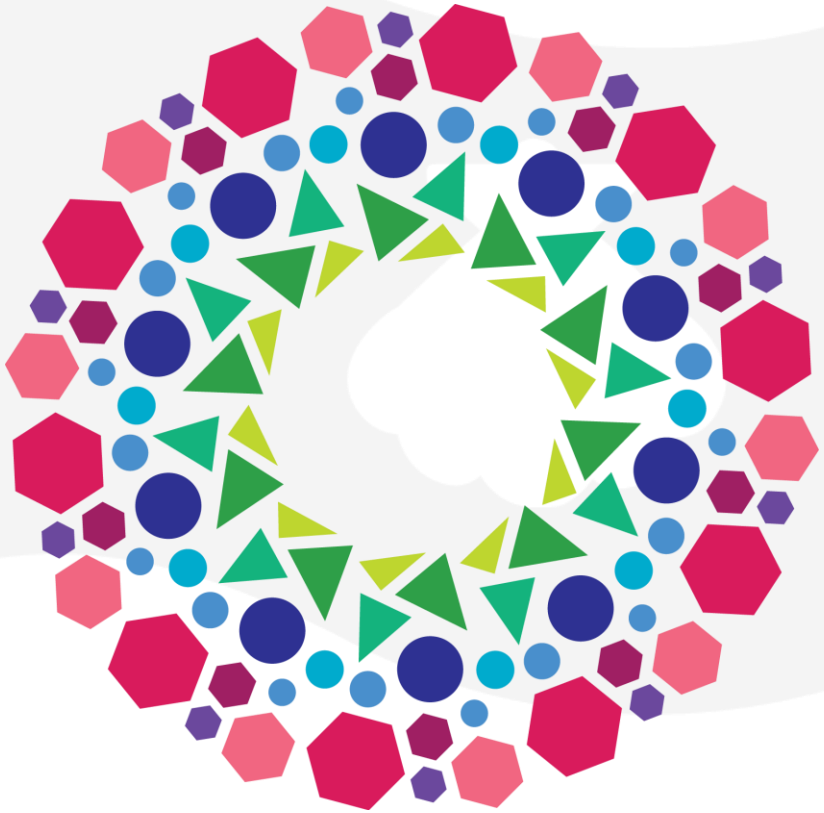
University Hospitals Plymouth NHS Trust

South West Liver Unit

Assessing tumour antigens in archived liver samples: a clinical research experience.



Ollie Rupar

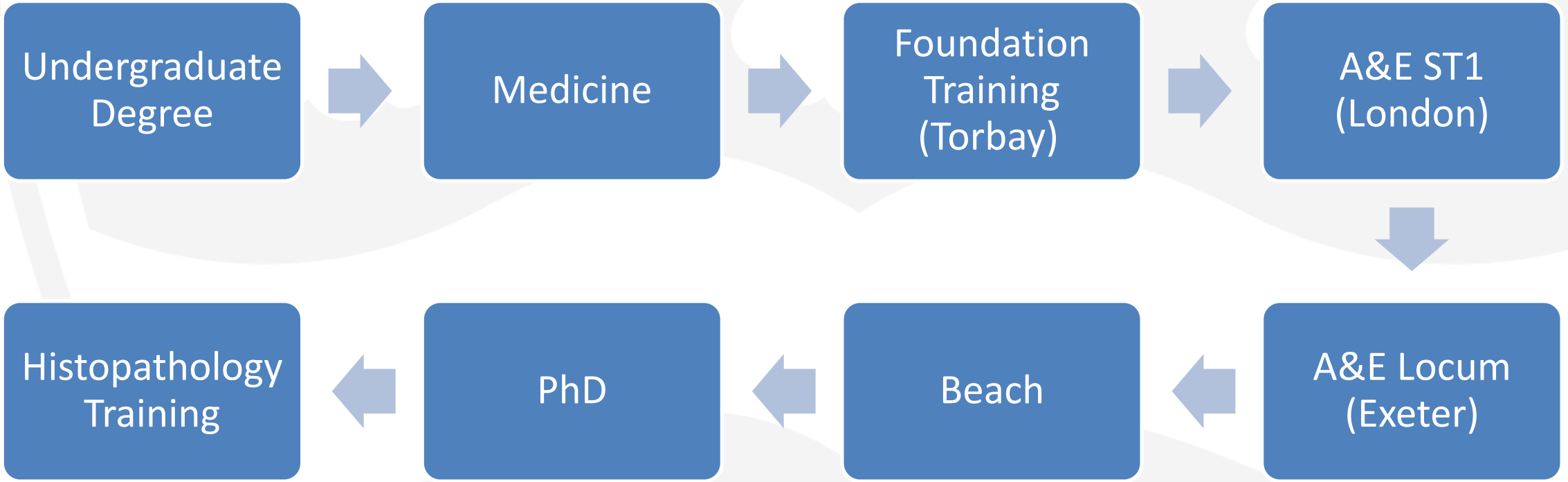
10th June 2022

Oliver.Rupar@nhs.scot

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

My Timeline



UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

Hepatology Research Group

Dedicated to improving the understanding and treatment of liver disease

The hepatology research group (HRG) is an integral part of the Institute of Health and Care Research: infection, immunity and inflammation.

We utilise state of the art laboratory facilities based in the Derriford Research Facility, and the world class clinical research strengths of the Faculty of Health and Plymouth Hospitals NHS Trust (PHNT).

We work in unison with the South West Liver Unit, at Plymouth Hospitals NHS Trust, providing a full range and secondary, tertiary and community Hepatology services to the South West region, including assessment for liver transplantation, TIPS and liver cancer therapy.

The research team run several commercially sponsored clinical trials in hepatitis C therapy, non-alcoholic steatohepatitis (NASH), primary biliary cholangiopathy (PBC), alcoholic liver disease and liver failure amongst others, through the clinical research facilities of The Lind Research Centre at Derriford Hospital.

Hepatology Research Group Annual Reports

- Download the 2017 report
- Download the 2018 report
- Download the 2019 report
- Download the 2020 report

The broad themes of the hepatology research group are:

- Protection from hepatitis C virus infection – (principal investigator) PI Professor Matthew Cramp
- Lipid metabolism and the pathogenesis and treatment of non-alcoholic steatohepatitis – PI Dr David Sheridan
- Alcoholic hepatitis – PI Ashwin Dhanda
- Molecular virology of hepatitis C and other hepatitis viruses – PI Dr Dan Felmlee

NHS
University Hospitals Plymouth
NHS Trust

South West Liver Unit

Research capability funding

NIHR research capability funding (RCF) is allocated to NHS organisations that undertake NIHR research, to help them maintain research capacity and capability.

What are the aims of research capability funding?

The aims of RCF funding are to:

- help research-active NHS organisations to act flexibly and strategically to maintain research capacity and capability
- support the appointment, development and retention of key staff undertaking or supporting people and patient-based based research
- contribute towards the costs of hosting NIHR-funded or 'adopted' research that are not currently fully covered across NIHR's programmes, and that are not met in other ways.

Documents

Research capability funding frequently asked questions

Research capability funding allocations 2022-23 (.XLSX - Excel)

Q&A on the outcome of the 2018 research capability funding review

Use of NIHR research capability funding in 2019-20

University Hospitals Plymouth NHS Trust

South West Liver Unit

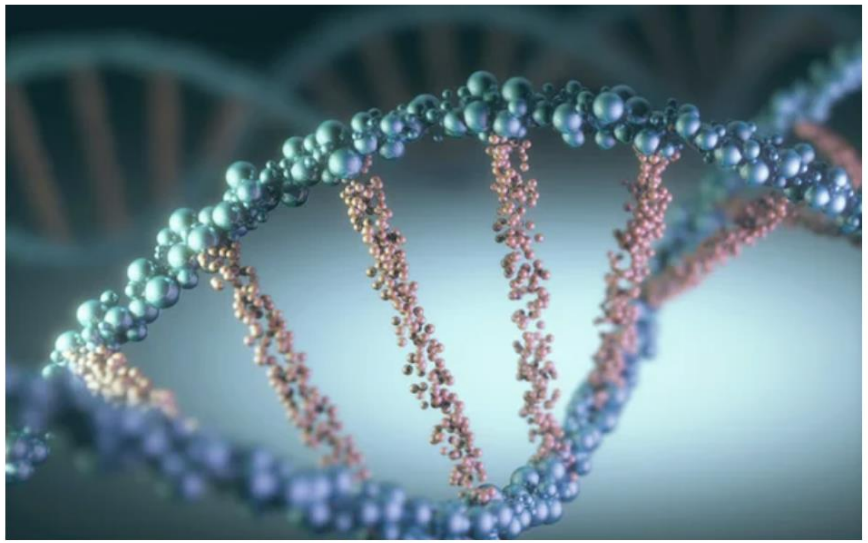
nature

BIOLOGY

Century-Old Tumors Offer Rare Cancer Clues

DNA sequences from 100-year-old tumor samples could bolster childhood cancer research

By Heidi Ledford, Nature magazine on May 11, 2017



Credit: Ktsdesign *Getty Images*

READ THIS NEXT

SPONSORED
Why Cancer Raises COVID Risks, and How Patients Can Stay Safe

PUBLIC HEALTH
Global Initiative Seeks 1,000 New Cancer Models
Heidi Ledford and Nature magazine

THE SCIENCES
Frederick Sanger, Father of DNA Sequencing, Dead at 95
Ewen Callaway and Nature News Blog

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

https://www.scientificamerican.com/article/century-old-tumors-offer-rare-cancer-clues/#

NHS Tayside

Survivin

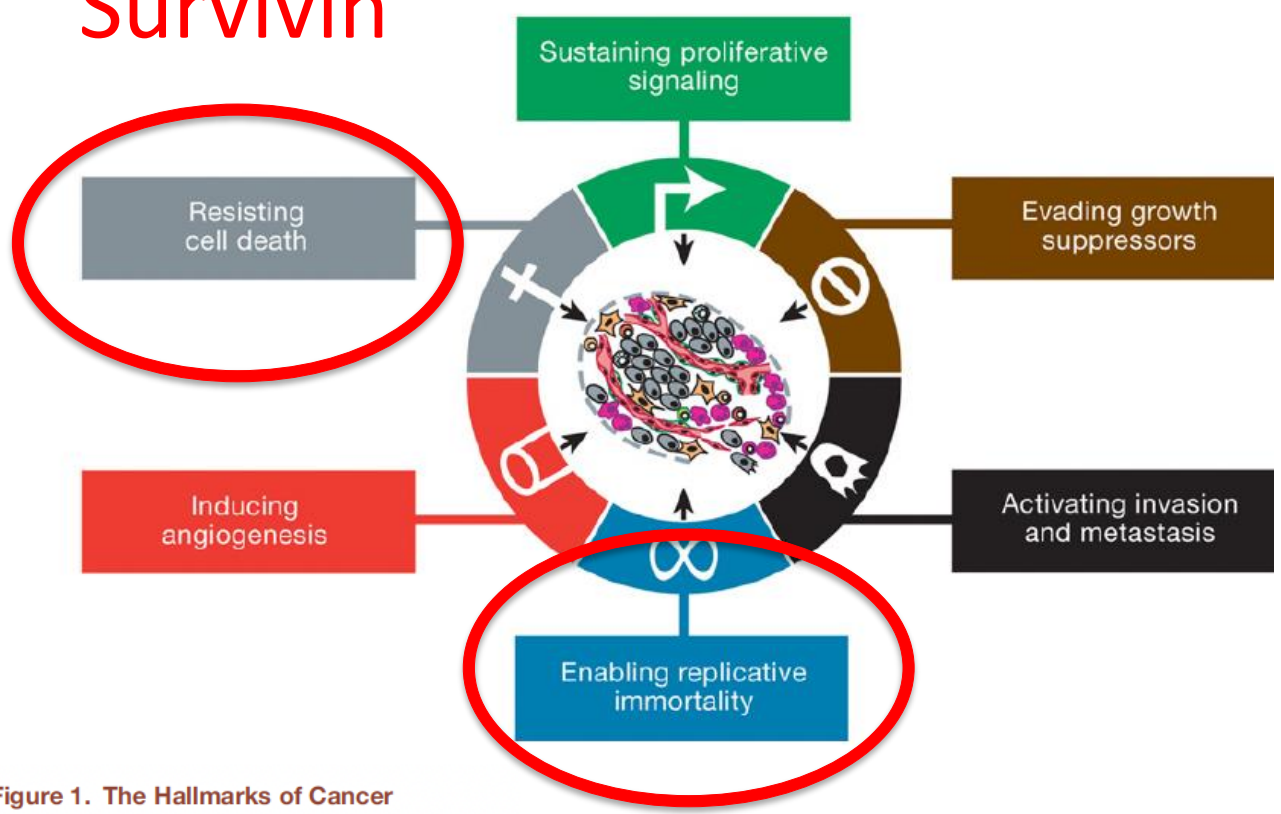


Figure 1. The Hallmarks of Cancer
This illustration encompasses the six hallmark capabilities originally proposed in our 2000 perspective. The past decade has witnessed remarkable progress toward understanding the mechanistic underpinnings of each hallmark.

Telomerase

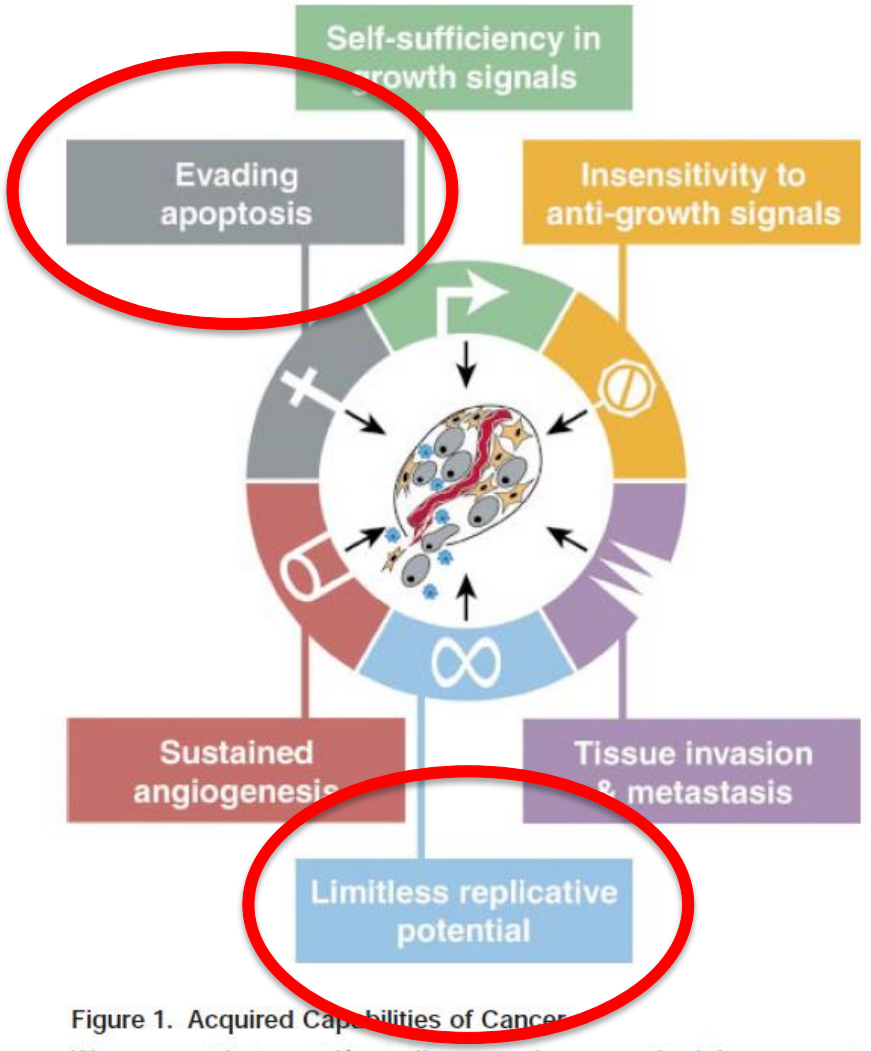


Figure 1. Acquired Capabilities of Cancer
We suggest that most if not all cancers have acquired the same set of functional capabilities during their development, albeit through various mechanistic strategies.

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

Hanahan & Weinberg, 2000 & 2011:

https://doi.org/10.1016/S0092-8674(00)81683-9,

https://doi.org/10.1016/j.cell.2011.02.013

NHS Tayside

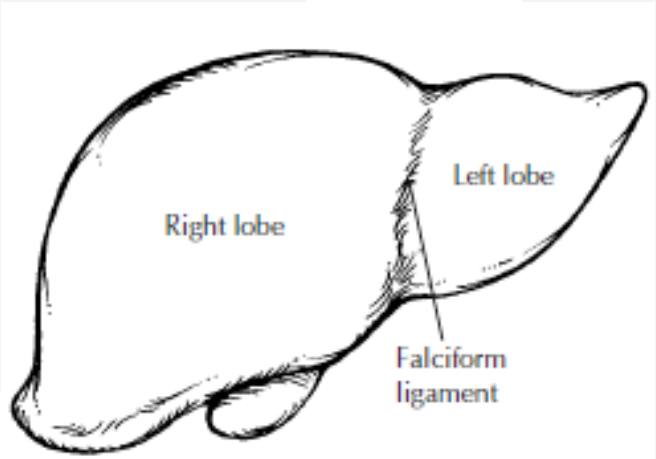
UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

https://ollieburton.com/feed-articles/2017/7/19/interview-preparation-four-pillars-of-medical-ethics

NHS Tayside

NHS Specimens

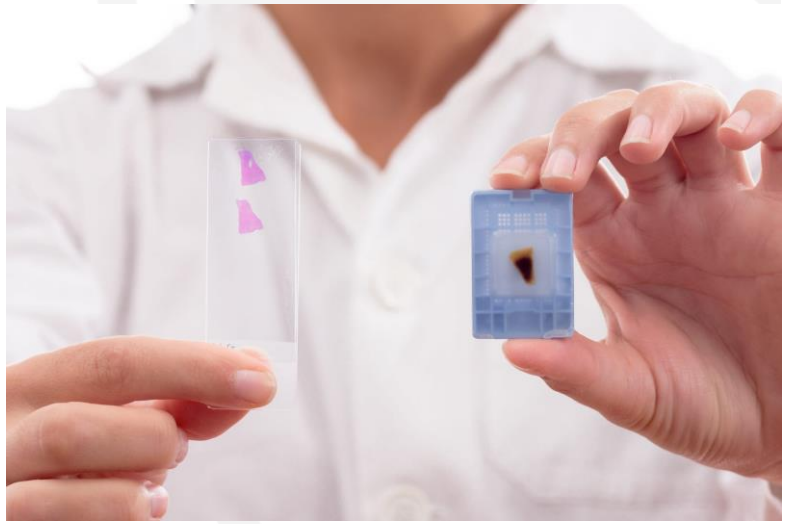
- Kept for 30 years
- Human Tissue Act
- Health Research Authority
- Research Ethics Committee (REC) approval
- Confidentiality Advisory Group (CAG) approval

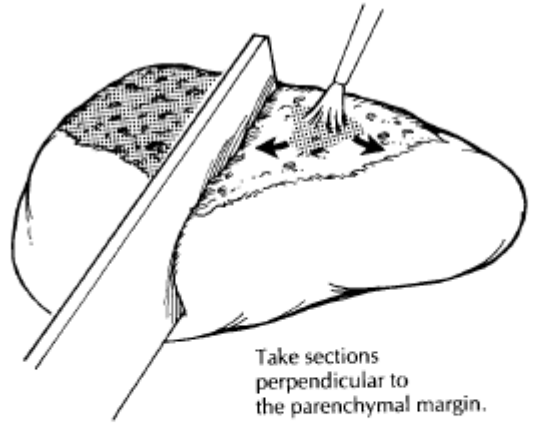


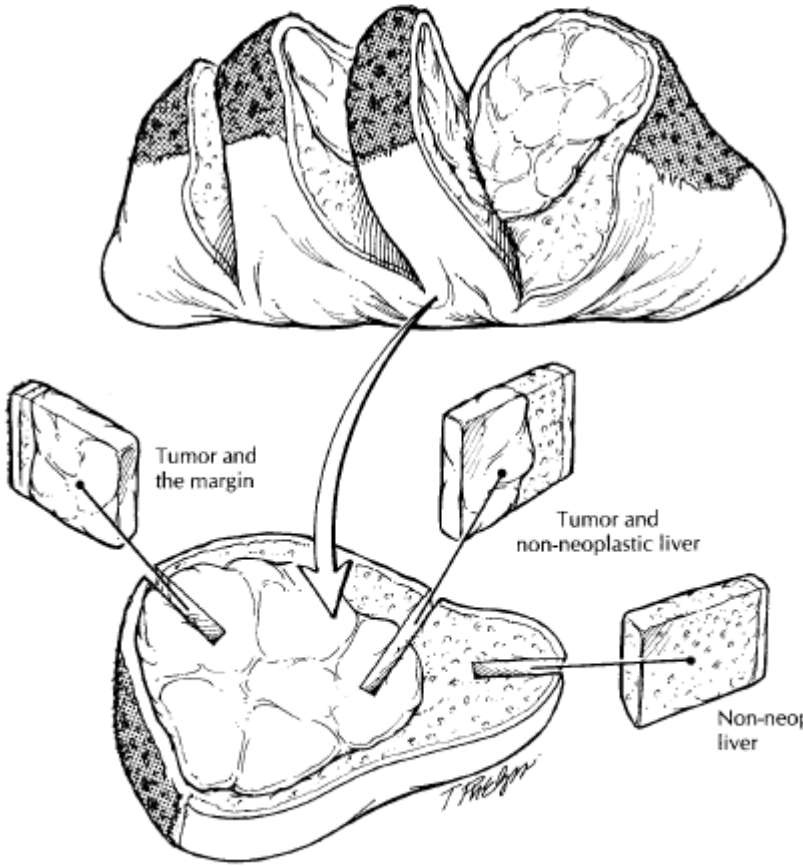
https://www.rcpath.org/uploads/assets/049ea966-df5c-4a9f-9353ba24a69bb808/The-retention-and-storage-of-pathological-records-and-specimens-5th-edition.pdf
Surgical Pathology Dissection: An Illustrated Guide, Second Edition. Ch 16, M Torbenson MD
https://patologi.com/dissection.pdf
https://www.geneticistinc.com/blog/importance-of-ffpe-in-modern-research
https://audubonbio.com/tissues/ffpe-tissue/

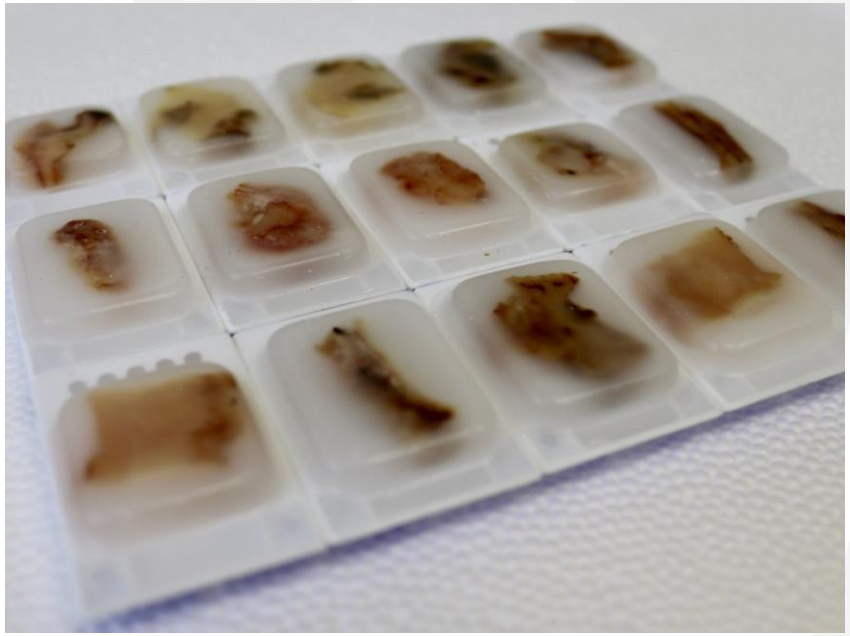
UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside



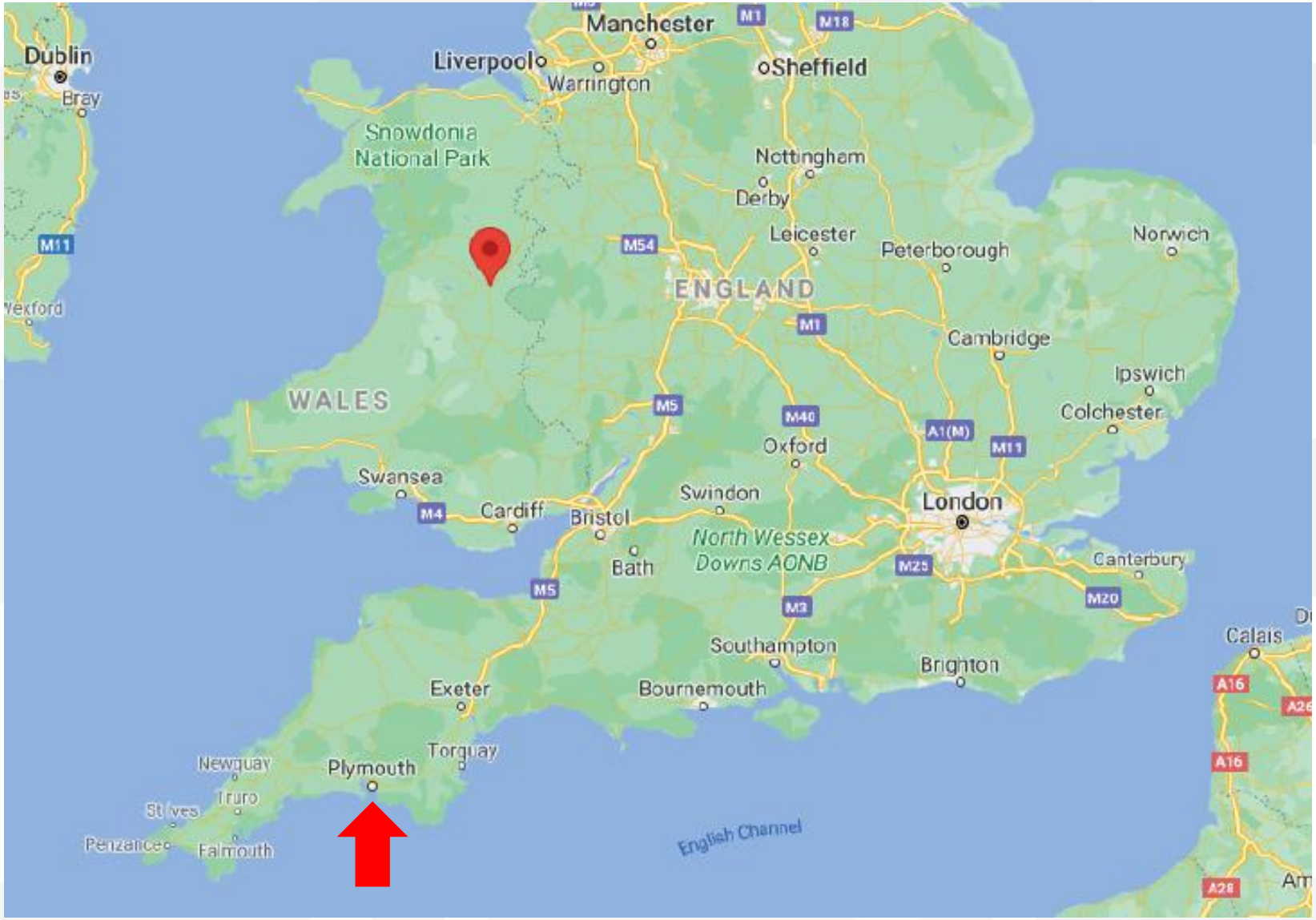






UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside



UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

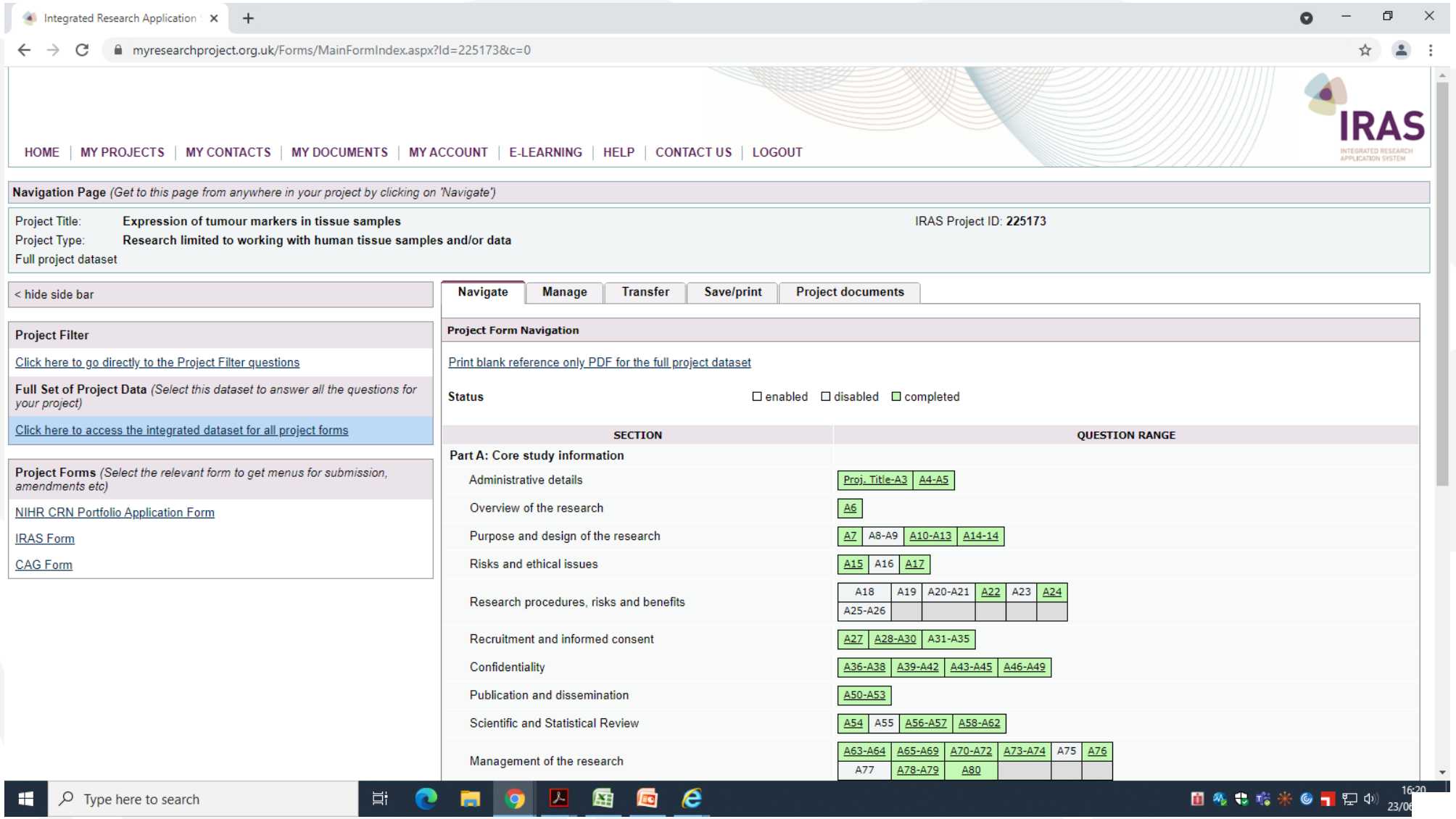
NHS Tayside

Integrated Research Application System

- Administration
  - CVs
  - Schedule of events
  - Statement of activities
  - Research Protocol
  - Sponsor (UoP)
    - Public liability
    - Professional indemnity
    - Insurance
  - Supporting document
    - British Liver Trust
    - Local patient interest group
- Confidentiality Advisory Group (CAG)
  - Caldicott Guardian input
- Research Ethics Committee (REC)
  - 17/NW/0457

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside



UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

Criteria

Inclusion

1. Tumour in the liver
2. Liver tissue at either UHP or KCH
3. >18 years old
4. Tissue surplus
5. Archived samples **only**
6. Research will not exhaust the archive

Exclusion

1. < 18 years old
2. Insufficient quantity of tissue
3. Explicit written evidence of the withdrawal of consent for research inclusion

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

NHS | HEART of ENGLAND NHS Foundation Trust

Consent Form 1

Patient Agreement to Investigation or Treatment

Patient details (or pre-printed label)

Patient's surname/family name ..... Patient's first names .....
Date of Birth ..... Male ☐ Female ☐
NHS number ..... PID .....
Responsible health professional .....
Job title ..... Registration number .....
Special requirements .....
(eg other language/other communication method)

Name of proposed procedure or course of treatment (include brief explanation if medical term not clear): .....

.....

Statement of health professional (to be filled in by health professional with appropriate knowledge of prop

I have read and und
I have explained th
The intended benef
.....
.....
The significant, una
.....
.....
Any extra procedur

☐ Blood transf

☐ Other procedures (please specify): .....
.....

I have also discussed what the procedure is likely to involve, the benefits and risks of any available alternative treatments (including no treatment) and any particular concerns of this patient.

☐ The following leaflet/CD/DVD has been provided .....

This procedure will involve:

☐ General anaesthesia ☐ Local anaesthesia ☐ Sedation

Signed ..... Date .....
Name (PRINT) ..... Job Title .....

Statement of interpreter (where appropriate)

I have interpreted the information above to the patient to the best of my ability and in a way in which I believe she/he can understand.

Signed ..... Date .....
Name (PRINT) .....

I consent/do not consent to the removal of my tissue and/or blood products during this operation and
I consent/do not consent to its use for (tick as applicable):

☐ Research in connection with disorders and/or the functioning of the human body
☐ Obtaining scientific or medical information about a living or deceased person which may be relevant to any other person (including a future person)

Patient's signature ..... Date .....

Name (PRINT) .....

Statement of patient

Please read this form carefully. If your treatment has been planned in advance, you should already have your own copy, which describes the benefits and risks of the proposed treatment. If not, you will be offered a copy now. If you have any further questions, do ask - we are here to help. You have the right to change your mind at any time, including after you have signed this form.

I agree to the procedure or course of treatment described on this form.

I understand that you cannot give me a guarantee that a particular person will perform the procedure. The person will, however, have appropriate experience.

I understand that I will have the opportunity to discuss the details of anaesthesia with an anaesthetist before the procedure, unless the urgency of my situation prevents this (this only applies to patients having general anaesthesia).

I understand that any procedure in addition to those described on this form will only be carried out if it is necessary to save my life or to prevent serious harm to my health.

I have been told about additional procedures which may become necessary during my treatment. I have listed here any procedures which I do not wish to be carried out without further discussion, even if I become at risk of death: .....
.....
is operation and
ich may be relevant to
.....
.....
er consent. Young people
.....
Name (PRINT) .....

Confirmation of consent (be completed by a health professional and the patient when the patient is admitted for the procedure, if the patient has signed the form in advance)

On behalf of the team treating the patient, I have discussed the treatment with the patient and answered any further questions or concerns. I have also confirmed with the patient that she/he has made an informed decision and wishes to go ahead.

Health Professional

Signed ..... Date .....
Name (PRINT) ..... Job Title .....

Patient

Signed ..... Date .....
Name (PRINT) .....

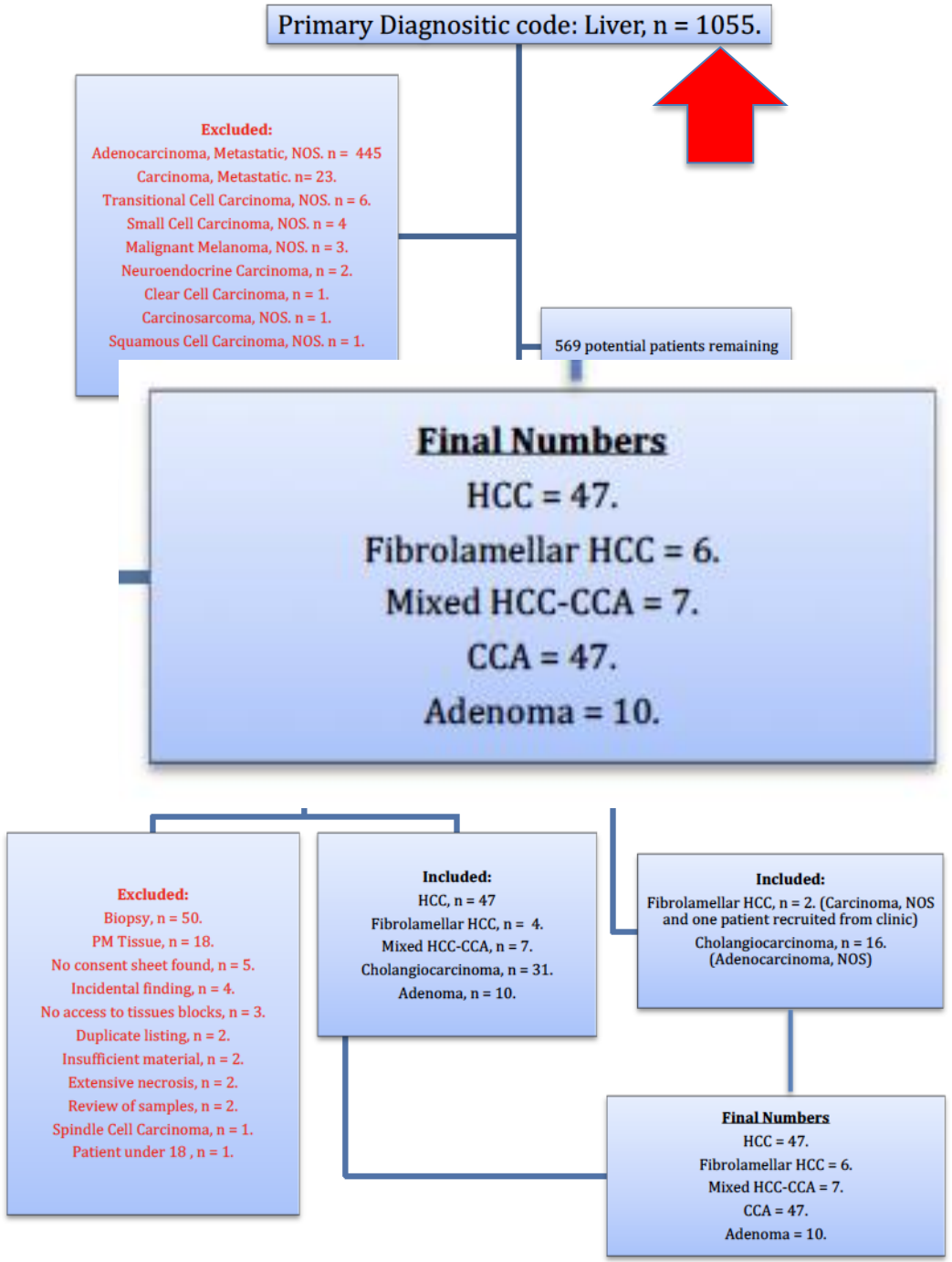
Important notes: (tick if applicable)

☐ See also advanced decision to refuse treatment/living will (e.g. Jehovah's witness form)
☐ Patient has withdrawn consent, patient to sign and date here to confirm .....

Copy accepted by patient: yes / no (please ring)
YELLOW COPY: CASE NOTES WHITE COPY: PATIENT

UNI
PLY
Institu
and Str

NHS Tayside



UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

Figure 5. Eppendorf tubes containing tissue curls extracted from FFPE archived materials.

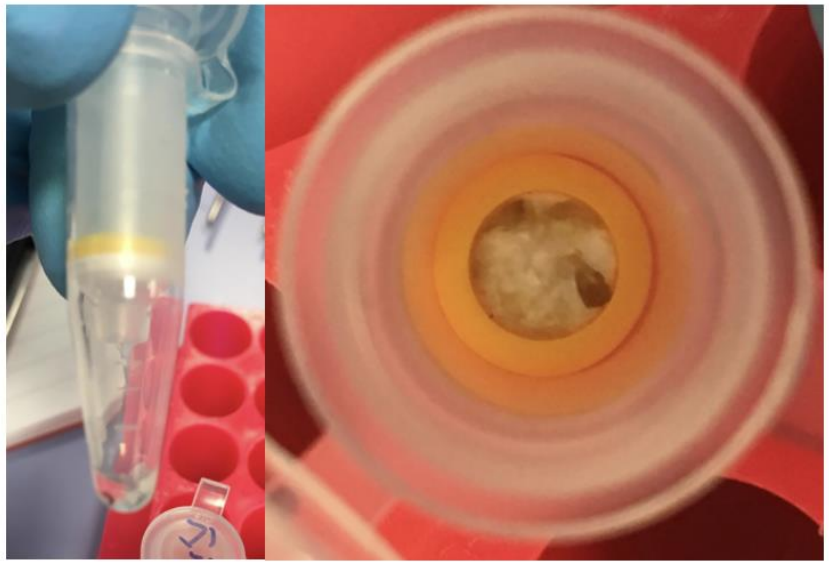


Figure 6 Spin columns used for DNA extraction from FFPE tissues. The clear eluate in left image. Some tissue remains in the filter after processing in the right image.

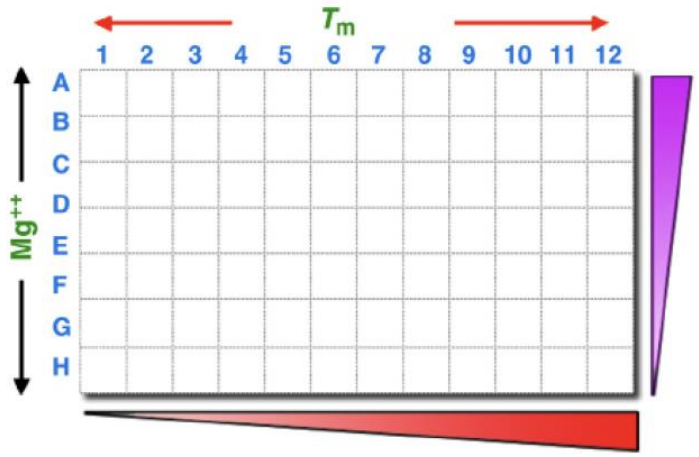


Figure 7. A grid pattern for altering two components of PCR simultaneously.

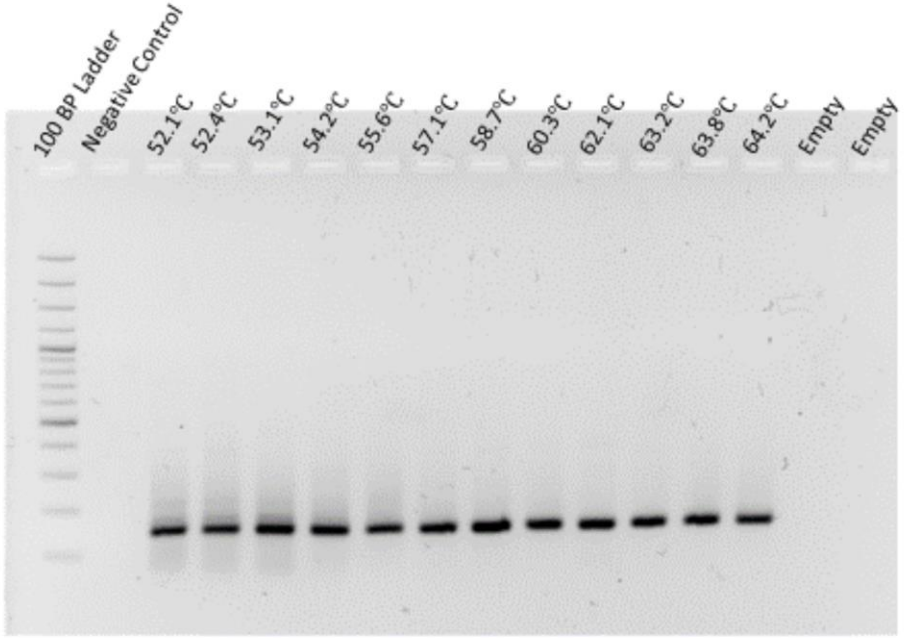


Figure 23. A 2% Agarose gel from a gradient PCR using the 'Surv F1R1' primers

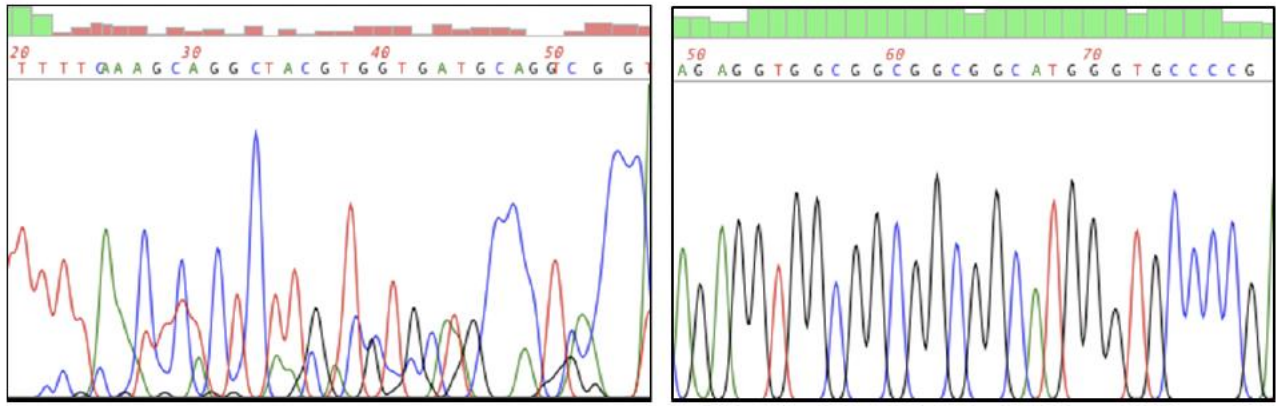


Figure 8. Chromatograms from MacVector with evidence of contamination (left) compared to an uncontaminated sequence. Note the low confidence (red bars, >1% error in the left image) compared to the high-confidence (green bars, <1% error in the right image) in base calls.

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

pTERT: All Malignancies		N	Mutant	WT	P value
Gender	**Female**	36	1	35	**0.001**
	Male	65	18	47	
Outcome	**Alive**	41	6	35	0.444
	Dead	60	13	47	
Margin	**R0**	58	14	44	0.196
	R1	41	5	36	
Fibrosis	-	59	6	53	**0.01**
	+	41	13	28	
Cirrhosis	-	77	10	67	**0.012**
	+	23	9	14	
Tumour Stage	**I & II**	65	9	56	0.221
	III & IV	26	7	19	
Grade	**Well Diff**	11	2	9	0.401
	Mild/Mod Diff	66	14	52	
	Poorly Diff	17	1	16	
Vascular Invasion	-	42	7	35	0.794
	+	52	10	42	
Tumour Type	**HCC**	35	17	52	**0.003**
	FL-HCC	6	1	5	
	CCA	36	0	36	
	Mixed HCC-CCA	7	1	6	

Table 35. Clinical measures are compared between mutants and wild type Telomerase promoter sequences across all malignancies.

pTERT: HCC only		N	Mutant	WT	P value
Gender	**Female**	12	0	12	**0.005**
	Male	40	17	23	
Outcome	**Alive**	23	4	19	**0.043**
	Dead	29	13	16	
Margin	**R0**	36	12	24	1.000
	R1	14	5	9	
Fibrosis	-	26	5	21	0.075
	+	26	12	14	
Cirrhosis	-	36	9	27	0.111
	+	16	8	8	
Tumour Stage	**I & II**	29	8	21	0.516
	III & IV	17	7	10	
Grade	**Well Diff**	8	2	6	0.689
	Mild/Mod Diff	35	13	22	
	Poorly Diff	5	1	4	
Vascular Invasion	-	29	6	23	**0.031**
	+	19	10	9	

Table 37. Across traditional HCCs, clinical measures are compared between mutants and wild type Telomerase promoter sequences.

Computational and Structural Biotechnology Journal 19 (2021) 852–859

ELSEVIER

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

QuPath: The global impact of an open source digital pathology system

M.P. Humphries [a], P. Maxwell [a], M. Salto-Tellez [a,b,*]

[a] Precision Medicine Centre of Excellence, The Patrick G Johnston Centre for Cancer Research, Queen's University, Belfast, UK
[b] Integrated Pathology Programme, Division of Molecular Pathology, The Institute of Cancer Research, London, UK

ARTICLE INFO

Article history:
Received 19 October 2020
Received in revised form 11 January 2021
Accepted 14 January 2021
Available online 21 January 2021

Keywords:
QuPath
Digital Pathology
Computational Pathology
Image Analysis
Artificial Intelligence

ABSTRACT

QuPath, originally created at the Centre for Cancer Research & Cell Biology at Queen's University Belfast as part of a research programme in digital pathology (DP) funded by Invest Northern Ireland and Cancer Research UK, is arguably the most wildly used image analysis software program in the world. On the back of the explosion of DP and a need to comprehensively visualise and analyse whole slides images (WSI), QuPath was developed to address the many needs associated with tissue based image analysis; these were several fold and, predominantly, translational in nature: from the requirement to visualise images containing billions of pixels from files several GBs in size, to the demand for high-throughput reproducible analysis, which the paradigm of routine visual pathological assessment continues to struggle to deliver. Resultantly, large-scale biomarker quantification must increasingly be augmented with DP. Here we highlight the impact of the open source Quantitative Pathology & Bioimage Analysis DP system since its inception, by discussing the scope of scientific research in which QuPath has been cited, as the system of choice for researchers.

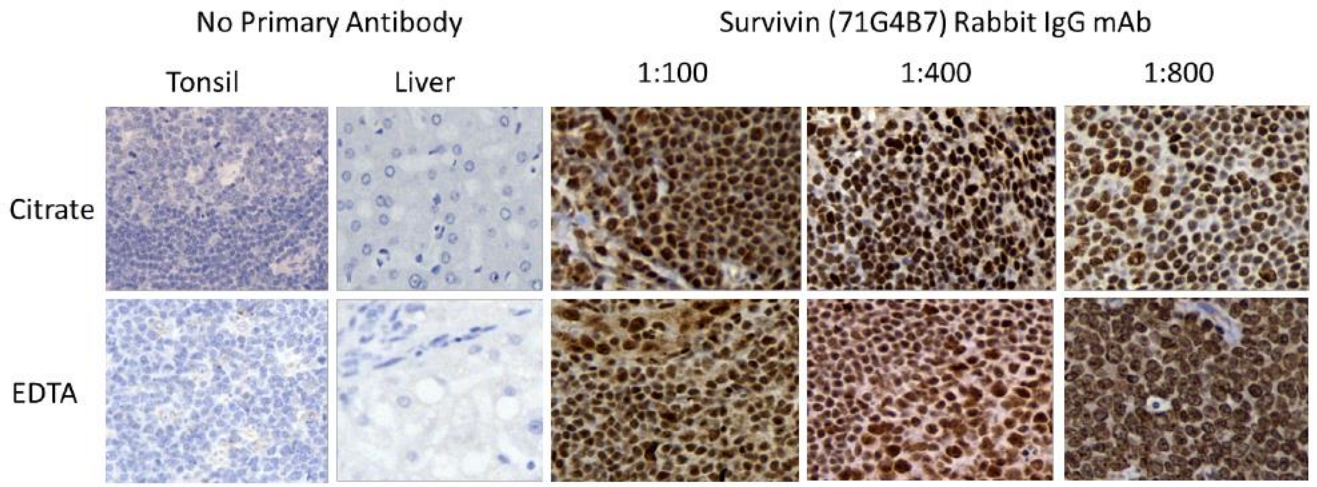


Figure 49. Optimisation steps for the Survivin primary antibody. Images shown at 200X magnification.

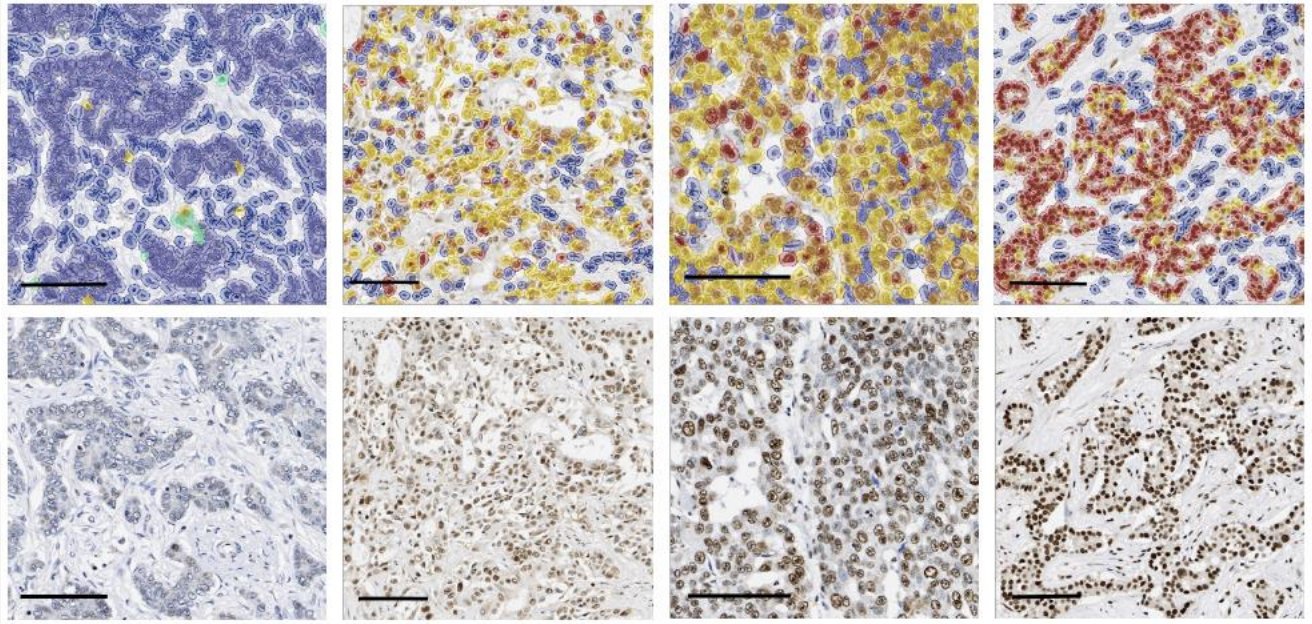
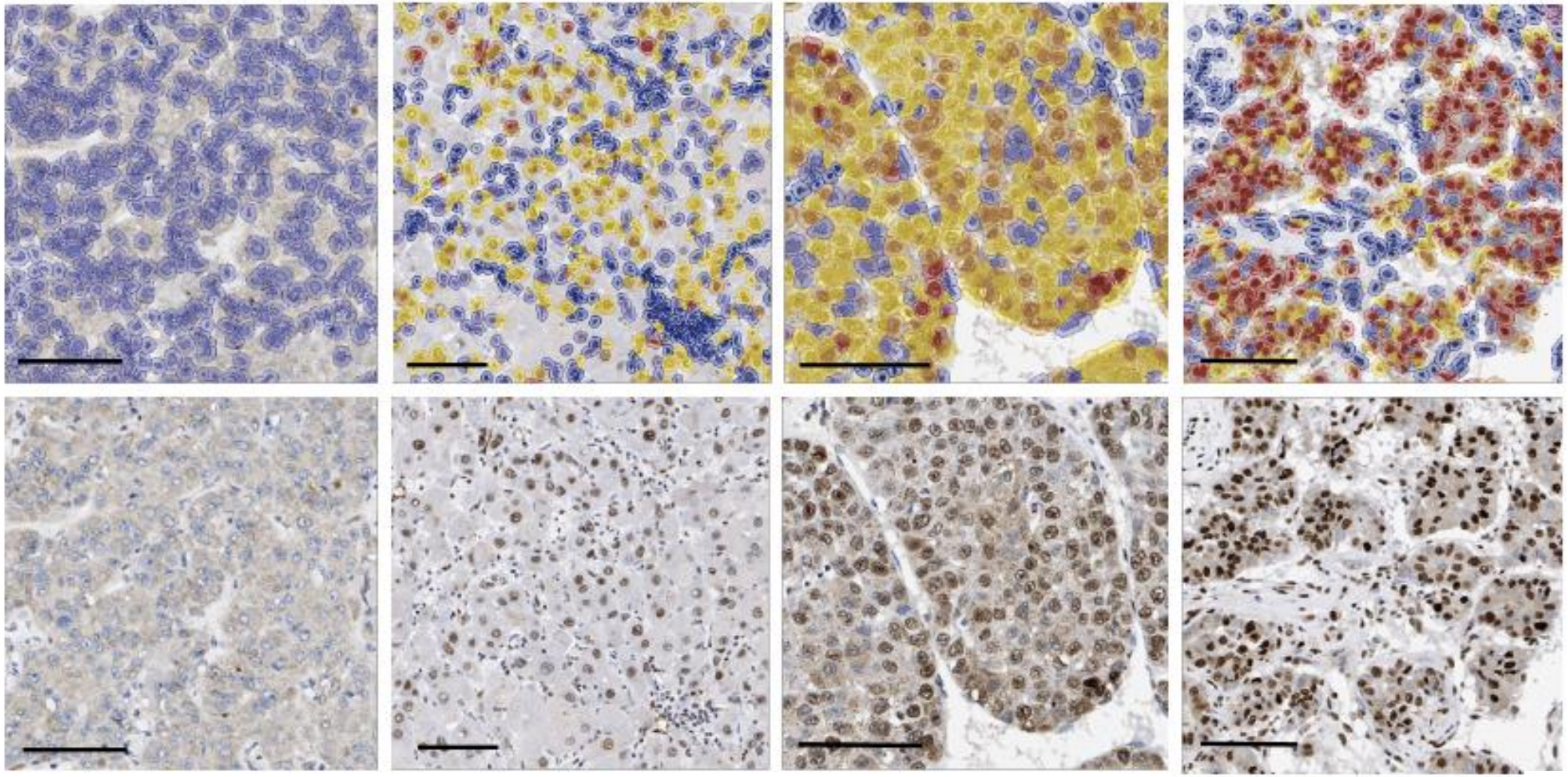


Figure 51 CCA tumours with varying degrees of nuclear positivity for Survivin. Scale bar: 100 microns.

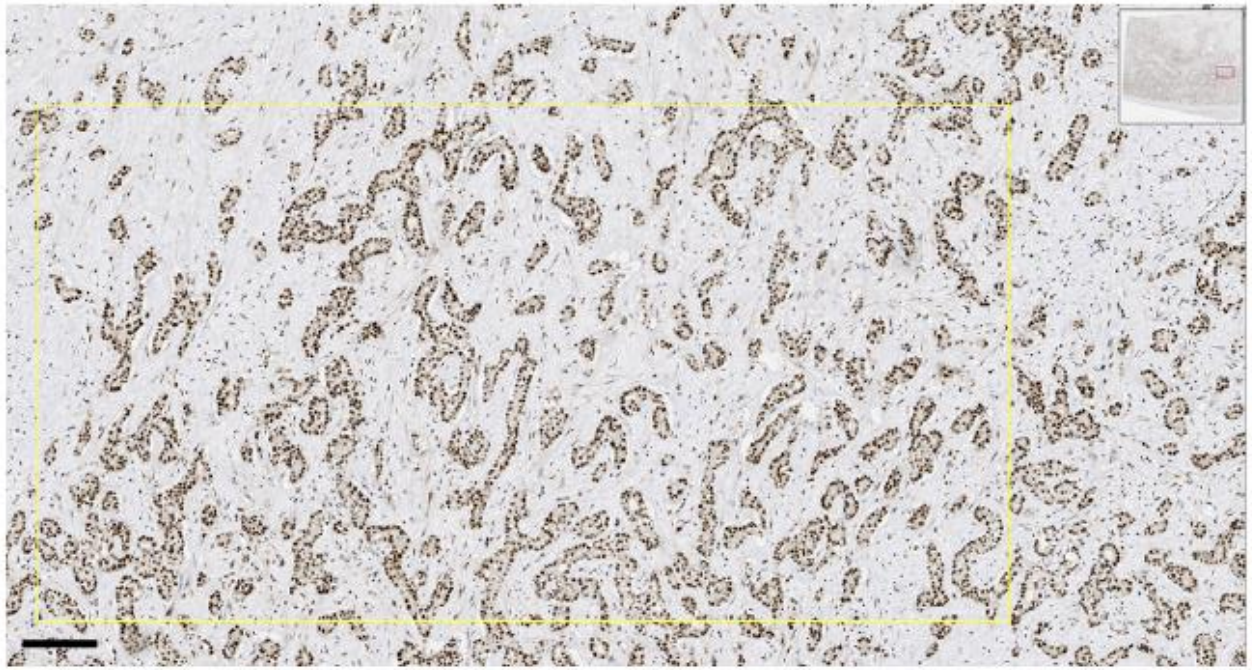
UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

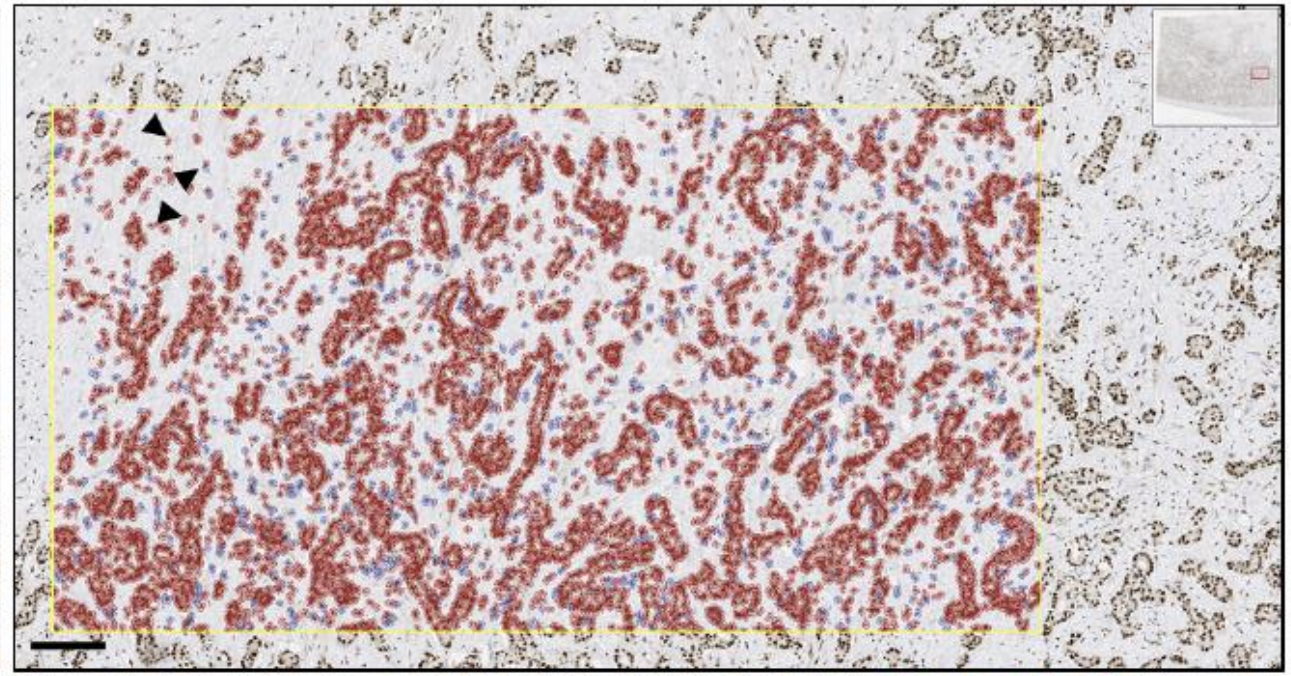
NHS Tayside

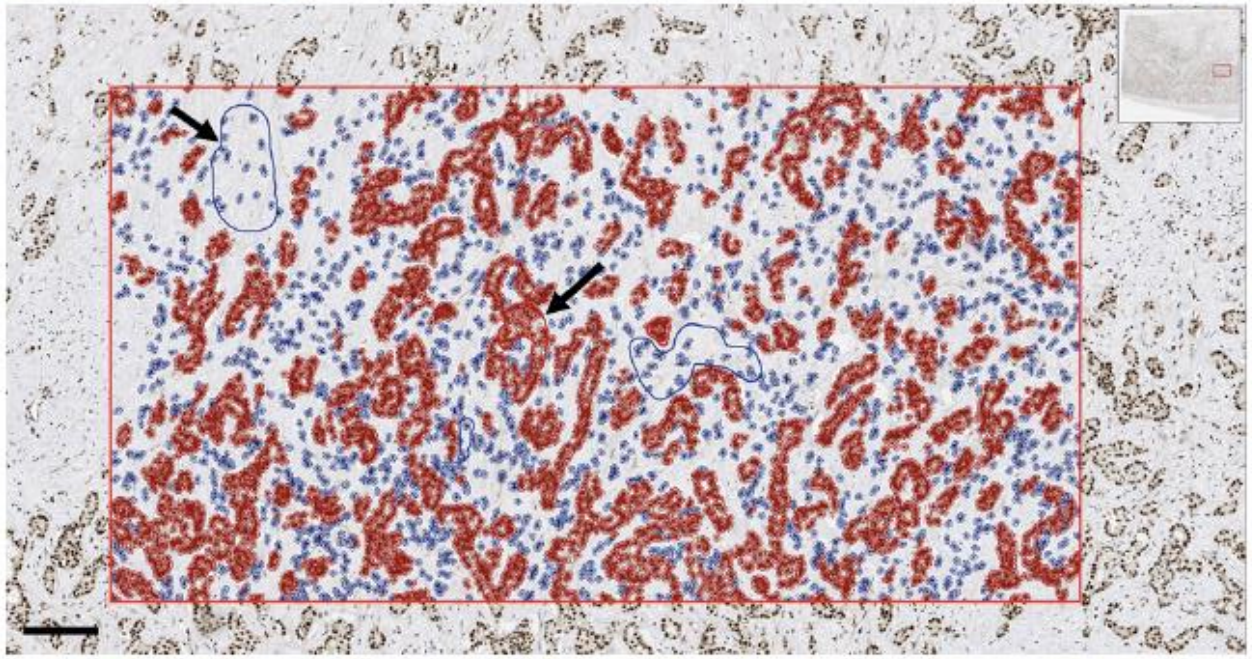


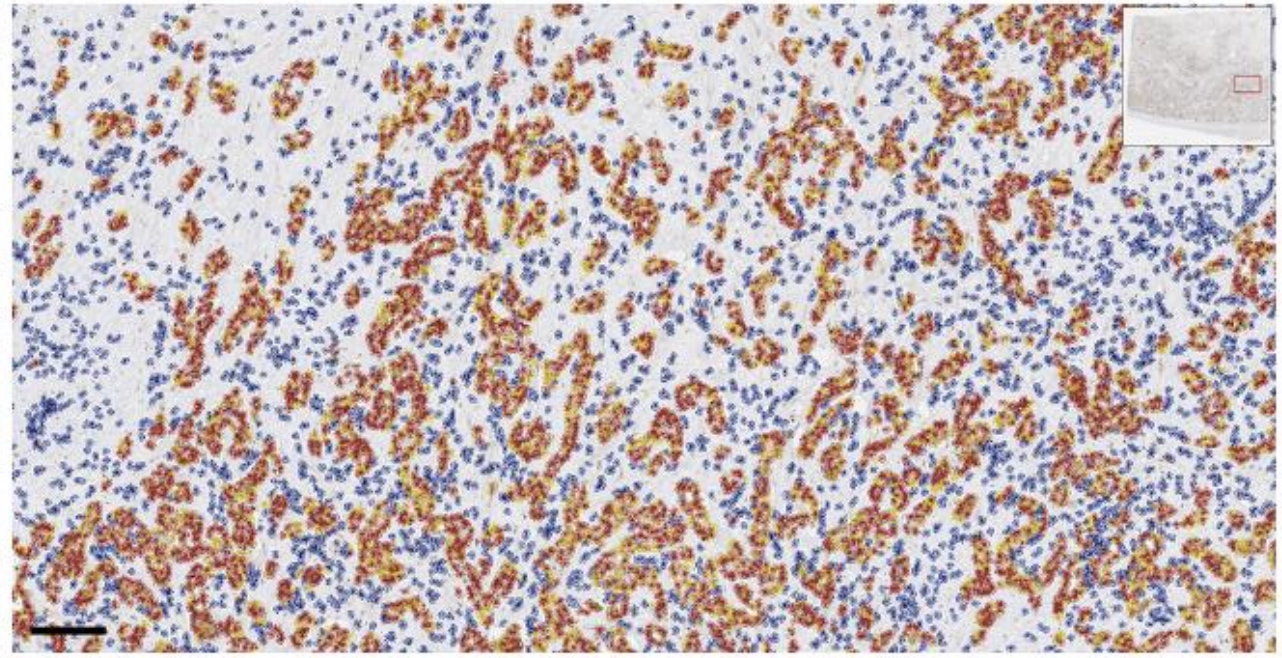
UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

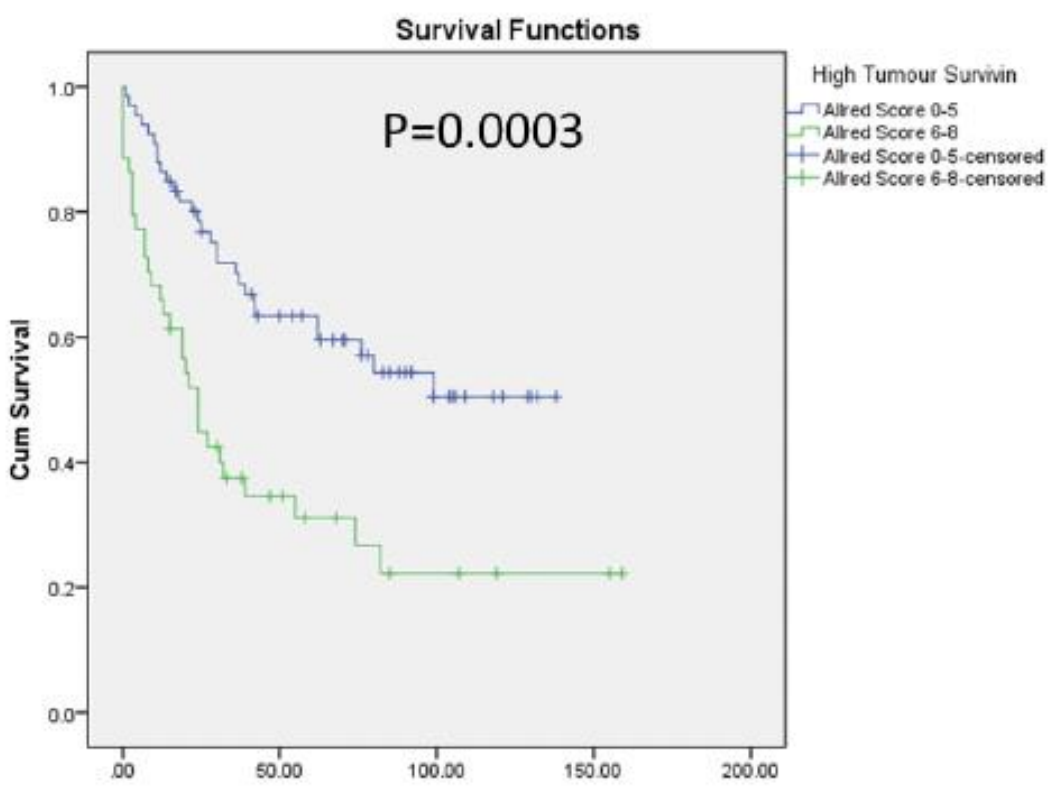




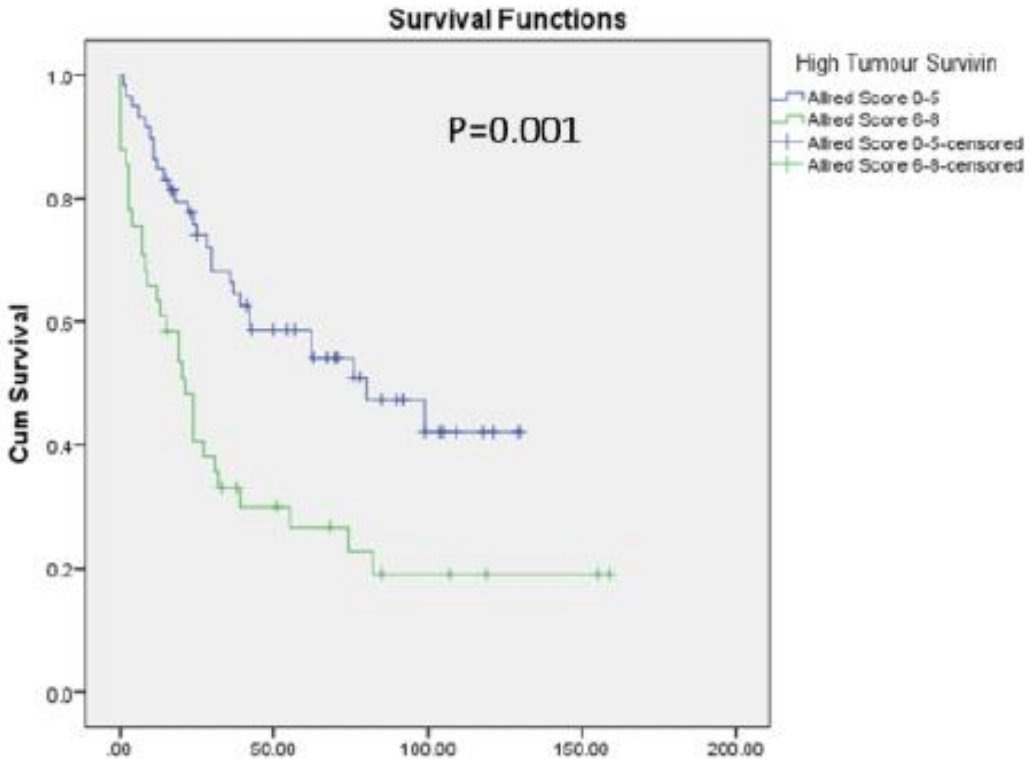




Neoplastic



Malignant



UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

Malignancy - Survivin		N	Survivin^{Low} % (n)	Survivin^{High} % (n)	P-value
Outcome	**Alive**	41	75.5 (31)	24.4 (10)	**0.007**
	Dead	59	47.5 (28)	52.5 (31)	
Margin	**R0**	58	67.2 (39)	32.8 (19)	0.062
	R1	40	47.5 (19)	52.5 (21)	
Fibrosis	-	59	57.6 (34)	42.4 (25)	0.680
	+	40	62.5 (25)	37.5 (15)	
Cirrhosis	-	77	54.5 (42)	45.5 (35)	0.084
	+	22	77.3 (17)	22.7 (5)	
Tumour Stage	**I & II**	65	67.7 (44)	32.3 (21)	**0.029**
	III & IV	25	40 (10)	60 (15)	
Tumour Stage	**I**	30	83.3 (25)	16.7 (5)	**0.007**
	II	35	54.3 (19)	45.7 (16)	
	GG	38	50 .0 (19)	50.0 (19)	

Table 49. High tumour Survivin correlates with the clinic-pathological measures.

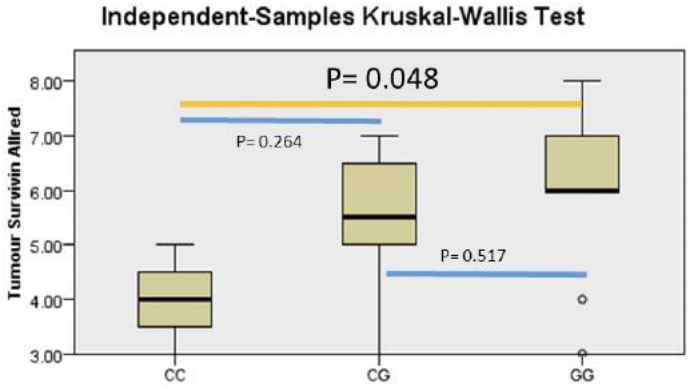


Figure 65. Survivin promoter variants differentially express Survivin protein In CCAs.

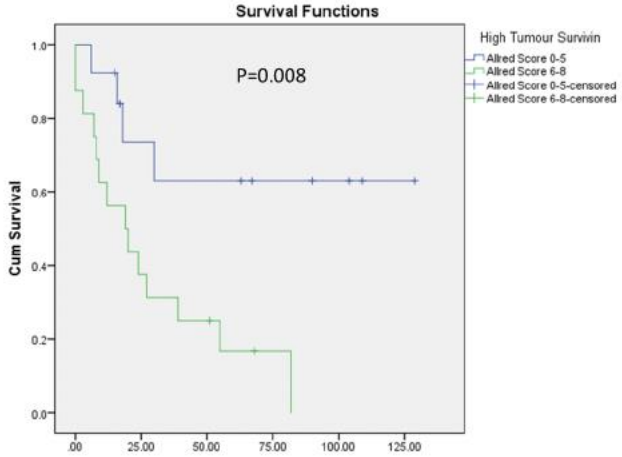


Figure 71. High Survivin expressing iCCA have a reduced cumulative survival.

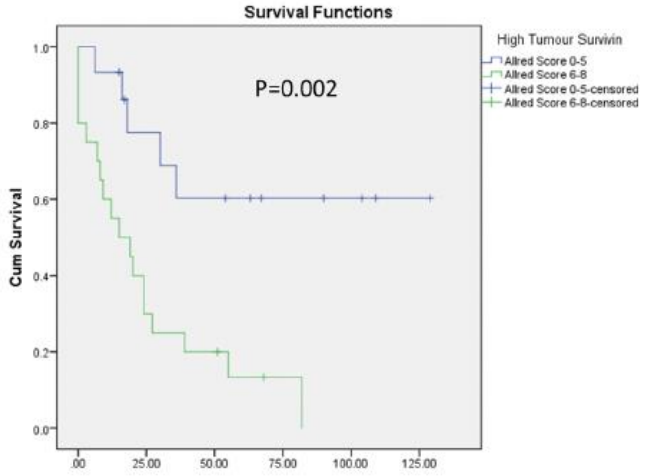


Figure 69. Cumulative survival differences based on Survivin expression in CCA.

Blood Work

- ELISA: Didn't work
- Exosomes: Optimised but then…COVID
- Dr David Sheridan, REC reference 15/LO/0948

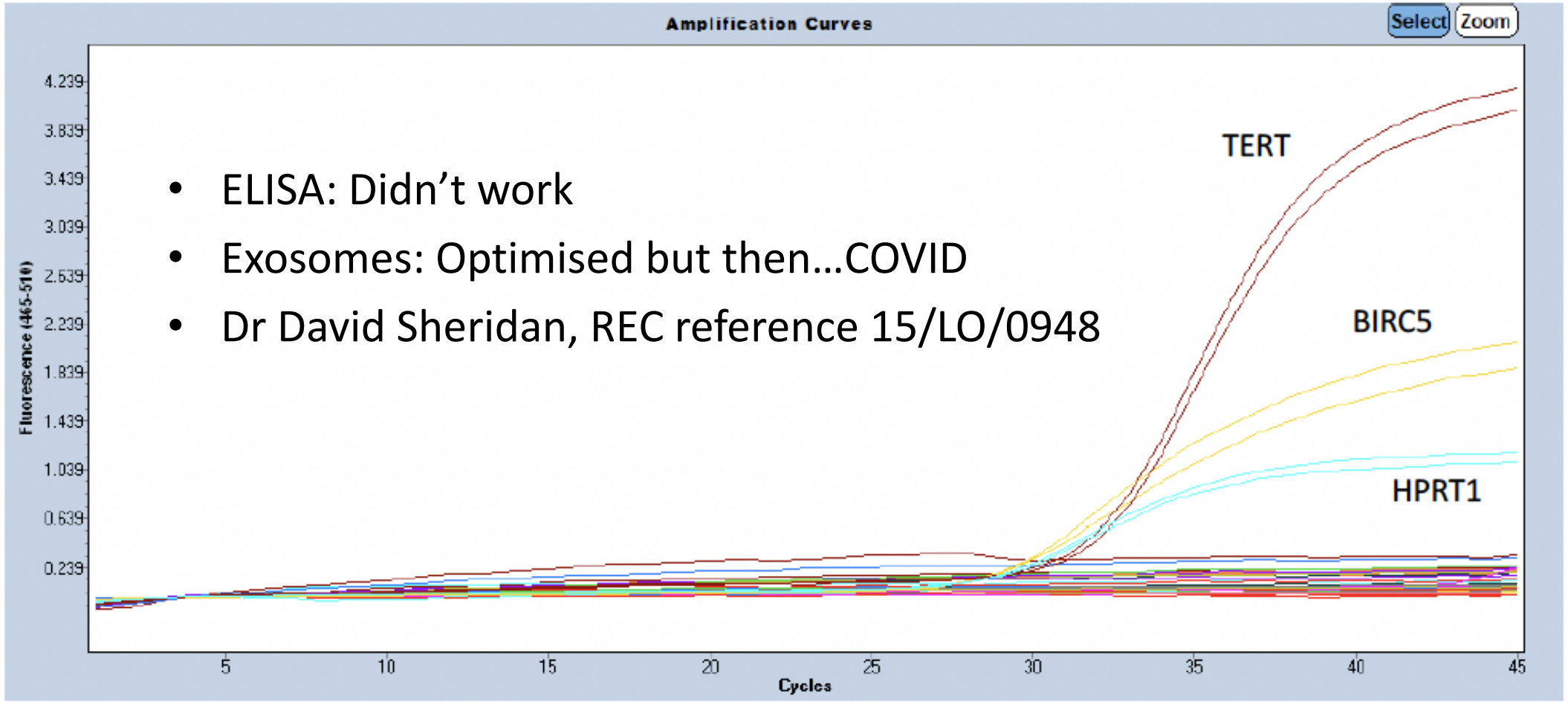


Figure 92. Quantitative PCR data from amplified Exosomal RNA. The calibrators (TERT and BIRC5) demonstrate the presence of this RNA from the Huh7.5 cell line and highlights that the reaction works. The internal control, HPRT1, is also detected.

NHS Tayside

Conclusions

- It WORKED!
- Funding…
- Supervision…
- Time consuming, but worthwhile.
- Unimaginably large potential research resource

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine

NHS Tayside

Thanks to:

Prof Matthew Cramp
Dr Michael Jarvis

Dr Jemimah Denson, Alec McLean, UHP Pathology.

The Hepatology Research Group, Plymouth, especially Dr David Sheridan.

Dr Jemma Dunn
Dr Aisling Murphy

Pam Baxter

UNIVERSITY OF PLYMOUTH
Institute of Translational and Stratified Medicine