gy	1.2	0.72 - 1.98	0.48	0.11
60 Gy & 57 Gy	0.82	0.48 - 1.4	0.47	0.5

* Odds ratios (OR) are adjusted for the matching variables and age at randomisation. As no values are significant, p-values for the BH adjustment with 10% False Discovery Rate are not included.

Table S15: Odds ratios for BCR estimated from univariate conditional logistic regression models without and with interaction terms between categorical variables (p53, p16, PTEN and Bcl-2) and fractionation schedules.

Schedules		OR	95 % CI (OR)	P value (OR)	P value for interaction*
p53					
74 Gy & 60 Gy	Wild type	1			
	Mutant	1	0.32 - 3.11	1	0.56
74 Gy & 57 Gy	Wild type	1			
	Mutant	1.4	0.44 - 4.42	0.57	0.92
60 Gy & 57 Gy	Wild type	1			
	Mutant	1.04	0.3 - 3.61	0.95	0.47
p16					
74 Gy & 60 Gy	Wild type	1			
	Mutant	0.61	0.34 - 1.1	0.1	0.89
74 Gy & 57 Gy	Wild type	1			
	Mutant	0.71	0.42 - 1.23	0.22	0.67
60 Gy & 57 Gy	Wild type	1			
	Mutant	0.72	0.4 - 1.3	0.28	0.67
PTEN					
74 Gy & 60 Gy	Wild type	1			
	Mutant	2.19	1.21 - 3.97	0.01	0.26
74 Gy & 57 Gy	Wild type	1			
	Mutant	2.74	1.53 - 4.9	0.0007	0.69
60 Gy & 57 Gy	Wild type	1			
	Mutant	1.94	1.09 - 3.43	0.02	0.42
Bcl-22					
74 Gy & 60 Gy	No increase	1			
	Increase	0.85	0.39 - 1.87	0.69	0.4
74 Gy & 57 Gy	No increase	1			
	Increase	0.68	0.28 - 1.65	0.39	0.74
60 Gy & 57 Gy	No increase	1			
	Increase	1.03	0.45 - 2.36	0.95	0.71

* Odds ratios (OR) are adjusted for the matching variables and age at randomisation