UNIVERSITY OF PLYMOUTH
Faculty of Medicine and Dentistry

Universal Tumour Antigens in Primary Liver Cancers

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14th November 2019

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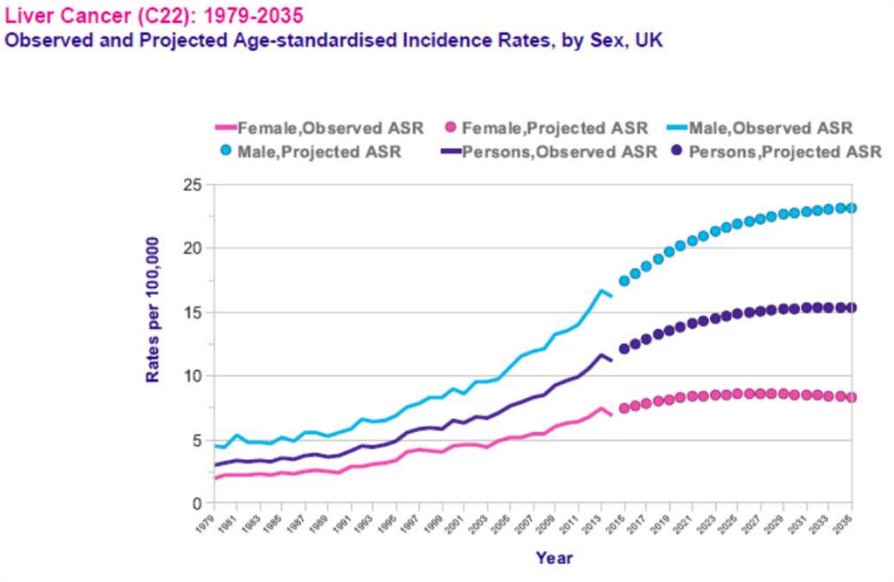
Burden of Disease



Cancer type	Estimated 2013 incidence total^	Estimated 2013 deaths total^	◀ DECREASING 0% INCREASING ▶
All Malignant Cancers	1,660,290	580,350	-24.5 -15.8
Head and Neck Region			
Brain & other nervous system	23,130	14,080	-11.7 -10.5
Oral cavity & pharyngeal	41,380	7,890	-35.1 -35.5
Laryngeal	12,260	3,630	-34.7 -22.2
Lung & bronchus	228,190	159,480	-32.0 +4.8
Breast	234,580	40,030	-32.9
Gastrointestinal (GI) Tract			
Esophageal	17,990	15,210	-14.4 +4.3
Stomach	21,600	10,990	-41.1
Liver & intrahepatic bile duct	30,640	21,670	+41.2 +62.8
Pancreatic	45,220	38,460	-1.3 +3.0
Small intestine	8,810	1,170	
Colon and Rectal†	142,820	50,830	-38.1 -35.7
Urogenital System			
Kidney & renal pelvis	65,150	13,680	-6.7 -9.4
Ovarian	22,240	14,030	-15.9
Uterine (non-cervical)	49,560	8,190	-3.9
Cervical (uterine)	12,340	4,030	-37.4
Urinary bladder	72,570	15,210	-3.8 -10.7
Prostate	238,590	29,720	-42.6
Testicular	7,920	370	
Melanoma (skin)	76,690	9,480	-9.6 +10.5
Hematological System			
Leukemia	48,610	23,720	-11.0 -15.0
Lymphoma			
Hodgkin lymphoma	9,290	1,180	-43.5 -36.0
Non-Hodgkin lymphoma	69,740	19,020	-18.6 -22.2 -12.4 -16.6
Myeloma	22,350	10,710	
Bones & joints	3,010	1,440	
Soft tissue (including heart)	11,410	4,390	

Male
Female

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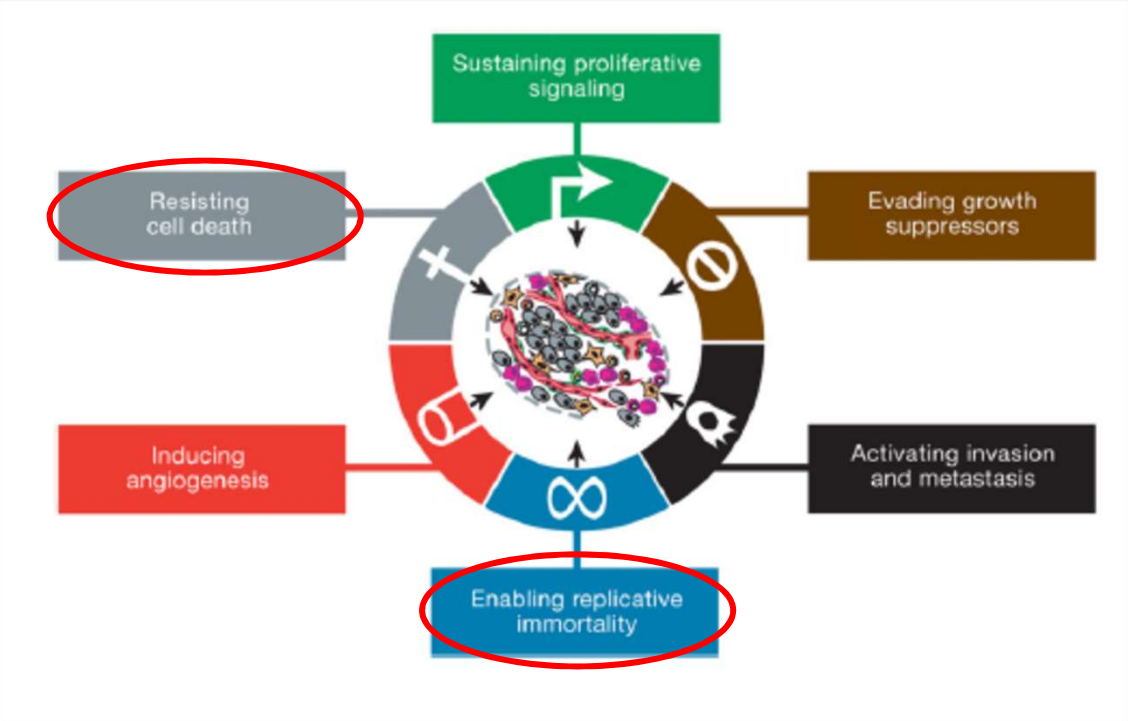
http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/liver-cancer/incidence#heading-Four

AACR Cancer Progress Report Writing Committee, C. L., Sawyers, C. L., Abate-Shen, C., Anderson, K. C., Barker, A., Baselga, J., … Weiner, G. J. (2013). AACR Cancer Progress Report 2013. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *19*(20 Suppl), S4-98. https://doi.org/10.1158/1078-0432.CCR-13-2107

Hallmarks of Cancer

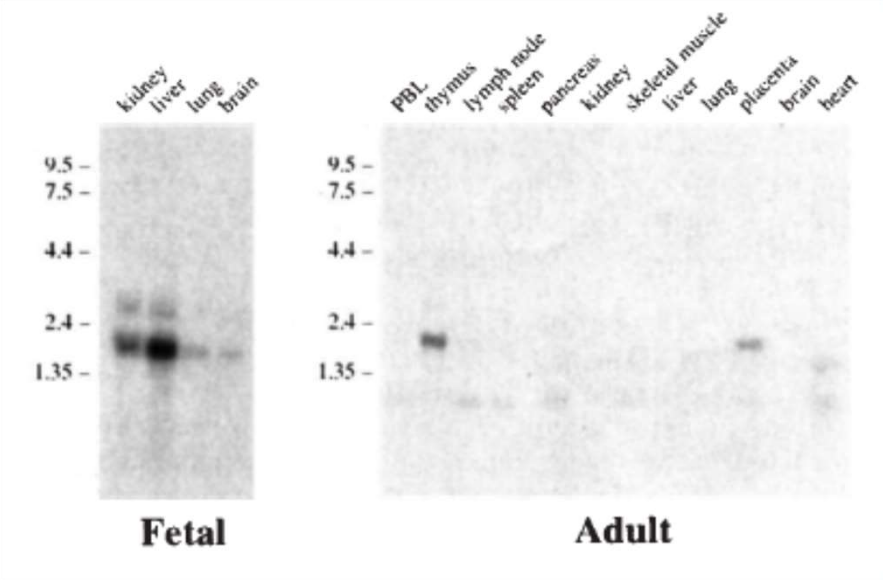


Hanahan, D. *et al.* (2011) 'Hallmarks of Cancer: The Next Generation', *Cell*. Arnold Constable, London, UK, 144(5), pp. 646–674. doi: 10.1016/j.cell.2011.02.013.

Universal Tumour Associated Antigens

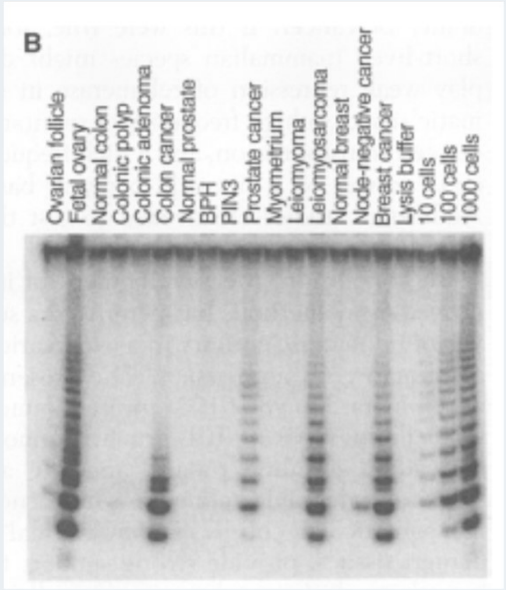
A novel anti-apoptosis gene, *survivin*, expressed in cancer and lymphoma

Grazia Ambrosini, Colette Adida & Dario C. Altieri



Specific Association of Human Telomerase Activity with Immortal Cells and Cancer

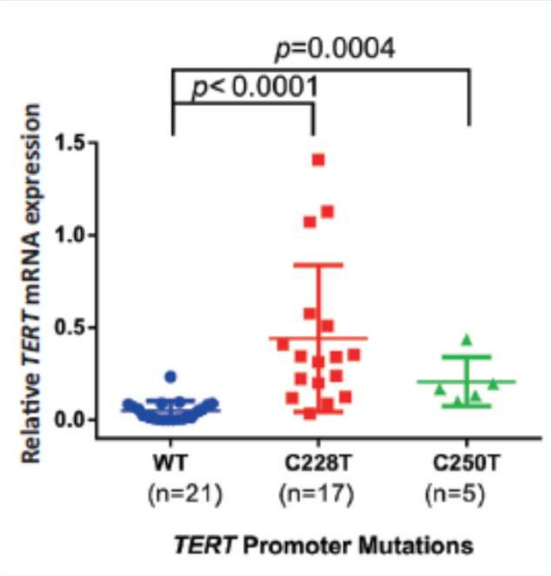
Nam W. Kim,* Mieczyslaw A. Piatyszek,* Karen R. Prowse, Calvin B. Harley, Michael D. West, Peter L. C. Ho, Gina M. Coviello, Woodring E. Wright, Scott L. Weinrich,*† Jerry W. Shay*†



Ambrosini, G., Adida, C. and Altieri, D. C. (1997) 'A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma.', *Nature medicine*, 3(8), pp. 917–21.
Nam W. Kim,* Mieczyslaw A. Piatyszek, * Karen R. Prowse *et al.* (1994) 'Specific Association of Human Telomerase Activity with Immortal Cells and Cancer', *Science*, 266(5193), pp. 2011–2015.

Promoter Regions

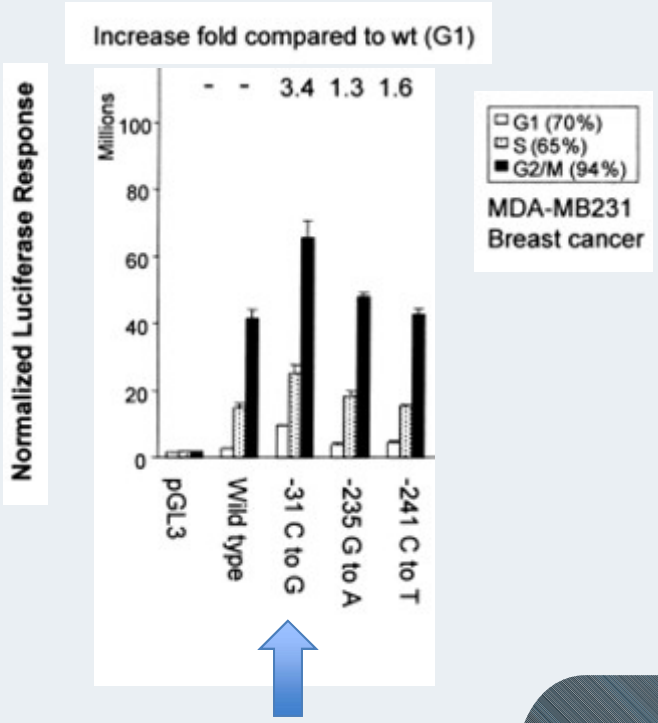
Telomerase SNPs



C228T = "-124"

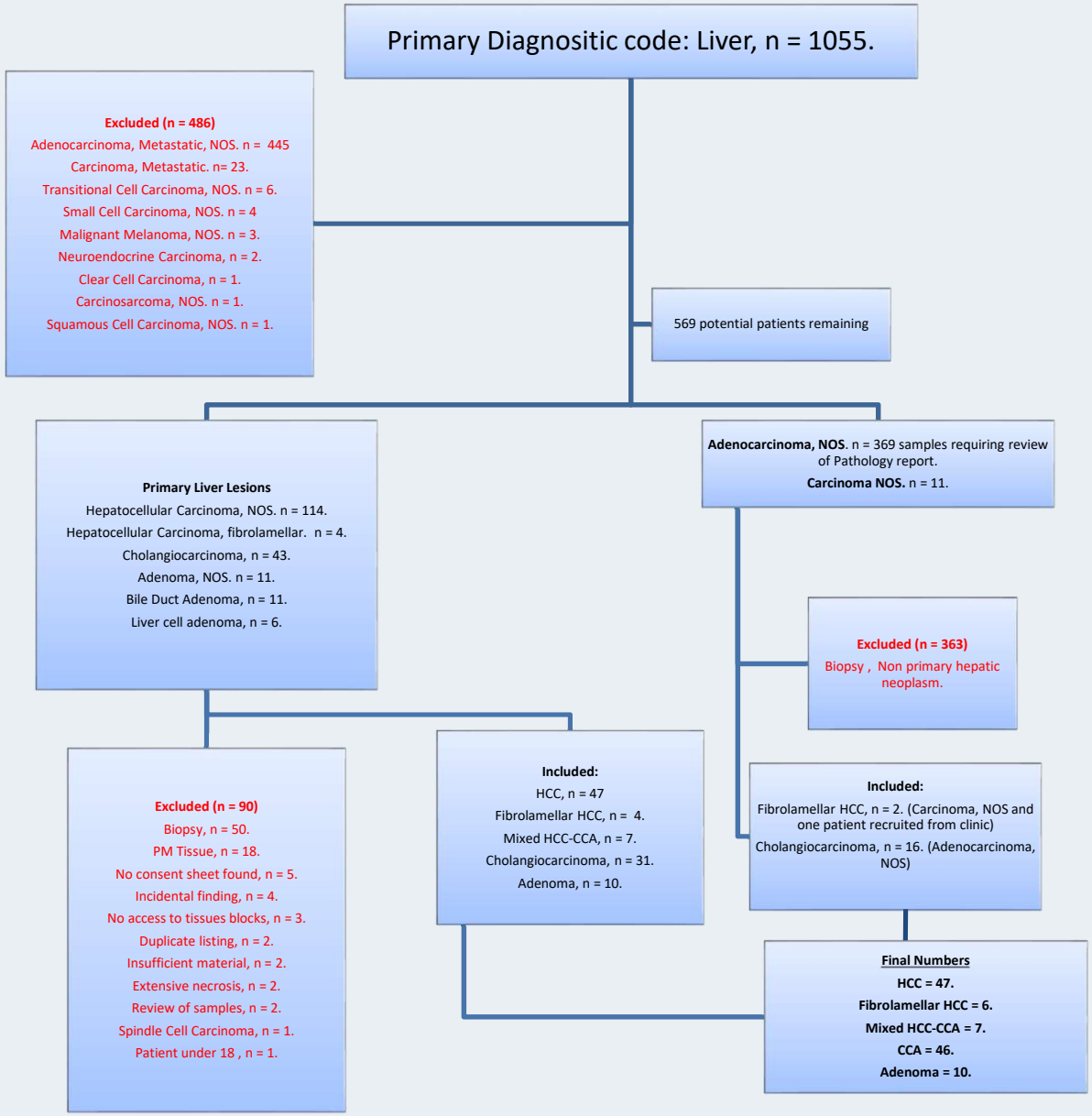
C250T = "-146"

Survivin -31G/C



Xu, Y., et al (2004). A Mutation Found in the Promoter Region of the Human Survivin Gene is Correlated to Overexpression of Survivin in Cancer Cells. *DNA and Cell Biology*, *23*(7), 419–429.

Huang D-S, et al. Recurrent TERT promoter mutations identified in a large-scale study of multiple tumour types are associated with increased TERT expression and telomerase activation. Eur J Cancer. 2015 May 1;51(8):969–76.



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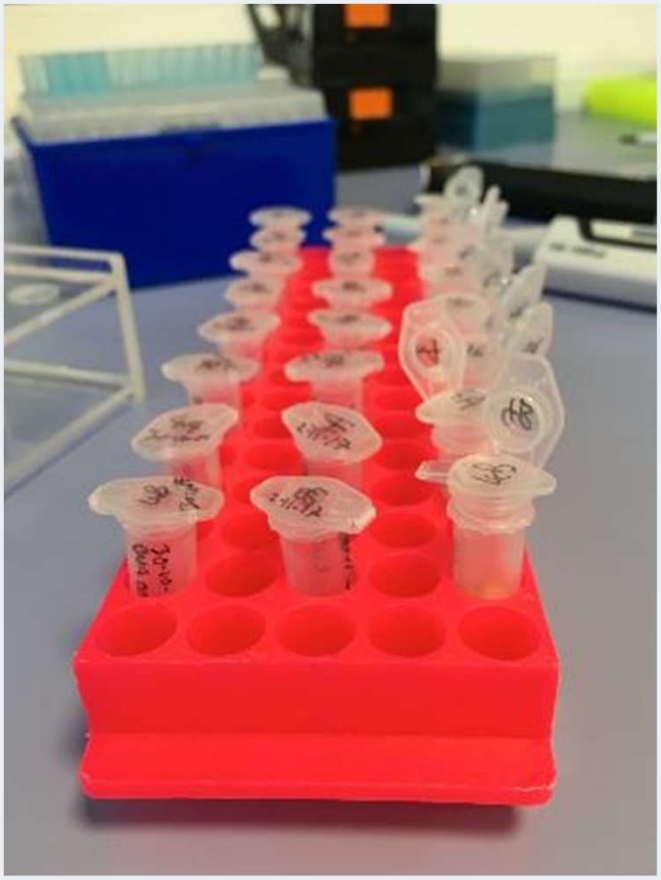
Further Refinement

Tumour	n
Hepatocellular Carcinoma	52
Fibrolamellar Hepatocellular Carcinoma	6
Intrahepatic-Cholangiocarcinoma	30
Perihilar-Cholangiocarcinoma	6
Mixed HCC-CCA	7
Total	**101**



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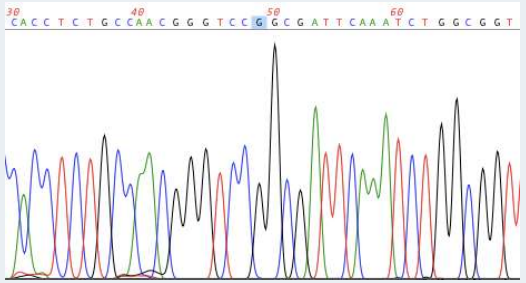
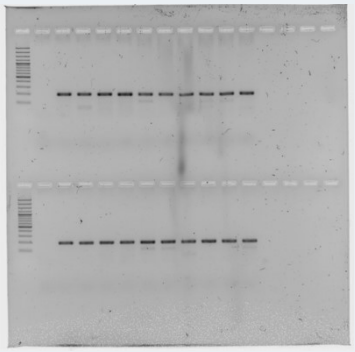
PCR Workflow



DNA from FFPE

Sanger Sequencing



PCR Amplification

Purification

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Telomerase Promoter

Hepatocellular Carcinoma

Study Number	7a	7b	8a	8c	9b	9x	10b	11a	11b	13a	13b	14a	14b	15a	15b	16a	16b	17a	17b	18a	18b	19a	19b	20a	20b	22a	22b	23a	23b	24a	24b	25a	25b	26a	26b	27a	27b	28a	28b	29a	29b	30a	30b	31a	31i	33a	33b	35a	35b	36a	36c	37a	37b	38a	38b
pTERT WT/Mut		-124										-124											-124				-124				-124				-124												-124								
BG/Tumour	BG	T	BG	T	BG	T	T	BG	T	T	BG	T	T	BG	T	T	BG	BG	T	T	BG	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	T	BG	BG	T	BG	T	T	BG	T	BG	T	BG	BG	T	BG	T

Study Number	39a	39c	41a	41b	42a	42b	42x	43a	43c	44a	44b	53b	54a	54b	55a	55b	56a	56b	57a	57b	64a	64b	69a	70a	70b	71a	71b	72a	72b	73a	73b	74a	74b	93a	93b	101a	101b	116a	116b	116x	117a	117b	118a	118b	119a	131a	131b	141a	141b	143a	143b	146a	146b	147x
pTERT WT/Mut							-124					-124							-124					-124					-124		-146				-146		-124			-124							-124		-124					
BG/Tumour	T	T	T	BG	BG	T	T	BG	T	T	BG	T	T	T	T	T	T	T	T	BG	T	BG	T	T	BG	BG	T	BG	T	BG	T	T	BG	BG	T	BG	T	BG	T	T	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T

Cholangiocarcinoma

Study Number	48a	48b	49a	49b	51a	51b	52d	52e	58a	58b	60c	60d	62a	62b	65a	65b	68a	76a	76b	77a	77b	78a	78b	79a	79b	80a	80b	83a	84a	87a	87b	90a
pTERT WT/Mut																																
BG/Tumour	BG	T	BG	T	BG	T	T	BG	BG	T	BG	T	BG	T	T	BG	T	T	BG	BG	T	BG	T	T	BG	T	BG	T	T	T	BG	T

Study Number	90b	99a	102a	102b	103a	103b	105a	107a	107b	113a	113b	112a	112b	120a	121a	122a	124a	124b	126a	126b	129a	129b	135a	135b	137a	137b	138a	138b	142a	142b	144a	144b
pTERT WT/Mut																																
BG/Tumour	BG	T	BG	T	T	BG	T	BG	T	BG	T	BG	T	BG	T	T	BG	T	BG	T	BG	T	T	BG	BG	T	BG	T	T	BG	BG	T

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Survivin Promoter

Hepatocellular Carincoma

Study Number	7a	7b	8a	8c	10b	14a	14b	15a	15b	17a	17b	19a	19b	22a	22b	23a	23b	25a	25b	27a	27b	28a	28b	35a	35b	41a	41b	43a	43c	44a	44b	64a	64b	69a	70a	70b	73a	73b	74a	74b	116a	116b	116x	131a	131b
pSurv SNP	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
BG/Tumour	BG	T	BG	T	T	T	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	T	BG	T	BG	BG	T	T	BG	T	BG	T	T	BG	BG	T	T	BG	BG	T	T	BG	T

Study Number	9b	9x	11a	11b	13a	13b	16a	16b	18a	18b	24a	24b	29a	29b	31a	31i	33a	33b	36a	36c	38a	38b	39a	39c	42x	42a	42b	53b	54a	54b	55a	55b	56a	56b	57a	57b	71a	71b	72a	72b	93a	93b	101a	101b	117a	117b	119a	141a	141b	146a	146b
pSurv SNP	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
BG/Tumour	BG	T	BG	T	T	BG	T	BG	T	BG	BG	T	T	BG	BG	T	T	BG	T	BG	BG	T	T	T	T	BG	T	T	T	T	T	T	T	T	BG	BG	T	BG	T	BG	T	BG	T	T	BG	T	T	BG	T	BG

Study Number	20a	20b	26a	26b	30a	30b	37a	37b	118a	118b	143a	143b	147x
pSurv SNP	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
BG/Tumour	BG	T	BG	T	BG	T	BG	T	T	BG	T	BG	T

Cholangiocarcinoma

Study Number	49a	49b	51a	51b	60c	60d	62a	62b	65a	65b	76a	76b	78a	78b	79a	79b	80a	80b	84a	87a	87b	103a	103b	105a	107a	107b	120a	121a	124a	124b	135a	135b	137a	137b	138a	138b	142a	142b
pSurv SNP	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
BG/Tumour	BG	T	BG	T	BG	T	BG	T	T	BG	T	BG	BG	T	T	BG	T	BG	T	T	BG	T	BG	T	BG	T	BG	T	BG	T	BG	T	T	BG	BG	T	BG	T	T	BG

Study Number	48a	48b	52d	52e	58a	58b	68a	83a	99a	102a	102b	113a	113b	122a	126a	126b	129a	129b	144a	144b	77a	77b	90a	90b	112a	112b
pSurv SNP	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	CC	CC	CC	CC	CC	CC
BG/Tumour	BG	T	T	BG	BG	T	T	T	T	BG	T	BG	T	T	BG	T	BG	T	BG	T	BG	T	T	BG	BG	T

Mixed Tumours

Study Number	34a	40a	40b	45a	45c	46a	47a	47b	50a	50b	125a	125b
pSurv SNP	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
pTERT WT/Mut										-124		
Background/Tumour	T	BG	T	BG	T	T	BG	T	BG	T	T	BG

Tumour	WT (%)	Mutant (%)
Hepatocellular Carcinoma	40 (69%)	18 (31%)
Cholangiocellular Carcinoma	36 (100%)	0 (0%)
Mixed HCC-CCA	6 (86%)	1 (14%)

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Telomerase Results - Pooled

Telomerase Promoter SNPs		N	WT	Mut	P
Tumour Type	HCC	58	40	18	<0.001
	CCA	36	36	0	
	Mixed	7	6	1	
Gender	Female	36	35	1	0.001
	Male	65	47	18	
Fibrosis	Absent	59	53	6	0.01
	Present	41	28	13	
Cirrhosis	Absent	77	67	10	0.012
	Present	23	14	9	
Outcome	Alive	45	38	7	0.610
	Dead	56	44	12	
Margin	R0	58	44	14	0.196
	R1	41	36	5	
Tumour Stage	I/II	64	55	9	0.229
	III/IV	27	20	7	
Grade	Well Diff	11	9	2	0.401
	Mild/Mod Diff	66	52	14	
	Poorly Diff	17	16	1	
Vascular Invasion	No VI	42	35	7	0.794
	VI	52	42	10	
Viral Status	No Viral Hep	86	72	14	0.151
	Viral Hep	15	10	5	
Metabolic Risk Factors	+	46	35	11	0.308
	-	55	47	8	
Genetic Liver Disease	+	10	8	2	1.00
	-	91	74	17	

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Telomerase Results – HCC only

	Telomerase Promoter Mutants	N	WT	Mut	P
Gender	Female	14	14	0	0.003
	Male	44	26	18	
Fibrosis	Absent	32	26	6	0.044
	Present	26	14	12	
Cirrhosis	Absent	42	32	10	0.066
	Present	16	8	8	
Outcome	Alive	29	23	6	0.155
	Dead	29	17	12	
Margin	R0	40	27	13	1.00
	R1	16	11	5	
Tumour Stage	I/II	31	23	8	0.528
	III/IV	19	12	7	
Grade	Well Diff	10	8	2	0.576
	Mild/Mod Diff	37	24	13	
	Poorly Diff	6	5	1	
Vascular Invasion	No VI	31	25	6	0.068
	VI	22	12	10	
Viral Status	No Viral Hep	49	35	14	0.438
	Viral Hep	9	5	4	
Metabolic Risk Factors	+	28	17	11	0.258
	-	30	23	7	
Genetic Liver Disease	+	6	4	2	1.00
	-	52	36	16	

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Survivin Results - Pooled

Survivin GG vs. CG+CC		N	CC+CG	GG	P
Gender	Female	35	23	12	0.666
	Male	64	38	26	
Outcome	Alive	45	29	16	0.680
	Dead	54	32	22	
Margin	R0	57	34	23	0.673
	R1	41	27	14	
Fibrosis	Absent	58	33	25	0.399
	Present	40	27	13	
Cirrhosis	Absent	76	42	34	0.027
	Present	22	18	4	
Tumour Stage	I/II	64	41	23	0.814
	III/IV	26	16	10	
Grade	Well Diff	11	7	4	1.00
	Mild/Mod Diff	65	41	24	
	Poorly Diff	17	11	6	
Vascular Invasion	No VI	42	27	15	1.00
	VI	51	32	19	
Viral Status	No Viral Hep	84	50	34	0.395
	Viral Hep	15	11	4	
Metabolic Risk Factors	+	45	28	17	1.00
	-	54	33	21	
Genetic Liver Disease	+	10	5	5	0.501
	-	89	56	33	
Tumour Type	HCC	56	30	26	0.043
	CCA	36	24	12	
	Mixed	7	7	0	

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Conclusions

- FFPE tissue is a potential source of DNA for genetic analysis of tumours.
  - Optimisation required.
  - Short sequences only.
  - Unlocks a potentially huge clinical resource
- Telomerase promoter mutations are:
  - Absent in Cholangiocarcinoma
  - Present in Hepatocellular tumours
    - Telomerase-dependent reactivation of senescent cells

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Future Work

- Quantify Protein levels with IHC
  - Correlate with pSurv & pTERT status
- For the Telomerase Promoter SNPs, clarify:
  - Inter-tumoural heterogeneity
  - Intra-tumoural heterogeneity
- Explore other methods of Telomerase reactivation:
  - Amplifications
  - Translocations
  - Viral Insertions

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Thanks to my team & for listening!

Professor Matthew Cramp (DoS)

Dr Michael Jarvis (Supervisor)

South West Liver Unit

Dr Jem Denson

Dr Louisa Vine

Mr David Stell
Mr Matthew Bowles
Mr Somaiah Aroori
Mr Chris Briggs

NHS University Hospitals Plymouth NHS Trust

Hepatology Research Group

Dr David Sheridan
Dr Dan Felmlee
Dr Ashwin Dhanda
Dr Kris Bennett
Paula Boeira
Euan Yates

The Jarvis Laboratory

Dr Aisling Murphy
Thekla Mauch

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Any Questions?

Hepatocellular Carcinoma

Study Number	7a	7b	8a	8c	10b	14a	14b	15a	15b	17a	17b	19a	19b	22a	22b	23a	23b	25a	25b	27a	27b	28a	28b	35a	35b	41a	41b	43a	43c	44a	44b	64a	64b	69a	70a	70b	73a	73b	74a	74b	116a	116b	116x	131a	131b
pSurv Variant	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
pTERT SNP		-124				-124							-124		-124									-124												-124				-146				-124			
Study Number	9b	9x	11a	11b	13a	13b	16a	16b	18a	18b	24a	24b	29a	29b	31a	31i	33a	33b	36a	36c	38a	38b	39a	39c	42x	42a	42b	53b	54a	54b	55a	55b	56a	56b	57a	57b	71a	71b	72a	72b	93a	93b	101a	101b	117a	117b	119a	141a	141b	146a	146b
pSurv Variant	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
pTERT SNP												-124													-124				-124							-124					-124			-146		-124							
Study Number	20a	20b	26a	26b	30a	30b	37a	37b	118a	118b	143a	143b	147x																																						
pSurv Variant	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC																																						
pTERT SNP				-124							-124																																							

Cholangiocarcinoma

Study Number	49a	49b	51a	51b	60c	60d	62a	62b	65a	65b	76a	76b	78a	78b	79a	79b	80a	80b	84a	87a	87b	103a	103b	105a	107a	107b	120a	121a	124a	124b	135a	135b	137a	137b	138a	138b	142a
pSurv Variant	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
pTERT SNP																																					
Study Number	48a	48b	52d	52e	58a	58b	68a	83a	99a	102a	102b	113a	113b	122a	126a	126b	129a	129b	144a	144b																	
pSurv Variant	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG																	
pTERT SNP																																					
Study Number	77a	77b	90a	90b	112a	112b																															
pSurv Variant	CC	CC	CC	CC	CC	CC																															
pTERT SNP																																					

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Mixed Tumours

Study Number	34a	40a	40b	45a	45c	46a	47a	47b	50a	50b	125a	125b
pSurv Variant	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
pTERT SNP										-124		

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Patient Characteristics

Tumour	Total	M	F	Age (range)	Dead (%)	Months Follow up (range)	Fibrotic	Cirrhotic	R1 Resection	Viral Hepatitis +	Metabolic Risk Factor +	Genetic Cause
Hepatocellular Carcinoma	58	44	14	63 (22 - 84)	29 (50%)	47 (0 - 150)	26	16	16	9	28	6
Cholangiocarcinoma	36	16	20	64 (44 - 83)	21 (58%)	31 (0 - 120)	9	1	24	2	15	2
Mixed HCC-CCA	7	5	2	60 (47 - 72)	6 (86%)	30 (3 - 76)	6	6	1	4	3	2

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