# Submission for the Award of Doctor of Science (DSc)

Orla Sheils MA, PhD, FRCPath

2022

# TABLE OF CONTENTS

## DECLARATION:

In submitting this application, I declare that:

- this thesis has not been submitted as an exercise for a degree at this or any other university.
- the listed publications are my original work, or work in which I had a significant role as part of a collaborative project.
- I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.
- I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

Signed: 

Prof Orla Sheils

Professor of Molecular Diagnostics

West Theatre

Trinity College Dublin, the University of Dublin

Dublin 2, Ireland.

T +353 1 896 1329

Date: 20th July 2022

# ACKNOWLEDGEMENTS

I would like to acknowledge the many people who have helped and supported me through my research career, beginning with Prof Eamon Sweeney who supervised my PhD.

John O'Leary has been a colleague, supporter, and advocate for more than two decades and we have shared many wonderful, and some scary research adventures together.

I have been lucky to have shared research benches and lab space with so many wonderful colleagues especially the late Frances Hogan and Yuri Volkov.

From the earliest days in the Sir Patrick Dun Research Laboratory with wonderful colleagues like Anne Murphy, Sharon O'Toole, Stephen Smith and Ross McManus to the more recent times in the Trinity Translational Medicine Institute with Aideen Long, Jacintha O'Sullivan, Derek Doherty Joe Keane, Frank Mangan, Bill McCormack, Eamon Breen and the amazing Anne-Marie Baird, I have been supported by friendship, loyalty and inspiration.

Throughout most of this journey I have had my wing-man Paul Smyth at my side. We have rejoiced and despaired through research projects. But we have forged a life-long friendship that is worth more than any publication.

I would not have achieved any outcomes at all without wonderful clinical colleagues including Stephen Finn, Richie Flavin, Esther O'Regan, Colm Bergin, RoseAnne Kenny, Marie-Louise Healy, Tom Ryan and Martina Hennessy.

I have been blessed to supervise so many wonderful PhD and MD candidates – each of you has made their mark and I will always be proud of your achievements.

I have had the fortune to work alongside so many wonderful industrial collaborators including Steve Picton, Patrick Kelly, Roland Wicki, Rosella Petrarolli, Richard Henfrey, Chris Streck, Alain Rico, Andy Felton, Raymond Samaha, Jon Sherlock and Simone Guenther. They have vindicated my belief in the importance of translational research.

To all the funders who placed their trust in my plans and proposals- a huge thank you!

None of this could have happened without the support of so many staff at Trinity College Dublin; people like Derry Shanley and Dermot Kelleher, but I want to especially thank the

inspirational Linda Doyle who has given me my dream job and allowed me to bask in some of her reflected glory.

From my earliest days, my biggest supporters were my late parents Eddie & Etta. They taught me the importance and value of hard work  and they deserve the biggest posthumous thank-you imaginable. I have also been uplifted by my brothers Eamon, Rory and Niall and their wives, Barbara, Anne and Denise.

And finally, to my best-friend, soulmate and love of my life David – this is for you.

# CONTEXT AND BACKGROUND:

"BASIC RESEARCH IS THE PACEMAKER OF TECHNOLOGICAL PROGRESS… . NEW PRODUCTS AND NEW PROCESSES DO NOT APPEAR FULL-GROWN. THEY ARE FOUNDED ON NEW PRINCIPLES AND NEW CONCEPTIONS, WHICH IN TURN ARE PAINSTAKINGLY DEVELOPED BY RESEARCH IN THE PUREST REALMS OF SCIENCE"

*1945 report by Vannevar Bush: Science, The Endless Frontier.*

In research and throughout my career, my commitment to translational medicine has been underpinned by the singular objective of using clinical research to advance human health. The underlying theme of my research is to understand the causes and molecular basis of the development of disease, with particular reference to cancer, and to apply this knowledge to improving disease prevention, detection, diagnosis, and treatment. Over many years, I have maintained a strong track record in developing novel molecular diagnostics and translating this research into clinical service.

I work closely with industry bringing novel technologies and applications to the translational research setting. My work is widely published, cited and has been referenced in seminal textbooks. Since joining Trinity College, I have held many senior leadership roles including, Director of Postgraduate Teaching & Learning of the School of Medicine, where I was responsible for more than 500 postgraduate students and 20 taught Masters and Diploma Courses spanning a broad spectrum of medical and scientific disciplines. During this time, I also introduced several online postgraduate courses.

In 2012, I was appointed chair of College's Research Ethics Policy Committee (REPC) having chaired the Faculty of Health Sciences Research Ethics Committee (REC) for the previous 7 years. In this role, I developed policy documents and was responsible for aligning Research Ethics Committees across College. I have served on the Joint SJH/TUH hospital REC for 15 years. During the pandemic I was deputy chair of the national REC for covid and was subsequently appointed as deputy chair of the national REC for clinical trials.

I am a key advisor of molecular diagnostics, medical ethics, and policy issues to various governmental and other groups at a National and International levels. I am recognised as a Key Opinion Leader internationally and I act as a scientific advisor to biotech partners (including Becton Dickinson, ThermoFisher, Illumina and Qiagen) and the European Commission (through my work on the Advisory Committee for SC-1 in H2020).

In 2016, I was appointed Director of the Trinity Translational Medicine Institute where I lead an exclusively health sciences-focused educational and research institution, embedded within the acute hospital setting. My philosophy at TTMI centred on the acquisition of knowledge to enhance human wellbeing and thus realise human benefit. It was underpinned by achieving excellence in research. TTMI's strategy of improving human health through translational research is predicated on clinical, laboratory-based and health service research informed by real world clinical bedside problems, and societal and global health challenges.

Throughout my career I have built a research infrastructure, networking nationally and internationally, which continues to make a real difference in people's lives who are affected by cancer. This research forms the bedrock of service provision and interdisciplinary innovation, and this work has contributed to the ambition for a Trinity St James' Hospital Comprehensive Cancer Centre. That ambition is well underway, and the recognition of research excellence is evident with OECI accreditation of the Trinity St James' Cancer Institute in place.

As genomics-focused pharmacology begins to play a greater role in cancer treatment, molecular diagnostics is emerging as a valuable method for obtaining a deeper and more accurate look into the molecular underpinnings of individual tumours.

These technologies have the potential to change the future of oncology and advance the promise of personalised or precision medicine and create new frontiers in health care. They will provide hope to meet a wide range of public health challenges. The essence of this approach, which aims to facilitate the application of basic scientific discoveries in clinical and community settings, is central to my research ethos and has underpinned my role in leading programmes including Trinity Translational Medicine Institute and the Trinity- St James's Cancer Institute.

For more than two decades I have contributed impactful research to the area of molecular diagnostics. My research outputs have been sustained, are held in high regard, and have influenced policy nationally and internationally, and it is on this basis that I have applied for this recognition of my activities.

# OVERVIEW OF NATURE AND EXTENT OF RESEARCH ACTIVITY

## GENERAL BACKGROUND:

My research career began in the diagnostic histopathology laboratory setting. As a novice researcher, I was charged with establishing a molecular pathology suite adjacent to the diagnostic service laboratory. In doing this my intention was to establish a translational research bridge, exploring areas that might impact on routine service delivery.

There are several facets to my research which can be seen over a 30-year timeframe, that demonstrate consistency and continued research outputs that are significant at local and international levels.

- Sample Preparation and Assay design.
- Functional Genomics including mRNA Gene and miRNA Expression analyses.
- Genomic analysis focussed on driver mutations of cancer progression
- Elucidation of key pathways underpinning cancer progression and metastasis.

## KEY ASPECTS OF RESEARCH

My research is of international consequence, and my name is synonymous with the design and development of novel molecular diagnostic assays. I have used these design skills to investigate biological changes that drive disease and have validated many of these assays for use in the clinical setting. I have procured grants worth (gross) >€35M, published >130 peer reviewed journal articles and presented my work on the international stage. In maintaining an excellent record of demonstrated ability, and high-calibre outputs throughout my 30-year career, I have contributed significantly to the development of preventive/therapeutic approaches to areas of unmet clinical need. My work epitomises the medical model that

proposes the customization of healthcare, with medical decisions, practices, or products being tailored to the individual patient – or precision medicine.

In that regard, my research is exclusively patient centred and is uniquely placed to develop and enhance translational research for the benefit of patients and to improve the health of the community. My research resonates at the cutting edge of evidence-based medicine with the integration of basic and clinical research, to advance human health. My position within the Department of Histopathology means that I understand the diagnostic and prognostic deficiencies within current clinical practice and many of the research innovations made under my supervision have been geared towards enhanced clinical diagnostic service delivery.

My research focuses on elucidating the underlying mechanisms of human disease with a focus on functional genomics. I have made seminal discoveries into the pathobiology of cancers including thyroid (see refs: 29, 32, 49, 59, 60, 66, 68, 70, 73, 79, 81, 82, 84, 85, 88, 90, 92, 96, 104, 106, 107, 108, 110, 113, 116, 118, 119, 123) and cervix (see refs: 41,50, 61,69,77,78,100,102, 105,109), which have resulted in major changes to the way patients are treated globally. I have also compiled an extensive body of work on prostate (see refs: 1,2,5,6,9,14,20,25,36,56,62,86,91,94,98) and ovarian cancers (see refs: 23,35,43,48, 53,54,57,64,67,74,75,80).

I have secured competitive and continuous grant funding over three decades with income exceeding €35M (total). This funding has been sourced nationally and internationally and from conventional funding agencies and industrial partnerships including, Irish Cancer Society, Health Research Board, Science Foundation Ireland, Enterprise Ireland, EU – though FP5,6 & 7 and Horizon 2020, and partnerships with industry including ThermoFisher Life Technologies, Becton Dickinson Life Sciences, Qiagen and Illumina.

I act in an advisory capacity for several biotech companies including Becton Dickinson, ThermoFisher, Illumina and Qiagen. As a result of my research in assay design and innovation, I procured European Reference Lab status from ThermoFisher. This industry recognition of my research ensures pre-commercial launch access to novel chemistries, designation as a

beta test site for new technology platforms and analysis kits, and access to substantial in-kind consumables and equipment.

The international significance of my research is further recognized by invitations to deliver plenary and keynote addresses at leading conferences in Pathology and Human Genetics, examples of which include American Society of Human Genetics (Salt Lake City), Chips to Hits (Boston), United States and Canadian Academy of Pathology (Chicago), European Association of Pathology (Barcelona), AACR (San Francisco), IonWorld (Amsterdam), Pathological Society of UK and Ireland.

A significant logjam in the adoption of molecular diagnostic techniques into routine usage in the 1990's was the inability to extract nucleic acids reliably and reproducibly from archival fixed tissue samples. To overcome this, I developed extraction protocols and worked on assay design to ensure degraded samples could effectively be interrogated and reliable results obtained. (e.g. refs 79, 114, 116, 118, 119, 121, 123, 124).

Having established techniques to successfully extract nucleic acids (DNA and RNA (mRNA and miRNA)), I worked with industry to develop new chemistries to be incorporated into fluorescent dyes for optimal detection of gene expression. This work continued to evolve by incorporating multiple dyes in single assays to yield multi-plex analyses (e.g. refs 69,73, 93,107), and ultimately to whole genome expression detection (e.g. refs 32, 37, 85, 88,90,92,106). These approaches led to seminal discoveries that elucidate critical components in carcinogenesis (e.g. refs 76, 102,105, 109, 119,123). This work was also the foundation upon which direct translation of many molecular diagnostic assays were incorporated into routine clinical diagnostics service provision.

The ability to successfully manipulate and investigate RNA species from archival tissues led to a further group of work examining the role of micro RNAs in driving or curtailing cancer progression (for example in refs 29, 49, 51, 70). My work with biotech partners has developed a suite of methodologies that enable enhanced extraction of nucleic acids from archival tissues, reproducible detection of gene expression from archival tissues and targeted detection of clinically relevant driver mutations. I have worked to develop a multi-omics approach that simultaneously detects mRNA transcripts and cell surface markers at a single cell level. These innovations have transformed the diagnostics laboratory's ability to

interrogate the patho-biology, and genetic evolution of disease states and target specific therapies for individual patients at a specific time course in their disease.

My credentials in manipulating and detecting rare transcripts in clinical samples led to several collaborations including work in sepsis and viral bronchiolitis (33), von Willebrand Disease (22, 27,28), psychotic depression (26) and allergic lung inflammation (37). Recently, I led an innovation to develop an application for multi-omics analysis as a predictor of outcome for sepsis patients. This work involved optimising a high-throughput single-cell multiomic analyser and included a microwell-based instrument platform equipped with quality monitoring features, an array of RNA and protein assays and software tools. This pilot and developmental study enabled the identification of relevant subsets of lymphocytes based on known surface marker signatures. The combination of single-cell multiomics and high-dimensional data analysis further enabled a clear resolution of distinct subsets of cells defined by unique protein and gene expression signatures. These likely represent unique differentiation states and potential prognosticators of disease severity. This proof of principle study has been adopted and expanded both within the Trinity SJH network and by the BD international alliance consortium members.

Invited to work as a lead on an EU funded consortium project, I used my skills in manipulating difficult/denatured biological samples and assay design to develop a microfluidics lab on a disk platform. This was used for rapid diagnosis of neonatal sepsis. This interdisciplinary work involving life sciences and engineering was expanded to explore methods to isolate single cells from mixed cell populations (examples listed in refs 39, 58). The prototype generated has been further developed for use in many near patient testing settings globally.

For almost three decades I have worked with industry partners to develop and optimise new chemistries and to clinically evaluate workflows. In this context my laboratory has acted as a reference laboratory for these partners, and this has enabled my researchers to access pre-commercial reagents and instruments. In collaborating with industry, I have also driven the establishment of multinational consortia to design, validate and implement panels of assays for early molecular diagnostics (examples OncoNetwork and Multi-Omics consortia – see refs including 3,8,19,21,24). I have continuously worked to design, develop, validate, and implement, discrete assays and panels of targeted assays for the early detection of cancer,

and for the prediction of those patients who may be directed to specific treatment regimens. In collaboration with these multinational consortia, I have developed a significant programme concerned with molecular diagnostics and prognostics. I worked to develop a simple next Generation Sequencing (NGS) data analysis and interpretation systems predicated on semiconductor sequencing to help understand the impact of genomic variants and rapidly process genomic data using predefined optimized workflows to identify genetic abnormalities and novel variants. This research has established novel sequencing technologies as routine diagnostic testing regimens in clinical laboratories worldwide. Additional transformational significance of this work has seen Trinity College affiliated St James's Hospital become Ireland's first accredited Cancer Molecular Diagnostics Laboratory.

My research on Human Papilloma Virus (HPV), Human Immunodeficiency Virus (HIV), human herpes Virus 8 (HHV-8) and virally driven cancers has informed national policy and has been used in the implementation of vaccination programmes internationally. This work has led to innovations in objectifying conventional screening based on markers identified in my lab that correlate with progression, and by elucidating the pathobiology of HPV in the context of virally driven cancers. I have worked in conjunction with pharma to validate iterations of vaccine and data from these projects has informed international policy regarding national vaccination and screening strategies (15, 30, 31, 41, 44, 45, 50, 69, 76, 97, 102, 105, 109, 117, 120, 122, 125, 126).

Over the past decade I have been researching the process of metastasis and this continues to be the focus of my research activity. I have discovered key elements of the metastatic process which enhance our understanding and offer the prospect of novel treatment options (refs 4,5,12,17,24,32,34, 57). I have designed protocols to identify and characterise biologically relevant circulating tumour cells, cell free DNA and tumour derived exosomes, and a novel algorithm for the integration of tumour derived analytes from peripheral blood for incorporation into early cancer diagnostics and treatment planning.

My research on metastasis and the role of host immune system during metastasis has identified novel potential therapeutics. This emerging field of liquid biopsy is set to change the face of personalised medicine and this element of my research is currently being expanded through a collaboration with Becton Dickinson and the support of Enterprise

Ireland. Metastasis is a major challenge of cancer and accounts for greater than 90% of total cancer lethality. Despite intense efforts to understand the underlying mechanisms of how cancer spreads, treatment of metastatic cancer has not improved. Most treatments and research efforts address basic changes that happen within the metastatic cancer, but an alternative is to study the metastatic process itself, and that has been a focus of my research in the past 10 years. I have shown that successful metastatic cancer cells interact with blood platelets for protection. My work has examined the interplay between tumour cells that are circulating through blood and platelets. I have also examined the mediators that facilitate this process and am currently interrogating a phenomenon called platelet education by tumour cells. The reason underpinning this body of work is to understand the process and then to identify targets at crucial points along the metastatic cascade that are amenable to treatment.

My experience in designing novel diagnostics was employed at the commencement of the pandemic. I designed a LAMP based assay for the detection of covid-19 in self-collected saliva samples. This methodology was used by Trinity to provide a twice weekly screening service for staff and students throughout 2020-2022. It formed the basis of a national programme funded by SFI to monitor incidence and prevent outbreaks in four Irish universities.

Many PIs who have a strong track record in ongoing research have little additional time for contributions to teaching or College service. However, I have achieved and maintained national and international stature and continuous funding while remaining committed to undergraduate and postgraduate teaching, including new course design and curricular development. I have also held and made significant contributions in major administrative roles, informing policy, facilitating enhanced research capacity and academic excellence.

Overall, my research has led to discoveries highlighting pivotal mutations in cancers and directly influencing improved treatment of patients. In essence my research has been the difference between life or death for many patients. My research outputs have been sustained, impactful and disruptive over many years. They have changed routine histopathology service delivery internationally and have enabled early detection and targeted treatment options for patients with a cancer diagnosis.

I believe these outputs to be indicative of a continued and esteemed contribution to the field of Molecular Diagnostics. My research is positioned in the translational space and juxtaposes novel basic biology discovery with implementation in a clinical setting to advance biomedicine and patient outcomes and wellbeing. My work in early cancer diagnostics and theranostics is recognised globally and has made tangible differences in the way patients are managed. The translation of basic research to clinically relevant assays has had an industry disruptive and transformative influence on molecular diagnostics.

I believe this application provides sufficient information to establish that I hold international standing in the field of molecular diagnostics as demonstrated by my publications and research network. My research outputs have been sustained for three decades and have led to major innovations and additions to knowledge within and beyond my field.

# SELECTED PUBLICATIONS

In this section I shall highlight a selection of publications to demonstrate:

- international standing
- sustained contribution
- major contribution to knowledge within the discipline
- impact on developments in the field

I have selected papers that span more than two decades to reinforce the constant rate of research outputs over this period, and that speak to the broad areas of impact introduced in the General Background section of this document. The selection has been compiled with an eye to including papers demonstrating technical advances which have opened new avenues for further research and those which have attempted to uncover mechanisms underpinning disease progression and elucidating potential therapeutic targets. The publication of each paper in this section has preceded invitations to deliver keynote lectures on their content. This reinforces the notion that they are recognised within the discipline of molecular diagnostics for their significance in elucidating molecular pathways of disease progression. The research has eradicated technical barriers to the reproducible analysis of archival tissues and the findings have been pivotal in the implementation of novel diagnostics for routine use in clinical laboratories.

## RET/PTC-1 ACTIVATION IN HASHIMOTO THYROIDITIS

INT JOURNAL OF SURGICAL PATHOLOGY 8(3):185-89, 2000

Thyroid cancer is the most frequent endocrine malignancy. Papillary thyroid carcinoma (PTC), which accounts for approximately 80% of cases, is the most frequent of all thyroid malignancies. In the wake of the Chernobyl nuclear accident in 1986, there was an exponential rise in the diagnosis of thyroid cancers among those exposed to radioactive fallout. This enabled a rapid and comprehensive discovery, and analysis of key mutations, principal among which are chimeric variants of ret/PTC. Papillary thyroid carcinoma (PTC) is frequently associated with *RET* gene rearrangements that generate the so-called *RET*/*PTC* oncogenes. The rearranged during transfection (RET) proto-oncogene, located on chromosome 10q11.2, was isolated in 1985 and shown to be activated by a DNA rearrangement (rearranged during transfection). It encodes a single-pass trans-membrane tyrosine kinase that functions as the receptor (receptor tyrosine kinase, RTK) for the growth factors of the glial cell line-derived neurotropic factor family. The prevalence of RET/PTC rearrangements in thyroid cancer varies widely probably reflecting not only the geographic variation but also the different procedures used to identify RET/PTC rearrangements.

This paper is important for two reasons. First, it detailed technical advances and novel assay design to enable reproducible and reliable extraction and detection of RNA from archival tissues, and further it applied this technical advance to elucidate the presence of ret/PTC-1 mutation among autoimmune thyroiditis specimens. This was the first time the mutation was described in benign albeit neoplastic tissues. It demonstrated the importance of ret/PTC-1 as a facilitator of malignant transformation rather than a driver of it, and the importance of myriad genetic aberrations in tumour heterogeneity.

The finding has been included in Rosai and Ackerman's Surgical Pathology textbook (11th Ed) as a seminal finding in deciphering the molecular pathology of thyroid neoplasia. Moreover, this paper set the stage for a corpus of research conducted under my supervision into thyroid neoplasia and MAP-Kinase signalling, including the concept the canonical WNT/β-catenin pathway plays a pivotal role in the development of thyroid neoplasia.

I was first author on this publication, and I was solely responsible for procuring funding for, designing the project, performing the laboratory work and drafting the manuscript.

International Journal of Surgical Pathology 8(3):185–189, 2000

# *ret*/PTC-1 Activation in Hashimoto Thyroiditis

O. M. Sheils,* J. J. O'Leary,† V. Uhlmann,† K. Lüttich,† and E. C. Sweeney*

Activation of *ret*/PTC-1 has been documented in a minority of papillary thyroid carcinomas (PTC). In a recent study, the authors' group detected the presence of *ret*/PTC-1 in association with a background of florid lymphocytic thyroiditis (LT) in 58% of cases of PTC studied, which prompted them to examine the incidence of RET/PTC-1 expression in 27 examples of various forms of nonlymphomatous lymphoid infiltration of the thyroid by using TaqMan RT-PCR. Overall, 21 cases (78%) were found to express the chimeric transcript of *ret*/PTC-1. Eighteen cases of Hashimoto thyroiditis were positive (95%), and, of these, three had concomitant PTC while the remainder had no histologic evidence of associated malignancy. Three cases of lymphocytic thyroiditis demonstrated activated *ret*/PTC-1 (43%), two having associated PTC. These data suggest either that *ret*/PTC-1 is an indicator of follicular thyroid cell activation or that *ret*/PTC-1 activation is an early event in malignant transformation. If the latter is the case, it may be that, in a defined subset of the cell population, *ret*/PTC-1 activation elicits an autoimmune response, which, while possibly curtailing the development of PTC in the majority of cases, results in destruction of the thyroid parenchyma. *Int J Surg Pathol 8(3):185–189, 2000*
**Key words:** Hashimoto thyroiditis, *ret*/PTC-1, TaqMan RT-PCR.

Hashimoto thyroiditis is by far the most common form of thyroiditis, with a female incidence some 20 times that of males [1]. The majority of patients are hypothyroid on presentation and have elevated serum thyrotropin concentrations. The histologic changes of diffuse lymphoplasmacytic infiltration of the thyroid with germinal center formation, Hürthle cell change, and variable fibrosis are well recognized. However, the trigger that induces the observed autoimmune response in these patients is unknown.

Several chromosomal translocations and inversions involving the c-*ret* protooncogene have been documented in papillary thyroid carcinoma (PTC) [2]. Activation is engendered by a genetic rearrangement where the tyrosine kinase domain of the oncogene is juxtaposed to a second gene. To date, seven characterized varieties of c-*ret* activation have been described in addition to one undefined rearrangement termed *ret*/x [3], with *ret*/PTC-1 occurring most frequently. This variant comprises the tyrosine kinase domain of c-RET and a partial sequence of a gene termed H4 [4].

The possibility that antigenic alterations induced by activation of c-*ret* (such as loss of ligand binding and transmembrane domains or adhesive junctions) are responsible for eliciting an immune response has been postulated [5]. In this study, using TaqMan RT-PCR, 95% of Hashimoto thyroiditis tissue samples showed evidence of *ret*/PTC-1 activation. The observations of Wirtschafter et al. [6], who found mRNA expression for *ret*/PTC-1 and /or *ret*/PTC-3 in 95% of patients categorized as having Hashimoto thyroiditis with use of RT-PCR, support the concept of *ret*/PTC-1 activation playing a central role in this phenomenon.

From the *Department of Histopathology, Trinity College, and the †Departments of Pathology, The Coombe Women's Hospital and Trinity College, Dublin, Ireland.
Reprint requests: Prof. E. C. Sweeney, Department of Histopathology, C.P.L., St. James's Hospital, Dublin 8, Ireland.

## Materials and Methods

Twenty-seven thyroiditis cases accessioned between 1992 and 1998 were included in the study. All tissues were fixed in 10% formal saline and embedded in paraffin. Diagnosis was confirmed on H&E-stained sections in all cases. In this study we evaluated 19 patients with Hashimoto thyroiditis (HT), three of whom had been found to have occult PTC, five cases of nonspecific lymphocytic thyroiditis (LT), two cases of LT associated with PTC, and one case of Graves' disease with heavy lymphocytic infiltration of the gland. Cases were analyzed for the presence of *ret*/PTC-1 activation by use of RT-TaqMan PCR.

As previously described [7], attention was given to standard anticontaminating procedures. A single 20 µm section was cut from each block and placed in a sterile Eppendorf tube. Sections were dewaxed in xylene, and RNA extracted by use of a PUREscript RNA Isolation Kit (Gentra Systems Inc. MN, USA), with previously described modifications.

Reverse transcription of extracted RNA was performed by means of a GeneAmp RNA PCR kit (Perkin Elmer, CA, USA) by following the supplied recommended reaction conditions. The derived cDNA was used as a template in the TaqMan reaction. Primers designed with use of Primer Express Software (PE-Biosystems) were as follows:

| | |
|---|---|
| *ret*/PTC-1 F | CGC GAC CTG CGC AAA |
| *ret*/PTC-1 R | CAA GTT CTT CCG AGG GAA TTC C |
| *ret*/PTC-1 Probe | CCA GCG TTA CCA TCG ACC ATC CAA AGT |

Primers for *ret*/PTC-1 detection were designed to flank the junction between the tyrosine kinase encoding region of c-*ret* and the 5′ terminal sequence of H4. The probe was designed to span the c-*ret*-H4 fusion point for *ret*/PTC-1. cDNA from the TPC-1 cell line was used as a positive control for *ret*/PTC-1 rearrangement. Primers and TaqMan probe for the housekeeping gene glyceraldehyde phosphate dehydrogenase (GAPDH) were used to confirm the integrity of extracted RNA. Polymerase chain reaction (PCR) was carried out with use of a PE 9600 thermal cycler with TaqMan Core Reagents (PE Applied Biosystems) followed by sequence detection using an ABI Prism 7200 Sequence Detector.

## Results

*ret*/PTC-1 chimeric transcripts were detected in 18 of 19 (95%) cases of Hashimoto thyroiditis, in addition to the control cell line (TPC-1). Sixteen of these cases (84%) had no evidence of malignancy, while the remaining three (16%) displayed concomitant PTC. mRNA extracted from both areas of PTC and Hashimoto thyroiditis showed activated *ret*/PTC-1 in each case. Seven cases of lymphocytic thyroiditis (LT) were examined for expression of the chimeric RET/PTC-1 transcript. Three of seven cases of LT had positive signals for *ret*/PTC-1. Of these cases, two had associated PTC. A single case of Graves' disease was analyzed and was negative for RET/PTC-1 expression (Fig. 1). All cases from HT and LT cohorts harboring PTC were positive for *ret*/PTC-1 activation (Fig. 2). Those cases of HT and LT with PTC were biochemically hypothyroid on presentation. Among the nonspecific LT group, three were euthyroid on presentation with the remainder hypothyroid, and the single case of Graves' disease was biochemically hyperthyroid (Table 1).

Statistical analysis comparing the incidence of *ret*/PTC-1 positivity with thyroiditis demonstrated a significant association ($p=0.0104$) (Table 2).

Successful amplification of the extracted RNA was achieved in all cases, as demonstrated by positive signals for GAPDH.

## Discussion

The *ret*/PTC oncogene, an activated form of c-*ret*, has been described as a specific phenomenon in some papillary thyroid carcinomas (PTC) [8]. In a recent study, our group demonstrated a statistically significant incidence of *ret*/PTC-1 activation with concomitant thyroiditis in PTC [5]. These findings prompted us to examine a series of tissues from various forms of nonlymphomatous lymphoid infiltration of the thyroid, among which we detected the *ret*/PTC-1 chimeric transcript in 78%.

The activation of the PTC oncogene has previously been thought to be restricted to PTC [9], although there are varying opinions as to whether it may be associated with indolent [10] or aggressive [11] variants of the disease. The detection of *ret*/PTC-1 in 94% of patients (14/15) with Hashimoto thyroiditis without evidence of malignancy was unexpected and poses a number of interesting questions: (1) Is it possible that Hashimoto thyroiditis may represent an early malignant (premicroscopic) state to which a subset of patients are able to mount an immune response, curtailing the development of PTC, albeit with the autoimmune destruction of thyroid tissue? (2) Is it possible that *ret*/PTC-1 is a marker for follicular cell activation rather than being linked with the specific development of PTC? The fact that follicular adenomas and

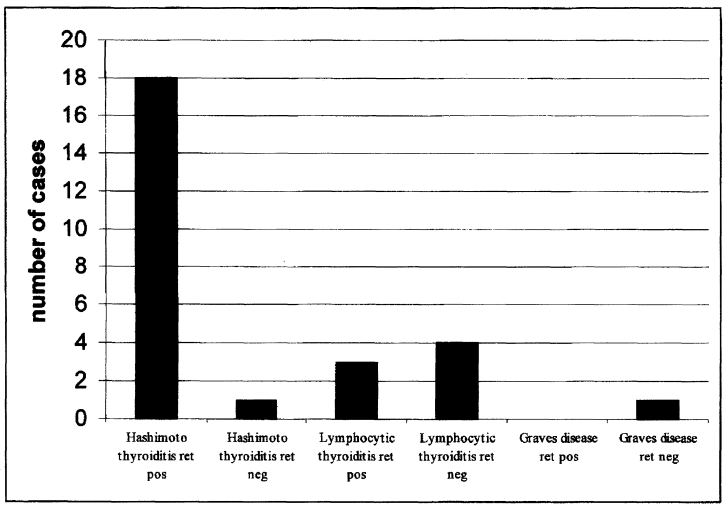



**Fig. 1.** RET/PTC-1 expression and associated thyroiditis.

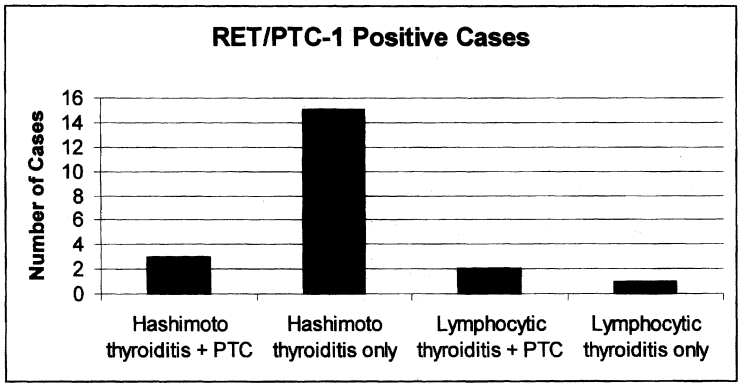



**Fig. 2.** Incidence of *ret*/PTC-1 activation and PTC in thyroiditis.

other benign lesions of the thyroid have not been shown to express RET/PTC-1 militates against the latter postulate.

Among lymphocytic thyroiditis cases examined, 43% (three of seven) demonstrated activated *ret*/PTC-1; in two of these cases this positivity could be ascribed to concomitant PTC.

Wirtschafter et al. [6] have documented the presence of chimeric transcripts of *ret*/PTC-1/3 in 100% of cases of Hashimoto thyroiditis. However, the criteria used by this group to assign a diagnosis of Hashimoto thyroiditis are unclear, for their study cohort comprised 11 cases of Hashimoto thyroiditis, seven of papillary thyroid carcinoma, and three fol-

**Table 1. Diagnosis, Biochemical, and *ret*/PTC-1 Status**

| Case # | Diagnosis | *ret*/PTC-1 Status | Biochemical Status |
|---|---|---|---|
| 1 | HT | Positive | Hypothyroid |
| 2 | HT | Positive | Hypothyroid |
| 3 | HT | Negative | Hypothyroid |
| 4 | HT | Positive | Hypothyroid |
| 5 | HT | Positive | Hypothyroid |
| 6 | HT | Positive | Hypothyroid |
| 7 | HT | Positive | Hypothyroid |
| 8 | HT | Positive | Hypothyroid |
| 9 | HT | Positive | Hypothyroid |
| 10 | HT | Positive | Hypothyroid |
| 11 | HT | Positive | Hypothyroid |
| 12 | HT | Positive | Hypothyroid |
| 13 | HT | Positive | Hypothyroid |
| 14 | HT | Positive | Hypothyroid |
| 15 | HT + PTC | Positive | Hypothyroid |
| 16 | HT | Positive | Hypothyroid |
| 17 | HT | Positive | Hypothyroid |
| 18 | HT + PTC | Positive | Hypothyroid |
| 19 | LT + PTC | Positive | Hypothyroid |
| 20 | HT + PTC | Positive | Hypothyroid |
| 21 | LT + PTC | Positive | Hypothyroid |
| 22 | LT | Negative | Euthyroid |
| 23 | LT | Negative | Euthyroid |
| 24 | LT | Negative | Euthyroid |
| 25 | Graves' disease | Negative | Hyperthyroid |
| 26 | LT | Negative | Hypothyroid |
| 27 | LT | Negative | Hypothyroid |

HT=Hashimoto thyroiditis, LT=lymphocytic thyroiditis, PTC=papillary thyroid carcinoma.

**Table 2. Statistical Analysis of Results**

| | *ret*/PTC-1 Positive | *ret*/PTC-1 Negative |
|---|---|---|
| Hashimoto thyroiditis | 18 | 1 |
| Lymphocytic thyroiditis | 3 | 4 |

Fisher's Exact Test. The two-sided p value is 0.0104. There is a significant association between rows and columns.

licular adenomas. Their conclusion, that expression of a chimeric transcript for either *ret*/PTC-1 or *ret*/PTC-3 was synonymous with the presence of microcarcinoma, even though microscopic examination of multiple sections did not reveal any tumor, is untenable. In addition, their data indicate activation of *ret*/PTC in follicular adenomas (FA). This finding is supported by the work of Bouancer et al. [12], who demonstrated RET rearrangements in 45% of FA. However, our study did not reveal *ret*/PTC-1 activation among the cohort of FA examined, a finding supported by other workers [2,13].

While we have found activation of *ret*/PTC-1 in 95% of Hashimoto thyroiditis cases, we believe that the mere presence of *ret*/PTC-1 transcripts does not automatically imply a diagnosis of PTC. Among the cases in our study cohort, only three were found to have concomitant PTC, the remainder (16/19), having no evidence of tumor on exhaustive examination. This caused us to speculate that *ret*/PTC-1 activation itself may trigger the autoimmune response characteristic of Hashimoto thyroiditis, linked either to the action of the constitutively activated TK moiety of RET or to altered expression of H4. H4 is the other gene affected in the generation of the *ret*/PTC-1 chimeric transcript, and it has been suggested that H4 codes for a cytoskeletal protein having structural homology with actin [14]. In a previous study [5], our group demonstrated a failure to express H4 when the *ret*/PTC-1 transcript is present. Furthermore, it has been suggested that *ret*/PTC-1 activation may influence the growth pattern in PTC [14,15] owing either to (1) failure of expression of

the cytoskeletal protein H4 leading to altered cellular structure or (2) the fact that RET/PTC-1 is constitutively phosphorylated. Yap et al. [14] have shown that increased tyrosine phosphorylation alters thyroid epithelial organization in culture by altering the assembly of actin-associated adhesive junctions. They have shown that under the influence of tyrosine phosphorylation, thyroid cells lose their capacity to form follicles and spread and migrate into confluent monolayers. It is possible, therefore, that similar cytomorphologic alterations with inherent antigenic modification occur *in vivo* in association with *ret*/PTC-1 activation and may contribute at least in part to the initiation of the autoimmune response characteristic of Hashimoto thyroiditis.

## Acknowledgments

We are grateful to Dr. Nobuo Satoh, Cancer Research Institute, Kanazawa University, for his permission to use the cell line TPC-1, and Dr. Mariwil Wong, Mt. Zion Medical Center of University of California San Francisco, for generously providing same. This work was supported by a grant from the Freemasons of Ireland Medical Research Fund.

## References

1. McGee JO'D, Isaacson P, Wright NA (eds), The thyroid gland, vol. 2b, ch. 26 in Oxford textbook of pathology. Oxford University Press, pp. 1951–1954, 1992
2. Grieco M, Santoro M, Berlingieri MT, et al. PTC is a novel rearranged form of the *ret* proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. Cell 60:557–563, 1990
3. Rabes HM, Demidchik EP, Siderow JD, et al. Pattern of radiation-induced RET and NTRK rearrangements in 191 post-Chernobyl papillary thyroid carcinomas: Biological, phenotypic, and clinical implications. Clin Cancer Res 6:1093–1103, 2000
4. Grieco M, Cerrato A, Santoro M, Fusco A, Melillo RM, Vecchio G. Cloning and characterization of H4 (D10S170), a gene involved in *RET* rearrangements in vivo. Oncogene 9:2531–2535, 1994
5. Sheils O, O'Leary J, Sweeney E. Involvement of *ret*/PTC-1 rearrangements in papillary carcinoma of the thyroid. J Pathol (in press).
6. Wirtschafter A, Schmidt R, Rosen D, et al. Expression of the RET/PTC fusion gene as a marker for papillary carcinoma in Hashimoto's thyroiditis. Laryngoscope 1:95–100, 1997
7. Sheils O, Sweeney E. TSH receptor status of thyroid neoplasms—TaqMan RT-PCR analysis of archival material. J Pathol 188:87–92, 1999
8. Jhiang SM, Mazzaferri EL. The *ret*/PTC oncogene in papillary thyroid carcinoma. J Lab Clin Med 123:331–337, 1994
9. Santoro M, Carlomagno F, Hay ID, et al. RET oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype. J Clin Invest 89:1517–1522, 1992
10. Soares P, Fonesca E, Wynford-Thomas D, Sobrinho-Simões M. Sporadic *ret*-rearranged papillary carcinoma of the thyroid: A subset of slow growing, less aggressive thyroid neoplasms. J Pathol 185:71–78, 1998
11. Sugg SL, Zheng L, Rosen IB, Freeman JL, Ezzat S, Asa SL. *ret*/PTC-1, -2, and -3 oncogene rearrangement in human thyroid carcinomas: Implications for metastatic potential. J Clin Endocrinol Metab 81:3360–3365, 1996
12. Bouancer A, Wicker R, Caillou B et al. High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumours from patients who had received external radiation. Oncogene 15:1263–1273, 1997
13. Lam AKY, Montone KT, Nolan KA, Livolsi VA. *Ret* activation in papillary thyroid carcinoma: Prevalence and implication on the histological parameters. Human Pathol 29:565–568, 1998
14. Yap AS, Stevenson BR, Cooper V, Manley S. Protein tyrosine phosphorylation influences adhesive junction assembly and follicular organization of cultured thyroid epithelial cells. Endocrinology 138:2315–2324, 1997
15. Fischer AH, Bond JA, Taysavang P, Battles OE, Wynford-Thomas D. Papillary thyroid carcinoma oncogene (RET/PTC) alters the nuclear envelope and chromatin structure. Am J Pathol 153:1443–1450, 1998

## P16INK4A AS A MARKER FOR CERVICAL DYSKARYOSIS: CIN AND CGIN IN CERVICAL BIOPSIES AND THINPREP™ SMEARS

J CLIN PATHOL 2003;56:56-63

Cervical cancer is one of the most common gynecological malignancies. In recent years, the implementation of cervical cancer screening has resulted in the effective control of cervical cancer incidence. However, many deficiencies still exist in the current screening techniques and strategies. With advancements in cervical cancer screening research, immunochemical staining to determine cervical cytology has shown a broader application prospect in the early screening for cervical cancer, especially for triage in cervical cancer screening. Among the various biomarkers established to facilitate early screening is the cell cycle regulatory protein known as $p16^{INK4a}$, which has demonstrated to be highly sensitive and specific marker of high-grade squamous and glandular neoplasia of the cervix due to its overexpression in cancerous and precancerous cervical neoplasia.

The cyclin-dependent protein kinase inhibitor $p16^{INK4a}$ is a negative regulator of the cell cycle pathway of cyclinD-CDK4/6-pRb-E2F that regulates the transition from the G1 to the S phase of the cell cycle and normally functions as a cell cycle brake or tumour suppressor. The expression of p16 is reduced in many cancers by mutation, deletion of the gene, or hypermethylation of its promoter. However, in the cases of cervical high-grade squamous intraepithelial lesion (HSIL) and carcinomas induced by persistent infection of high-risk HPV (HR-HPV) that contributes to neoplastic progression through the action of E6 and E7 viral oncoproteins, the product of p16 has been shown to be overexpressed as a result of functional inactivation of retinoblastoma protein (pRb) by the HPV E7 protein, which due to that the loss of pRb function should result in the release of the P16 gene from negative transcriptional feedback control.

This manuscript documented the capacity for $p16^{INK4a}$ to serve as a marker of dysplastic squamous and glandular cells of the cervix with a sensitivity of 99.9% and a specificity of 100%. The study clearly indicated the utility of this biomarker as a reliable marker for dysplastic squamous and glandular cervical cells in tissue sections and in cervical ThinPreps. The use of p16INK4a immunohistochemical analysis as a complement to conventional

screening programmes has contributed to a reduction of false positive and false negative results, has contributed to cost efficiency and increased public confidence in cervical cancer screening programmes.

I was Co-PI on this project and was responsible for procuring funding, designing the study, supervising the laboratory research, and drafting the manuscript.

ORIGINAL ARTICLE

# p16$^{INK4A}$ as a marker for cervical dyskaryosis: CIN and cGIN in cervical biopsies and ThinPrep™ smears

**N Murphy, M Ring, A G Killalea, V Uhlmann, M O'Donovan, F Mulcahy, M Turner, E McGuinness, M Griffin, C Martin, O Sheils, J J O'Leary**

*J Clin Pathol* 2003;**56**:56–63

See end of article for authors' affiliations

Correspondence to: Ms N Murphy, Department of Pathology, Coombe Women's Hospital, Dublin 8, Ireland; ncmurphy@tcd.ie

Accepted for publication 23 May 2002

**Aim:** To examine the potential of p16$^{INK4A}$ as a biomarker for dysplastic squamous and glandular cells of the cervix in tissue sections and ThinPrep™ smears.
**Methods:** Immunocytochemical analysis of p16$^{INK4A}$ expression was performed on 22 normal cervical tissue samples, five cervical glandular intraepithelial neoplasia (cGIN), 38 cervical intraepithelial neoplasia 1 (CIN1), 33 CIN2, 46 CIN3, and 10 invasive cancer cases (eight squamous and two adenocarcinomas). All samples were formalin fixed and paraffin wax embedded, and immunohistochemical analysis was carried out using a mouse monoclonal anti-p16$^{INK4A}$ antibody after antigen unmasking. The staining intensity was assessed using a 0 to 3 scoring system. In addition, the expression status of p16$^{INK4A}$ was examined in 12 normal ThinPrep smears, one smear exhibiting cGIN, and a total of 20 smears exhibiting mild, moderate, and severe dyskaryosis. Human papillomavirus (HPV) detection was carried out using a modified SYBR green assay system. Fluorogenic polymerase chain reaction (PCR) and solution phase PCR were used for specific HPV typing.
**Results:** p16$^{INK4A}$ immunoreactivity was absent in all normal cervical tissues examined. Dysplastic squamous and glandular cells were positive for p16$^{INK4A}$ expression in all cases included in this study, except for one CIN3 case. p16$^{INK4A}$ expression was mainly nuclear in CIN1 cases, and both nuclear and cytoplasmic in CIN2, CIN3, cGIN, and invasive cases. All cases positive for HPV expressed the p16$^{INK4A}$ protein, although not all cases found positive for p16$^{INK4A}$ were HPV positive. In general, the p16$^{INK4A}$ staining intensity was lower in cases negative for HPV or those containing a low risk HPV type.
**Conclusion:** This pattern of overexpression demonstrates the potential use of p16$^{INK4A}$ as a diagnostic marker for cervical squamous and also glandular neoplastic lesions. In addition, the technique can be used to identify individual dyskaryotic cells in ThinPrep smears. Thus, p16$^{INK4A}$ is a useful marker of cervical dyskaryosis.

Cervical cancer is one of the most common forms of cancer in women worldwide. In developing countries, cancer of the uterine cervix is ranked second, with a relative frequency of 15% of all cancers in women, whereas in developed countries cervical cancer is ranked fifth, with a relative frequency of 4.4%.[1] The Papanicolaou (Pap) test, as described by G Papanicolaou, is a cytological staining technique, which allows the identification of asymptomatic women who have preneoplastic lesions or early cancer of the uterine cervix. Although the introduction of mass screening programmes in developed countries has been effective in reducing cervical cancer mortality and morbidity rates, the success of the Pap smear test is limited with respect to sensitivity and specificity. False negative rates for cervical premalignant lesions and cervical cancer lie between 15% and 50% and false positive rates of approximately 30% have been reported.[2] This failure may reflect the subjectivity of cytological diagnosis. In addition, histological analysis of biopsy samples taken from women with abnormal smears can (as with cytology) be affected by interobserver discrepancies.[3] The failure of the Pap test to eradicate this potentially preventable disease outlines the limitations of current screening programmes and emphasises the need for the identification of specific biomarkers for dysplastic epithelial cells of the cervix to aid in primary screening and lesion diagnosis. The use of specific markers of dysplasia of the cervical epithelium in conjunction with current cytological or histological procedures could greatly improve the accuracy, precision, and sensitivity of cervical cancer screening programmes.

> "The failure of the Pap test to eradicate this potentially preventable disease outlines the limitations of current screening programmes and emphasises the need for the identification of specific biomarkers for dysplastic epithelial cells of the cervix"

A wide array of immunohistochemical markers have been tested to evaluate their specificity in staining dysplastic cells in either biopsies or cytological smears.[4] Two proteins involved in the regulation of DNA replication, Cdc6 and Mcm5, are specific markers for active replication. Cdc6 and Mcm5 have been shown to mark dysplastic cells, although their clinical usefulness is limited because they are unable to differentiate precisely between proliferating dysplastic cells and normal proliferating cells.[5] The MN antigen, a transmembrane glycoprotein containing a carbo-anhydrase domain, has also been described as a potential diagnostic marker for dysplastic and cancerous cervical epithelial cells. However, immunoreactivity of MN has been seen in normal columnar and squamous cells in immature metaplasia, and several cases with dysplastic lesions were negative for MN immunoreactivity.[6] Thus, the identification of a definitive marker for dysplastic cervical epithelial cells is still required to aid in the future diagnosis,

**Abbreviations:** BSA, bovine serum albumin; cdk, cyclin dependent kinase; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; Pap, Papanicolaou test; PBST, phosphate buffered saline/Tween 20; PCR, polymerase chain reaction; pRb, retinoblastoma protein; TBS, Tris buffered saline

**Table 1** Primers and probes used in human papillomavirus (HPV) detection and typing

| HPV | Primer or probe | Sequence |
|---|---|---|
| DgHPV | Primer 1 | TTT GTT ACT GTG GTA GAT AC |
| | Primer 2 | GAA AAA TAA ACT GTA AAT CAG C |
| HPV-16 L1A | U primer 6564 | CCT TAT TGG TTA CAA CGA GCA C |
| | L primer 7012 | GCG TCC TAA AGG AAA CTG ATA TA |
| | U probe 6862 | CCC CAG GAG GCA CAC TAG AAG AT |
| HPV-18 L1A | U primer 6548 | GTT ACA TAA GGC ACA GGG TCA T |
| | L primer 6993 | CGT CCA AGG GGA TAT TGA TC |
| | U probe 6902 | AAA GGA TGC TGC ACC GGC T |
| HPV-31 L1A | U primer 6490 | GAT GCA ACG TGC TCA GGG A |
| | L primer 6930 | GCG ACC CAG TGG AAA CTG ATC TA |
| | U probe 6852 | CCC AAA AGC CCA AGG AAG ATC |
| HPV-33 L1A | U primer 6490 | GGT TAC TTC CGA ATC TCA GTT ATT T |
| | L primer 6964 | TCC CAA AGG AAA CTG ATC TAA A |
| | L probe 6787 | TGT TAA ACC AAA TTG CCA ATC TTC T |
| HPV-6B | Primer 1 | CCT GTT TCG AGG CGG CTA TCC ATA |
| | Primer 2 | GTA CAA TTT AGC TTT ATG AAC CGC GCC TTG GTT |
| HPV-11B | Primer 1 | TGT GTG GCG AGA CAA CTT TCC CTT |
| | Primer 2 | TGG TTA TTT AGT TTT ATG AAG CGT GCC TTT CCC |

A primer pairs were designed by Swan *et al*.[18] B primer pairs were designed based on the following GenBank accession numbers: HPV-6, AF12648, X00203.1; HPV-11, NC 001525.1, M14119.1.

prognosis, and treatment of cervical intraepithelial lesions and cervical cancer.

The important role of human papillomaviruses (HPV) infection in cervical carcinogenesis is now well established. Indeed, HPV infection has been detected in almost all preneoplastic and neoplastic lesions of the cervix.[7] HPV is the most diverse group of DNA viruses involved in human disease, with more than 80 different types identified, approximately 30 of which can infect the cervical epithelium and give rise to various lesions of the cervix. HPV subtypes are subdivided into three categories according to risk: high, intermediate, and low. High risk HPV types, in particular HPV types 16 and 18, have been identified in more than 99% of cervical cancers.[8] HPV contributes to neoplastic progression predominantly through the action of two viral oncoproteins, namely E6 and E7, which interact with various cell cycle regulatory proteins.[9]

One such regulatory protein is the retinoblastoma gene product (pRb). pRb is a tumour suppressor, which inhibits the progression of cells into S phase and is regulated via phosphorylation by cyclin D1, complexed with cyclin dependant kinases (cdks).[10] Progressive and prolonged phosphorylation of the Rb protein leads to its inactivation and reduction of its growth suppressive activity.[11] The HPV E7 protein specifically binds to and inactivates pRb. This inactivation is mediated by the release of E2F-like transcription factors from pRB, which allows the activation of cdk and transcriptional activation of target promoters.[12 13]

The CDKN2A gene product, the p16INK4A protein, is a tumour suppressor protein that inhibits cdk4 and cdk6, which phosphorylate the Rb protein. A reciprocal relation between p16INK4A and pRb expression has been seen, suggesting the presence of a negative feedback loop allowing pRb to limit the concentration of p16INK4A.[14 15] p16INK4A overexpression has been demonstrated in cervical cancers as a result of functional inactivation of pRb by the HPV E7 protein.[16] This overexpression highlights the possible potential of p16INK4A as a marker for cervical intraepithelial lesions and cervical cancer.

In our study, we examined the potential of p16INK4A as a biomarker for dysplastic squamous and glandular cells of the cervix. Immunocytochemical analysis of p16INK4A expression was carried out using an anti-p16INK4A mouse monoclonal antibody on a large number of formalin fixed and paraffin wax embedded samples of normal, cervical intraepithelial neoplasia 1 (CIN1), CIN2, CIN3, cervical glandular intraepithelial neoplasia (cGIN), and invasive cancer cases. The expression status of p16INK4A was also examined on several ThinPrep™ smears exhibiting mild, moderate, and severe dyskaryosis. p16INK4A immunoreactivity was absent in all normal cervical tissues examined. Neoplastic squamous and glandular cells were positive for p16INK4A expression in all cases included in our study, except for one CIN3 case. This expression was nuclear in a small number of CIN1 cases, although the remaining CIN2, CIN3, cGIN, and invasive cases showed a combination of nuclear and cytoplasmic staining. As previously reported by Klaes *et al*,[17] our results demonstrate that p16INK4A is a reliable and sensitive marker of dysplastic squamous cells of the cervix. Our results also indicate that p16INK4A is a sensitive and specific marker of glandular intraepithelial neoplasia and adenocarcinoma of the cervix. In addition, we found that the immunocytochemical assay for p16INK4A can be performed successfully on ThinPrep slides. In our study, p16INK4A identified exfoliated cells exhibiting CIN and cGIN in ThinPrep smears. No non-specific staining of normal squamous cells was seen in the normal smears examined; however, sporadic staining of inflammatory and metaplastic cells was identified.

## MATERIALS AND METHODS

### Tissues and cytology samples

Cervical biopsy samples were selected from the pathology files of the Coombe Women's Hospital, Dublin. All samples were fixed in formalin and embedded in paraffin wax by conventional techniques. Haematoxylin and eosin stained slides of all samples were reviewed and classified by a certified pathologist. A total of 22 normal cases were selected, in addition to five cGIN, 38 CIN1, 33 CIN2, and 46 CIN3 cases. Eight invasive squamous and two adenocarcinomas were also selected. A total of 12 normal ThinPrep smears, in addition to one smear exhibiting cGIN and a total of 20 smears exhibiting mild, moderate, and severe dyskaryosis, were also included in our study. Cervical samples were collected using the ThinPrep cytology collection system (Cytyc Corporation, Boxborough, Massachusetts, USA), according to the manufacturer's protocol.

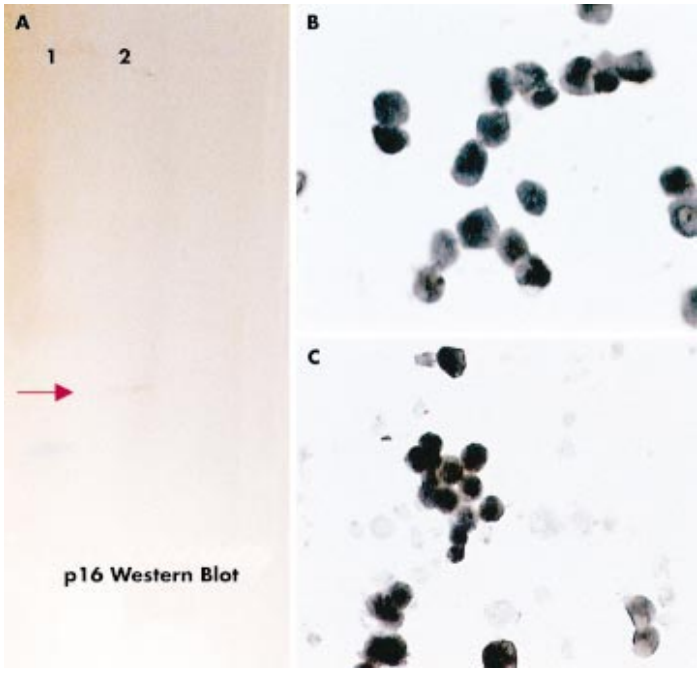



**Figure 1** Western blot analysis of $p16^{INK4A}$ protein extracted from CaSki cells using mouse monoclonal anti-$p16^{INK4A}$ antibody (clone G175–405). (A) Lane 1, molecular weight standards; lane 2, anti-$p16^{INK4A}$ clone G175–407. (B,C) Immunocytochemical analysis of cell line cytospins using anti-$p16^{INK4A}$ antibody (clone G175–405). Strong immunoreactivity was seen both in (B) CaSki and (C) C33A cervical carcinoma cell lines.

### HPV detection and typing

After dewaxing of paraffin wax embedded sections, DNA extraction was carried out using a Gentra DNA isolation kit (Puregene, Minneapolis, Minnesota, USA), according to the manufacture's protocol. Absolute measurement of extracted DNA was carried out using a Taqman real time quantitative polymerase chain reaction (PCR) assay for β actin (Applied Biosystems, Foster City, California, USA). HPV detection was then carried out using a modified SYBR green assay system (Applied Biosystems). A general HPV "degenerate" primer set that detects sequences within the L1 open reading frame of at least HPV types 6, 11, 16, 18, 30, 31, 32, 33, and 39 was used (table 1). Each 25 µl PCR reaction contained 1× SYBR buffer, 4.5mM $MgCl_2$, 200nM deoxynucleoside triphosphates, 150nM each primer (GAP1 and 2), 1.25 U TaqGold polymerase, and 100 ng template DNA. After denaturation for 10 minutes at 95°C, amplification conditions were as follows: 40 cycles (each) of 15 seconds at 95°C, 30 seconds at 42°C for primer annealing, and 30 seconds extension at 72°C. Amplification was carried out in a 7700 sequence detection system (Applied Biosystems). Interpretation of results was performed using Applied Biosystems' sequence detector software.

Fluorogenic PCR was used for specific HPV typing. Each 50 µl PCR reaction contained 10mM Tris (pH 8.3), 50mM KCl, 4.5mM $MgCl_2$, 200µM deoxynucleoside triphosphates, 0.3µM each primer, 50nM each fluorogenic probe (probe and primer pair sequences for HPV 16, 18, 31, and 33 are shown in table 1), 0.025 U Taq polymerase/µl, and 10 µl of template DNA. After template denaturation for two minutes at 95°C, amplification conditions were as follows: 40 cycles (each) of 30 seconds at 94°C, 10 seconds at 60°C, and two minutes at 65°C.[18] Amplification was carried out in a 7700 sequence detection system (Applied Biosystems). Interpretation of results was performed using Applied Biosystems' sequence detector software.

Solution phase PCR was used for HPV-6 and HPV-11 typing. Each 50 µl PCR reaction contained 10mM Tris/HCl (pH 8.3), 50mM KCl, 1.5mM $MgCl_2$, 0.01% gelatin, 200µM of each dNTP, 1.0µM of each primer, 2.5 U Taq polymerase, and 5 µl of template DNA. Samples were subjected to 40 cycles of PCR in a Perkin Elmer Gene Amp PCR system 2400. Each PCR cycle consisted of one minute at 94°C, two minutes at 55°C, and three minutes at 72°C (extended to 10 minutes in the final cycle).

### Antibodies

Several commercial $p16^{INK4A}$ specific monoclonal antibodies are currently available. These include clone DCS-50 (Oncogene, Research Products, Cambridge, Massachusetts, USA), clone ZJ11, and clone JC8 (Neomarkers, Freemount, California, USA). Clone G175–405 (PharMingin, San Diego, California, USA) was chosen for our study based on performance results published by Geradts *et al*.[19]

### Characterisation of $p16^{INK4A}$ antibody by western blot analysis

CaSki cells were homogenised in RIPA buffer (Santa Cruz Biotechnology, San Diego, California, USA). The protein extract was then separated by electrophoresis on a 15% sodium dodecyl sulfate polyacrylamide electrophoresis gel and then transferred to a nitrocellulose membrane using transfer buffer containing 25mM Tris/HCl, pH 8.3, 192mM glycine, and 20% methanol. The blot was preblocked with 3% Marvel in TTBS (20mM Tris/HCl, pH 7.5, 0.5 NaCl, and 0.05% vol/vol Tween 20). The blot was then incubated with purified mouse antihuman $p16^{INK4A}$ antibody (Clone G175–405, PharMingin). After three 10 minute washes in phosphate buffered saline/Tween 20 (PBST; 136mM NaCl, 26mM KCl, 15mM $KH_2PO_4$, 82mM $Na_2HPO_4$, and 0.05% vol/vol Tween 20) the blot was then incubated in biotinylated universal secondary antibody (Vectastain

ABC kit; Vector Laboratories, Burlingame, California, USA) for 30 minutes. This was followed by three washes in PBST and incubation in an avidin–biotin complex (Vectastain ABC kit; Vector Laboratories) for 30 minutes. Immunoreactive bands were detected with diaminobezaminidine (Vector Laboratories) (fig 1A).

### Immunocytochemistry on paraffin wax embedded tissues

Sections (4 μm thick) were cut from formalin fixed, paraffin wax embedded biopsy samples and mounted on saline coated glass slides. Sections were dewaxed by passage through xylene and then rehydrated in graded alcohol. Endogenous peroxidase activity was blocked by incubating the sections in 0.3% $H_2O_2$/methanol for 30 minutes. Antigen retrieval was performed in 0.01M citrate buffer (pH 6) using a pressure cooker method. After rinsing sections in Tris buffered saline (TBS; pH 7.4) non-specific antibody binding was reduced by incubating the sections in 0.1% bovine serum albumin (BSA) for 30 minutes. After decanting excess serum, sections were incubated for one hour at room temperature with a purified mouse antihuman p16INK4A monoclonal antibody (PharMingen, Becton Dickinson) at a 1/75 dilution with 0.1% BSA in TBS). After washing thoroughly with TBS, the sections were incubated with biotinylated universal secondary antibody (Vectastain ABC kit; Vector Laboratories) for 30 minutes. This was followed by incubation with the avidin–biotin complex (Vectastain; Vector Laboratories) for 30 minutes. Slides were developed with diaminobezaminidine (Vector Laboratories) for approximately one minute and counterstained lightly with haematoxylin.

### Immunocytochemistry on ThinPrep smears

The procedure for p16INK4A immunocytochemical analysis on ThinPreps was identical to the above described procedure for immunohistochemical analysis except that the dewaxing step in xylene was omitted and a 1/100 dilution of purified mouse antihuman p16INK4A monoclonal antibody in 0.1% BSA in TBS was used for all ThinPrep samples. ThinPreps of CaSki and C33A cells (ECACC number 87020501) were used as positive controls to evaluate the specificity of each staining run.

### Interpretation of p16INK4A expression in biopsy tissues

All formalin fixed, paraffin wax embedded sections that showed either strong nuclear or cytoplasmic staining were considered positive. A certified pathologist then graded all sections qualitatively according to the following arbitrary scale: 0 (no positive staining), 1 (< 10% positive staining), 2 (> 10% but < 50% positive staining), and 3 (> 50% positive staining).

## RESULTS

### HPV detection and typing

SYBR green HPV analysis found the CaSki cell line positive for HPV. Further specific HPV typing by fluorogenic PCR found the cell line to contain HPV-16 DNA sequences. The C33A cervical cancer cell line was found to be negative for HPV DNA by SYBR green HPV analysis.

All cases positive for HPV using SYBR green PCR were also HPV positive for type specific PCR. No case included in our study was found to be positive for HPV-31 or HPV-33. This is not unusual in an Irish population. Table 2 shows the results of HPV typing.

**Table 2** Comparison between p16INK4A staining intensity and human papillomavirus (HPV) status

| | N | P16INK4A staining intensity | HPV -ive | HPV-6/11 | HPV-16 | HPV-18 | HPV-31 | HPV-33 |
|---|---|---|---|---|---|---|---|---|
| Normal | 20 | 0 | 18/20 (90%) | 2/20 (10%) | – | – | – | – |
| | | 1 | – | – | – | – | – | – |
| | | 2 | – | – | – | – | – | – |
| | | 3 | – | – | – | – | – | – |
| Total | | | 90% | 10% | | | | |
| cGIN | 3 | 0 | – | – | – | – | – | – |
| | | 1 | – | – | – | – | – | – |
| | | 2 | – | – | – | – | – | – |
| | | 3 | – | – | 3/3 (100%) | – | – | – |
| Total | | | | | 100% | | | |
| CIN 1 | 29 | 0 | – | – | – | – | – | – |
| | | 1 | 2/29 (7%) | 3/29 (10%) | – | – | – | – |
| | | 2 | 2/29 (7%) | 1/29 (4%) | – | – | – | – |
| | | 3 | – | – | 21/29 (72%) | – | – | – |
| Total | | | 14% | 14% | 72% | | | |
| CIN2 | 28 | 0 | – | – | – | – | – | – |
| | | 1 | 3/28 (11%) | 2/28 (7%) | – | – | – | – |
| | | 2 | – | – | 8/28 (28%) | 1/28 (4%) | – | – |
| | | 3 | – | – | 14/28 (50%) | – | – | – |
| Total | | | 11% | 7% | 78% | 4% | | |
| CIN3 | 36 | 0 | 1/36 (3%) | – | – | – | – | – |
| | | 1 | 1/36 (3%) | 1/36 (3%) | – | – | – | – |
| | | 2 | 1/36 (3%) | – | 8/36 (22%) | 2/36 (5%) | – | – |
| | | 3 | – | – | 20/36 (56%) | 2/36 (5%) | – | – |
| Total | | | 9% | 3% | 78% | 10% | | |
| Invasive | 9 | 0 | – | – | – | – | – | – |
| | | 1 | – | – | – | – | – | – |
| | | 2 | – | – | – | – | – | – |
| | | 3 | – | – | 9/9 (100%) | – | – | – |
| Total | | | | | 100% | | | |

Not all cases included in our study were evaluated for HPV status. Staining intensity: 0, no positive p16INK4A staining; 1, <10% positive p16INK4A staining; 2, >10% but <50% positive p16INK4A staining; 3, >50% positive p16INK4A staining.
CIN, cervical intraepithelial neoplasia; cGIN, cervical glandular intraepithelial neoplasia; N, number of cases tested for HPV.

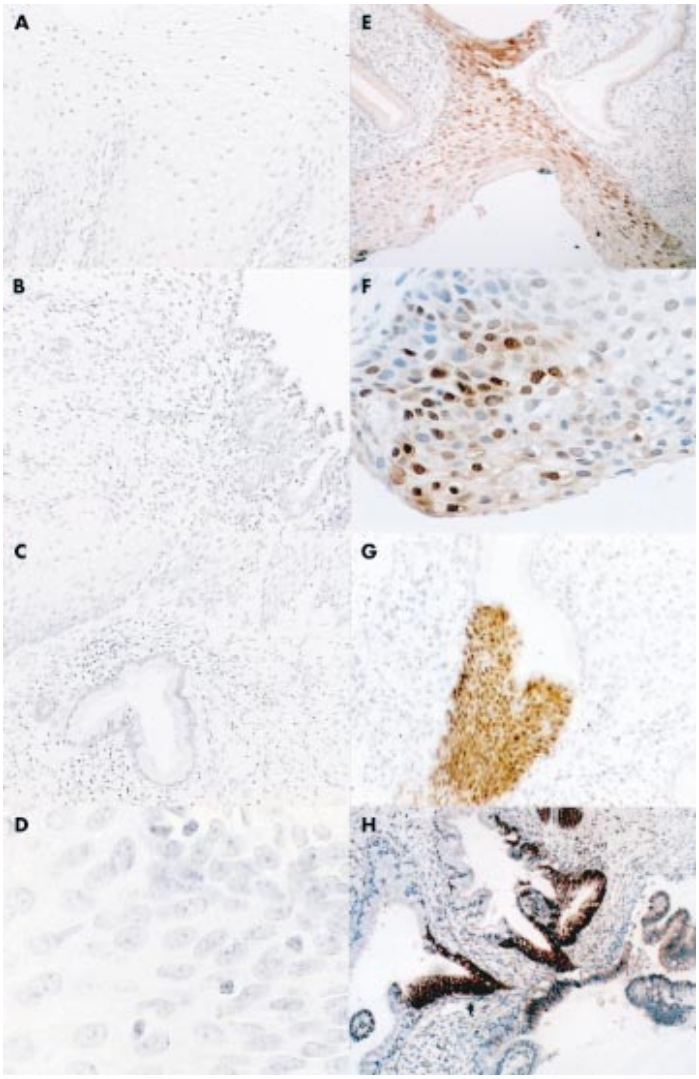



**Figure 2** Immunohistochemical staining for p16$^{INK4A}$ in histologically normal tissue samples. (A) Normal epithelium, (B) squamous metaplasia, (C) normal endocervical gland, and (D) reactive epithelium showing chronic inflammation. Immunohistochemical staining for p16$^{INK4A}$ in cervical genital lesions. (E) CIN1, (F) CIN2, (G) CIN3 in an endocervical gland, and (H) cGIN.

### Immunocytochemical staining for p16$^{INK4A}$ in cell lines

To check the specificity of clone G175–405 (PharMingin) we performed immunostaining for p16$^{INK4A}$ in CaSki (HPV-16 positive) and C33A (HPV negative) cytospins. The HPV-16 positive CaSki cells exhibited strong immunopositivity for p16$^{INK4A}$ protein. Unexpectedly, the HPV negative C33A cell line (ATCC) was also strongly positive for p16$^{INK4A}$ protein expression. Staining was predominantly cytoplasmic in both cell lines (fig 1B,C).

### Immunocytochemical staining for p16$^{INK4A}$ in histologically normal tissue samples

Using a monoclonal antibody to p16$^{INK4A}$ (clone G175–405; PharMingin), immunocytochemical analysis was performed in histologically normal tissue samples. In all cases, normal epithelial, stromal, metaplastic reactive, and inflammatory cells were not stained (fig 2A,B,D). Negative immunostaining with anti-p16$^{INK4A}$ was also seen in surface and glandular endocervical epithelium (fig 2C). In addition, normal areas adjacent to CIN lesions showed no detectable p16$^{INK4A}$ expression.

### Immunocytochemical staining for p16$^{INK4A}$ in cervical genital lesions

In 117 CIN lesions, five cGIN lesions, and 10 invasive cervical carcinomas (eight squamous and two adenocarcinoma), p16$^{INK4A}$ expression was generally very strong in dysplastic epithelial cells. A clear distinction was seen between dysplastic cells and adjacent normal cells. Interestingly, a small number of CIN1 lesions exhibited exclusively nuclear staining,

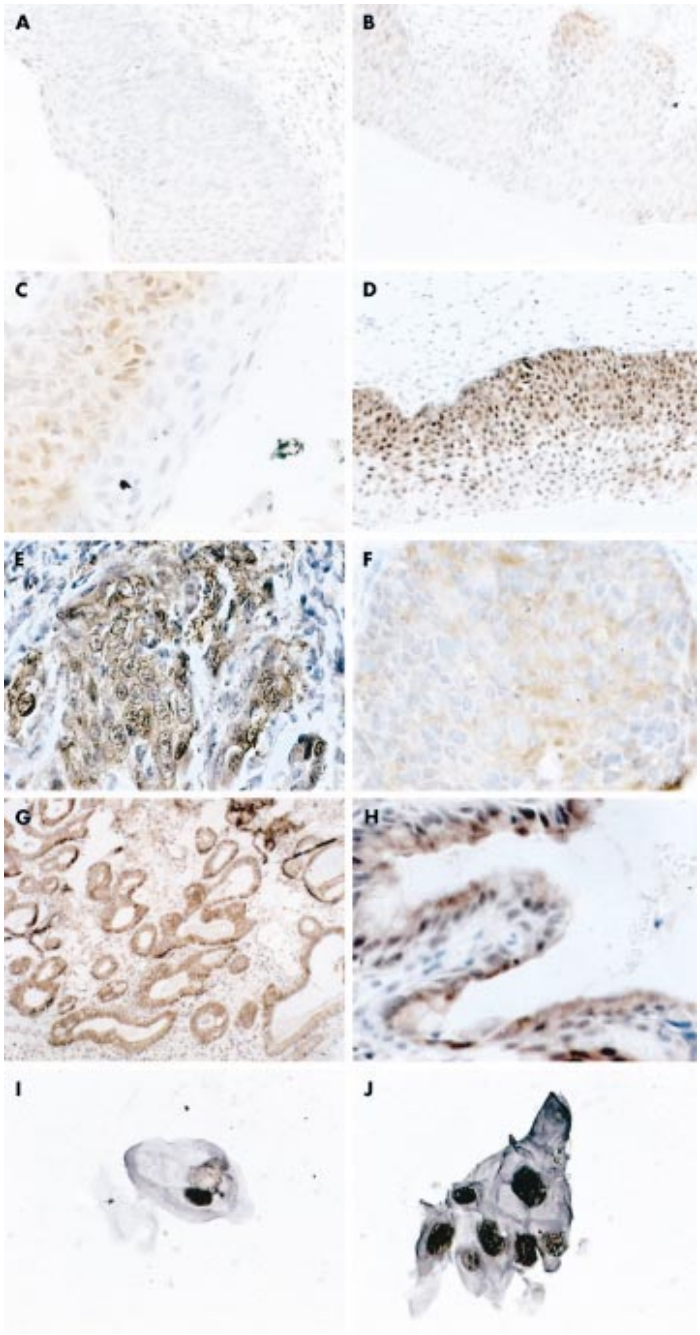



**Figure 3** Scoring system for p16INK4A immunostaining in CIN3 lesions. (A) 0, (B) 1, (C) 2, and (D) 3. Immunohistochemical staining of p16INK4A in invasive squamous cell carcinoma; (E) nuclear staining, (F) cytoplasmic staining, (G) adenocarcinoma, and (H) cGIN. Immunocytochemical staining of exfoliated dysplastic cells in ThinPrep smears using p16INK4A specific antibody; (I) p16INK4a expression in a mild dyskaryotic cell; (J) mild to moderately dyskaryotic cell cluster exhibiting p16INK4a immunopositivity.

**Table 3** Results of immunocytochemical analysis of p16$^{INK4A}$

| | N | Score 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|
| Normal | 21 | 21 (100%) | 0 (0%) | 0 (0%) | 0 (0%) |
| cGIN | 5 | 0 (0%) | 0 (0%) | 1 (20%) | 4 (80%) |
| CIN 1 | 38 | 0 (0%) | 3 (8%) | 11 (29%) | 24 (63%) |
| CIN 2 | 33 | 0 (0%) | 9 (27%) | 9 (27%) | 15 (46%) |
| CIN 3 | 46 | 1 (2%) | 3 (7%) | 14 (30%) | 28 (61%) |
| Invasive | 10 | 0 (0%) | 0 (0%) | 0 (0%) | 10 (100%) |

Score: 0, no positive staining; 1, <10% positive staining; 2, >10% but <50% positive staining); 3, >50% positive staining. Examples of 0, 1, 2, and 3 are shown in fig 3A–D.
CIN, cervical intraepithelial neoplasia; cGIN, cervical glandular intraepithelial neoplasia.

whereas in the remaining cGIN, CIN1, CIN2, and CIN3 lesions a predominantly cytoplasmic pattern of staining was seen (fig 2E–H). All invasive squamous carcinomas and adenocarcinomas exhibited strong nuclear and cytoplasmic staining (fig 3E–G). All lesions included in our study were qualitatively graded according to the following criteria: 0 (no positive staining), 1 (< 10% positive staining), 2 (> 10% but< 50%), 3 (> 50%positive staining) (table 3; fig 3A–D).

### Immunocytochemical staining for p16$^{INK4A}$ in ThinPrep slides

To evaluate the potential of p16$^{INK4A}$ as a biomarker for dysplastic exfoliated cells in cytological samples, immunocytochemical analysis using monoclonal anti-p16$^{INK4A}$ was carried out on a series of ThinPrep slides. The p16$^{INK4A}$ antibody assay was positive in all five smears exhibiting mild dyskaryosis, six of seven smears exhibiting moderate dyskaryosis, and all eight ThinPrep smears showing severe dyskaryosis. Exfoliated cells exhibiting cGIN were also positive for p16$^{IK4a}$ expression. The p16$^{INK4a}$ antibody assay was negative in all 12 normal smears examined. Figure 3I and J shows the immunocytochemical analysis of the p16$^{INK4A}$ antigen on ThinPrep slides. This example demonstrates the ability of p16$^{INK4A}$ to identify dysplastic exfoliated epithelial cells. In all ThinPrep slides analysed, no non-specific staining of normal squamous cells was seen, although sporadic staining of morphologically normal metaplastic cells and inflammatory cells was identified (in one case).

## DISCUSSION

Despite the success of conventional screening programmes questions have arisen concerning the reliability of conventional cervical cytology and histology.[20] These concerns have highlighted the need for improved screening technologies and prompted investigation into the possible usefulness of tumour associated antigen markers as an adjunct to conventional Pap testing. One such potential biomarker is the p16$^{INK4A}$ tumour suppressor gene. In our study, we wished to examine the potential usefulness of p16$^{INK4A}$ as a diagnostic marker for dysplastic squamous and glandular cells of the cervix.

We have extensively analysed the immunocytochemical distribution of p16$^{INK4A}$ in normal and neoplastic tissue of the cervix. In all normal cervical tissues examined all epithelial, metaplastic, endocervical, reactive, and inflammatory regions were not stained with the monoclonal anti-p16$^{INK4A}$ antibody. In addition, all normal regions adjacent to CIN lesions showed no detectable p16$^{INK4A}$ expression. In 117 cases (including CIN1, CIN2, and CIN3, five cGIN, and 10 invasive cancer cases) all but one CIN3 lesion exhibited overexpression of the p16$^{INK4A}$ gene product. Failure of this isolated CIN3 case to express p16$^{INK4A}$ could not be explained because this case demonstrated immunoreactivity for common leucocyte antigen and epithelial membrane antigen. All squamous cell carcinomas and adenocarcinomas examined exhibited strong overexpression of the p16$^{INK4A}$ protein. Although a small number of CIN1 cases showed exclusive nuclear staining, interestingly, the remaining CIN1, CIN2, CIN3, and invasive cancer cases showed a combination of nuclear and cytoplasmic staining. p16$^{INK4A}$ protein localisation is thought to be nuclear. The presence of p16$^{INK4A}$ in the cytoplasm may result from a type of post transcriptional modification or, more simply, overproduction of the protein may force its transfer into the cytoplasm. These findings clearly support previous studies confirming the hypothesis that p16$^{INK4A}$ is overexpressed in dysplastic cells of the cervix.[12 13 15]

In our study we performed p16$^{INK4A}$ immunohistochemical analysis on a series of ThinPrep slides. Smears categorised as normal, in addition to those showing mild, moderate, and severe dyskaryosis were analysed. We found that the anti-p16$^{INK4A}$ monoclonal antibody specifically stains exfoliated dysplastic cells within ThinPrep smears. The p16$^{INK4a}$ antibody assay also identified exfoliated cells exhibiting cGIN. Non-specific staining of normal squamous cells was not seen, although sporadic staining of inflammatory cells and morphologically normal metaplastic cells was identified (in one case). We found ThinPrep prepared monolayer slides easily adaptable to the p16$^{INK4A}$ immunohistochemical assay. The methanol based fixative used by the ThinPrep system is capable of morphological preservation and maintaining the integrity of cellular protein.[21] ThinPrep smears also have reduced amounts of blood and mucous, which have in conventional smears been reported to reduce immunostaining efficiency.

> "We found ThinPrep prepared monolayer slides easily adaptable to the p16$^{INK4A}$ immunohistochemical assay"

All cases positive for HPV expressed p16$^{INK4A}$ protein, although not all cases found positive for p16$^{INK4A}$ protein expression were HPV positive. Table 2 compares the p16$^{INK4A}$ staining intensity with HPV type. In general, the p16$^{INK4A}$ staining intensity was lower in cases containing a low risk HPV type or those negative for HPV. In addition, the HPV negative cell line C33A is p16$^{INK4A}$ positive; this clearly indicates that a non-HPV dependent p16$^{INK4A}$ expression pathway may also exist.

In conclusion, p16$^{INK4A}$ marks dysplastic squamous and glandular cells of the cervix with a sensitivity of 99.9% and a specificity of 100%. The results of our study clearly indicate that p16$^{INK4A}$ is a reliable marker for dysplastic squamous and glandular cervical cells in tissue sections and in cervical ThinPreps. The use of p16$^{INK4a}$ immunohistochemical analysis as a complement to conventional screening programmes will aid in

**Take home messages**

- p16$^{INK4A}$ marks dysplastic squamous and glandular cells of the cervix with a sensitivity of 99.9% and a specificity of 100%
- All cases positive for human papillomavirus (HPV) expressed the p16$^{INK4A}$ protein, although not all cases found positive for p16$^{INK4A}$ were HPV positive. In general, the p16$^{INK4A}$ staining intensity was lower in cases negative for HPV or those containing a low risk HPV type
- p16$^{INK4A}$ is a reliable marker for dysplastic squamous and glandular cervical cells both in tissue sections and in cervical ThinPreps
- p16$^{INK4a}$ immunohistochemical analysis would provide a useful adjunct to conventional screening programmes and would help reduce false positive and false negative results, which could reduce patient anxiety and the overall cost of cervical cancer screening programmes

the reduction of false positive and false negative results. This will ultimately result in a reduction in patient anxiety and the overall cost of cervical cancer screening programmes.

## ACKNOWLEDGEMENTS

This work was supported by the Irish Cancer Society, the Health Research Board of Ireland, and the Royal City of Dublin Hospital Trust.

### Authors' affiliations

**M Ring, A G Killalea, V Uhlmann, C Martin, J J O'Leary,** Department of Pathology, Coombe Women's Hospital, Dublin 8, Ireland
**N Murphy, M O'Donovan, O Sheils,** Trinity College Dublin, Dublin 2, Ireland
**E McGuinness, M Turner,** Department of Obstetrics and Gynaecology, The Coombe Women's Hospital
**F Mulcahy,** Department of Genitourinary Medicine, St James's Hospital, Dublin, Ireland
**M Griffin,** Department of Histopathology, St James Hospital

## REFERENCES

1 **Parkin DM**, Pasini P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int J Cancer* 1999;**54**:827–41.
2 **Jacobs MV**, Snijders PJF, Voorhorst FJ, *et al*. Reliable high risk HPV DNA testing by polymerase chain reaction: an intermethod and intramethod comparison. *J Clin Pathol* 1999;**52**:498–503.
3 **McCluggage WG**, Walsh MY, Thornton CM, *et al*. Inter- and intra-observer variation in the histopathological reporting of cervical intraepithelial lesions using a modified Bethesda grading system. *Br J Obstet Gynaecol* 1995;**105**:206–10.
4 **Keating JT**, Ince T, Crum CP. Surrogate biomarkers of HPV infection in cervical neoplasia screening and diagnosis. *Adv Anat Pathol* 2001;**8**:83–92.
5 **Williams GH**, Piotr R, Morris L, *et al*. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. *Proc Natl Acad Sci U S A* 1998;**99**:14932–7.
6 **Liao SY**, Brewer C, Zavada J, *et al*. Identification of the MN antigen as a diagnostic marker of cervical intraepithelial squamous and glandular neoplasia and cervical carcinomas. *Am J Pathol* 1994;**145**:598–609.
7 **Zur Hausen H**. Papillomavirus infections: a major cause of human cancers. *Biochim Biophys Acta* 1996;**1288**:F55–78.
8 **Walboomers JM**, Jacobs MV, Manos MM, *et al*. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;**189**:12–19.
9 **Giannoudis A**, Herrington SC. Human papillomavirus variants and squamous neoplasia of the cervix. *J Pathol* 2001;**193**:295–302.
10 **Dublin EA**, Patel NK, Gillett CE, *et al*. Their relationship to cyclin D1 and histopathological parameters. *Int J Cancer* 1998;**79**:71–5.
11 **Kato JY**, Matsushume H, Hiebert SW, *et al*. Direct binding of cyclin D to the retinoblastoma gene product (pRB) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. *Genes Dev* 1993;**7**:331–42.
12 **Sano T**, Oyama T, Kashibwabara K, *et al*. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. *Am J Pathol* 1998;**153**:1741–8.
13 **Sano T**, Oyama T, Kashiwabara K, *et al*. Overexpression of p16 protein associated with intact retinoblastoma protein expression in cervical cancer and cervical intraepithelial neoplasia. *Pathol Int* 1998;**48**:580–8.
14 **Li Y**, Nichols MA, Shay JW, *et al*. Transcriptional repression of the D-type cyclin dependent kinase inhibitor p16 by retinoblastoma susceptibility gene product pRb. *Cancer Res* 1994;**54**:6078–82.
15 **Tam SW**, Shay JW, Pagano M. Differential expression and cell cycle regulation of the cyclin dependent kinase inhibitor p16INK4A. *Cancer Res* 1994;**54**:5816–20.
16 **Sakaguchi M**, Fujii Y, Hirabayashi H, *et al*. Inversely correlated expression of p16 and Rb protein in non-small cell lung cancers: an immunohistochemical study. *Int J Cancer*1996;**65**:442–5.
17 **Klaes R**, Friedrick T, Spitkovsky D, *et al*. Overexpression of p16INK4A as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int J Cancer* 2001;**92**:276–84.
18 **Swan DC**, Tucker RA, Holloway BP, *et al*. A sensitive, type-specific, fluorogenic probe assay for detection of human papillomavirus DNA. *J Clin Microbiol* 1997;**35**:886–91.
19 **Geradts J**, Hruban RH, Schutte M, *et al*. Immunohistochemical p16INK analysis of archival tumour with deletion, hypermethylation, or mutation of the CDKN2/MTS1 gene. A comparison of four antibodies. *Appl Immunohistochem Mol Morphol* 2000;**1**:71–9.
20 **Hutchinson ML**, Isenstein ML, Goodman A, *et al*. Homogeneous sampling accounts for increased accuracy using the ThinPrep™ processor. *Am J Clin Pathol* 1994;**101**:215–19.
21 **Lin WM**, Ashfaq R, Michalopulos EA, *et al*. Molecular Papanicolaou tests in the twenty first century: molecular analysis with fluid based technology. *Am J Obstet Gynecol* 2000;**183**:39–45.

## COMPARISON OF MIRNA EXPRESSION PATTERNS USING TOTAL RNA EXTRACTED FROM MATCHED SAMPLES OF FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) CELLS AND SNAP FROZEN CELLS

BMC BIOTECHNOLOGY 2007,7:36

MicroRNAs (miRNAs) are essential regulators of biological activity, suppressing gene expression at the posttranscriptional level. MiRNA expression alterations profoundly affect cell differentiation and metabolic activity, and they are often involved in the pathogenesis of human diseases. MicroRNAs (miRNAs) have diagnostic and prognostic potential for many diseases, most notably for cancer.

Worldwide, there are an estimated one billion archived tissue samples, most of which are formalin-fixed and paraffin-embedded (FFPE). These tissue samples harbour valuable research materials. Storing FFPE specimens is more economical and practicable than storing frozen samples, and the histological structure can be preserved almost permanently. However, formalin fixation and paraffin embedding inevitably lead to nucleic acids degradation in these tissues. Fragmented and chemically modified DNA and RNAs have frustrated the routine application of transcriptomic analyses in archived tissues. Many of these samples are associated with long-term clinical follow-up data. This, coupled with increased insights into role of regulatory RNAs was the driver for this body of work with the ambition of developing novel approaches for exploiting FFPE archives.

This paper categorically demonstrated the utility of miRNA profiling in archival FFPE samples and provided a mechanism for robust and reproducible interrogation of older, more recalcitrant tissue samples. It showed that archival FFPE RNA, that is severely degraded and empirically incompatible with transcriptome expression methods, can nonetheless yield meaningful biology when combined with miRNA expression profiling using carefully tailored assay design. The paper has been cited more than 400 times since its publication and continues to be a key reference some 15 years after its publication.

I was the Principal Investigator on this study. I procured the funding and supervised the lab work to complete it and assisted in writing the manuscript.

BMC Biotechnology

BioMed Central

Research article

Open Access

# Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells

Jinghuan Li[1], Paul Smyth[1], Richard Flavin[1], Susanne Cahill[1], Karen Denning[1], Sinead Aherne[1], Simone M Guenther[2], John J O'Leary[1] and Orla Sheils*[1]

Address: [1]Deptment of Histopathology, University of Dublin, Trinity College, Dublin, Ireland and [2]Applied Biosystems, Foster City, CA, USA

Email: Jinghuan Li - jinghual@tcd.ie; Paul Smyth - smythpa@tcd.ie; Richard Flavin - flavinr@tcd.ie; Susanne Cahill - sucahill@tcd.ie; Karen Denning - Denningk@tcd.ie; Sinead Aherne - ahernesi@tcd.ie; Simone M Guenther - Simone.M.Guenther@eur.appliedbiosystems.com; John J O'Leary - olearyjj@tcd.ie; Orla Sheils* - osheils@tcd.ie

* Corresponding author

Published: 29 June 2007

*BMC Biotechnology* 2007, **7**:36 doi:10.1186/1472-6750-7-36

Received: 12 March 2007
Accepted: 29 June 2007

This article is available from: http://www.biomedcentral.com/1472-6750/7/36



## Abstract

**Background:** Archival formalin-fixed paraffin-embedded (FFPE) tissues have limited utility in applications involving analysis of gene expression due to mRNA degradation and modification during fixation and processing. This study analyzed 160 miRNAs in paired snap frozen and FFPE cells to investigate if miRNAs may be successfully detected in archival specimens.

**Results:** Our results show that miRNA extracted from FFPE blocks was successfully amplified using Q-RT-PCR. The levels of expression of miRNA detected in total RNA extracted from FFPE were higher than that extracted from snap frozen cells when the quantity of total RNA was identical. This phenomenon is most likely explained by the fact that larger numbers of FFPE cells were required to generate equivalent quantities of total RNA than their snap frozen counterparts.

**Conclusion:** We hypothesise that methylol cross-links between RNA and protein which occur during tissue processing inhibit the yield of total RNA. However, small RNA molecules appear to be less affected by this process and are recovered more easily in the extraction process. In general miRNAs demonstrated reliable expression levels in FFPE compared with snap frozen paired samples, suggesting these molecules might prove to be robust targets amenable to detection in archival material in the molecular pathology setting.

## Background

MicroRNAs (miRNAs) are small non-coding sequences of RNA, approximately 20 to 22 nucleotides long, which play important roles in the regulation of target genes by binding to complementary regions of messenger transcripts to repress their translation or regulate degradation [1]. This regulation appears to be involved in many fundamental cellular processes, including development, differentiation, proliferation, apoptosis, stress response, fat metabolism and insulin secretion [2]. Although the total number of different miRNA sequences in humans might approach 1000 based on the estimation of computer sim-

ulations [3], only 300 to 400 of them have been studied on fresh or snap-frozen samples to date. To discover the full regulatory impact of miRNA species and to understand individual biological functions within disease settings, larger scale analysis needs to be performed in a robust and reliable manner.

Formalin-Fixed, Paraffin-Embedded (FFPE) tissue samples are the most readily available archival material. They generally may be retrieved with documented clinico-pathological histories. Thus they represent an invaluable source for the study of human disease. However, these tissues have not been widely used in molecular biology due to the poor quality of RNA extracted from FFPE blocks [4] which is degraded to fewer than 300 bases in length [5] and also chemically modified by methylol groups during formalin fixation [6]. Thus, the value of FFPE materials in molecular setting has been shadowed by the technical difficulties limiting extensive analysis of gene expression. Interestingly, miRNAs are a class of small RNAs whose survivability and expression level in FFPE blocks compared with fresh tissues are largely unknown.

In this study we examined the reliability of miRNA detection in formalin fixed paraffin embedded blocks by interrogation of 160 miRNA assays in paired RNA extracts from fresh and FFPE samples using a cell line model.

## Results

### *RNA extraction*

To achieve 50 ng of total RNA for each RT reaction, 10,000 ng of total RNA (for 200 assays) was extracted from approximately $2 \times 10^6$ of FFPE cells and from approximately $1.7 \times 10^5$ snap frozen cells. Analysis using an Agilent 2100 Bioanalyser showed that the RNA Integrity Number (RIN Number) was 9.1 for the snap frozen cell preparations and 6.4 for the corresponding FFPE preparation.

### *miRNA expression*

There was a good correlation of miRNA expression pattern in between FFPE and snap frozen cells, with $R^2 > 0.95$ (Figure 1). The mean of ΔCts was -1.04107. The median of ΔCts was -1.152, (126 below 0 and 28 above 0) with $p < 0.0001$. The sign test of median showed that miRNA exhibited approximately two fold higher expression with the total RNA extracted from the FFPE cells than that extracted from the snap frozen cells.

65.58% of ΔΔCts (101 out of 154 determined assays), were between +1 and -1. Furthermore the abundance of some individual miRNAs changed in FFPE cells with a total of 23 miRNAs displaying increased expression and 30 miRNAs decreased expression (Table 1 and Figure 2).

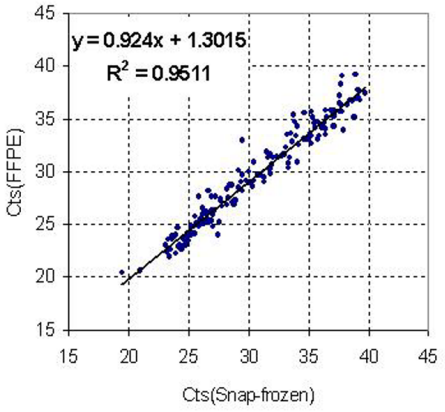



**Figure 1**
**Comparison of Ct values of 154 miRNA assays from paired FFPE and Snap-frozen cell lines**. Identical amount of total RNA was employed in each assay. $R^2$ is over 95% between two cell lines.

## Discussion

Since their discovery, miRNA analysis has generally been performed on snap-frozen or fresh samples, using variable techniques including microarray, northern blot analysis and PCR [2]. FFPE tissues, as a readily available source, could be invaluable in performing miRNA expression analysis if their expression were maintained following processing. In this study we compared miRNA profiling performed on fresh samples and FFPE using stem-loop RT-PCR quantification techniques in a cell line model. We found that miRNA profiling could be performed on routinely fixed FFPE blocks.

### *miRNA abundance in FFPE*

Some laboratories have examined mRNA gene expression profiles using real-time RT-PCR in paired snap-frozen and FFPE tissue samples [7-10]. The general consensus is that mRNA detection from archival material is limited due to the labile nature of mRNA and the deleterious effects of enzymatic fragmentation during long periods of storage and RNA modification induced by formalin fixation. Subsequently, it has been suggested that small amplicons [11] (shorter than ~ 130 [7,9] nucleotides) could have utility as robust markers in gene expression studies using FFPE tissues. Indeed, our own experiments (data not shown) confirmed this phenomenon using mRNA targets over a range of amplicon sizes in this cell line model. For example, FFPE extracts produced Cts 4 to 10 cycles higher than their

**Table 1: Sorted expression levels of 160 miRNA between FFPE and snap frozen cells using ΔΔCts. 65.58% of ΔΔCts (101 out of 154 determined assays), were between +1 and -1**

| Decreased expression | Increased expression | ΔΔCts between +/-1 | | | | Undeter-mined |
|---|---|---|---|---|---|---|
| mir-30b | mir-302b* | mir-9 | mir-133b | mir-200a | mir-370 | c-lin-4 |
| mir-130a | mir-302a | mir-10a | mir-134 | mir-200b | mir-371 | mir-104 |
| mir-218 | let-7b | mir-15a | mir-137 | mir-200c | mir-372 | mir-122a |
| mir-30e | mir-184 | mir-17-3p | mir-138 | mir-203 | mir-373 | mir-144 |
| mir-34a | mir-183 | mir-17-5p | mir-140 | mir-204 | let-7d | mir-302b |
| mir-135a | mir-211 | mir-23a | mir-142-5p | mir-210 | let-7e | mir-325 |
| mir-20 | mir-128b | mir-23b | mir-145 | mir-213 | mir-2 | |
| mir-15b | mir-189 | mir-25 | mir-147 | mir-214 | let-7g | |
| mir-135b | mir-128a | mir-26b | mir-148a | mir-215 | let-7i | |
| mir-31 | mir-154 | mir-27a | mir-149 | mir-216 | let-7a | |
| mir-9* | mir-198 | mir-27b | mir-150 | mir-219 | mir-16 | |
| mir-338 | mir-139 | mir-28 | mir-151 | mir-221 | | |
| mir-190 | mir-373* | mir-30a-3p | mir-152 | mir-222 | | |
| mir-133a | mir-100 | mir-30c | mir-154* | mir-223 | | |
| mir-29a | mir-323 | mir-30d | mir-155 | mir-224 | | |
| mir-142-3p | mir-125b | mir-34b | mir-181a | mir-296 | | |
| mir-141 | mir-105 | mir-34c | mir-181b | mir-299 | | |
| mir-335 | mir-182* | mir-92 | mir-181c | mir-302c | | |
| mir-29c | mir-129 | mir-96 | mir-182 | mir-302c* | | |
| mir-26a | mir-159a | mir-98 | mir-185 | mir-320 | | |
| mir-220 | mir-199a | mir-99a | mir-186 | mir-324-5p | | |
| mir-374 | mir-367 | mir-103 | mir-187 | mir-326 | | |
| mir-95 | mir-107 | mir-106a | mir-191 | mir-328 | | |
| mir-21 | | mir-124a | mir-193 | mir-330 | | |
| mir-302d | | mir-124b | mir-194 | mir-331 | | |
| mir-29b | | mir-125a | mir-195 | mir-337 | | |
| mir-301 | | mir-126 | mir-197 | mir-339 | | |
| mir-205 | | mir-127 | mir-199a* | mir-340 | | |
| mir-19a | | mir-130b | mir-199b | mir-342 | | |
| mir-146 | | mir-132 | mir-199-s | mir-368 | | |

snap frozen counterparts depending on the amplicon sizes used (62 to 164 bp). Cts between FFPE and snap frozen were closer for small amplicons than that for large amplicons. For example analysis of GAPDH using a target amplicon of 67 bp displayed a mean difference of 4.28 cycles, whereas an assay designed for the same gene (GAPDH) using a target amplicon size of 122 bp displayed a mean difference of 6.51 Cts between FFPE and snap frozen material.

miRNAs have the advantage of small size, being only approximately 20 to 22 nucleotides long. In addition, they are protein protected by the RISC complex. Consequently they may not be as susceptible to RNA degradation as mRNA in FFPE tissues. Our results showed that the amount of miRNA in total RNA extracted from FFPE was greater than that extracted from snap frozen cells when the input amounts of total RNA were identical. The average quantity of miRNAs derived from total RNA extracted from FFPE was double (one Ct lower) that in snap frozen cells which is most likely a consequence of methylol cross-links between RNA and protein introduced during processing.

We extracted identical quantities of total RNA (10,000 ng) for analysis. In practical terms this required input of almost ten times the number of FFPE cells ($2 \times 10^6$ cells) compared to snap frozen ($1.7 \times 10^5$ cells). This difference in extracted yields was consistent with previous reports. This suggests the amount of RNA that can be extracted from FFPE tissue represents only a fraction of that which is obtainable from fresh-frozen tissue [9]. The residual cross-links in every RNA molecule that have not been removed by proteinase K digestion prevent this RNA being extracted (Figure 3). The longer an RNA molecule is, the greater the likelihood that a cross-link still exists after the proteinase K digestion procedure. Therefore, small RNA molecules are more amenable to extraction than larger mRNA molecules resulting to a higher expression of miRNA in FFPE compared to that in snap frozen.

### *Reliability of miRNA in FFPE*

It is plausible to anticipate that miRNA species are less susceptible to RNA degradation associated with tissue processing than occurs with mRNA, and this formed the hypothesis to be tested in this study. We found a good correlation of miRNA expression levels between FFPE and

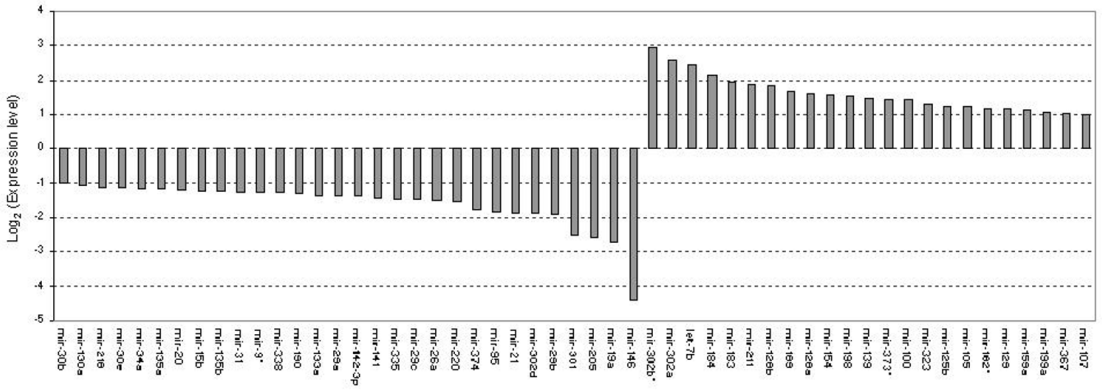



**Figure 2**
**Sorted $Log_2$(Expression level) shows 30 miRNAs with decreased expression and 23 with increased expression in FFPE**. The most significantly altered expression was seen in mir-146 with decreased expression and mir-302b* with increased expression.

snap frozen cells with $R^2 > 0.95$. Our data demonstrated that, for majority of miRNAs, the expression in FFPE is comparable with snap frozen cells. 65.58% of miRNAs displayed ΔΔCts in a range between +/-1 indicating that these normalized profiles were essentially identical between the two samples.

However, there was some outlying data where there was poor correlation between expression profiles for the paired snap-frozen and FFPE samples. The most significant of these was mir-146 with decreased expression and mir-302b* with increased expression. These changes could possibly occur during formalin fixation procedure or could also be caused by post fixation handling. mir-146 overexpression has been found in PTC tissues [12] and was also suggested to be involved in cellular stress [13] and innate immune responses [14]. Interestingly, we found it was decreased in FFPE extracted Nthy-ori cells. For those overexpressed miRNAs, it is possible that precursors of miRNA might have been cleaved by RNase to produce positive signals because FFPE blocks are often stored at room temperature in the absence of an RNase free environment. Alternatively, increased cellular stress following harvesting and during the fixation process may have contributed to the altered expression patterns in specific miRNAs. In these cases, the FFPE material could still be used to compare relative miRNA expression patterns if

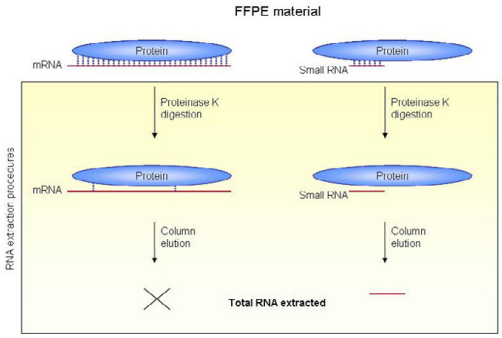



**Figure 3**
**A schematic representation of the impact of cross-links on RNA extraction**. In FFPE materials, RNA has been chemically modified by methylol groups to form cross-links with protein. Digestion with proteinase K [6] followed by column elution is the common method used to extract RNA from FFPE. However, a fraction of RNA remains impervious to extraction because of un-removed cross-links. The longer an RNA molecule is, the more likely cross-links will remain after the digestion procedure. Therefore, it is easier to extract small RNA molecules than larger ones from archival material.

a series of known blocks were fixed and handled simultaneously or in the same manner.

## Conclusion

We analyzed 160 miRNAs expression levels in freshly fixed FFPE by comparing to snap frozen in a cell line model. Although the RNA extracted from FFPE blocks is often compromised, we demonstrated the robustness of miRNA profiles in FFPE material which could provide a source of study material for large scale or retrospective studies. This study has confirmed the proof of principle that miRNA species may be successfully extracted and analysed from archival sources. Further work may be required to determine precise effects of FFPE on miRNA expression profiles across different tissue samples.

## Methods

### *Cell culture and formalin fixation*

Nthy-ori 3-1 (ECACC, Wiltshire, UK) is a normal thyroid follicular epithelial cell line derived from adult thyroid tissue that has been transfected with a plasmid encoding for the SV40 large T gene [15].

This cell line was grown to confluence in a humidified atmosphere containing 5% $CO_2$ at 37°C in the following plating medium: RPMI 1640 with 2 mM L-glutamine, 10% Foetal calf serum (FCS), Penicillin (100 U/ml) and Streptomycin (100 μg/ml). Tripsinized cells were counted with a hemocytometer. Suspended cells were aliquot and were pelleted (a) snap frozen and (b) formalin fixed and paraffin embedded into a cell block. When formalin fixation was required, a cohesive solid cell pellet was constructed using 20% agar. The cells were centrifuged in an eppendorf tube, and the supernatant was removed using a pipette. Approximately 30 μl of pre-warmed agar (60°C) was added to each tube. The solid cell pellet was formed within a few seconds. Cell blocks were fixed following standard tissue processing which included 10% buffered formalin fixation for 4 hours, on a Tissue-Tek® V.I.P.™ tissue processor. The pellets were subsequently paraffin embedded.

### *RNA extraction*

RNA was extracted from fresh cells using mirVana™ miRNA Isolation kit (Ambion Ltd., Cambridgeshire, UK) and from FFPE cells using RecoverAll™ Total Nucleic Acid Isolation kit (Ambion Ltd., Cambridgeshire, UK) following the manufacturer's protocol. For snap-frozen extraction, one extraction was performed using approximately 4 × $10^5$ cells. For FFPE extraction, 4 extractions were performed in parallel with one pellet (1 × $10^6$ cells) in each extraction. The entire pellet was dissected from each block and was finely minced using a scalpel. These preparations were then deparaffinized, followed by proteinase K digestion for 3 hours at 50°C, on column DNase digestion and elution as described in the protocol. At that point all 4 FFPE extracts were combined into one tube designated the FFPE sample. The concentrations of these two samples were measured using a NanoDrop® ND-1000 Spectrophotometer (Wilmington, USA) and extracts were diluted to 10 ng/μl. RNA quality was measured using the RNA 6000 Pico LabChip® Kit on an Agilent 2100 Bioanalyser (Agilent technologies, Waldbronn, Germany).

### *miRNA assays*

Applied Biosystems TaqMan® microRNA (miRNA) assays (designed for mature miRNA quantification using Applied Biosystems Real Time PCR instruments) were utilised in this study. The human panel early access kit (P/N: 4365381, Applied Biosystems) used in this study contained 160 individual assays and comprised two steps: Reverse Transcription (RT) and real time PCR. The stem-loop RT primer specifically hybridizes to a miRNA molecule and is reverse transcribed with a MultiScribe reverse transcriptase [16]. The RT products are then quantified using real-time TaqMan® PCR.

Applied Biosystems High-Capacity cDNA Archive Kit (P/N: 4322171, Applied Biosystems, CA, USA) was used following manufacturer's protocol for reverse transcription. Each RT reaction contained 50 ng of extracted total RNA, 50 nM stem-looped RT primer, 1 × RT buffer, 0.25 mM each of dNTPs, 3.33 U/μl Multiscribe reverse transcriptase and 0.25 U/μl RNase Inhibitor. The 15 μl reactions were incubated in an Applied Biosystems Thermocycler in a 96-well plate for 30 min at 16°C, 30 min at 42°C, 5 min at 85°C and then held at 4°C.

For the Real-time PCR step, amplification was carried out using sequence specific primers on the Applied Biosystems 7900 HT Real-Time PCR system. The 20 μl reaction included 1.33 μl RT product, 1 × TaqMan® Universal PCR Master Mix with no UNG (P/N: 4324018, Applied Biosystems) and 1 × TaqMan® MicroRNA assays. The reactions were incubated in a 96-well optical plate at 95°C for 10 min, following by 40 cycles of 95°C for 15 s and 60°C for 1 min. The real-time PCRs for each miRNA were run in triplicate. hsa-let-7a was included as an endogenous control and cel-lin-4 was incorporated as a negative control.

### *Statistical analysis*

Replicates were omitted if Ct standard deviation was greater than 1.5. All 160 miRNAs were detectable with the exception of c-lin-4 in FFPE and c-lin-4, mir-104, mir-122a, mir-144, mir-302b and mir-325 in snap frozen sample. The data was collected using Microsoft Excel and was statistical analyzed using MINITAB® 14 on ΔCts with the formulas below:

$$\Delta Ct = Ct\_Mean(FFPE) - Ct\_Mean(Snap\ frozen)$$

$$\Delta\Delta Ct = \Delta Ct - \Delta Ct\_Mean$$

$$\text{Expression level} = 2^{-\Delta\Delta Ct}$$

## Authors' contributions

JL performed the RNA extraction and miRNA analysis and wrote original and final versions of the manuscript. PS, RF, SC helped with the miRNA analytical analysis of miRNA data and helped draft the manuscript. KD, SA carried out cell culture. SG helped with the analysis of the miRNA data. JOL and OS conceived the study and helped write the original and final versions of this manuscript. All authors read and approved the final manuscript.

## Acknowledgements

We would like to thank staff in both the Central Pathology Laboratory, St. James's Hospital, for assistance with fixation and processing of cell blocks, and in the Statistics Department, Trinity College Dublin, for assistance with MINITAB® 14 statistical analyses.

## References

1. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ: **miRBase: microRNA sequences, targets and gene nomenclature.** *Nucleic Acids Res* 2006, **34:**D140-4.
2. Wijnhoven BP, Michael MZ, Watson DI: **MicroRNAs and cancer.** *Br J Surg* 2007, **94(1):**23-30.
3. Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RH, Cuppen E: **Phylogenetic shadowing and computational identification of human microRNA genes.** *Cell* 2005, **120(1):**21-4.
4. Srinivasan M, Sedmak D, Jewell S: **Effect of fixatives and tissue processing on the content and integrity of nucleic acids.** *Am J Pathol* 2002, **161(6):**1961-71.
5. Cronin M, Pho M, Dutta D, Stephans JC, Shak S, Kiefer MC, Esteban JM, Baker JB: **Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay.** *Am J Pathol* 2004, **164(1):**35-42.
6. Masuda N, Ohnishi T, Kawamoto S, Monden M, Okubo K: **Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples.** *Nucleic Acids Res* 1999, **27(22):**4436-43.
7. Godfrey TE, Kim SH, Chavira M, Ruff DW, Warren RS, Gray JW, Jensen RH: **Quantitative mRNA expression analysis from formalin-fixed, paraffin-embedded tissues using 5' nuclease quantitative reverse transcription-polymerase chain reaction.** *J Mol Diagn* 2000, **2(2):**84-91.
8. Specht K, Richter T, Muller U, Walch A, Werner M, Hofler H: **Quantitative gene expression analysis in microdissected archival formalin-fixed and paraffin-embedded tumor tissue.** *Am J Pathol* 2001, **158(2):**419-29.
9. Abrahamsen HN, Steiniche T, Nexo E, Hamilton-Dutoit SJ, Sorensen BS: **Towards quantitative mRNA analysis in paraffin-embedded tissues using real-time reverse transcriptase-polymerase chain reaction: a methodological study on lymph nodes from melanoma patients.** *J Mol Diagn* 2003, **5(1):**34-41.
10. Koch I, Slotta-Huspenina J, Hollweck R, Anastasov N, Hofler H, Quintanilla-Martinez L, Fend F: **Real-time quantitative RT-PCR shows variable, assay-dependent sensitivity to formalin fixation: implications for direct comparison of transcript levels in paraffin-embedded tissues.** *Diagn Mol Pathol* 2006, **15(3):**149-56.
11. Antonov J, Goldstein DR, Oberli A, Baltzer A, Pirotta M, Fleischmann A, Altermatt HJ, Jaggi R: **Reliable gene expression measurements from degraded RNA by quantitative real-time PCR depend on short amplicons and a proper normalization.** *Lab Invest* 2005, **85(8):**1040-50.
12. He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A: **The role of microRNA genes in papillary thyroid carcinoma.** *Proc Natl Acad Sci USA* 2005, **102(52):**19075-80.
13. Marsit CJ, Eddy K, Kelsey KT: **MicroRNA responses to cellular stress.** *Cancer Res* 2006, **66(22):**10843-8.
14. Taganov KD, Boldin MP, Chang KJ, Baltimore D: **NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses.** *Proc Natl Acad Sci USA* 2006, **103(33):**12481-6.
15. Cahill S, Smyth P, Finn SP, Denning K, Flavin R, O'Regan EM, Li J, Potratz A, Guenther SM, Henfrey R, O'Leary JJ, Sheils O: **Effect of ret/PTC 1 rearrangement on transcription and post-transcriptional regulation in a papillary thyroid carcinoma model.** *Mol Cancer* 2006, **5:**70.
16. Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, Mahuvakar VR, Andersen MR, Lao KQ, Livak KJ, Guegler KJ: **Real-time quantification of microRNAs by stem-loop RT-PCR.** *Nucleic Acids Res* 2005, **33(20):**e179.

## DEVELOPMENT OF A SEMI-CONDUCTOR SEQUENCING-BASED PANEL FOR GENOTYPING OF COLON AND LUNG CANCER BY THE ONCONETWORK CONSORTIUM

BMC CANCER 2015,15:26

I have selected this paper as a representative of my consortium work. I was a founder member of the OncoNetwork consortium which was facilitated by industrial partner ThermoFisher Scientific. In this regard, I was involved in the selection of markers and design of panels to be utilised in the detection of key driver mutations in lung and colorectal cancers. This is an impactful and important paper as it paved the way for the implementation of targeted panels for the detection of driver mutations. It consolidated the application of genomic analysis of archival tissues as companion diagnostics, to inform the use of personalised treatment options for cancer patients by identifying treatment options that may be appropriate based on the unique drivers of their individual cancer. It demonstrates some of my work in the area of personalised medicine and relates to the broader concept of patient centred care. It refers to a medical model using characterisation of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention. The ability to select patients for treatment with targeted agents based on specific molecular alterations within their cancer cells has led to novel drugs for molecularly selected patient populations, with a view to improving treatment outcome, while minimising side effects.

The paper describes the use of targeted Next-Generation Sequencing (NGS) allowing the sequencing of several genomic regions in a single test, on a single platform and in samples with degraded DNA content. (NGS) utilizes massively parallel sequencing to generate high throughput and efficient analysis of patients' samples. Our panel comprised multiplex PCR targeting 87 hotspot regions across 22 genes that are of clinical interest for lung and/or colorectal cancers. The gene-panel was tested by 7 different labs, each in their own clinical setting, allowing cross institutional validation of results. The technique employed semiconductor chips, Ion Torrent next-generation sequencing technology and we developed a fast and simple workflow that could be scaled to the needs of routine cancer clinical diagnostics.

The consortium was subsequently expanded giving wider global coverage of partners to design a panel for the detection of chromosomal translocations and gene fusion products that are essential drivers and targets in lung tumour subtypes. Hence, this was the prototype consortium for the development of a series of patient centred diagnostic panels to identify novel therapeutic targets for tailored treatment of cancers.

Tops et al. BMC Cancer (2015) 15:26
DOI 10.1186/s12885-015-1015-5

BMC Cancer

RESEARCH ARTICLE Open Access

# Development of a semi-conductor sequencing-based panel for genotyping of colon and lung cancer by the Onconetwork consortium

Bastiaan BJ Tops[1*], Nicola Normanno[2,3*], Henriette Kurth[4], Eliana Amato[5], Andrea Mafficini[5], Nora Rieber[6], Delphine Le Corre[7], Anna Maria Rachiglio[2], Anne Reiman[8], Orla Sheils[9], Christoph Noppen[4], Ludovic Lacroix[10], Ian A Cree[8], Aldo Scarpa[5,11], Marjolijn JL Ligtenberg[1,12] and Pierre Laurent-Puig[7]

## Abstract

**Background:** The number of predictive biomarkers that will be necessary to assess in clinical practice will increase with the availability of drugs that target specific molecular alterations. Therefore, diagnostic laboratories are confronted with new challenges: costs, turn-around-time and the amount of material required for testing will increase with the number of tests performed on a sample. Our consortium of European clinical research laboratories set out to test if semi-conductor sequencing provides a solution for these challenges.

**Methods:** We designed a multiplex PCR targeting 87 hotspot regions in 22 genes that are of clinical interest for lung and/or colorectal cancer. The gene-panel was tested by 7 different labs in their own clinical setting using ion-semiconductor sequencing.

**Results:** We analyzed 155 samples containing 112 previously identified mutations in the *KRAS*, *EGFR* en *BRAF* genes. Only 1 sample failed analysis due to poor quality of the DNA. All other samples were correctly genotyped for the known mutations, even as low as 2%, but also revealed other mutations. Optimization of the primers used in the multiplex PCR resulted in a uniform coverage distribution over the amplicons that allows for efficient pooling of samples in a sequencing run.

**Conclusions:** We show that a semi-conductor based sequencing approach to stratify colon and lung cancer patients is feasible in a clinical setting.

**Keywords:** Next-generation sequencing, Semi-conductor sequencing, Colorectal cancer, Non-small cell lung cancer, Multiplex PCR, Ion Torrent

## Background

Although molecular mechanisms underlying carcinogenesis have been the subject of medical research for decades, only in the last few years has this information translated into the clinical setting through 'smart' drugs targeting specific molecular aberrations of neoplastic cells [1]. Target based agents were initially explored in unselected cohorts of cancer patients, and this approach led to failure of the majority of clinical trials with these new agents. More recently, the value of selecting patients to be treated with targeted agents on the basis of specific molecular alterations of neoplastic cells has been acknowledged [2,3]. This new therapeutic approach has led to the approval of novel drugs for molecularly selected patient populations. These agents have revolutionized the treatment of cancer patients, improving treatment outcome, while minimizing side effects.

The number of predictive biomarkers that are assessed in clinical practice is rapidly increasing with the availability of drugs that target specific molecular alterations [4]. In some tumor types, such as non-small-cell lung carcinoma (NSCLC) and colorectal carcinoma (CRC), a new classification based on the identification of specific

* Correspondence: bastiaan.tops@radboudumc.nl; nicnorm@yahoo.com
[1]Department of Pathology, Radboud University Medical Centre (Radboudumc), PO box 9101, 6500 HB Nijmegen, The Netherlands
[2]Cell Biology and Biotherapy Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione Giovanni Pascale", IRCCS, 80131 Naples, Italy
Full list of author information is available at the end of the article

predictive and/or prognostic molecular alterations has emerged in the past decade [5,6]. However, molecular diagnostics is faced with its own specific challenges. Costs per test, turn-around-time and the amount of material required for testing increase with the number of tests performed on a sample. In particular, the availability of tissue is limiting in NSCLC, which is often diagnosed on small histological or cytological samples yielding limited amounts of DNA [7].

Comprehensive molecular characterization of tumor tissue in clinical practice will rely on the development of high throughput technologies allowing this process to be accomplished in a cost-effective and timely manner and by using a limited amount of tissue. Two different approaches, genotyping assays and next generation sequencing (NGS) have been proposed for molecular screening [4,8]. NGS has the advantage to providing information on known and novel molecular alterations. Indeed, by using NGS-based techniques multiple genes can be sequenced simultaneously, and benchtop sequencers, like the Ion PGM™ (Life Technologies), are fast and relatively inexpensive. In this context, the 'OncoNetwork consortium', a European collaborative effort developed and tested the performance of a next-generation sequencing approach in a clinical setting. The advantages of a multi-lab collaboration are that methods can be quickly validated, facilitating availability, and that tests are evaluated in different laboratory settings, assessing robustness. As a proof-of-principle we chose to focus on CRC and NSCLC, two frequent tumor types for which there are known mutations associated with treatment decisions. We decided to design an Ion AmpliSeq custom gene-panel as a single multiplex PCR requiring only 10 ng of input DNA for the detection of the most frequent hot spot mutations in NSCLC and CRC. We opted for a relatively small, focused gene-panel, instead of a broader panel (like the AmpliSeq Cancer panel). Since a smaller gene-panel requires less sequence capacity more samples can be pooled in a single sequence run resulting in lower costs per sample and an increased throughput compatible with a diagnostic activity. Finally, we assessed the performance of the panel by semiconductor sequencing of 155 CRC and NSCLC formalin-fixed, paraffin-embedded (FFPE) cancer samples containing mutations previously identified by alternative genotyping methods.

## Methods

### Tumor specimens and DNA isolation

FFPE tumor samples were retrieved from the archives of the collaborating institutes and anonymised. Given the study was interpreted by all institutions (Radboud university medical centre, Istituto di ricovero e cura a carattere scientifico (IRCCS), VIOLLIER, Applied Research On Cancer Centre (ARC-NET), Institut national de la santé et de la recherche médicale (INSERM), Trinity College, Institut Gustave-Roussy and University Hospitals Coventry and Warwickshire) as a service improvement, it did not require specific research ethics committee approval as stated in the EU Clinical Trials Directive (2001/20/EC). Specific approval was obtained from the SJH/AMNCH Joint Research Ethics Committee of Trinity College, Dublin. Approval was procured for the current study as part of a wider study involving molecular analysis of a wider cohort and for research purposes that does require approval from an ethics committee. A cover letter to this effect is available.

Tissue areas for DNA extraction were micro-dissected and quantified for the percentage of neoplastic cells by a pathologist. More details regarding sample characteristics are depicted in Additional file 1: Figure S1. DNA extraction differed per institute. Most laboratories (6/7) isolated DNA using the QIAamp DNA FFPE Tissue Kit, while one lab purified DNA by overnight digestion in a proteinase K/Chelex-100 solution.

### Ion AmpliSeq™ primer design

Ion AmpliSeq™ primer pools were designed by Life Technologies (Additional file 2: Table S1 and Additional file 3: Table S2). To minimize the risk of allelic drop-out during the PCR due to primers that are not able to prime, no confirmed SNPs with a frequency >0.5% were allowed in the 5 most 3' nucleotides. For the same reason low-frequency SNPs at other positions were kept as low as possible, with a maximum of four. Primer designs were confirmed by SNPCheck v3 analysis (www.snpcheck.net/, April 2012). The resulting oncopanel primer pool contained 87 primer pairs with an average amplicon length of 203 bp (Additional file 2: Table S1).

### AmpliSeq enrichment and Ion Torrent sequencing

Library generation was performed according to the manufacturer's protocol. In short, 10 ng of DNA per pool was amplified in 21 cycles by PCR using the Ion AmpliSeq™ mastermix, followed by barcode and adapter ligation. Amplified products were purified with Agencourt AMPure XP beads (Beckman Coulter Genomics, High Wycombe, UK). The library was diluted to 20 pM. Emulsion PCR was performed using the Ion OneTouch™ 200 Template kit following the protocol of the Ion OneTouch™ System. Next, Ion Sphere Particles (ISPs) were recovered and enriched for template positive ISPs using Dynabeads MyOne Streptavidin C1 beads (Life Technologies) in the Ion OneTouch™ ES instrument (Life Technologies). ISP enrichment was quantified using the Qubit 2.0 fluorometer (Life Technologies). Sequencing primer and polymerase were added to the final enriched spheres before loading onto an Ion 316 chip according to the Ion PGM™ 200 sequencing kit protocol.

### Data analysis

Sequence data were directly uploaded from the Ion Torrent Suite Software to the IonReporter™ Software version 1.2 and data were analyzed using a pre-configured workflow specific for detecting low frequency somatic variants. The allele frequency cut-off was set at 0.04. In addition, the data were analyzed in parallel using a 'hotspot bed file' containing hotspot mutations in the regions of our gene-panel derived from the COSMIC database. The allele frequency cut-off for hotspot analysis was set at 0.02. These cutoffs were chosen following a preliminary phase in which, starting from samples with known sequence, the best bioinformatic algorithm ensuring the highest sensitivity and specificity was identified. The workflow includes mapping statistics, measurements of the quality of all reads, on and off target statistics, SNP and INDEL calling and comprehensive annotation using public databases (dbSNP version 137, COSMIC version 60, OMIM version 08012012, Gene Ontology version 1.218). The variant caller algorithm was used and variants that were identified for each sample per lab in each phase were further filtered. We applied quality filtering by discarding reads with a quality score <40 and checking against strand bias. We filtered against variants with a variant allele count less than 10, variants that showed up in every sample of one lab with the same allele frequency, the synonymous and non-coding variants and variants that were known as polymorphisms or with a Minor Allele Frequency (MAF) greater than 35%. As a result, a variant knowledgebase was generated with the variants detected in those samples. A specific workflow for this panel was configured in the online Ion Reporter™ Software and was used to perform analysis for data generated using the Colon/Lung panel.

## Results

### Panel design and performance

The panel was designed in order to contain well-known predictive markers in the receptor tyrosine kinase (RTK) pathway, such as mutations of the *EGFR* (for NSCLC) and *KRAS* (for NSCLC and CRC) genes, but also included genes that might serve as targets in the near future or have a prognostic relevance, like *BRAF* [9,10], *AKT1* [11], *DDR2* [12,13] and *ERBB2* [14-16] (Table 1). Selection of the gene regions was based on their mutation frequencies, based on the COSMIC database (Table 1). In particular, the entire gene-panel targets 87 hotspot regions for the following 22 genes: RTKs (*ALK*, *EGFR*, *ERBB2*, *ERBB4*,

**Table 1 Mutation frequency of genes for which hotspot regions are present in the gene-panel**

| Gene | Chr | RefSeq | Function | % Mutated CRC[#] | % Mutated lung cancer[#] |
|---|---|---|---|---|---|
| *AKT1* | 14 | NM_005163.2 | Oncogene | 0.64 | 0.42 |
| *ALK* | 2 | NM_004304.3 | Oncogene | 2.74 | 5.25 |
| *BRAF* | 7 | NM_004333.4 | Oncogene | 12.19 | 2.46 |
| *CTNNB1* | 3 | NM_001904.3 | Oncogene | 4.61 | 3.13 |
| *DDR2* | 1 | NM_006182.2 | Oncogene | 0 | 0.21 |
| *EGFR* | 7 | NM_005228.3 | Oncogene | 2.75 | 26.71 |
| *ERBB2* | 17 | NM_004448.2 | Oncogene | 1.48 | 1.80 |
| *ERBB4* | 2 | NM_005235.2 | Oncogene | 3.84 | 8.35 |
| *FGFR1* | 8 | NM_023110.2 | Oncogene | 0.55 | 1.62 |
| *FGFR2* | 10 | NM_000141.4 | Oncogene | 0.42 | 1.68 |
| *FGFR3* | 4 | NM_000142.4 | Oncogene | 0.53 | 0.92 |
| *KRAS* | 12 | NM_004985.3 | Oncogene | 34.51 | 16.23 |
| *MAP2K1* | 15 | NM_002755.3 | Oncogene | 0.15 | 1.14 |
| *MET* | 7 | NM_001127500.1 | Oncogene | 0.73 | 3.03 |
| *NOTCH1* | 9 | NM_017617.3 | Oncogene | 0.13 | 3.44 |
| *NRAS* | 1 | NM_002524.3 | Oncogene | 3.37 | 0.88 |
| *PIK3CA* | 3 | NM_006218.2 | Oncogene | 11.90 | 4.08 |
| *FBXW7* | 4 | NM_0033632.2 | Tumor suppressor | 6.83 | 3.00 |
| *PTEN* | 10 | NM_000314.4 | Tumor suppressor | 3.86 | 3.59 |
| *SMAD4* | 18 | NM_005359.5 | Tumor suppressor | 7.89 | 2.68 |
| *STK11* | 19 | NM_000455.4 | Tumor suppressor | 1.28 | 8.40 |
| *TP53* | 17 | NM_000546.5 | Tumor suppressor | 41.75 | 37.64 |

[#]Data was extracted from the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) February 4th 2013.

*FGFR1, FGFR2, FGFR3, MET, DDR2*); RTK signaling genes (*KRAS, PIK3CA, BRAF, AKT1, PTEN, NRAS, MAP2K1, STK11*); and other well known cancer-related genes (*NOTCH1, CTNNB1, SMAD4, FBXW7, TP53*).

Dilution experiments with an artificial control sample containing 10 hotspot mutations in the *BRAF, EGFR, KRAS, NRAS* and *PIK3CA* genes (Horizon Diagnostics) demonstrated that hotspot mutations could be confidentially identified as low as 2% mutant alleles provided that the coverage was >500× (results not shown). Preliminary testing of the lung/colon cancer primer pool showed that up to 5 samples could be pooled on a 316 chip (Ion PGM™ Sequencer) with a minimal average read-depth of 500× (results not shown).

#### Experimental design

The above described panel, from now on called version 1 (v1), was used to test 155 FFPE tissue samples that contained mutations previously identified by other methods. In particular, the performance of the panel was assessed in three phases (Figure 1). The first phase aimed at setting up the AmpliSeq protocols, workflow and data analysis and to define accuracy and precision of the panel. To this end, 7 consortia labs tested 5 control samples in an inter-laboratory 'ring-trial', i.e. 2 AcroMetrix® controls (FFPE colon cancer cell lines, A12 and A13), 2 FFPE xenograft colon tumours (X23 and X32) and one FFPE lung sample (L1). All labs correctly identified in the control samples the mutations previously detected by Sanger sequencing (Table 2).

By using the panel, all 7 labs also identified 6 new variants in the two AcroMetrix® control samples (Table 2). To confirm that these new variants are not sequencing artifacts, we re-analyzed these positions by conventional Sanger sequencing. All 6 new variants were confirmed suggesting the specificity of our workflow (data not shown). The two xenograft samples were not analyzed for new variants since off-target amplification of mouse DNA hampered data analysis.

In the second phase, we designed a ring-trial in which 6 labs selected 10 FFPE specimens (5 lung and 5 colon carcinoma FFPE samples) previously tested in a diagnostic setting (for sample characteristics see Additional file 1: Figure S1A). These samples were tested, in the blind, by a second consortium lab. In total, the selected 60 samples contained 47 previously identified mutations (42 missense and 5 indels) in the *KRAS*, *EGFR*, *BRAF* and *CTNNB1* genes (Table 3). All previously detected mutations were detected using our oncopanel v1 and the IonReporter variant caller.

In the third and final phase, each lab selected 15 in-house samples representative of the type of samples encountered in a diagnostic clinical setting and that are problematic to analyze due to paucity of material, such as small biopsies or cytological material, low percentage of neoplastic cells and poor DNA quality (for sample characteristics see Additional file 1: Figure S1B). In total 29 CRC and 61 NSCLC samples were selected. In these samples, 56 mutations in the *KRAS*, *EGFR* and *BRAF* genes (46 missense and 10 indels) were previously identified in 54 unique samples using different methods (Additional file 4: Table S3). DNA of 1 sample failed to amplify due to low quality (technical failure 1.1%). All other samples could be analyzed and all known variants were identified. Two additional samples were excluded from further in-depth analyses (2.2%), since these samples contained a high number of variants (>15) with an allele frequency of 4-7%, probably due to (over) fixation with formalin [17]. In the remaining 87 assessable samples, we identified 92 new variants in regions that were not analyzed with the

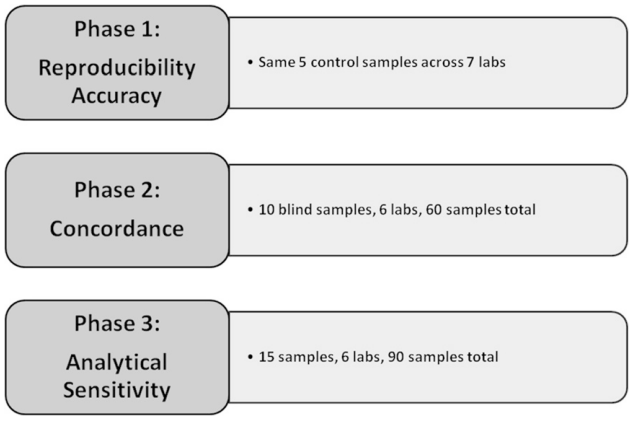



**Figure 1** Schematic scheme of the 3 phases to assess the performance of the gene-panel.

**Table 2 Variants identified in the 5 control samples**

| Sample | Known mutations | Mutations identified by NGS[‡] | Allele frequency[#] |
|---|---|---|---|
| L1 | *KRAS*: p.Gly12Cys | *KRAS*: p.Gly12Cys | 0.44 - 0.54 |
| A12 | *KRAS*: p.Gly12Ala | *KRAS*: p.Gly12Ala | 0.66 - 0.67 |
| | | *FBXW7*: p.His460Tyr | 0.46 - 0.49 |
| | | *TP53*: p.Ala159Asp | 0.74 - 0.75 |
| A13 | *KRAS*: Gly13Asp | *KRAS*: Gly13Asp | 0.50 - 0.56 |
| | | *PIK3CA*: p.Asp549Asn | 0.43 - 0.46 |
| | | *DDR2*: p.Thr98Ala | 0.47 - 0.56 |
| | | *FGFR1*: p.Ala268Ser | 0.50 - 0.66 |
| | | *NOTCH1*: p.Pro1581Leu | 0.48 - 0.54 |
| X23 | *KRAS*: p.Gly12Asp | *KRAS*: p.Gly12Asp | 0.65 - 0.75 |
| | *PIK3CA*: p.Glu545Lys | *PIK3CA*: p.Glu545Lys | 0.40 - 0.51 |
| X32 | *KRAS*: p.Gly12Asp | *KRAS*: p.Gly12Asp | 0.54 - 0.69 |
| | *PIK3CA*: p.Glu542Lys | *PIK3CA*: p.Glu542Lys | 0.44 - 0.46 |
| | *FBXW7*: p.Arg465His | *FBXW7*: p.Arg465His | 0.46 - 0.55 |
| | *TP53*: p.Gly244Asp | *TP53*: p.Gly244Asp | 0.32 - 0.35 |

[‡]Newly identified variants were verified by conventional Sanger sequencing.
[#]Indicated is the range of the allele frequencies over the different laboratories.

previously used alternative method (mutations and low frequency SNPs with a MAF <0.04). Most of these new variants were identified in the RTK-signaling genes *BRAF*, *EGFR*, *KRAS* and *PIK3CA* (24 mutations) and in *TP53* (30 mutations) (Figure 2 and Additional file 5: Table S4). Furthermore, when taking the percentage of neoplastic cells in individual samples and the allelic frequency of the variants into account, in contrast to most mutations in *KRAS*, *EGFR* and *BRAF*, a large proportion of the newly identified mutations appear to be present in only a subset of tumor cells (Figure 2).

**Table 3 Known mutations present in the 60 samples that were analyzed in the blind during phase 2 of the panel validation**

| Mutation | Unique samples | Identified |
|---|---|---|
| BRAF: p.Val600Glu | 3 | Yes |
| CTNNB1: p.Thr41Ile | 1 | Yes |
| CTNNB1: p.Asp32Asn | 1 | Yes |
| EGFR: p.Glu746_Arg748del | 1 | Yes |
| EGFR: p.Glu746_Ala750del | 4 | Yes |
| EGFR: p.Glu746_Ser752del | 1 | Yes |
| EGFR: p.Leu858Arg | 5 | Yes |
| KRAS: p.Gln61Arg | 1 | Yes |
| KRAS: p.Gly12Arg | 2 | Yes |
| KRAS: p.Gly12Cys | 5 | Yes |
| KRAS: p.Gly12Asp | 8 | Yes |
| KRAS: p.Gly12Ala | 2 | Yes |
| KRAS: p.Gly12Val | 7 | Yes |
| KRAS: P.Gly13Cys | 1 | Yes |
| KRAS: p.Gly13Asp | 5 | Yes |

Thus, combining the data of all experiments, we analyzed 155 unique samples containing 112 previously identified mutations. All specimens were correctly genotyped, except one that could not be analyzed due to poor quality of the DNA, demonstrating excellent performance of the gene panel.

### Sequencing efficiency and panel re-design

While the level of concordance for mutational analysis using this panel compared to classical Sanger sequencing is excellent, we could 'only' multiplex 5 samples on a 316 chip to reach a minimal read depth of 500× per amplicon. To determine if we could reduce the cost per sample by multiplexing more samples per chip, we assessed the sequencing efficiency by determining the read distribution over the individual amplicons. While the average coverage over the amplicons was reproducible among different labs and runs (Figure 3A and not shown), the difference between the lowest and highest covered amplicons was substantial (average coverage of 3274 ± 2470 reads). While this in itself is not a problem, the available sequencing capacity of the chip is not efficiently used. Since an improved primer design algorithm was introduced by Life Technologies after the design of panel v1, the panel primers were re-designed to attempt to optimize the read distribution over the amplicons (Additional file 3: Table S2). Moreover, the amplicons were designed to be smaller to facilitate the amplification of DNA extracted from FFPE specimens (91 amplicons, average size 158 bp). As shown in figure 3B, the read distribution of v2 over the

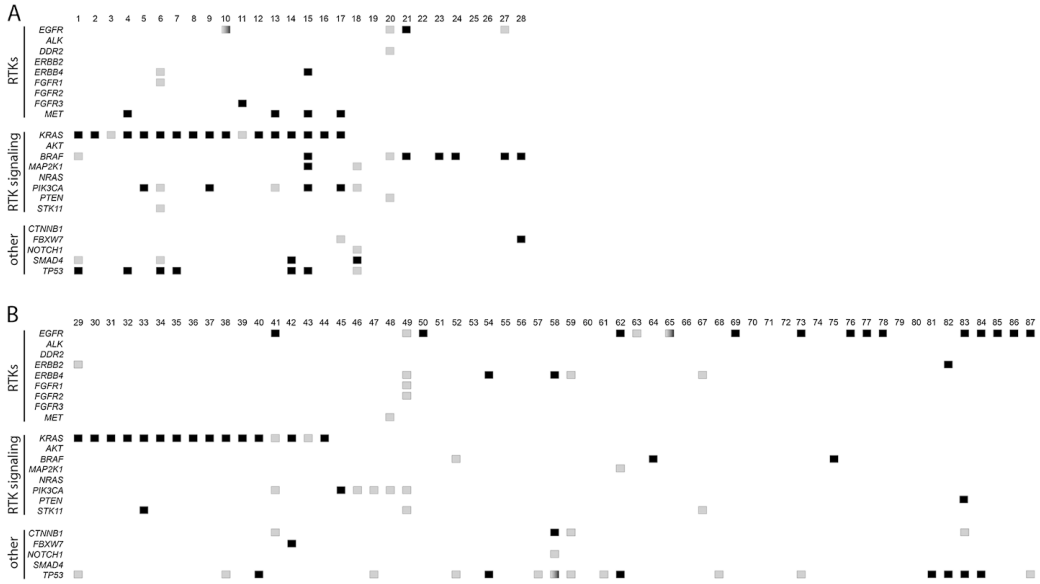



**Figure 2 Schematic representation of the results of phase 3 of the panel validation.** Indicated are the variants per sample identified in the 28 CRC **(A)** and 59 NSCLC **(B)** samples. Variant frequencies were extrapolated to 100% neoplastic cells and/or related to other variants present in the sample (intra-sample comparison). Variants in black correspond to 'driver' mutations (present in >35% of neoplastic alleles), while variants in grey are present in only a minority of the neoplastic cells (<35% of neoplastic alleles or an allele frequency 0.5x that of the driver mutation present in the sample). Genes containing >1 variant in different frequencies are indicated in grey/black boxes.

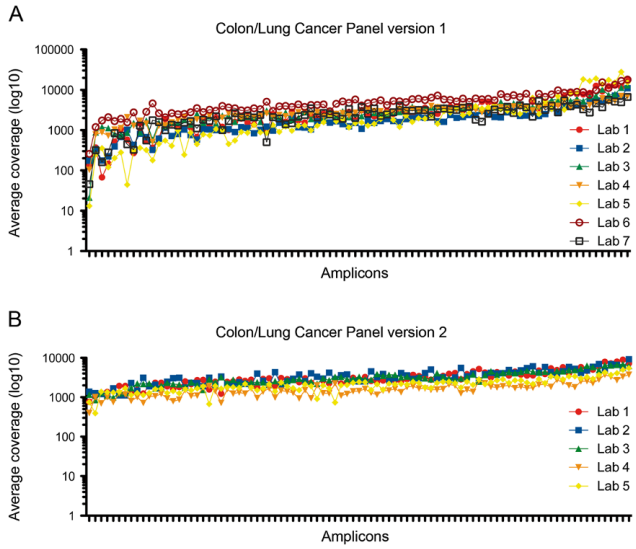



**Figure 3 Average coverage distribution over the individual amplicons.** The coverage distribution plot over the individual amplicons in gene-panel v1 **(A)** and v2 **(B)**. For panel v1 5 samples are pooled per 316 chip; with panel v2 8 samples are pooled per 316 chip **(B)**. Note the logarithmic scale of the y-axis.

individual amplicons was superior to v1 (lowest and highest covered amplicons within 10 fold range). Due to the improved read distribution, up to 8 samples could be pooled on a 316 chip with an average read depth of 2751 ± 1107 reads.

To check the performance of the panel, the 5 control samples of phase 1 were-resequenced by all 7 laboratories, and the expected variants as well as the new variants identified in the two Accrometrix control samples were detected using panel v2. In addition, the new panel was used by 5 labs to re-sequence their 15 in-house samples (75 samples in total) previously tested with panel v1, and 6 labs also tested 5 samples not previously analyzed (not all labs were able to participate). All mutations previously detected with panel v1 were again identified using panel v2 (data not shown), demonstrating that this panel performs as well as v1 for these mutations. However, since 8 samples can be multiplexed on a 316 chip with panel v2, instead of 5 samples using v1, the sequencing costs per sample are significantly reduced.

## Discussion

Specification of the molecular class of NSCLC and CRC is mandatory to optimize personalized medicine for these diseases. However, a comprehensive molecular characterization of the tumor is faced with a number of challenges, including cost per test, turn-around-time and the limited amount of material available for genotyping. NGS-based approaches can overcome these challenges by providing in a single analysis information on tumor inter- and intra-heterogeneity that can be relevant in a clinical setting. In this respect, a number of NGS panels for tumor profiling have become commercially available. However, these panels lack of validation in clinical samples and their inter-laboratory reproducibility has not been shown. In this study we propose a novel model of collaboration between clinical research laboratories and NGS-companies that led to the development of a tool that is ready for application in a clinical setting. Indeed, the validation of panels through a consortium of academic institutions represents a novel approach to speed the development of new diagnostic tools, being able to provide information on a significant number of clinical specimens and on inter-laboratory reproducibility.

We must acknowledge that there are still several issues that need to be addressed to implement NGS panels in the clinic. Our panel included 22 genes, although the approved predictive biomarkers in colon and lung cancer are quite few (*KRAS, NRAS, EGFR, ALK*). Nevertheless, in many cancer comprehensive centers clinical trials with new drugs are open and molecular pathologists are requested to screen for a number of biomarkers that are included in the Colon and Lung cancer panel that we developed. For example, among the 61 difficult NSCLC samples analyzed in the third phase, we identified 18 *EGFR*, 7 *PIK3CA* and 3 *BRAF* mutants. Since drugs targeting these latter molecular alterations are in clinical trials, the availability of this information might significantly improve the possibility of these patients to receive a personalized therapy [5]. It is possible that novel biomarkers will be discovered in the future. In this respect, one advantage of this panel is that it can be easily adapted by adding new amplicons/genes of interest. Of course, a re-validation on a minimum number of samples is recommended for any modification of the panel.

The performance of a new test is a key element in evaluating the possibility to introduce it in clinical diagnostics. In this regard, we chose to analyze our data with an allele frequency cut-off of 4% for all variants and 2% for known hotspot mutations corresponding to 5-10% neoplastic cells carrying heterozygous mutations. This estimate is in agreement with a previous study that assessed somatic mutations in FFPE material by using semiconductor-based massive parallel sequencing [18]. The allele frequency cut-off required for the detection of somatic mutations is debatable, but samples with <10% neoplastic cells are also difficult to diagnose by classical histomorphological methods. Using this allele-frequency cut-off we tested 155 clinical FFPE samples that were previously analyzed by conventional methods. A substantial number of these specimens were specifically selected based on the fact that they were difficult to analyze with routine techniques. Even with this bias in sample selection we were still able to correctly analyze 154 of the 155 samples. Although our sample set was selected for certain mutations, our specimens contained variants that represent the full spectrum of mutations that are identified in NSCLC and CRC specimens. These include a variety of indel and missense mutations of which also hotspot mutations present in only 2% of the alleles were readily detected. Other studies have previously suggested that semiconductor-based sequencing of FFPE tissue is indeed feasible [18-20]. However, this is the first study that evaluated a so large collection of FFPE samples.

Other mutation-detection techniques, like quantitative PCR (qPCR) or high resolution melting (HRM), can also reach this level of sensitivity, but lack information regarding the allele-frequency of the mutation. For example, EGFR mutations showed allelic frequencies ranging between 20% and 210% (amplification), after normalization for the percentage of neoplastic cells. This information could be clinically relevant in the near future as suggested by a report showing that the duration of the response to target based agents might be related to the relative content of mutant alleles in the tumor [21]. A quantitative assessment of the different mutant clones in polyclonal tumors will also be useful for treatment decision, as recently shown for EGFR sensitizing and resistance mutations in NSCLC [22]. In addition,

genotyping techniques can only provide information on known mutations whereas sequencing-based methods can also detect novel and rare mutations. Some of the new variants we identified might not be true somatic mutations. Variants with high allelic frequencies could also represent infrequent SNPs, while especially non-hotspot variants with low allelic frequencies could be false positive calls. These are difficult to confirm however with standard methods like Sanger sequencing. It must be emphasized that during this project we used IonReporter Software version 1.2, while currently version 1.6 is available with improved mutation calling, especially in homopolymer regions.

Conventional methods, like Sanger sequencing or qPCR, are singleplex tests and, as the number of targets increases, the amount of DNA required for multiple tests is a limiting factor. This can be problematic especially in lung cancer, since molecular analyses are often performed on fine needle aspiration (FNA) biopsies or cytology samples yielding limited amounts of DNA. Our single multiplex PCR, consisting of 87 amplicons targeting 22 genes and requiring as little as 10 ng of input DNA, overcomes this problem. Analyzing a similar number of amplicons by conventional methods would require 500–1000 ng of DNA, clearly demonstrating the advantage of NGS.

Turn-around-time (TAT) is an important variable to take into account in clinical diagnostics. We opted for the IonPGM in our institutes since benchtop sequencers, like the Ion PGM or the MiSeq only need hours to generate sequence data. In combination with a sample preparation that is fast, like the Ion AmpliSeq™ gene-panel presented here, the TAT from DNA isolation to results is between 48 and 72 hours. While this may not be as fast as some conventional methods, it is adequate for most routine clinical applications.

An additional important consideration is cost. NGS in general is cheap if costs are calculated per sequenced base. However, clinical diagnostic laboratories do not need to sequence hundreds of genes (yet), and neither can they afford to batch numerous samples because of the short TAT needed for clinical specimens. It is therefore important that the sequencing capacity of the NGS platform is flexible and can be adjusted based on the requirements per experiment (e.g. the number of clinical specimens may vary from day to day). A second consideration is that the available sequence capacity on the chip is efficiently used. Since our custom panel specifically investigates genetic aberrations of interest for CRC and NSCLC, the required sequencing capacity for our panel is smaller compared to commercial panels that have been designed for a broad spectrum of cancers. We estimate the bill of materials costs between €130 and €175 per sample (excluding sales tax, depreciation and overhead costs) using the redesigned panel. While this may be higher compared to a single conventional diagnostic test, it is actually a lot cheaper considering that the NGS approach replaces multiple conventional tests (Additional file 6: Table S5 and [18]).

## Conclusion

The rapidly changing landscape in the field of tumor molecular characterization requires the analysis of an increasing amount of targets. With the availability of fast bench top sequencers such as the Ion Torrent PGM, massive parallel sequencing becomes available for clinical laboratories. Here we showed that the development and validation of new tests is feasible with a multi-lab effort, and believe it will facilitate the introduction of these tests into clinical settings. We also demonstrated that implementation of massive parallel sequencing using dedicated gene-panels can be fast, cost-effective and accurate. More importantly, this novel approach might facilitate the adoption of a molecular classification of lung and colon carcinoma in clinical research, thus improving the possibility to identify tumors carrying actionable molecular alterations. Finally, we want to highlight that organization of consortia similar to the one that we established in this study might represent a novel approach to have a rapid, un-biased validation among academic Institutions of new testing methods in a clinical scenario.

## Additional files

**Additional file 1: Figure S1.** Depicted are the characteristic s for the samples used in phase 2 (A) and phase 3 (B) of the study. Indicated are the neoplastic cell content of the samples (information provided by 6/7 labs), the type of tissue (biopsy or resection), origin of tumor tissue (primary or metastasis), if the tumor tissue was micro-dissected, the method previously used to determine mutation-status and for the samples in phase 3 the reason for inclusion.

**Additional file 2: Table S1.** Gene-panel version 1.

**Additional file 3: Table S2.** Gene-panel version 2.

**Additional file 4: Table S3.** Known mutations present in the 90 samples that were analyzed during phase 3 of the panel validation.

**Additional file 5: Table S4.** All variants identified in the 90 samples that were analyzed during phase 3 of the panel validation. Indicated are the identified variants and the allele frequency.

**Additional file 6: Table S5.** Approximate indication of hands on time and costs of Sanger sequencing and Next Generation Sequencing of the genetic regions covered by the described gene panel.

#### Competing interests

Reagents used during this study were purchased at discount from Life Technologies.

#### Authors' contributions

All authors either performed the experiments or analyzed the data. NR performed the bio-informatical analyses. BT, NM, HK, CN, AS, IC, OS, LL, ML and PLP designed the study. BT and NM drafted the manuscript. All authors read and approved the final manuscript.

#### Acknowledgements

We would like to thank Chrysanthi Ainali, Alain Rico, Mark Andersen and Rosella Petraroli from Life Technologies for their expertise and helpful discussion. This work is supported by: AIRC 5xmille (grant n. 12182) and the

Italian Cancer Genome Project (FIRB RBAP10AHJB) to AS. AIRC also supports the laboratory of NN (Grant number: IG12118).

**Author details**
[1]Department of Pathology, Radboud University Medical Centre (Radboudumc), PO box 9101, 6500 HB Nijmegen, The Netherlands. [2]Cell Biology and Biotherapy Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione Giovanni Pascale", IRCCS, 80131 Naples, Italy. [3]Pharmacogenomic Laboratory, CROM – Centro Ricerche Oncologiche di Mercogliano, Mercogliano, 83013 Avellino, Italy. [4]VIOLLIER, Department of Genetics/Molecular Biology, Basle, Switzerland. [5]ARC-NET Miriam Cherubini Research Centre, University of Verona, Verona, Italy. [6]Division of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany. [7]Paris Sorbonne Cité; INSERM UMR-S775, Bases moléculaires des la réponses aux xénobiotiques, Paris, France. [8]Department of Pathology, Warwick Medical School, University Hospitals Coventry and Warwickshire, Coventry CV2 2DX, UK. [9]Department of Histopathology, The University of Dublin, Trinity College, Dublin, Ireland. [10]Department of Medical Biology and Pathology, Translational research Laboratory and Biobank (UMS3655 CNRS/US23 INSERM), INSERM Unit U981, Gustave Roussy, Villejuif, France. [11]Department of Pathology and Diagnostics, University of Verona, Verona, Italy. [12]Department of Human Genetics, Radboud University Medical Centre (Radboudumc), PO box 9101, 6500 HB Nijmegen, The Netherlands.

Received: 1 July 2014 Accepted: 8 January 2015
Published online: 31 January 2015

**References**

1. Mendelsohn J. Personalizing oncology: perspectives and prospects. J Clin Oncol. 2013;31(15):1904–11.
2. Douillard J-Y, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med. 2013;369(11):1023–34.
3. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361(10):947–57.
4. Normanno N, Rachiglio AM, Roma C, Fenizia F, Esposito C, Pasquale R, et al. Molecular diagnostics and personalized medicine in oncology: Challenges and opportunities. J Cell Biochem. 2013;114(3):514–24.
5. Garraway LA. Genomics-Driven Oncology: Framework for an Emerging Paradigm. J Clin Oncol. 2013;31(15):1806–14.
6. Li TH, Kung HJ, Mack PC, Gandara DR. Genotyping and Genomic Profiling of Non-Small-Cell Lung Cancer: Implications for Current and Future Therapies. J Clin Oncol. 2013;31(8):1039–49.
7. Coghlin CL, Smith LJ, Bakar S, Stewart KN, Devereux GS, Nicolson MC, et al. Quantitative analysis of tumour in bronchial biopsy specimens. J Thorac Oncol. 2010;5(4):448–52.
8. Dienstmann R, Rodon J, Barretina J, Tabernero J. Genomic medicine frontier in human solid tumors: prospects and challenges. J Clin Oncol. 2013;31(15):1874–84.
9. Tian S, Simon I, Moreno V, Roepman P, Tabernero J, Snel M, et al. A combined oncogenic pathway signature of BRAF, KRAS and PI3KCA mutation improves colorectal cancer classification and cetuximab treatment prediction. Gut. 2013;62(4):540–9.
10. Yokota T, Ura T, Shibata N, Takahari D, Shitara K, Nomura M, et al. BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. Br J Cancer. 2011;104(5):856–62.
11. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007;448(7152):439–44.
12. Day E, Waters B, Spiegel K, Alnadaf T, Manley PW, Buchdunger E, et al. Inhibition of collagen-induced discoidin domain receptor 1 and 2 activation by imatinib, nilotinib and dasatinib. Eur J Pharmacol. 2008;599(1-3):44–53.
13. Hammerman PS, Sos ML, Ramos AH, Xu C, Dutt A, Zhou W, et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. Canc Discov. 2011;1(1):78–89.
14. Arcila ME, Chaft JE, Nafa K, Roy-Chowdhuri S, Lau C, Zaidinski M, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Canc Res. 2012;18(18):4910–8.
15. De Greve J, Teugels E, Geers C, Decoster L, Galdermans D, De Mey J, et al. Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. Lung Cancer. 2012;76(1):123–7.
16. Mazieres J, Peters S, Lepage B, Cortot AB, Barlesi F, Beau-Faller M, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol. 2013;31(16):1997–2003.
17. Williams C, Ponten F, Moberg C, Soderkvist P, Uhlen M, Ponten J, et al. A high frequency of sequence alterations is due to formalin fixation of archival specimens. Am J Pathol. 1999;155(5):1467–71.
18. Endris V, Penzel R, Warth A, Muckenhuber A, Schirmacher P, Stenzinger A, et al. Molecular diagnostic profiling of lung cancer specimens with a semiconductor-based massive parallel sequencing approach: feasibility, costs, and performance compared with conventional sequencing. The Journal of molecular diagnostics. 2013;15(6):765–75.
19. Beadling C, Neff TL, Heinrich MC, Rhodes K, Thornton M, Leamon J, et al. Combining highly multiplexed PCR with semiconductor-based sequencing for rapid cancer genotyping. The Journal of molecular diagnostics: JMD. 2013;15(2):171–6.
20. Hadd AG, Houghton J, Choudhary A, Sah S, Chen L, Marko AC, et al. Targeted, high-depth, next-generation sequencing of cancer genes in formalin-fixed, paraffin-embedded and fine-needle aspiration tumor specimens. The Journal of molecular diagnostics: JMD. 2013;15(2):234–47.
21. Zhou Q, Zhang XC, Chen ZH, Yin XL, Yang JJ, Xu CR, et al. Relative abundance of EGFR mutations predicts benefit from gefitinib treatment for advanced non-small-cell lung cancer. J Clin Oncol. 2011;29(24):3316–21.
22. Morabito A, Costanzo R, Rachiglio AM, Pasquale R, Sandomenico C, Franco R, et al. Activity of Gefitinib in a Non-Small-Cell Lung Cancer Patient with Both Activating and Resistance EGFR Mutations. J Thorac Oncol. 2013;8(7):e59–60.

## SUPPRESSION OF NATURAL KILLER CELL NKG2D AND CD226 ANTI-TUMOUR CASCADES BY PLATELET CLOAKED CANCER CELLS: IMPLICATIONS FOR THE METASTATIC CASCADE

## PLOS ONE MARCH 25,2019

In order to complete the metastatic cascade, cancer cells must detach from the primary tumour, intravasate into the circulatory and lymphatic systems, evade immune attack, extravasate at distant capillary beds, and invade and proliferate in distant organs. Metastatic cells also establish a microenvironment that facilitates angiogenesis and proliferation, resulting in macroscopic, malignant secondary tumours. Although systemic metastasis is responsible for about 90% of cancer deaths, most research in cancer does not involve metastasis in the *in-vivo* state. The fact that about 1,500 people continue to die each day from advanced cancer further attests to the failure in managing the disease once it disseminates through the body.

Primary tumour cells that intravasate into the peripheral circulation are called circulating tumour cells (CTCs) and are the functional moderators of the metastatic process. CTCs represent a promising target for anti-cancer screening and therapy. However, to efficiently detect and target CTCs, a greater understanding of their biology, particularly as it relates to their evasion of the immune system is essential. In the circulation, CTCs must overcome physiological barriers. They are subject to mechanical shear stress, immunological surveillance by immune cells in the peripheral circulation and cellular checkpoints for apoptosis and senescence. One mechanism by which they overcome these challenges is the adoption of a cloak of platelets onto their cellular surface. Platelets are essential components of haemostasis. Due to a plethora of factors released on activation, platelet functions are also connected to tumour growth, notably by acting on angiogenesis, contributing to the poor outcome of cancer patients occurs during hematogenous dissemination of cancer cells. CTCs bind to platelets and induce a cascade of platelet activation resulting in CTC-platelet clusters that protect CTCs from mechanical and biological clearance.

The findings in this study indicated a profound and systematic inhibition of innate immune surveillance induced by platelet cloaking or the elaboration of platelet releasate from cancer-primed platelets. It is an important finding in the context of immune evasion and cancer progression. I was Co-PI on this broad study. I procured the funding for the NK aspect of the work. I designed the study with co-PIs and provided lab supervision and assistance in drafting the manuscript.

PLOS ONE

RESEARCH ARTICLE

# Suppression of Natural Killer cell NKG2D and CD226 anti-tumour cascades by platelet cloaked cancer cells: Implications for the metastatic cascade

**Christopher D. Cluxton**[1,2,3]*, **Cathy Spillane**[1,2,4], **Sharon A. O'Toole**[1,4,5], **Orla Sheils**[1,4☯], **Clair M. Gardiner**[3☯], **John J. O'Leary**[1,2,4☯]

**1** Department of Histopathology, Trinity College Dublin, Dublin, Ireland, **2** Emer Casey Research Laboratory, Coombe Women and Infants University Hospital, Dublin, Ireland, **3** Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, **4** The Trinity Translational Medicine Institute, Trinity College Dublin, Dublin, Ireland, **5** Department of Obstetrics and Gynaecology, Trinity College Dublin, Dublin, Ireland

☯ These authors contributed equally to this work.
* cluxtoc@tcd.ie



OPEN ACCESS

**Citation:** Cluxton CD, Spillane C, O'Toole SA, Sheils O, Gardiner CM, O'Leary JJ (2019) Suppression of Natural Killer cell NKG2D and CD226 anti-tumour cascades by platelet cloaked cancer cells: Implications for the metastatic cascade. PLoS ONE 14(3): e0211538. https://doi.org/10.1371/journal.pone.0211538

**Editor:** Salvatore V. Pizzo, Duke University School of Medicine, UNITED STATES

**Received:** August 13, 2018

**Accepted:** January 16, 2019

**Published:** March 25, 2019



**Data Availability Statement:** All relevant data are within the manuscript and its Supporting Information files.

**Funding:** This work was funded by SFI (SFI CSET [10/CE/B1821]; JOL received this award; www.sfi.ie), Friends of the Coombe (Friends of the Coombe grant – no grant number; JOL; http://www.friendsofthecoombe.ie) and the Emer Casey Foundation (Emer Casey Foundation grand – no grant number; JOL;

## Abstract

Tumour cell immune evasion is a principal hallmark of successful metastasis. Tumour cells in the vasculature adopt a platelet cloak that efficiently suppresses the innate immune system by directly inhibiting Natural Killer (NK) cells, which normally function to neutralise spreading cancers. Here we describe two novel mechanisms of tumour cell evasion of NK cell anti-tumour functions. The first, an 'immune decoy' mechanism in which platelets induce the release of soluble NKG2D ligands from the tumour cell to mask detection and actively suppress NK cell degranulation and inflammatory cytokine (IFNγ) production, concomitantly. This represents a double-hit to immune clearance of malignant cells during metastasis. The second mechanism, a platelet-derived TGFβ-mediated suppression of the CD226/CD96-CD112/CD155 axis, is a novel pathway with poorly understood anti-cancer functions. We have demonstrated that platelets robustly suppress surface expression of CD226 and CD96 on the NK cell surface and their associated ligands on the tumour cell to further enhance NK cell suppression. These highly evolved mechanisms promote successful tumour immune evasion during metastasis and provide a unique opportunity for studying the complexity of cellular interactions in the metastatic cascade and thus novel targets for cancer immunotherapy.

## Introduction

Cancer is a leading cause of death in the developed world, second only to cardiovascular disease [1]. Greater than 90% of all cancer-associated deaths are caused by metastasis [1], and by extension, metastasised cancer is effectively an incurable disease. Primary tumour cells that intravasate into the peripheral circulation are called circulating tumour cells (CTCs).

http://www.emercaseyfoundation.com). No commercial companies were involved in the project. Authors did not receive a salary. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

CTCs represent a promising target for anti-cancer screening and therapy. However, to efficiently detect and target CTCs, a greater understanding of their biology, particularly as it relates to their evasion of the immune system is essential. As such, our study attempts to understand the biology of CTCs by examining how platelets function to promote their evasion of the immune system using *in vitro* models of established tumour cell lines and Natural Killer cells.

In the circulation, cancer cells must overcome physiological barriers. Cancer cells are subject to mechanical shear stress, immunological surveillance by immune cells in the peripheral circulation and cellular checkpoints for apoptosis and senescence. One mechanism by which they overcome these challenges is the adoption of a cloak of platelets onto their cellular surfaces [2]. Cancer cells bind to platelets by GPIIb-IIIa-fibrinogen binding and up-regulate P-selectin on activated platelets [3]. This induces a cascade of platelet activation, resulting in cancer cell-platelet clusters that protect the cancer cells from mechanical and biological clearance [4]. The complex molecular processes of protection are, however, not fully understood. It is believed that this protection is, in part, mediated by the physical barrier of the platelet cloak as many anti-tumour actions require direct contact between the immune cell and the cancer cell target [5]. However, recent data indicates that activated platelets can transfer their major histocompatibility complex (MHC) class I to cancer cells by trogocytosis, which allows the cancer cell to escape immune detection via the newly acquired 'self' signal [6]. Moreover, cancer cells activate platelets, inducing degranulation and release of a plethora of immune modulators including potent immune suppressant cytokines, such as transforming growth factor β (TGFβ) [7].

Natural killer (NK) cells are immune cells that function to lyse tumour cells without prior sensitization [8]. They play an important role in the immunosurveillance of tumours by recognizing and eliminating malignant cells, thereby preventing both local tumour progression and metastatic spread [8]. NK cell reactivity is guided by the principles of "missing-self" and "induced-self," which asserts that cells with low or absent expression of MHC class I (missing-self) and/or stress-induced expression of ligands for activating NK receptors (induced-self) are preferentially recognized and eliminated [9]. These functions of NK cells are complex and are regulated by a range of activating and inhibitory receptors expressed on the NK cell surface [9] and it is a balance of activating and inhibitory signals mediated by these receptors that determines whether NK cell responses will proceed. In the context of cancer cell surveillance and clearance, natural-killer group 2, member D (NKG2D), CD226 (DNAM-1) and CD96 (TACTILE) bind to ligands of cellular stress often overexpressed on malignantly transformed cells [9]. In response, NK cells degranulate and produce pro-inflammatory cytokines to stimulate an inclusive immune response to the transiting tumour cells [10].

In this study, we report that platelet cloaking of tumour cells promotes the loss of the NKG2D ligands, MICA and MICB, from the surface of tumour cells, inducing their secretion into the tumour microenvironment where they suppress NKG2D receptor expression on NK cells. This represents a sophisticated two-hit mechanism for platelet-mediated tumour cell evasion from immune defences and reveals an important therapeutic target to disrupt cancer cell functions during metastasis. Additionally, we describe the novel mechanism of platelets and their soluble molecules downregulating the receptors and ligands of the CD226/CD96-CD155/CD112 axis, further inhibiting NK cell functions and promoting tumour cell survival. This represents a potent mechanism of immune-evasion in cancer. We therefore suggest that better understanding of the molecular basis of NK cell immune evasion by tumour cells in circulation will enable the development of future therapeutics targeting the metastatic cascade in patients with late-stage disease.

## Materials and methods

### Ethics statement

Recruitment of participant blood donors for this study was approved by the School of Biochemistry & Immunology, Level 1 REC in Trinity College Dublin and written informed consent was obtained from all donors prior to phlebotomy. Additionally, all experiments were performed in accordance with relevant guidelines and regulations.

### Reagents

Anti-CD3-PerCP, CD56-PE, CD107a-FITC and CD42B-APC, including their IgG controls, were obtained from BD Pharmingen (San Diego, CA). Anti-IFNgamma-PE-Cy7, NKG2D-APC were obtained from eBioscience (San Diego, CA). Anti-MICA (AMO1) and MICB (BMO2) were obtained from BAMOMAB (Germany). Anti-mouse alexa-fluor-488 was obtained from Life Technologies (California, USA). Anti-CD226-FITC, Anti-CD96-PE, anti-CD155-FITC and anti-CD112-APC were obtained from Biolegend (California, USA). A TGF-β-1 neutralising antibody and recombinant TGF-β-1 was obtained from R&D systems (Minnesota, USA). Recombinant MICA and MICB were obtained from R&D systems (Minnesota, USA). Recombinant Human IgG1 Fc Protein, CF from R&D systems (Minnesota, USA). Thrombin receptor-activated peptide (TRAP) was obtained from Sigma-Aldrich (USA).

### Cell lines

Ovarian 59M and SKOV3 cells and melanoma SK-Mel-28 cells were used as a model system and cultured as previously described [11, 12]. K562 control cells were established and cultured as previously described [13].

### Preparation of peripheral blood mononuclear cells

For each experiment, Peripheral blood mononuclear cells (PBMCs) from healthy donors were used. Each experimental replicate is a representative of different donor. PBMCs were isolated from peripheral blood. Briefly, whole blood was layered onto LymphoPrep (Axis-Shield) and centrifuged at 600 x g for 30 minutes. PBMCs, which formed a white cell layer, were taken and washed with PBS. Red cells were eliminated by incubating the cells in red blood cell lysis buffer (Life Technologies) for 5 minutes at room temperature and washed again in PBS. Washed PBMCs were counted and plated according to each assay's specifications.

### Preparation of washed platelets

Platelets were donated by healthy donors. Each experimental replicate is representative of a different healthy donor. Blood was collected from donors by venepuncture through a 19-gauge butterfly needle without a tourniquet, to avoid platelet activation. Platelets were prepared as previously described [11].

### Platelet cloaking

The platelet adhesion assay was performed as previously described [11]. Briefly, washed platelets were co-incubated with tumour cells at a ratio of 1000:1 in RPMI media at room temperature under gentle rocking for 1 hour to obtain platelet cloaked tumour cells. To eliminate soluble factors, cloaked tumour cells were washed (3x) with PBS and co-incubated with PMBCs. To obtain soluble platelet cloaked tumour cell releasate, cloaked tumour cells were

centrifuged at 1000xg for 5 minutes and the supernatants were used with PBMCs for functional analysis.

## Staining of cell surface molecules for flow cytometric analysis

NK cells were incubated with optimal concentrations of the following anti-human Abs: CD56-PE, CD3-PerCP, NKG2D-APC, CD226-FITC, CD96-PE or the corresponding isotype control Abs (all from BD Pharmingen) in 100 μl of 1% FCS/PBS at 4˚C for 20 min.

Tumour cells, with and without platelets, were incubated with anti-human MICA, MICB unconjugated antibodies and alexa-fluor-488 secondary antibody. Staining of tumour cells using primary and secondary antibodies was performed for 1 hour at 4˚C. Tumour cells were also stained with CD112-APC and CD155-FITC antibodies and isotype controls in 100 μl of 1% FCS/PBS at 4˚C for 20 min.

Cells were washed twice with PBS and acquired on a Cyan flow cytometer (Beckman Coulter, Brea, CA, USA). Events were stored and analysed on the FlowJo software (TreeStar). NK cells were gated from PBMC populations as distinct CD3-CD56+ cells (S1 Fig).

## CD107a staining

A total of $1 \times 10^6$ PBMCs were stimulated with 500U hrIL-2 for 18 h in 96-round-bottom well plates. Freshly re-suspended tumour cells ($2 \times 10^6$) and anti-CD107a-FITC (2 μl/well) or IgG1-FITC (2 μl/well), as a control, were then added. Plates were incubated for 1 h, before the addition of GolgiStop (BD Pharmingen). Plates were incubated for further 3 h before extracellular staining and flow cytometric analysis. All incubations were performed at 37˚C.

## IFN-γ intracellular staining for flow cytometric analysis

Cells were stimulated with 500U hrIL-2 for 18 h, the last 4 h in the presence of Golgi-Plug (BD Pharmingen). Cells were washed once in PBS and FcRs were blocked by incubating with 10% human AB serum in 1% FCS/PBS. Cell surface staining was performed as above, followed by intracellular staining with IFN-γ-PE-Cy7 or IgG2a/b-PE-Cy7 (both from BD Pharmingen) as control using the Cytofix/Cytoperm Plus kit (BD Pharmingen) according to the manufacturer's instructions.

## Functional assays

NK cell activation assays were performed with PBMCs at a PBMC to target cell ratio of 5:1. PBMCs yielded 10% NK cells on average. Functional studies were performed in the presence and absence of blocking antibodies to NKG2D, CD226 or CD96 on NK cells and MICA, MICB, CD155, CD112 tumour cell surfaces respectively. Here, cells were incubated in 1ug/mL solutions of either anti-NKG2D (R&D clone# 149810), anti-MICA (R&D clone# 159227), anti-MICB (R&D clone# 236511), anti-CD226 (BioLegend clone# 11A8), anti-CD96 (BioLegend clone# 92.39), anti-CD155 (BioLegend clone# SKII.4) and anti-CD112 (BioLegend clone# TX.31) for 1 hour at room temperature and washed to remove excess soluble antibody. Recombinant MICA and MICB were used at a final concentration of 10μg/mL for 30 minutes with PBMCs. IgG controls were used for each experiment.

Recombinant TGFbeta was used to pre-treat PBMCs for 1 hour at room temperature prior to functional assay in relevant experiments.

Activated platelets were obtained by incubating washed platelets with 125μg/mL thrombin receptor-activated peptide (TRAP) for 30 minutes at room temperature under gentle rocking

conditions. Activated platelets, and their releasate, were then used in functional assays as indicated within the text.

### ELISA

Detection of soluble MICA and MICB was performed using DuoSet ELISA development system from R&D Systems, according to the manufacturer's instructions. All concentrations are expressed as mean ± SEM of triplicates.

### Real time PCR

Cancer cells were incubated for 24 hr in standard cell culture conditions with media alone or with media containing washed platelets, with a final cancer cell-platelet ratio of 1:1000. Total RNA was extracted from the samples using the miRVana Kit (Life Technologies, Foster City, CA, USA), according to manufacturer's protocol. ADAM10, ADAM17, ADAM19 and GAPDH mRNA expression levels were evaluated by TaqMan RT-PCR. The data was analysed using the comparative Ct method; where ADAM10, ADAM17 and ADAM19 mRNA expression was normalised to that of GAPDH and calibrated to that of untreated cells to establish the relative level of mRNA expression.

## Results

### Platelet cloaking facilitates evasion of ovarian and melanoma cancer cells from NK cell anti-tumour activity

To establish cloaking of tumour cells, the ovarian cell lines 59M and SKOV3 and the melanoma tumour cells SK-Mel-28 were incubated with platelets and CD42b platelet antigen was measured on tumour cell populations. The K562 cell line, an established target cell of NK cells, was chosen as a positive control. CD42b antigen was detected on tumour cell populations indicating efficient platelet cloaking for each cell line (Fig 1A and 1B; S1 Fig). We subsequently demonstrated robust anti-tumour functions of CD3-CD56+ NK cells (S1 Fig) when challenged with each tumour cell line, quantified by CD107a expression on the NK cell surface and intracellular IFNγ production (Fig 1C and 1D). Ovarian and melanoma tumour cells induced a strong NK interferon gamma production, while melanoma cells poorly induced the CD107a response (Fig 1C and 1D). The presence of the platelet cloak significantly reduced NK cell anti-tumour activity to ovarian and melanoma tumour cells (Fig 1C and 1D). These results suggest that platelet cloaking of tumour cells potently inhibits NK cell effector functions in *in vitro* epithelial cancer models.

### Immune modulation is mediated by both soluble and contact factors

Given that both cell contact factors, such as HLA class-I, and platelet derived soluble molecules, such as TGFβ, are known to alter NK cell effector functions, we addressed the respective roles of soluble and contact factors in ovarian and melanoma tumour cell immune evasion. To study contact factors, PBMCs were incubated with platelet cloaked ovarian and melanoma tumour cells, and our K562 controls, which were washed to remove platelet soluble factors. Washing platelet-cloaked ovarian tumour cells to remove the platelet releasate partially restored NK cell activity and IFNγ production (Fig 1E and 1F). For melanoma tumour cells, CD107a activity was completely restored in the absence of platelet releasate, while IFNγ was unaffected (Fig 1E and 1F). This suggests a role for membrane bound cell-contact factors for ovarian and melanoma tumour cell lines. To examine soluble factors, PBMCs were incubated with tumour cells suspended in platelet releasate (platelet cloaked tumour cell supernatants) in

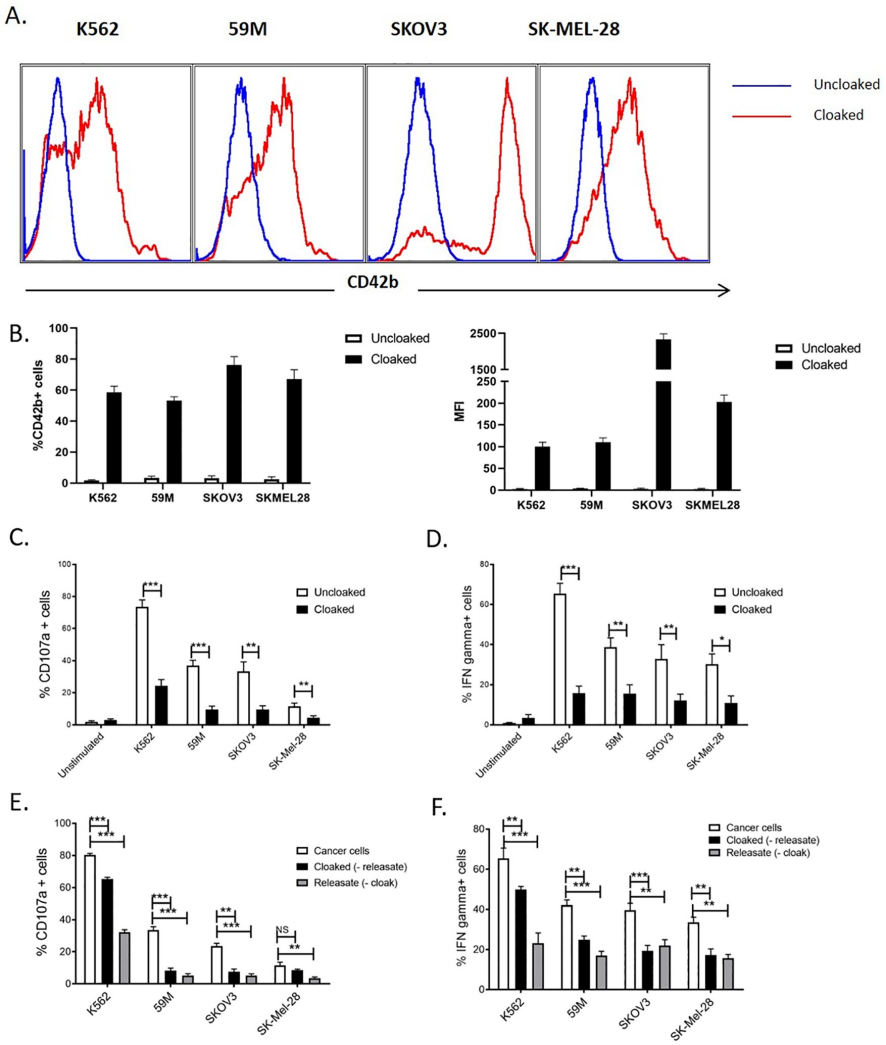



**Fig 1. Platelet cloaking inhibits NK cell functions.** (A,B) Quantification of platelet cloaking of ovarian and melanoma tumour cells, and the myelogenous leukaemia control cell line K562. Tumour cell lines were co-incubated with and without platelets and analysed for the surface expression of the CD42b platelet specific marker. Expression data are represented by histogram (A), as a percentage of total cells and by mean fluorescent intensity (MFI; B). (C-F) Analysis of the function consequences of platelet cloaking on NK cell functions. NK cell anti-tumour assays were performed by co-incubating PBMCs with tumour cells (cloaked and uncloaked) for 4 hours and measuring CD107a (C,E) and IFNgamma production (D,F) as markers of activation. (E,F) To dissect the respective roles of platelet contact factors (cloaked (minus the releasate)) and soluble factors (releasate (minus the platelet cloak)) platelets and releasate were isolated and used to treat NK cells in NK activation assays as previously described. (C-F) Data analysed by ANOVA—each experiment represents mean±S.E.M. of at least three independent experiments. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

https://doi.org/10.1371/journal.pone.0211538.g001

the absence of platelets. Soluble factors potently inhibit NK cell functions for all cell lines. This data demonstrates that both cell contact factors and the platelet releasate induced by both ovarian and melanoma tumour cells is a potent inhibitor of NK cell activity (Fig 1E).

## Platelet cloaking drives an immune decoy mechanism via ADAM proteases

The NKG2D-MICA/MICB receptor-ligand system is well established in tumour immune surveillance. We hypothesised that the platelet cloak modulates both receptor and ligand to promote tumour immune evasion. Initially, we confirmed that the axis is functional in NK targeting of our cell lines by NK cell functional assays in the presence/absence of neutralising antibodies to NKG2D (S2 Fig) and the MICA and MICB tumour expressed ligands (S2 Fig). Neutralising each component significantly decreased NK cell anti-tumour activity against each epithelial cancer. We subsequently defined the role of platelet cloaked tumour cells, platelets and platelet releasate (from TRAP activated platelets) in regulating NKG2D expression and NK cell functions. Initial results reveal that both cloaked and uncloaked tumour cells actively suppress NKG2D on the NK cell surface (Fig 2A). Importantly, the decrease in detectable NKG2D on NK cells when co-incubated with tumour cells over 24 hours is well established phenomenon, which we have further demonstrated here. We have therefore dissected the functions of activated platelets and soluble factors in the absence of tumour cells. PBMCs were incubated with either activated platelets (by TRAP) and/or platelet releasate and significantly decreased NKG2D on the surface of NK cells was observed (Fig 2B).

We then examined the effect of the platelet cloak on the tumour cell and its expression of NKG2DL. Each tumour cell line expressed varying levels of MICA and MICB ligand on unstimulated cells (Fig 2C). The expression of surface MICA and MICB was markedly reduced for each epithelial cancer cell line when cloaked with tumour cells (Fig 2D and 2E). The strongest effect was observed for MICA expression on the melanoma cell line and for MICB on the 59M ovarian cells, which expressed the highest basal levels of their respective ligands. To examine the temporal relationship of platelets with decreased expression of tumour ligands, tumour cells were incubated for 4 hours and 24 hours with platelets to examine direct masking of ligand and/or proteolytic cleavage by the platelet (4h) and transcriptional changes in the tumour cell line (24h) (Fig 2D and 2E). Our results suggest that while there is some loss of MICA and MICB from the tumour cell at 4 hours (did not reach statistical significance) the majority of ligand is lost up to 24 hours suggesting that the mechanism is not a rapid steric occlusion but occurs more gradually (Fig 2D and 2E).

A known mechanism of NK cell inhibition is the induced suppression of the NKG2D receptor by soluble NKG2D ligands, specifically MICA and MICB. We incubated NK cells with recombinant MICA and MICB and observed significantly decreased surface NKG2D ligand expression in our system (S3 Fig). It is important to note that the presence of the recombinant molecule in the binding site of the NKG2D receptor may artificially decrease detection with antibody should they compete for binding sites. We therefore characterised the downstream effect of ligand binding to support our hypothesis. NK cells that were pre-treated with recombinant MICA and MICB had decreased anti-tumour functions compared with the IgG-Fc control protein (S3 Fig). This is supported by previous data in which NK cell functions were inhibited when NKG2D receptor functions were neutralised (S2 Fig).

Given that MICA and MICB are lost from the tumour cell surface in a platelet-dependent manner, we hypothesised a role for the platelet in promoting the release of soluble NKG2D ligands into the microenvironment of the cloaked tumour cells. To quantify this, supernatants from tumour cell cultures in the presence or absence of platelets, and platelets alone, were analysed by ELISA for soluble MICA and MICB molecules (Fig 2F). MICA was produced by

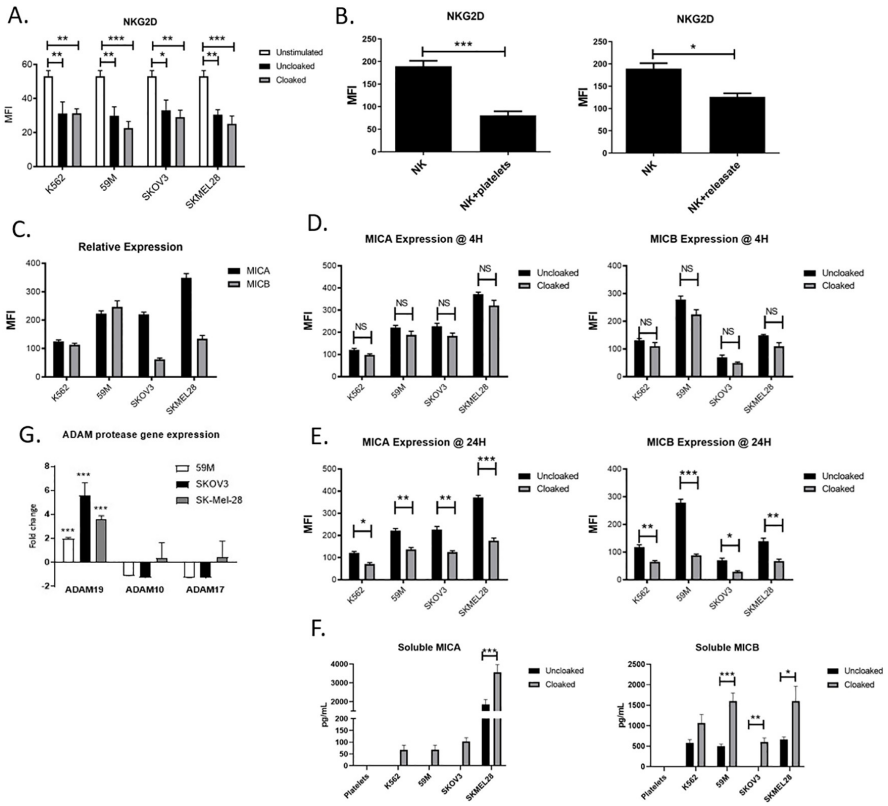



**Fig 2. Platelets modulate NKG2D receptor expression by inducing the release of NKG2DL MICA and MICB from the surface of tumour cell lines.** (A,B,C) To examine the role of platelet cloaked tumour cells, platelets and TRAP-induced platelet releasate in regulating NKG2D expression, quantification of NKG2D was performed by flow cytometry of PBMCs in the presence or absence of tumour cells, platelet cloaked tumour cells, platelets or platelet releasate for 24 hours. (C) Relative expression of MICA and MICB on tumour cell lines was quantified by MICA/MICB mAbs and flow cytometry (n = 3) (D,E) The role of the platelet cloak on the expression on known NK cell activating ligands MICA and MICB on tumour cells was investigated by measuring baseline expression (clear bars) and comparing this to platelet cloaked tumour cells (filled bars). Tumour cells were incubated for 4 (D) or 24 (E) hours in the presence or absence of platelets and ligand expression was quantified by flow cytometry. (F) Analysis of soluble MICA and MICB in the supernatants of tumour cell lines incubated for 24 hours in the presence or absence of platelets by ELISA. (G) Taqman analysis of platelet cloaked and uncloaked ovarian/melanoma tumour cells for ADAM19, ADAM10 and ADAM17 genes. Values shown are cloaked cells relative to uncloaked cells. (A,B,D) Data analysed by ANOVA—each experiment represents mean±S.E.M. of at least three independent experiments. (G) Data analysed by t-test and represented as standard deviation of at least three independent experiments, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

https://doi.org/10.1371/journal.pone.0211538.g002

melanoma cells in the absence of platelets but was not detected for ovarian or control cells. (Fig 1C). Platelet cloaking induced the release of MICA from all cell lines, however this only reached significance for our melanoma cells. Similarly, the presence of the platelet cloak induced secretion of soluble MICB from all cell lines, most notably from the 59M and SKOV-3 ovarian cell lines, demonstrating a platelet-dependent immune evasion mechanism.

The temporal relationship of cloaking with sNKG2DL release indicates tumour cell gene regulation as mechanistically important. Taqman relative PCR of cloaked and uncloaked

tumour ovarian cells revealed that ADAM19 is upregulated in platelet cloaked ovarian and melanoma cells compared with uncloaked cells (Fig 2G). Interestingly, ADAM10 and ADAM17 are not upregulated in the tumour cell, suggesting a novel and distinct process to regulate NKG2DL cleavage on the tumour cell surface. Our results demonstrate that platelets specifically upregulate genes of a protease family known to cleave NKG2DL ligands and suggests a potential role for tumour derived ADAM proteases in NKG2DL cleavage.

By treating releasate from platelet cloaked tumour cells that contained platelet soluble factors plus tumour cell soluble factors, with neutralising antibodies to MICA and MICB the suppression of NKG2D (Fig 3A), degranulation (Fig 3B) and pro-inflammatory cytokine (Fig 3C) production was partially rescued. Interestingly, rescue with anti-MICA antibody was only achieved for the melanoma cells, consistent with our finding that MICA is released only from melanoma cells in significant quantities. Additionally, rescue was observed for all cell types when MICB was neutralised as MICB was present in the releasate from each cell line.

Together these results reveal a decoy mechanism to promote tumour cell evasion from NK cell surveillance (Fig 3D).

## Platelet cloaking disrupts the CD226/CD96-CD112/CD155 tumour recognition axis

Like NKG2D, CD226 and CD96 are receptors expressed on the surface of NK cells known to be important for tumour immune surveillance. The ligands for the CD226 and CD96 receptors on the tumour cell are CD112 and CD155. We confirmed and quantified their expression on the ovarian and melanoma cell lines by immunofluorescence using anti-CD112 and anti-CD155 specific monoclonal antibody based flow cytometry and each of our ovarian and melanoma tumour cell lines express both CD112 and CD155 (S4 Fig).

To investigate if the CD226/CD96-CD155/CD112 tumour cell recognition system is functional in the context of our ovarian and melanoma tumour cells we blocked each component with commercial mAb and performed NK cell functional assays (Fig 4 and S4 Fig). Blocking CD226 on the NK cell induced a marked inhibition of NK functions for both activity and cytokine production, with strongest effects seen for the epithelial cancer cell lines (Fig 4A). Neutralising CD96 on the NK cell (Fig 4A), or the ligands CD155 or CD112 (S4 Fig) on the tumour cell, did not disrupt NK cell targeted killing of our tumour cell lines. However, dual blocking of CD155 and CD112 ligands demonstrated marked inhibition of NK anti-tumour functions (Fig 4A). This highlights complexity and redundancy within the system.

Given the role of platelets in regulating tumour cell expressed ligands and NK cell expressed receptors in the NKG2D-MICA/MICB system, we hypothesised that the CD226/CD96-CD112/CD155 axis would be regulated in a similar manner. We observed that tumour cell lines cloaked with platelets had a significant decrease in expression of CD112 and CD155 (Fig 4B). As previously shown, platelet cloaked tumour cells are less immunogenic than uncloaked cells and by blocking access to tumour cell ligands (using monoclonal antibodies) NK cell degranulation and cytokine synthesis are inhibited (Fig 4A and S4 Fig). This immune evasion mechanism is mediated by platelets and actively disrupts the CD226/CD96-CD112/CD155 axis.

We further confirmed that the platelet cloak downregulates NK cell CD226 and CD96 (Fig 4C). CD226 was inhibited when incubated with uncloaked tumour cells alone, a phenomenon that has been published previously (Fig 4C). There is a paradoxical increase in CD226 with platelet cloaked tumour cells, an artefact from CD226+ activated platelets sticking to NK cells (Fig 4C). Interestingly, NK activity was unaffected at 24 hours compared with 4 hours incubation suggesting that decreased CD226 in not mechanistic in inhibiting NK cells in this

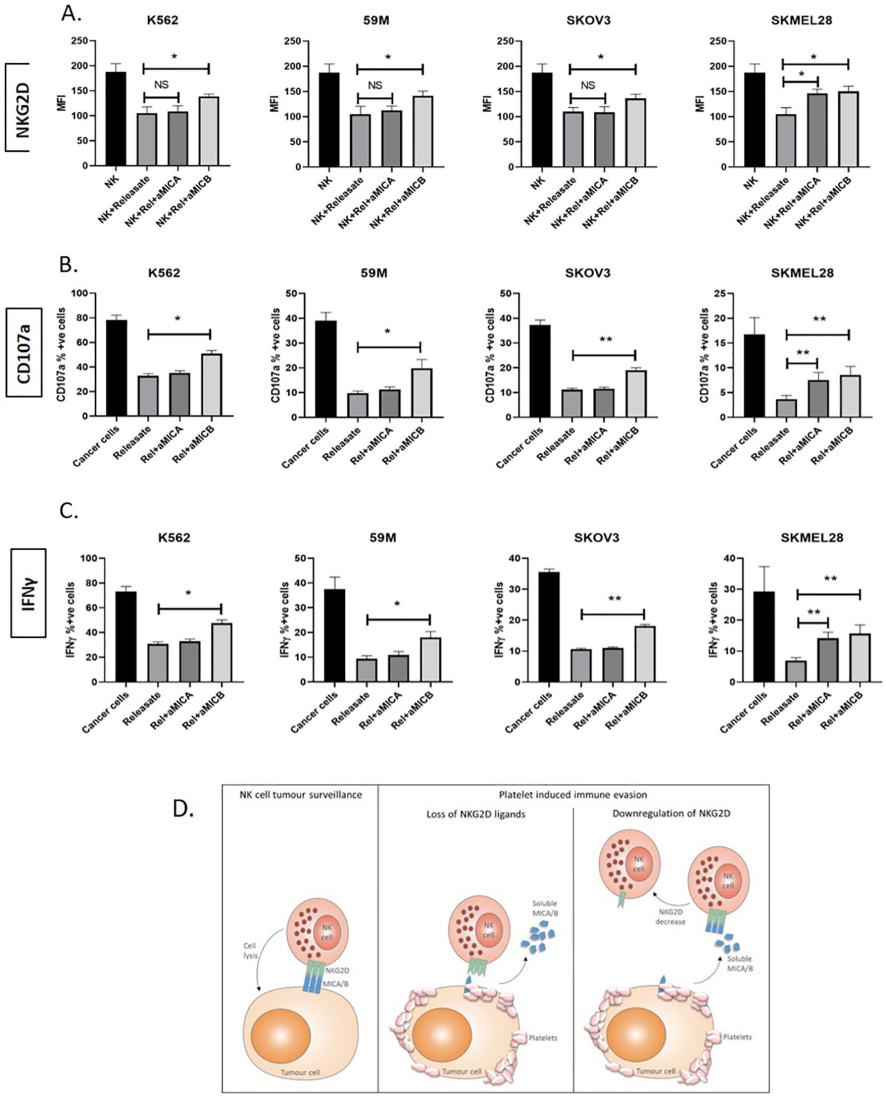



**Fig 3. MICA and MICB in the releasate modulate NKG2D expression and NK cell functions.** (A) To examine the role of MICA and MICB from the platelet releasate (from platelet cloaked tumour cells) in regulating NKG2D and NK cell functions, the platelet releasate was neutralised using monoclonal antibodies against MICA and MICB as previously described. NKG2D expression (A) and NK cell functions (CD107a (B) and IFNgamma (C)) were quantified following standard 4h NK cell function assays by flow cytometry and results are expressed as the mean fluorescent intensity (MFI; A) or as a percentage of unstimulated or IgG control (B and C). (D) Schematic representation of the platelet sNKG2DL mediated decoy mechanism of tumour immune evasion (A-C) Data analysed by ANOVA—each experiment represents mean±S.E.M. of at least three independent experiments. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

https://doi.org/10.1371/journal.pone.0211538.g003

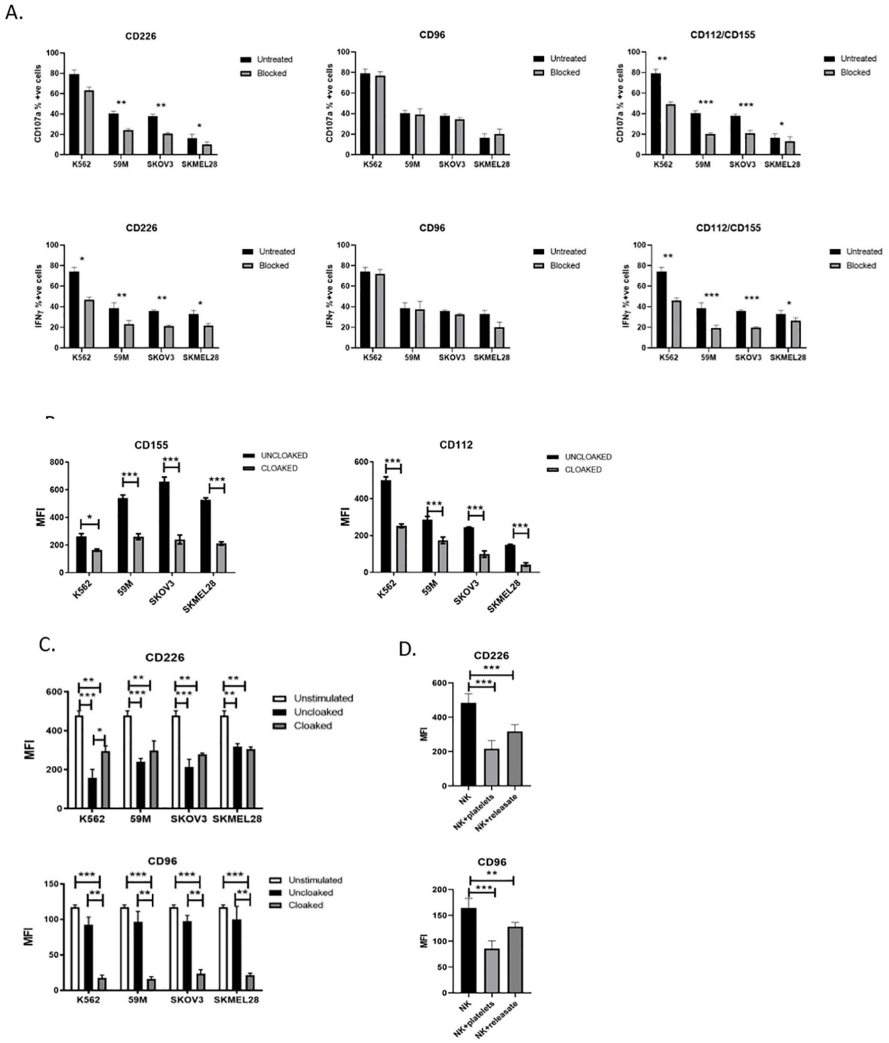



**Fig 4. Platelets disrupt the CD226/CD96-CD112/CD155 NK axis for NK cell targeting of tumour cell lines.** (A) Blocking assays using mAb against CD226, CD96 or CD155/CD112 in standard anti-tumour assays (CD107a surface expression and IFNγ production) to analyse the role of each molecule in NK cell targeting of tumour cell lines. (B) The role of the platelet cloak on the expression on known NK cell activating ligands CD112 and CD155 on tumour cells was investigated by measuring baseline expression (clear bars) and comparing this to platelet cloaked tumour cells (filled bars). Tumour cells were incubated for 24 hours in the presence or absence of platelets and ligand expression was quantified by flow cytometry. (C) Analysis of the effect of tumour cells with and without the platelet cloak on expression of the NK cell receptors CD226 and CD96. PBMCs (clear bars) were co-incubated with uncloaked (filled bar) and cloaked (grey bars) tumour cells for 24 hours and expression of CD226 and CD96 were quantified by flow cytometry. (D) To dissect the role of soluble factors, experiments were repeated as above with TRAP-activated degranulating platelets (+platelets group) and releasate from TRAP-activated degranulated platelets that was cleared of platelet cellular material (+releasate group). (A-D) Data analysed by ANOVA—each experiment represents mean±S.E.M. of at least three independent experiments. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

https://doi.org/10.1371/journal.pone.0211538.g004

scenario. To dissect further the role of the activated platelet and releasate, PBMCs were co-incubated with either TRAP activated platelets or platelet releasate and NK receptor expression quantified by flow cytometry (Fig 4D). CD226 expression on the cell surface was decreased by both activated platelets and platelet releasate (Fig 4D). More potent inhibition with platelet material suggests that molecules from the platelet surface have regulatory functions while soluble molecules are also active as demonstrated by a potent suppression of CD226 by the releasate (Fig 4D). A similar effect was observed for CD96 with significantly decreased expression in response to platelet cloaked tumour cells, platelets and releasate suggesting a role for both in CD96 suppression, suggesting that CD226 and its CD96 co-receptor are suppressed in concert (Fig 4C and 4D). The regulation of CD96 on the NK cell is, unlike CD226, independent of the suppressive effects of the tumour cell alone.

### TGFβ from platelets inhibits CD226 but not CD96 on NK cells

TGFβ has established functions in NKG2D receptor down-regulation and NK cell anti-tumour functions. TGFβ is released from activated platelets as demonstrated by ELISA assays performed on tumour cells in the presence or absence of platelets (Fig 5A). Recombinant TGFβ downregulated CD226 expression on NK cells. Interestingly, there was a significant increase in CD96 expression (Fig 5B). Additionally, by neutralising TGFβ in the platelet releasate (from platelet cloaked tumour cells) using anti-TGFβ antibody we observed rescue of CD226 suppression, supporting TGFβ dependant regulation of this receptor. No significant effect was observed for CD96 indicating that this receptor is regulated independently of TGFβ. By treating NK cells with recombinant TGFβ their functions could be suppressed (Fig 5C), which was supported further by neutralising TGFβ in the releasate to rescue NK cell suppression (Fig 5D and 5E). Together these data demonstrate that TGFβ suppresses NK cell functions via downregulation of CD226 on the NK cell surface.

## Discussion

In the bloodstream, cancer cells (also known as circulating tumour cells or CTCs) encounter many challenges that are overcome, in part, by the formation of CTC-platelet clusters mediated by coagulation factors released both by the tumour cell and the activated platelets [14]. CTCs must evade immune clearance by NK cells in peripheral circulation. NK cells express receptors that efficiently bind tumour cell antigens and release cytotoxic granules to neutralise tumour cells in the blood. However, as previously reported, CTCs induce the release of immune-suppressive molecules from platelets to inhibit NK cells and evade detection [7, 15]. Furthermore, platelets have been shown to transfer their major histocompatibility complex class I to tumour cells, thereby conferring 'self' status and contributing to immune evasion [6]. The interactions between tumour cells and platelets are complex, particularly the role of platelets in facilitating immune evasion of CTCs, similar to that previously identified for germ cell, prostate and colon cell lines [7].

In this study, we examined the effects of platelets and their releasate on two different models of metastatic disease; using low trafficking ovarian cancer cells that spread primarily through the abdominal cavity in ascitic fluid and less commonly through peripheral vasculature, and high trafficking malignant melanoma cells that spread predominantly via the lymphatic and vascular systems. We predicted that cells with such disparate metastatic strategies would vary in their basic molecular anti-immune functions. Using flow cytometry, we confirmed the capacity of ovarian and melanoma tumour cells to efficiently activate and adopt a cloak of platelets and induce the release of modulatory molecules, including TGFβ. We have subsequently shown that the molecules contained within the platelet releasate are potent inhibitors

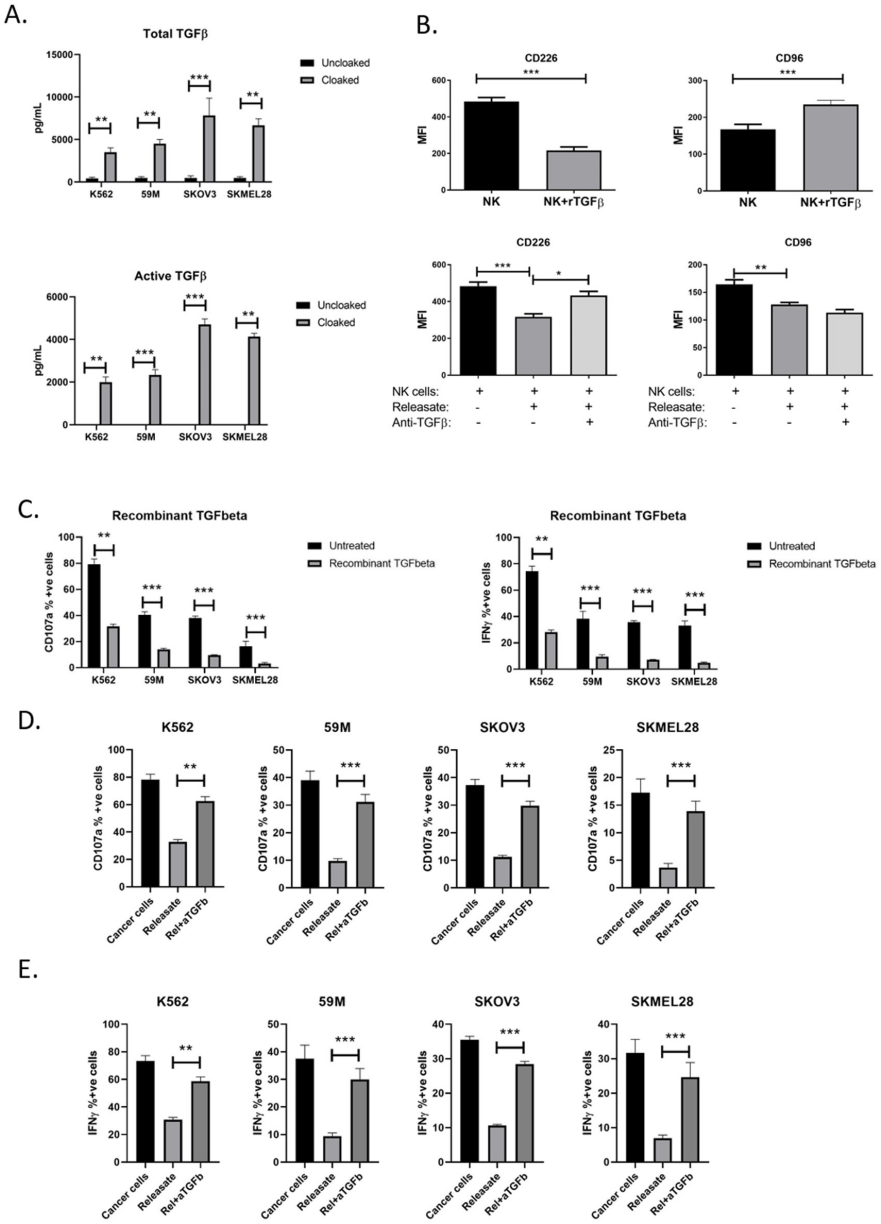



**Fig 5. TGFbeta is an active soluble molecule in platelet derive immune evasion of circulating tumour cells.** (A) Quantification of TGFbeta (total and active) released from cloaked tumour cells (filled box) compared with uncloaked cells (clear box) using ELISA duo-set analysis of supernatants of tumours cells incubated for 4 hours in the presence or absence of platelets. (B) To dissect the role of TGFbeta, recombinant TGFbeta1 molecule was used to pre-treat NK cells for 24 hours and CD226 and CD96 receptor expression was quantified by flow cytometry. To confirm the in vitro role of platelet derived TGFbeta, neutralising antibody against TGFbeta was used to pre-clear supernatants and receptor expression was compared with untreated

cells. (C) Functional assays to confirm the effect of TGFbeta on NK cell anti-tumour functions were performed by pre-treating NK cells with recombinant TGFbeta and measuring CD107a surface expression and IFNgamma production as NK cell activation markers. (D,E) Analysis of the role of TGFbeta in platelet releasate on CD107a expression (D) and IFNgamma production (E) by comparison of the effect of TRAP-induced platelet releasate versus releasate that has been cleared of TGFbeta by neutralising antibody. (C-E) Results represent the detection of the activation markers as a percentage of the average response of untreated NK cells to each individual tumour cell line. (A-E) Data analysed by ANOVA—each experiment represents mean±S.E.M. of at least three independent experiments. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

https://doi.org/10.1371/journal.pone.0211538.g005

of NK cells. We also demonstrated the conserved roles of contact factors from cell-cell interaction and soluble factors from the platelet releasate in suppressing NK cell anti-tumour functions to tumour cells that spread haematogenously (high trafficking) and transcoelomically (low trafficking).

The interaction between NKG2D on the NK cell and NKG2DL (MICA and MICB) on the tumour cell activates NK cell anti-tumour functions. As NKG2DL are expressed only on stressed cells, including malignantly transformed cells, this axis represents a targeted mechanism for immune clearance of cancer cells. Interestingly, prolonged exposure of NK cells to ligand expressing tumour cells results in decreased levels of NKG2D on the NK cell. This was an important consideration in our study and we therefore examined the functions of platelets and the soluble factors in isolation from tumour cells. In our study, platelet releasate from cloaked ovarian and melanoma tumour cells suppressed NKG2D on NK cells, a phenomenon previously reported for prostate and colon cancer cells [7]. Platelets acted on the tumour cell to decrease detectable surface NKG2DL, altering the 'stressed or non-self' to 'self' phenotype. Platelet cloaking subsequently reduced the immunogenicity of tumour cells to NK cells, promoting immune evasion. We supported this finding by demonstrating decreased NK cell recognition and reactivity to tumour cells that were neutralised with antibodies against the NKG2DL, MICA and/or MICB.

TGFβ from the platelet is a well-established mechanism of suppression of NKG2D receptor surface expression and NK cell function. However, we observed that NKG2D, and thus NKG2D function, is more potently suppressed by the platelet releasate than by recombinant TGFβ (S2 Fig). We, therefore, hypothesised that solubilised NKG2DL contributes to NKG2D suppression. In our study, ovarian and melanoma tumour cells with a platelet cloak could be induced to release soluble NKG2DL into the tumour cell microenvironment and that NKG2D was actively suppressed using recombinant MICA and MICB proteins. A recent study demonstrates the impact of platelet cloaking on the shedding of NKG2D ligands, which supports these findings [16]. Additionally, by neutralising soluble MICA and MICB in the platelet releasate we partially restored NKG2D suppression and function. Neutralising MICA was effective only for melanoma cells, which release soluble MICA in high doses in a platelet-independent manner. This perhaps suggests that the high trafficking melanoma cells release high concentrations of soluble MICA to evade NK cell detection and attack, providing an explanation for the lesser capacity of NK cells to mount an immune response to this cell line. Soluble MICA for ovarian cancer cells, while induced by platelet cloaking, saw only modest increases in levels in the microenvironment and is therefore unsurprising that neutralising sMICA in the releasate has no measurable effect. Conversely, however, neutralising MICB in the releasate restored NKG2D expression for each cell line which correlates with the platelet induced release of soluble MICB from tumour cells, previously described. This suggests a conserved role for MICB in high and low-trafficking cells, while MICA appears functional only in the high-trafficking cell type.

A role for the ADAM proteases in the shedding of MICA and MICB has been shown previously [16, 17]. To examine the role of platelet-surface ADAM proteases we defined the

temporal relationship of platelet cloaking with shedding of MICA and MICB from the surface. We found that while low levels of shedding are detectable at shorted time intervals (attributed to direct cleavage by platelet-surface ADAM10 and ADAM17), a much stronger effect was observed at 24 hours incubation. We subsequently examined ADAM10, ADAM17 and ADAM19 expression in the tumour cell and found that while ADAM10/17 are not induced by platelet cloaking, ADAM19 is potently induced. We suggest that ADAM19 plays an important functional role in MICA and MICB cleavage and we aim to address this further in future studies. Indeed, the detection of soluble MICA and MICB in the serum of patients with advanced hepatocellular carcinoma [18] and oral squamous cell carcinoma [19] respectively represents a marker of poorer prognosis, while increased soluble MICA and decreased NKG2D levels are poor prognostic markers in pancreatic cancer [20]. Our results demonstrate an immune decoy mechanism for tumour cell escape from Natural Killer cells in the peripheral circulation and identify a novel mechanistic target in the metastatic cascade for cancer immunotherapy.

NK cell immunoglobulin receptors CD226 and CD96 that interact with the nectin-like ligand CD112 and the polio-virus receptor CD155 on target cells are emerging as important mediators of NK cell anti-cancer functions [21–23]. The function of CD226 in recognition of tumour cells by NK cells has been previously examined [24]. In our study, we initially demonstrated that CD226 was functional in NK targeting of our melanoma and ovarian tumour cell lines. This was achieved by observing the negative functional impact of blocking the CD226 receptor with commercial mAb. Interestingly, neutralising CD96, a known adhesion molecule had no significant effect on NK cell functions. This is supported by previously published data [24]. CD96 is less well understood. Its primary ligand is CD155 and recent studies of murine CD96 have shown it to be a competitive inhibitor of CD226, competing for binding to CD155 with a stronger binding affinity for the ligand and directly inhibiting NK cell degranulation and cytokine production as a result [21]. However, CD96 acts as an adhesion molecule facilitating NK activity in the human context, and is therefore described as an NK cell activating receptor [25]. Evolutionarily divergent functions of the murine and human CD96 molecule has been considered by experts as an explanation for the differing reports and more work is required to fully define CD96 in the human context. The functional complexities of CD96 in tumour immune surveillance are intriguing, however, they are beyond the scope of this study and we intend to focus on these in further studies. Supporting a role for CD226 and CD96 in the context of tumour evasion, a study of patients with pancreatic cancer demonstrated that CD226+CD96+ NK cells are deficient in patients versus healthy controls, while TIGIT was not altered [26]. Additionally, CD226 and CD96 upregulation was observed on NK cells of relapse free breast cancer patients and CD226 was found to be decreased on anergic NK cells associated with lung cancer [27–29].

Given that the axis is active in immune surveillance of both melanoma and ovarian tumour cells, we established that platelet cloaked tumour cells, activated platelets and platelet releasate all function to suppress expression of CD226 and CD96 on the NK cell surface. Furthermore, we observed that platelet cloaked tumour cells have significantly decreased expression of the CD112 and CD155 ligands that activate CD226/CD96. This mimics the dual functionality of the platelet in the NKG2D/NKG2DL system to inhibit both immune receptors and the tumour cell marker. The role of TGFβ in modulating NK cell receptor function is established for NKG2D [7], and herein we have described a similar role for CD226. Recombinant TGFβ suppressed CD226 expression and NK cell functions and this was supported by neutralising TGFβ in the platelet releasate to partially rescue suppression. TGFβ significantly increased CD96 expression on NK cells, suggesting that CD96 is regulated independently of TGFβ. This novel role for TGFβ may aid in the development of successful anti-TGFβ or anti-platelet strategies in battling metastatic disease.

Taken together, these findings indicate a profound and systematic inhibition of innate immune surveillance by platelet cloaking or elaboration of platelet releasate from cancer-primed platelets and provide potential future chemotherapeutic targets for metastatic disease.

## Supporting information

**S1 Fig. Representative gating strategy.** (A) Gating of tumour cell populations from free platelets. FSC/SCC plots of SKOV3 cells, platelets and cloaked SKOV3 cells demonstrates two distinct populations. (B) Tumour cell gate (P11) stained with CD42b APC antibody. Isotype control, SKOV3, platelet and cloaked SKOV3 samples demonstrate the low free platelet contamination in the tumour cell population and the positive and negative populations for cloaked tumour cells. (C) A lymphocyte gate was applied to whole PBMCs from healthy donors. Lymphocytes were gated for NK cells (CD3-CD56+ cells). (D) CD3-CD56+ NK cells were treated with cancer cells and cloaked cancer cells (cancer cells + platelets) and analysed for CD107a expression.
(TIF)

**S2 Fig. Neutralising the NKG2D-NKG2DL axis inhibits NK cell functions.** (A) Quantifying the capacity of monoclonal antibodies to neutralise NKG2D receptor on NK cells and MICA and MICB ligands on tumour cell lines. PBMCs and tumour cell lines were incubated for 1 hour at room temperature in the presence or absence of 1μg/mL of respective mAb and subsequently stained with fluorescent antibodies to quantify molecular blockade compared with untreated cells. For tumour cell lines, the clear box represents staining in the absence of mAb blockade and the filled box represents neutralised cells. (B) Given the satisfactory neutralisation of surface molecules, the cells were used in standard anti-tumour assays (CD107a surface expression and IFNgamma production) to analyse the role of each molecule (and indeed, a combination of molecules) in NK cell targeting of tumour cell lines. (C) Expression of NKG2D on NK cells. NKG2D was potently suppressed but both platelet releasate and TGFbeta recombinant protein, with significant inhibition with releasate compared with recombinant protein. (A,B,C) Each experiment represents mean±S.E.M. of at least three independent experiments. $^{*}$ = $p<0.05$, $^{**}$ = $p<0.01$, $^{***}$ = $p<0.001$.
(TIF)

**S3 Fig. The role of soluble MICA and MICB in NKG2D expression and NK cell functions.** (A) Expression of NKG2D on NK cells post-treatment with recombinant MICA or MICB for 24 hours. (B and C) NK cells were also functionally analysed for CD107a expression and IFNy production. Results are expressed as a percentage of control in the presence of IgG control for each cell line. (A-C) Data analysed by ANOVA—each experiment represents mean±S.E.M. of at least three independent experiments. $^{*}$ = $p<0.05$, $^{**}$ = $p<0.01$, $^{***}$ = $p<0.001$.
(TIF)

**S4 Fig. Quantifying expression and function of CD112 and CD155 ligands on tumour cell lines.** (A) Quantifying CD112 and CD155 ligands on tumour cell lines using fluorescent mAb and flow cytometry (B) Monoclonal antibodies against CD155 or CD112 were used to block NK cell targeting of tumour cell lines. NK cells were co-incubated with tumour cells in the presence or absence of tumour cells that were pre-treated with neutralising antibodies and degranulation and cytokine production was quantified. Results are expressed as a percentage increase or decrease of neutralised conditions compared with untreated cells. (C) 24 hour timepoint for NK reactivity. CD107a and IFN gamma quantification of NK cells that were incubated for 24 hours with either tumour cells alone or with cloaked tumour cells (A,B,C)

Data analysed by ANOVA—each experiment represents mean±S.E.M. of at least three independent experiments. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.
(TIF)

## Acknowledgments

We would like to thank Dr. Ciara Keane for assisting in the development of the NK cell functional assays.

## Author Contributions

**Conceptualization:** Cathy Spillane, Sharon A. O'Toole, Orla Sheils, Clair M. Gardiner, John J. O'Leary.

**Data curation:** Christopher D. Cluxton, Cathy Spillane.

**Formal analysis:** Christopher D. Cluxton, Cathy Spillane.

**Funding acquisition:** Orla Sheils, John J. O'Leary.

**Investigation:** Christopher D. Cluxton, Cathy Spillane, Clair M. Gardiner, John J. O'Leary.

**Methodology:** Christopher D. Cluxton, Cathy Spillane, Orla Sheils, Clair M. Gardiner, John J. O'Leary.

**Project administration:** Christopher D. Cluxton, Cathy Spillane, Sharon A. O'Toole, Orla Sheils, Clair M. Gardiner, John J. O'Leary.

**Resources:** Sharon A. O'Toole, Orla Sheils, Clair M. Gardiner, John J. O'Leary.

**Supervision:** Cathy Spillane, Sharon A. O'Toole, Orla Sheils, Clair M. Gardiner, John J. O'Leary.

**Validation:** Christopher D. Cluxton.

**Visualization:** Christopher D. Cluxton.

**Writing – original draft:** Christopher D. Cluxton.

**Writing – review & editing:** Christopher D. Cluxton, Cathy Spillane, Sharon A. O'Toole, John J. O'Leary.

## References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100(1):57–70. PMID: 10647931
2. Egan K, Cooke N, Kenny D. Living in shear: platelets protect cancer cells from shear induced damage. Clin Exp Metastasis. 2014; 31(6):697–704. https://doi.org/10.1007/s10585-014-9660-7 PMID: 24942131
3. Leblanc R, Peyruchaud O. Metastasis: new functional implications of platelets and megakaryocytes. Blood. 2016; 128(1):24–31. https://doi.org/10.1182/blood-2016-01-636399 PMID: 27154188
4. Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell. 2014; 158(5):1110–22. https://doi.org/10.1016/j.cell.2014.07.013 PMID: 25171411
5. Vivier E, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting natural killer cells and natural killer T cells in cancer. Nat Rev Immunol. 2012; 12(4):239–52. https://doi.org/10.1038/nri3174 PMID: 22437937
6. Placke T, Örgel M, Schaller M, Jung G, Rammensee HG, Kopp HG, et al. Platelet-derived MHC class I confers a pseudonormal phenotype to cancer cells that subverts the antitumor reactivity of natural killer immune cells. Cancer Res. 2012; 72(2):440–8. https://doi.org/10.1158/0008-5472.CAN-11-1872 PMID: 22127925

7. Kopp HG, Placke T, Salih HR. Platelet-derived transforming growth factor-beta down-regulates NKG2D thereby inhibiting natural killer cell antitumor reactivity. Cancer Res. 2009; 69(19):7775–83. https://doi.org/10.1158/0008-5472.CAN-09-2123 PMID: 19738039
8. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011; 331(6013):44–9. https://doi.org/10.1126/science.1198687 PMID: 21212348
9. Gasser S, Raulet DH. Activation and self-tolerance of natural killer cells. Immunol Rev. 2006; 214:130–42. https://doi.org/10.1111/j.1600-065X.2006.00460.x PMID: 17100881
10. Vahlne G, Becker S, Brodin P, Johansson MH. IFN-gamma production and degranulation are differentially regulated in response to stimulation in murine natural killer cells. Scand J Immunol. 2008; 67(1):1–11. https://doi.org/10.1111/j.1365-3083.2007.02026.x PMID: 18028287
11. Egan K, Crowley D, Smyth P, O'Toole S, Spillane C, Martin C, et al. Platelet adhesion and degranulation induce pro-survival and pro-angiogenic signalling in ovarian cancer cells. PLoS One. 2011; 6(10):e26125. https://doi.org/10.1371/journal.pone.0026125 PMID: 22022533
12. Smyth T, Paraiso KHT, Hearn K, Rodriguez-Lopez AM, Munck JM, Haarberg HE, et al. Inhibition of HSP90 by AT13387 delays the emergence of resistance to BRAF inhibitors and overcomes resistance to dual BRAF and MEK inhibition in melanoma models. Mol Cancer Ther. 2014; 13(12):2793–804. https://doi.org/10.1158/1535-7163.MCT-14-0452 PMID: 25349308
13. Fraietta JA, Beckwith KA, Patel PR, Ruella M, Zheng Z, Barrett DM, et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. Blood. 2016; 127(9):1117–27. https://doi.org/10.1182/blood-2015-11-679134 PMID: 26813675
14. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science. 2013; 339(6119):580–4. https://doi.org/10.1126/science.1228522 PMID: 23372014
15. Amo L, Tamayo-Orbegozo E, Maruri N, Eguizabal C, Zenarruzabeitia O, Riñón M, et al. Involvement of platelet-tumor cell interaction in immune evasion. Potential role of podocalyxin-like protein 1. Front Oncol. 2014; 4:245.
16. Maurer S, Kropp KN, Klein G, Steinle A, Haen SP, Walz JS, et al. Platelet-mediated shedding of NKG2D ligands impairs NK cell immune-surveillance of tumor cells. Oncoimmunology. 2018; 7(2):e1364827. https://doi.org/10.1080/2162402X.2017.1364827 PMID: 29308299
17. Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, Ludwig A, et al. Tumor-associated MICA is shed by ADAM proteases. Cancer Res. 2008; 68(15):6368–76. https://doi.org/10.1158/0008-5472.CAN-07-6768 PMID: 18676862
18. Li JJ, Pan K, Gu MF, Chen MS, Zhao JJ, Wang H, et al. Prognostic value of soluble MICA levels in the serum of patients with advanced hepatocellular carcinoma. Chin J Cancer. 2013; 32(3):141–8. https://doi.org/10.5732/cjc.012.10025 PMID: 22704489
19. Tamaki S, Kawakami M, Ishitani A, Kawashima W, Kasuda S, Yamanaka Y, et al. Soluble MICB serum levels correlate with disease stage and survival rate in patients with oral squamous cell carcinoma. Anticancer Res. 2010; 30(10):4097–101. PMID: 21036725
20. Chen J, Xu H, Zhu XX. Abnormal expression levels of sMICA and NKG2D are correlated with poor prognosis in pancreatic cancer. Ther Clin Risk Manag. 2016; 12:11–8. https://doi.org/10.2147/TCRM.S96869 PMID: 26730197
21. Chan CJ, Martinet L, Gilfillan S, Souza-Fonseca-Guimaraes F, Chow MT, Town L, et al. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. Nat Immunol. 2014; 15(5):431–8. https://doi.org/10.1038/ni.2850 PMID: 24658051
22. Pauken KE, Wherry EJ. TIGIT and CD226: tipping the balance between costimulatory and coinhibitory molecules to augment the cancer immunotherapy toolkit. Cancer Cell. 2014; 26(6):785–7. https://doi.org/10.1016/j.ccell.2014.11.016 PMID: 25490444
23. Pende D, Bottino C, Castriconi R, Cantoni C, Marcenaro S, Rivera P, et al. PVR (CD155) and Nectin-2 (CD112) as ligands of the human DNAM-1 (CD226) activating receptor: involvement in tumor cell lysis. Mol Immunol. 2005; 42(4):463–9. https://doi.org/10.1016/j.molimm.2004.07.028 PMID: 15607800
24. Carlsten M, Björkström NK, Norell H, Bryceson Y, van Hall T, Baumann BC, et al. DNAX accessory molecule-1 mediated recognition of freshly isolated ovarian carcinoma by resting natural killer cells. Cancer Res. 2007; 67(3):1317–25. https://doi.org/10.1158/0008-5472.CAN-06-2264 PMID: 17283169
25. Fuchs A, Cella M, Giurisato E, Shaw AS, Colonna M. Cutting edge: CD96 (tactile) promotes NK cell-target cell adhesion by interacting with the poliovirus receptor (CD155). J Immunol. 2004; 172(7):3994–8. PMID: 15034010

26. Peng YP, Xi CH, Zhu Y, Yin LD, Wei JS, Zhang JJ, et al. Altered expression of CD226 and CD96 on natural killer cells in patients with pancreatic cancer. Oncotarget. 2016; 7(41):66586–94. https://doi.org/10.18632/oncotarget.11953 PMID: 27626490

27. Ascierto ML, Idowu MO, Zhao Y, Khalak H, Payne KK, Wang XY, et al. Molecular signatures mostly associated with NK cells are predictive of relapse free survival in breast cancer patients. J Transl Med. 2013; 11:145. https://doi.org/10.1186/1479-5876-11-145 PMID: 23758773

28. Gras Navarro A, Björklund AT, Chekenya M. Therapeutic potential and challenges of natural killer cells in treatment of solid tumors. Front Immunol. 2015; 6:202. https://doi.org/10.3389/fimmu.2015.00202 PMID: 25972872

29. Li T, Yang Y, Hua X, Wang G, Liu W, Jia C, et al. Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. Cancer Lett. 2012; 318(2):154–61. https://doi.org/10.1016/j.canlet.2011.12.020 PMID: 22182446

# CURICULUM VITAE

## PERSONAL INFORMATION

Name Orla Sheils

**Researcher unique identifier** (e.g. ORCID, Research ID, etc.) 0000-0002-4493-9496

Date of birth: 18/03/65

Nationality: Irish

Website URL: http://people.tcd.ie/osheils

## EDUCATION

| | |
|---|---|
| 07/00 | PhD- Dept of Histopathology, School of Medicine, Trinity College Dublin, |
| | PhD supervisor – Prof Eamonn Sweeney |
| 10/06 | Masters - Centre for Medical Ethics & Law/Dept of Law, |
| | Kings College London, UK |

## CURRENT OR MOST RECENT POSITION

| | |
|---|---|
| 09/22 | Vice Provost/Chief Academic Officer, Trinity College Dublin |

## PREVIOUS POSITION(S)

| | |
|---|---|
| 04/19 | Dean of Faculty of Health Sciences, Trinity College Dublin |
| 10/17 | Professor of Molecular Diagnostics |
| | Faculty of Health Sciences, School of Medicine, Discipline of Histopathology, TCD |
| 02/16 - 14/19 | Director Trinity Translational Medicine Institute |
| 10/11 -10/17 | Professor in Molecular Pathology |
| | Faculty of Health Sciences, School of Medicine, Discipline of Histopathology, TCD, Ireland |

## SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS

Successfully completed theses:

School of Medicine Trinity College Dublin:

| MD | PhD | Masters | Post-Doctoral Fellows |
|---|---|---|---|
| 12 | 28 | 22 | 15 |

## TEACHING ACTIVITIES

Professor of Molecular Diagnostics and Director of Medical Ethics School of Medicine, TCD

**Courses**-

UG Medicine (modules over 4 years)

PG Taught (MSc) Modules in MSc in Molecular Medicine, Translational Oncology, Bio-Engineering, Pharm Med, Medicine, Global Brain Health Institute

## INSTITUTIONAL RESPONSIBILITIES

| **Details** | **Capacity** | **Date From** | **Date To** |
|---|---|---|---|
| Vice-Provost-/Chief Academic Officer | VP | 09/2021 | present |
| Board | VP | 09/2021 | present |
| Dean – Faculty of Health Sciences | Dean/ VP | 04/2019 | present |
| Finance Committee | Member | 2019 | present |
| Council | Member | 2019 | present |
| Quality Committee | Chair | 2021 | present |
| Marino Institute of Education Governing Body | Member | 2021 | present |

| | | | |
|---|---|---|---|
| Dublin Dental University Hospital Board | Member | 2019 | present |
| DDUH – Strategic Oversight Committee | Member | 2019 | present |
| Trinity Translational Medicine Institute | Director | 2016 | 2019 |
| Trinity/St James's Liaison Group | College representative | 2017 | present |
| LERU- LEAR Policy Group | Steering Committee Member | 2021 | present |
| LERU -Biomedicine / Life Sciences (BIOM) Policy Group | Member | 2017 | 2021 |
| School of Medicine Curriculum Committee | Member | 2003 | 2019 |
| TCD Research Ethics Policy Group | Chair | 2012 | 2019 |
| National CoVid-19 Clinical REC | Member | 2020 | present |
| SJH/Tallaght Research Ethics Committee | Member (Deputy Chair) | 2012 | present |
| Chairperson of Faculty of Health Science (TCD) Research Ethics Committee | Chairperson | 2004 | 2012 |
| Director of Postgraduate Teaching & Learning | School of Medicine | 2012 | 2015 |
| College Working Group on Plagiarism | Member | 2012 | 2015 |
| Post Graduate Advisor | School of Medicine | 2015 | 2020 |
| Director of Medical Ethics | School of Medicine | 2007 | present |
| Acting Head of Discipline (during periods of illness/ extended leave) | Histopathology & Morbid Anatomy | 2010 | 2019 |
| Cancer Strategy Planning Group (St James's Hospital) | Member | 2016 | present |
| Patient Ethics Group (St James's Hospital) | Member | 2016 | 2019 |

| | | | |
|---|---|---|---|
| School of Medicine First Academic Court and Court of Appeal (Alternate years) | Chairperson | 2009 | 2018 |
| College Tutor | | 2010 | 2016 |
| Selected Steering Committees<br>1. EngAGE<br>2. Cancer institute | Member | 2013 | present |

## RECENT FUNDED GRANTS:

**>€35M total grant income.**

**Recent grants:**

| Funding Body Name | Grant Type/Reference number | Year of award | Amount of award to recipient |
|---|---|---|---|
| SFI | Multi-site study to develop a SARS-CoV-2 Infection Surveillance System for Third Level Students and Staff in Republic of Ireland (UniCoV). | 2021 | €500,000 |
| SFI | funded Investigator -Covid related research- to develop and implement a self-collected saliva sample for detection of SARS CoV-2 using RT-LAMP in student populations. | 2020 | €100,000 |
| SFI | Co- PI, (with Kingston Mills; joint bid with TTMI & TBSI). Next generation flow cytometry and single cell gene analysis, SFI Research Infrastructure award, | 2019 | €628,705.00 |

| | | | |
|---|---|---|---|
| Enterprise Ireland | IP20170616 | 2017 | €691,486 |
| Thermo Fisher | Industry grant – develop Oncomine Diagnostic NGS panel for solid tumor diagnostics | 2013 -2018 | €250,000 |
| Becton Dickinson | BD-Multi-omics Alliance | 2019 | €250,000 |

## EVENT ORGANISATION

Primary organiser for 20 conferences (National & International) (>100 registrants) in Ireland, UK and Europe. Symposium organiser – Europe and US.

## COMMISSIONS OF TRUST

| **Details** | **Date** |
|---|---|
| Member - National Medical Leaders Forum – Sub-group NPHET | 2020-21 |
| Member National REC -Covid | 2020-21 |
| Deputy Chair – National REC Clinical trials | 2021-22 |
| Irish Universities Association – Member of Registrars Group | 2021-present |
| Central Applications Office (CAO) - Director | 2021-present |
| CAO Audit Committee | 2022-present |
| Member of the Advisory Committee -H2020 Societal Challenge 1, Health & Demographic Change. European Commission | 2013-2017 |
| Key Opinion Leader and Scientific Advisor – Becton Dickinson | 2018-present |

| | |
|---|---|
| Board Member - European Institute of Women's Health | 2017-2022 |
| Associate Editor Irish Journal of Medical Science | 2017 |
| National workgroup on Bio-banking | 2012/17 |
| Member, CRDI Cancer Principal Investigators, Trans-institutional grouping of Principal Investigators leading research on Cancer themes | 2010/18 |
| Principal Investigator on ICAT clinician scientist training programme | |
| Associate Editor BMC Cancer | 2010/17 |
| Member, Core Technology PI's, DMMC Principal Investigators leading the development of core technology platforms. | 2006/18 |
| Wellcome Trust - Reviewer | 2005/18 |
| Reviewer for South African Medical Research Council | 2004/18 |
| Scientific Advisor - advisory capacity for ThermoFisher - planning and development of molecular diagnostic tools. | 2010-present |
| Reviewer for Hong Kong Earmarked Research Grants [Research Grants Council] | 2005 - present |
| Member of Editorial Board of Journal of Endocrine Pathology | 2009 - present |
| Advisor to the National Council with expertise in Medical Teaching – USI Academic Affairs Advisory Panel. | 2015/18 |
| Reviewer for journals including Oncogene, Nature Biotechnology, Nature Medicine, Cancer, Journal of Clinical Endocrinology and Metabolism, Molecular Cancer, Molecular Endocrinology, Journal of Pathology, Modern Pathology, International Journal of Surgical Pathology, DU Law Review | 2003 - present |

## PROFESSIONAL MEMBERSHIPS

2001 – present Member of the Pathological Society of Great Britain and Ireland

2007 – present FRCPath

2006 – present Member of Medico-legal Society of Ireland

2016- present Fellow Irish Academy of Biomedical Sciences

## DISTINCTIONS & AWARDS

- >80 international invited lectures,
- Provost's Teaching Award 2012
- FTCD – 2009
- President 134th session of the Biological Society – Dublin University – 2008
- Procured Status of Applied Biosystems European Reference Laboratory – 2004
- Young Researcher Award – EU High Level Scientific Conference – 2000

## EXAMPLES OF SOME INVITED LECTURES:

The list below is indicative of invited presentations. It is not exhaustive and is intended to convey a sense of the international relevance of my research outputs over a sustained period of time.

- IBC **USA International Microtechnology Conference** 'Chips to hits', Boston, MA, USA 2005 – Transcriptomic Profiling in Thyroid Cancer
- **American Society of Human Genetics** - Salt Lake City, UT, USA, 2005, – Applications for Transcriptomic Profiling in cancer
- **3rd Conference of the Consortium for Post-Genome Science** 'Genomes to Systems' - Manchester, UK, 2006 - Activating Pathways in Thyroid Neoplasia
- **European Association for Cancer Research** – Budapest, HU, 2006, -Advances in TaqMan Chemistries and Applications in Diagnostic Laboratories
- **International Congress of Human Genetics-** Brisbane, AU, 2006 – Translational Molecular Pathology

- **European Congress of Cytology**, Venice, IT, 2006 [Molecular Classification and Biomarkers in Thyroid Cancer]
- **Société Belge de Cytologie Clinique**, Brussels. 10th December 2011 – Mol Path thyroid
- **AACR** 2012, Chicago Illanois - Quantifying KRAS alterations with TaqMan™ Mutation Detection Assays on Open Array Platform
- **International qPCR symposium** - Amsterdam 2011 - RAS gene mutation detection in colorectal cancer
- **30th German Cancer Congress** – Berlin 2012- New technologies applied to critical pathways of anti-EGFR therapy resistance in colorectal cancer
- **Anatomical Society of Great Britain & Ireland** Meeting 2013- Ethical Issues regarding Consent
- **Ion World** -2015 Dublin HPV prevalence and genotypes among a cohort of HIV positive MSM
- **International Health Conference,** Tbilisi, Georgia, 2016 -The emerging roles of Liquid Biopsy & Circulating Tumour Cells in monitoring the Metastatic Cascade
- **European Institute of Women's Health** - Brussels, December 2017 - How precise is Precision Medicine?
- **Trinity Alumni Weekend** 2017 - Liquid Biopsy – Moving the goalposts in Cancer Diagnostics
- **Ion World 2017** – Amsterdam - Clinical Solutions – Can we make Cancer a Chronic Disease?
- **ThermoFisher (BID Marketing)** Gothenburg 2019- A new World of Research tools
- **NUIG Curam Conference** -2015 Circulating Tumour Cells, Metastasis and Evasion of Immune Surveillance
- **Single Cell Sympoisium (Fluidigm) -Madrid 2016** - Nanofluidic PCR solutions to facilitate the elucidation of the mRNA targets of miR222 and miR-25 in thyroid disease.
- **Diagnostics-4-Future Conference -** Lake Constanz -2018 - Diagnosing Cancer-Challenges and Progress
- ***U*K BD single-cell Multi-Omics** user group meeting - 2019 Winnersh, UK

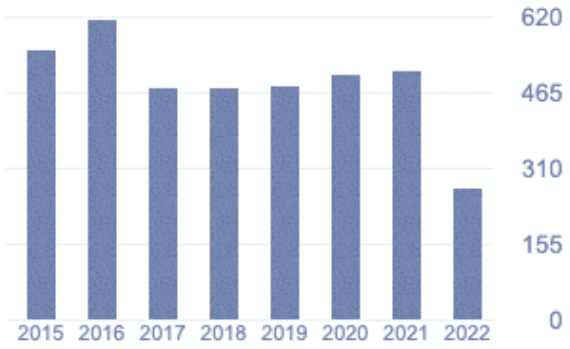
## APPLIED RESEARCH ACTIVITY-COMMERCIALISATION OF RESEARCH OUTPUTS

- micro RNA profiling in cervical cancer [2013] – pending;
- Autoantibody profiling in early ovarian cancer [2013] – pending.
- European Patent Application No. 16188421.8 (PL Ref: P11964EP00; TCD Ref: JO04-586 and JO04-587) – Inventions related to the use of CD112 and TIGIT that have potential as a diagnostic target and poor-prognostic biomarker for metastatic cancer.
- Spin out company – UniGenetics (2000-2003)
-

## RESEARCH INDICES

**Source- Google Scholar**

| Cited by | | VIEW ALL |
|---|---|---|
| | All | Since 2017 |
| Citations | 7782 | 2716 |
| h-index | 45 | 29 |
| i10-index | 110 | 69 |




## PUBLICATION RECORD

| Total Publications:<br><br>#>500 | Senior Author Publications:<br><br>#112 | | | | |
|---|---|---|---|---|---|
| Journal Articles: | Reviews: | Book Chapters: | Books: | Conference associated publications: | Other: |
| #135 | #15 | #13 | #2 | #450 | #5 |

## JOURNAL ARTICLES

1. HSa_circ_0001275 Is One of a Number of circRNAs Dysregulated in Enzalutamide Resistant Prostate Cancer and Confers Enzalutamide Resistance In Vitro.

   Lim MCJ, Baird AM, Greene J, McNevin C, Ronan K, Podlesniy P, Sheils O, Gray SG, McDermott RS, Finn SP
   Cancers (Basel), 13(24):6383, 20 Dec 2021

   Cited by: 0 articles | PMID: 34945002 | PMCID: PMC8715667

2. PATHOGENIC BRCA VARIANTS AS BIOMARKERS FOR RISK IN PROSTATE CANCER.

   McNevin CS, Cadoo K, Baird AM, Murchan P, Sheils O, McDermott R, Finn S
   Cancers (Basel), 13(22):5697, 14 Nov 2021

   Cited by: 0 articles | PMID: 34830851 | PMCID: PMC8616097

3. DEVELOPING A HEALTH PROMOTING UNIVERSITY IN TRINITY COLLEGE DUBLIN-OVERVIEW AND OUTLINE PROCESS EVALUATION.

   Darker CD, Mullin M, Doyle L, Tanner M, McGrath D, Doherty L, Dreyer-Gibney K, Barrett EM, Flynn D, Murphy P, Ivers JH, Burke E, Ryan M, McCarron M, Murphy P, Sheils O, Hevey D, Leen A, Keogh L, [...] Barry JM
   Health Promot Int, daab180, 05 Nov 2021

   Cited by: 0 articles | PMID: 34738107

4. THE INDUCTION OF A MESENCHYMAL PHENOTYPE BY PLATELET CLOAKING OF CANCER CELLS IS A UNIVERSAL PHENOMENON.

   Spillane CD, Cooke NM, Ward MP, Kenny D, Blackshields G, Kelly T, Bates M, Huang Y, Martin C, Skehan S, Canney A, Gallagher M, Smyth P, Brady N, Clarke A, Mohamed B, Norris L, Brooks DA, Brooks RD, [...] O'Leary JJ
   Transl Oncol, 14(12):101229, 27 Sep 2021

   Cited by: 0 articles | PMID: 34592589 | PMCID: PMC8488306

5. CIRCULATING TUMOUR CELL NUMBERS CORRELATE WITH PLATELET COUNT AND CIRCULATING LYMPHOCYTE SUBSETS IN MEN WITH ADVANCED PROSTATE CANCER: DATA FROM THE EXPECT CLINICAL TRIAL (CTRIAL-IE 15-21).

   Hayes B, Brady L, Sheill G, Baird AM, Guinan E, Stanfill B, Dunne J, Holden D, Vlajnic T, Casey O, Murphy V, Greene J, Allott EH, Hussey J, Cahill F, Van Hemelrijck M, Peat N, Mucci LA, Cunningham M, [...] Finn S
   Cancers (Basel), 13(18):4690, 18 Sep 2021

   Cited by: 1 article | PMID: 34572916 | PMCID: PMC8466183

6. CORRELATION OF INTEGRATED ERG/PTEN ASSESSMENT WITH BIOCHEMICAL RECURRENCE IN PROSTATE CANCER.

   Brady L, Carlsson J, Baird AM, Casey O, Vlajnic T, Murchan P, Cormican D, Costigan D, Gray S, Sheils O, O'Neill A, Watson RW, Andren O, Finn S
   Cancer Treat Res Commun, 29:100451, 02 Sep 2021

   Cited by: 0 articles | PMID: 34507017

7. DEEP LEARNING OF HISTOPATHOLOGICAL FEATURES FOR THE PREDICTION OF TUMOUR MOLECULAR GENETICS.

   Murchan P, Ó'Brien C, O'Connell S, McNevin CS, Baird AM, Sheils O, Ó Broin P, Finn SP
   Diagnostics (Basel), 11(8):1406, 03 Aug 2021

   Cited by: 2 articles | PMID: 34441338 | PMCID: PMC8393642

8. THE ROLE OF HUMAN PAPILLOMA VIRUS IN DICTATING OUTCOMES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA.

   Brennan S, Baird AM, O'Regan E, Sheils O
   Front Mol Biosci, 8:677900, 23 Jun 2021

   Cited by: 2 articles | PMID: 34250016 | PMCID: PMC8262095

9. DIFFERENTIAL CIRCRNA EXPRESSION SIGNATURES MAY SERVE AS POTENTIAL NOVEL BIOMARKERS IN PROSTATE CANCER.

   Greene J, Baird AM, Lim M, Flynn J, McNevin C, Brady L, Sheils O, Gray SG, McDermott R, Finn SP
   Front Cell Dev Biol, 9:605686, 25 Feb 2021

Cited by: 4 articles | PMID: 33718350 | PMCID: PMC7946979

10. THE ROLE OF CANCER STEM CELLS IN DRUG RESISTANCE IN GASTROESOPHAGEAL JUNCTION ADENOCARCINOMA.

Dinneen K, Baird AM, Ryan C, Sheils O
Front Mol Biosci, 8:600373, 08 Feb 2021

Cited by: 0 articles | PMID: 33628765 | PMCID: PMC7897661

11. EPIGENETIC MODIFIER UHRF1 MAY BE A POTENTIAL TARGET IN MALIGNANT PLEURAL MESOTHELIOMA.

Baird AM, Finn SP, Gray SG, Sheils O
J Thorac Oncol, 16(1):14-16, 01 Jan 2021

Cited by: 0 articles | PMID: 33384056

12. PLATELET CLOAKING OF CIRCULATING TUMOUR CELLS IN PATIENTS WITH METASTATIC PROSTATE CANCER: RESULTS FROM EXPECT, A RANDOMISED CONTROLLED TRIAL.

Brady L, Hayes B, Sheill G, Baird AM, Guinan E, Stanfill B, Vlajnic T, Casey O, Murphy V, Greene J, Allott EH, Hussey J, Cahill F, Van Hemelrijck M, Peat N, Mucci L, Cunningham M, Grogan L, Lynch T, [...] Finn S
PLoS One, 15(12):e0243928, 18 Dec 2020

Cited by: 3 articles | PMID: 33338056 | PMCID: PMC7748139

13. THE FEASIBILITY OF IMPLEMENTING AN EXERCISE PROGRAMME FOR DECONDITIONED CANCER SURVIVORS IN A NATIONAL CANCER CENTRE: FIXCAS STUDY

Devenney K, Murphy N, Ryan R, Grant C, Kennedy J, Manecksha RP, Sheils O, McNeely ML, Hussey J, Sheill G
HRB Open Res, 18 Dec 2020

Cited by: 0 articles | PPR: PPR254842

14. PROSTATE CANCER-DERIVED HOLOCLONES: A NOVEL AND EFFECTIVE MODEL FOR EVALUATING CANCER STEMNESS.

Flynn L, Barr MP, Baird AM, Smyth P, Casey OM, Blackshields G, Greene J, Pennington SR, Hams E, Fallon PG, O'Leary J, Sheils O, Finn SP
Sci Rep, 10(1):11329, 09 Jul 2020

Cited by: 3 articles | PMID: 32647229 | PMCID: PMC7347552

15. ANAL CANCER IN PEOPLE LIVING WITH HIV: A CASE SERIES.

Sadlier C, Lynam A, Kerr C, Sheils O, Bergin C
Int J STD AIDS, 31(1):82-84, 16 Dec 2019

Cited by: 0 articles | PMID: 31842694

16. THE FEASIBILITY OF IMPLEMENTING AN EXERCISE PROGRAMME FOR DECONDITIONED CANCER SURVIVORS IN A NATIONAL CANCER CENTRE: FIXCAS STUDY.

Devenney K, Murphy N, Ryan R, Grant C, Kennedy J, Manecksha RP, Sheils O, McNeely ML, Hussey J, Sheill G
HRB Open Res, 2:24, 30 Sep 2019

Cited by: 0 articles | PMID: 33870087 | PMCID: PMC8030104

17. SUPPRESSION OF NATURAL KILLER CELL NKG2D AND CD226 ANTI-TUMOUR CASCADES BY PLATELET CLOAKED CANCER CELLS: IMPLICATIONS FOR THE METASTATIC CASCADE.

Cluxton CD, Spillane C, O'Toole SA, Sheils O, Gardiner CM, O'Leary JJ
PLoS One, 14(3):e0211538, 25 Mar 2019

Cited by: 19 articles | PMID: 30908480 | PMCID: PMC6433214

18. POTENTIALLY IMPORTANT MIRNAS IN ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA PATHOGENESIS: A PILOT STUDY.

Clarke L, Adduri RS, Smyth P, Quinn F, Jeffers M, Dunne B, O'Leary J, McKiernan S, Vandenberghe E, Pyne S, Bashyam MD, Sheils O, Flavin R
Leuk Res Rep, 10:52-54, 16 Oct 2018

Cited by: 0 articles | PMID: 30989051 | PMCID: PMC6446659

19. SIMULTANEOUS DETECTION OF LUNG FUSIONS USING A MULTIPLEX RT-PCR NEXT GENERATION SEQUENCING-BASED APPROACH: A MULTI-INSTITUTIONAL RESEARCH STUDY.

Vaughn CP, Costa JL, Feilotter HE, Petraroli R, Bagai V, Rachiglio AM, Marino FZ, Tops B, Kurth HM, Sakai K, Mafficini A, Bastien RRL, Reiman A, Le Corre D, Boag A, Crocker S, Bihl M, Hirschmann A, Scarpa A, [...] Laurent-Puig P
BMC Cancer, 18(1):828, 16 Aug 2018

Cited by: 6 articles | PMID: 30115026 | PMCID: PMC6097211

20. INTEGRATING BIOMARKERS ACROSS OMIC PLATFORMS: AN APPROACH TO IMPROVE STRATIFICATION OF PATIENTS WITH INDOLENT AND AGGRESSIVE PROSTATE CANCER.

Murphy K, Murphy BT, Boyce S, Flynn L, Gilgunn S, O'Rourke CJ, Rooney C, Stöckmann H, Walsh AL, Finn S, O'Kennedy RJ, O'Leary J, Pennington SR, Perry AS, Rudd PM, Saldova R, Sheils O, Shields DC, Watson RW
Mol Oncol, 12(9):1513-1525, 07 Aug 2018

Cited by: 16 articles | PMID: 29927052 | PMCID: PMC6120220

21. DIAGNOSIS, MONITORING AND PREVENTION OF EXPOSURE-RELATED NON-COMMUNICABLE DISEASES IN THE LIVING AND WORKING ENVIRONMENT: DIMOPEX-PROJECT IS DESIGNED TO DETERMINE THE IMPACTS OF ENVIRONMENTAL EXPOSURE ON HUMAN HEALTH.

Budnik LT, Adam B, Albin M, Banelli B, Baur X, Belpoggi F, Bolognesi C, Broberg K, Gustavsson P, Göen T, Fischer A, Jarosinska D, Manservisi F, O'Kennedy R, Øvrevik J, Paunovic E, Ritz B, Scheepers PTJ, Schlünssen V, [...] Casteleyn L
J Occup Med Toxicol, 13:6, 05 Feb 2018

Cited by: 11 articles | PMID: 29441119 | PMCID: PMC5800006

22. A NOVEL ROLE FOR THE MACROPHAGE GALACTOSE-TYPE LECTIN RECEPTOR IN MEDIATING VON WILLEBRAND FACTOR CLEARANCE.

Ward SE, O'Sullivan JM, Drakeford C, Aguila S, Jondle CN, Sharma J, Fallon PG, Brophy TM, Preston RJS, Smyth P, Sheils O, Chion A, O'Donnell JS
Blood, 131(8):911-916, 27 Dec 2017

Cited by: 19 articles | PMID: 29282218

23. MIR-223 POTENTIALLY TARGETS SWI/SNF COMPLEX PROTEIN SMARCD1 IN ATYPICAL PROLIFERATIVE SEROUS TUMOR AND HIGH-GRADE OVARIAN SEROUS CARCINOMA.

Arts FA, Keogh L, Smyth P, O'Toole S, Ta R, Gleeson N, O'Leary JJ, Flavin R, Sheils O
Hum Pathol, 70:98-104, 24 Oct 2017

Cited by: 7 articles | PMID: 29079174

24. THE EVIDENCE BASE FOR CIRCULATING TUMOUR DNA BLOOD-BASED BIOMARKERS FOR THE EARLY DETECTION OF CANCER: A SYSTEMATIC MAPPING REVIEW.

Cree IA, Uttley L, Buckley Woods H, Kikuchi H, Reiman A, Harnan S, Whiteman BL, Philips ST, Messenger M, Cox A, Teare D, Sheils O, Shaw J, UK Early Cancer Detection Consortium
BMC Cancer, 17(1):697, 23 Oct 2017

Cited by: 44 articles | PMID: 29061138 | PMCID: PMC5654013

25. THE EXPECT (EXAMINING EXERCISE, PROSTATE CANCER AND CIRCULATING TUMOUR CELLS) TRIAL: STUDY PROTOCOL FOR A RANDOMISED CONTROLLED TRIAL.

Sheill G, Brady L, Guinan E, Hayes B, Casey O, Greene J, Vlajnic T, Cahill F, Van Hemelrijck M, Peat N, Rudman S, Hussey J, Cunningham M, Grogan L, Lynch T, Manecksha RP, McCaffrey J, Mucci L, Sheils O, [...] Finn S
Trials, 18(1):456, 04 Oct 2017

Cited by: 4 articles | PMID: 28978344 | PMCID: PMC5628461

26. PERIPHERAL BLOOD MICRORNA AND VEGFA MRNA CHANGES FOLLOWING ELECTROCONVULSIVE THERAPY: IMPLICATIONS FOR PSYCHOTIC DEPRESSION.

Kolshus E, Ryan KM, Blackshields G, Smyth P, Sheils O, McLoughlin DM
Acta Psychiatr Scand, 136(6):594-606, 04 Oct 2017

Cited by: 12 articles | PMID: 28975998

27. N-LINKED GLYCAN TRUNCATION CAUSES ENHANCED CLEARANCE OF PLASMA-DERIVED VON WILLEBRAND FACTOR.

O'Sullivan JM, Aguila S, McRae E, Ward SE, Rawley O, Fallon PG, Brophy TM, Preston RJ, Brady L, Sheils O, Chion A, O'Donnell JS
J Thromb Haemost, 14(12):2446-2457, 09 Dec 2016

Cited by: 15 articles | PMID: 27732771

28. N-LINKED GLYCANS WITHIN THE A2 DOMAIN OF VON WILLEBRAND FACTOR MODULATE MACROPHAGE-MEDIATED CLEARANCE.

Chion A, O'Sullivan JM, Drakeford C, Bergsson G, Dalton N, Aguila S, Ward S, Fallon PG, Brophy TM, Preston RJ, Brady L, Sheils O, Laffan M, McKinnon TA, O'Donnell JS
Blood, 128(15):1959-1968, 23 Aug 2016

Cited by: 13 articles | PMID: 27554083

29. ALTERED EXPRESSION OF MIR-222 AND MIR-25 INFLUENCES DIVERSE GENE EXPRESSION CHANGES IN TRANSFORMED NORMAL AND ANAPLASTIC THYROID CELLS, AND IMPACTS ON MEK AND TRAIL PROTEIN EXPRESSION.

Aherne ST, Smyth P, Freeley M, Smith L, Spillane C, O'Leary J, Sheils O
Int J Mol Med, 38(2):433-445, 22 Jun 2016

Cited by: 9 articles | PMID: 27353001 | PMCID: PMC4935456

30. HPV VACCINE ACCEPTABILITY IN HIV-INFECTED AND HIV NEGATIVE MEN WHO HAVE SEX WITH MEN (MSM) IN IRELAND.

Sadlier C, Lynam A, O'Dea S, Delamere S, Quinlan M, Clarke S, Sheils O, Bergin C
Hum Vaccin Immunother, 12(6):1536-1541, 06 May 2016

Cited by: 8 articles | PMID: 27153289 | PMCID: PMC4964722

31. RESPONSE TO LETTER: LIMITATIONS OF HUMAN PAPILLOMAVIRUS DNA TESTING IN MEASURING PREVIOUS EXPOSURE AND VACCINE PROTECTION.

Sadlier C, Sheils O, Bergin C, HPV MAPS research group (Human Papilloma Virus in Men, Awareness, Prevention Surveillance)
HIV Med, 17(7):557-558, 09 Feb 2016

Cited by: 0 articles | PMID: 26857554

32. PLURIPOTENCY MARKERS ARE DIFFERENTIALLY INDUCED BY MEK INHIBITION IN THYROID AND MELANOMA BRAFV600E CELL LINES.

Dorris ER, Blackshields G, Sommerville G, Alhashemi M, Dias A, McEneaney V, Smyth P, O'Leary JJ, Sheils O
Cancer Biol Ther, 17(5):526-542, 01 Feb 2016

Cited by: 5 articles | PMID: 26828826 | PMCID: PMC4910922

33. INTERLEUKIN-15 IS ASSOCIATED WITH DISEASE SEVERITY IN VIRAL BRONCHIOLITIS.

Leahy TR, McManus R, Doherty DG, Grealy R, Coulter T, Smyth P, Blackshields G, Sheils O, Carr MJ, Purandare N, Geary M, Hodemaekers HM, Janssen R, Bont L, Slattery D, Ryan T
Eur Respir J, 47(1):212-222, 05 Nov 2015

Cited by: 9 articles | PMID: 26541527

34. ASPIRIN AND P2Y12 INHIBITION ATTENUATE PLATELET-INDUCED OVARIAN CANCER CELL INVASION.

Cooke NM, Spillane CD, Sheils O, O'Leary J, Kenny D
BMC Cancer, 15:627, 09 Sep 2015

Cited by: 30 articles | PMID: 26353776 | PMCID: PMC4565001

35. IDENTIFYING NOVEL HYPOXIA-ASSOCIATED MARKERS OF CHEMORESISTANCE IN OVARIAN CANCER.

McEvoy LM, O'Toole SA, Spillane CD, Martin CM, Gallagher MF, Stordal B, Blackshields G, Sheils O, O'Leary JJ
BMC Cancer, 15:547, 25 Jul 2015

Cited by: 16 articles | PMID: 26205780 | PMCID: PMC4513971

36. ENHANCED REGULATION OF CELL CYCLE AND SUPPRESSION OF OSTEOBLAST DIFFERENTIATION MOLECULAR SIGNATURES BY PROSTATE CANCER STEM-LIKE HOLOCLONES.

Gallagher MF, Salley Y, Spillane CD, Ffrench B, El Baruni S, Blacksheilds G, Smyth P, Martin C, Sheils O, Watson W, O'Leary JJ
J Clin Pathol, 68(9):692-702, 02 Jun 2015

Cited by: 3 articles | PMID: 26038242

37. LIGATION OF TLR7 ON CD19(+) CD1D(HI) B CELLS SUPPRESSES ALLERGIC LUNG INFLAMMATION VIA REGULATORY T CELLS.

Khan AR, Amu S, Saunders SP, Hams E, Blackshields G, Leonard MO, Weaver CT, Sparwasser T, Sheils O, Fallon PG
Eur J Immunol, 45(6):1842-1854, 14 Apr 2015

Cited by: 18 articles | PMID: 25763771

38. DEVELOPMENT OF A SEMI-CONDUCTOR SEQUENCING-BASED PANEL FOR GENOTYPING OF COLON AND LUNG CANCER BY THE ONCONETWORK CONSORTIUM.

Tops BB, Normanno N, Kurth H, Amato E, Mafficini A, Rieber N, Le Corre D, Rachiglio AM, Reiman A, Sheils O, Noppen C, Lacroix L, Cree IA, Scarpa A, Ligtenberg MJ, Laurent-Puig P
BMC Cancer, 15:26, 31 Jan 2015

Cited by: 27 articles | PMID: 25637035 | PMCID: PMC4318366

39. RAPID AND FULLY AUTOMATED BACTERIAL PATHOGEN DETECTION ON A CENTRIFUGAL-MICROFLUIDIC LABDISK USING HIGHLY SENSITIVE NESTED PCR WITH INTEGRATED SAMPLE PREPARATION.

Czilwik G, Messinger T, Strohmeier O, Wadle S, von Stetten F, Paust N, Roth G, Zengerle R, Saarinen P, Niittymäki J, McAllister K, Sheils O, O'Leary J, Mark D
Lab Chip, 15(18):3749-3759, 01 Jan 2015

Cited by: 45 articles | PMID: 26235430

40. AN ASSOCIATION BETWEEN MICRORNA-21 EXPRESSION AND VITAMIN D DEFICIENCY IN CORONARY ARTERY DISEASE.

Sheane BJ, Smyth P, Scott K, Aziz R, Buckley M, Lodge E, Kiely N, Kingston M, McGovern E, Healy M, Walsh JB, Sheils O, Cunnane G
Microrna, 4(1):57-63, 01 Jan 2015

Cited by: 12 articles | PMID: 25882990

41. HUMAN PAPILLOMAVIRUS DNA AND MRNA PREVALENCE AND ASSOCIATION WITH CERVICAL CYTOLOGICAL ABNORMALITIES IN THE IRISH HIV POPULATION.

Loy A, McInerney J, Pilkington L, Keegan H, Delamere S, Martin CM, Sheils O, O'Leary JJ, Mulcahy F
Int J STD AIDS, 26(11):789-795, 25 Sep 2014

Cited by: 0 articles | PMID: 25258395

42. AN INTEGRATED ANALYSIS OF THE SOX2 MICRORNA RESPONSE PROGRAM IN HUMAN PLURIPOTENT AND NULLIPOTENT STEM CELL LINES.

Vencken SF, Sethupathy P, Blackshields G, Spillane C, Elbaruni S, Sheils O, Gallagher MF, O'Leary JJ
BMC Genomics, 15:711, 25 Aug 2014

Cited by: 13 articles | PMID: 25156079 | PMCID: PMC4162954

43. THE MYD88+ PHENOTYPE IS AN ADVERSE PROGNOSTIC FACTOR IN EPITHELIAL OVARIAN CANCER.

d'Adhemar CJ, Spillane CD, Gallagher MF, Bates M, Costello KM, Barry-O'Crowley J, Haley K, Kernan N, Murphy C, Smyth PC, O'Byrne K, Pennington S, Cooke AA, Ffrench B, Martin CM, O'Donnell D, Hennessy B, Stordal B, Finn S, [...] O'Leary JJ
PLoS One, 9(6):e100816, 30 Jun 2014

Cited by: 29 articles | PMID: 24977712 | PMCID: PMC4076208

44. HUMAN PAPILLOMAVIRUS (HPV) AND THE USEFULNESS OF THE HPV VACCINE FOR MEN WHO HAVE SEX WITH MEN.

Sadlier C, Rowley D, Higgins D, Sheils O, Bergin C
J Infect Dis, 210(10):1679, 08 May 2014

Cited by: 5 articles | PMID: 24812047

45. PREVALENCE OF HUMAN PAPILLOMAVIRUS IN MEN WHO HAVE SEX WITH MEN IN THE ERA OF AN EFFECTIVE VACCINE; A CALL TO ACT.

Sadlier C, Rowley D, Morley D, Surah S, O'Dea S, Delamere S, O'Leary J, Smyth P, Clarke S, Sheils O, Bergin C
HIV Med, 15(8):499-504, 24 Mar 2014

Cited by: 14 articles | PMID: 2465589

46. COMPARING ION TORRENT WITH PYROSEQUENCING AND SANGER SEQUENCING FOR THE DETECTION OF TP53 MUTATIONS IN COLORECTAL CANCER

Doyle B, Lee C, Harkins T, Petraroli R, Smyth P, Sheahan K, O'Leary J, Sheils O
The EPMA Journal, 5(suppl 1):A32-A32, 01 Jan 2014

Cited by: 0 articles | PMCID: PMC4125828

47. ACTIVATED EOSINOPHILS IN ASSOCIATION WITH ENTERIC NERVES IN INFLAMMATORY BOWEL DISEASE.

Smyth CM, Akasheh N, Woods S, Kay E, Morgan RK, Thornton MA, O'Grady A, Cummins R, Sheils O, Smyth P, Gleich GJ, Murray FM, Costello RW
PLoS One, 8(5):e64216, 22 May 2013

Cited by: 23 articles | PMID: 23717571 | PMCID: PMC3661526

48. PRE-TREATMENT OF PLATINUM RESISTANT OVARIAN CANCER CELLS WITH AN MMP-9/MMP-2 INHIBITOR PRIOR TO CISPLATIN ENHANCES CYTOTOXICITY AS DETERMINED BY HIGH CONTENT SCREENING.

Laios A, Mohamed BM, Kelly L, Flavin R, Finn S, McEvoy L, Gallagher M, Martin C, Sheils O, Ring M, Davies A, Lawson M, Gleeson N, D'Arcy T, d'Adhemar C, Norris L, Langhe R, Saadeh FA, O'Leary JJ, O'Toole SA
Int J Mol Sci, 14(1):2085-2103, 22 Jan 2013

Cited by: 14 articles | PMID: 23340649 | PMCID: PMC3565367

49. MIR141 EXPRESSION DIFFERENTIATES HASHIMOTO THYROIDITIS FROM PTC AND BENIGN THYROCYTES IN IRISH ARCHIVAL THYROID TISSUES.

Dorris ER, Smyth P, O'Leary JJ, Sheils O
Front Endocrinol (Lausanne), 3:102, 03 Sep 2012

Cited by: 10 articles | PMID: 22969748 | PMCID: PMC3432448

50. DIFFERENCES IN HUMAN PAPILLOMAVIRUS TYPE DISTRIBUTION IN HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASIVE CERVICAL CANCER IN EUROPE.

Tjalma WA, Fiander A, Reich O, Powell N, Nowakowski AM, Kirschner B, Koiss R, O'Leary J, Joura EA, Rosenlund M, Colau B, Schledermann D, Kukk K, Damaskou V, Repanti M, Vladareanu R, Kolomiets L, Savicheva A, Shipitsyna E, [...] HERACLES/SCALE Study Group
Int J Cancer, 132(4):854-867, 24 Jul 2012

51. MICRORNA-31 MODULATES TUMOUR SENSITIVITY TO RADIATION IN OESOPHAGEAL ADENOCARCINOMA.

Lynam-Lennon N, Reynolds JV, Marignol L, Sheils OM, Pidgeon GP, Maher SG
J Mol Med (Berl), 90(12):1449-1458, 17 Jun 2012

Cited by: 59 articles | PMID: 22706599

52. EPITOPE PRESENTATION IS AN IMPORTANT DETERMINANT OF THE UTILITY OF ANTIGENS IDENTIFIED FROM PROTEIN ARRAYS IN THE DEVELOPMENT OF AUTOANTIBODY DIAGNOSTIC ASSAYS.

Murphy MA, O'Connell DJ, O'Kane SL, O'Brien JK, O'Toole S, Martin C, Sheils O, O'Leary JJ, Cahill DJ

J Proteomics, 75(15):4668-4675, 05 Mar 2012

Cited by: 12 articles | PMID: 22415278

53. SUPPRESSION OF CANCER STEMNESS P21-REGULATING MRNA AND MICRORNA SIGNATURES IN RECURRENT OVARIAN CANCER PATIENT SAMPLES.

Gallagher MF, Heffron CC, Laios A, O'Toole SA, Ffrench B, Smyth PC, Flavin RJ, Elbaruni SA, Spillane CD, Martin CM, Sheils OM, O'Leary JJ
J Ovarian Res, 5(1):2, 19 Jan 2012

Cited by: 6 articles | PMID: 22260314 | PMCID: PMC3285047

54. LOW MAD2 EXPRESSION LEVELS ASSOCIATE WITH REDUCED PROGRESSION-FREE SURVIVAL IN PATIENTS WITH HIGH-GRADE SEROUS EPITHELIAL OVARIAN CANCER.

Furlong F, Fitzpatrick P, O'Toole S, Phelan S, McGrogan B, Maguire A, O'Grady A, Gallagher M, Prencipe M, McGoldrick A, McGettigan P, Brennan D, Sheils O, Martin C, W Kay E, O'Leary J, McCann A
J Pathol, 226(5):746-755, 17 Jan 2012

Cited by: 36 articles | PMID: 22069160 | PMCID: PMC3593171

55. CLINICAL UTILITY OF USING C-REACTIVE PROTEIN AND PROCALCITONIN AS BIOMARKERS FOR A NOVEL NEONATAL SEPSIS DIAGNOSTIC PLATFORM (ASCMICROPLAT)

McAllister K, Sheridan-Pereira M, O'Sullivan N, O'Kelly R, Mark D, Czilwik G, Martin C, Sheils O, O'Leary J
Crit Care, 16(suppl 3):P106-P106, 01 Jan 2012

Cited by: 0 articles | PMCID: PMC3504907

56. THE ROLE OF SECRETED FRIZZLED-RELATED PROTEIN 2 EXPRESSION IN PROSTATE CANCER.

O'Hurley G, Perry AS, O'Grady A, Loftus B, Smyth P, O'Leary JJ, Sheils O, Fitzpatrick JM, Hewitt SM, Lawler M, Kay EW
Histopathology, 59(6):1240-1248, 01 Dec 2011

Cited by: 19 articles | PMID: 22175903 | PMCID: PMC7377364

57. PLATELET ADHESION AND DEGRANULATION INDUCE PRO-SURVIVAL AND PRO-ANGIOGENIC SIGNALLING IN OVARIAN CANCER CELLS.

Egan K, Crowley D, Smyth P, O'Toole S, Spillane C, Martin C, Gallagher M, Canney A, Norris L, Conlon N, McEvoy L, Ffrench B, Stordal B, Keegan H, Finn S, McEneaney V, Laios A, Ducrée J, Dunne E, [...] O'Leary J
PLoS One, 6(10):e26125, 12 Oct 2011

Cited by: 95 articles | PMID: 22022533 | PMCID: PMC3192146

58. INKJET-LIKE PRINTING OF SINGLE-CELLS.

Yusof A, Keegan H, Spillane CD, Sheils OM, Martin CM, O'Leary JJ, Zengerle R, Koltay P
Lab Chip, 11(14):2447-2454, 09 Jun 2011

Cited by: 56 articles | PMID: 21655638

59. RET PROTEIN EXPRESSION IN PAPILLARY RENAL CELL CARCINOMA.

Flavin R, Finn SP, Choueiri TK, Ingoldsby H, Ring M, Barrett C, Rogers M, Smyth P, O'Regan E, Gaffney E, O'Leary JJ, Loda M, Signoretti S, Sheils O
Urol Oncol, 30(6):900-905, 10 Mar 2011

Cited by: 7 articles | PMID: 21396847 | PMCID: PMC8445588

60. BRAFV600E: IMPLICATIONS FOR CARCINOGENESIS AND MOLECULAR THERAPY.

Cantwell-Dorris ER, O'Leary JJ, Sheils OM
Mol Cancer Ther, 10(3):385-394, 01 Mar 2011

Cited by: 201 articles | PMID: 21388974

61. MYBL2 (B-MYB) IN CERVICAL CANCER: PUTATIVE BIOMARKER.

Astbury K, McEvoy L, Brian H, Spillane C, Sheils O, Martin C, O'Leary JJ
Int J Gynecol Cancer, 21(2):206-212, 01 Feb 2011

Cited by: 8 articles | PMID: 21270603

62. EVALUATION OF ZINC-A-2-GLYCOPROTEIN AND PROTEASOME SUBUNIT B-TYPE 6 EXPRESSION IN PROSTATE CANCER USING TISSUE MICROARRAY TECHNOLOGY.

O'Hurley G, O'Grady A, Smyth P, Byrne J, O'Leary JJ, Sheils O, William R, Watson G, Kay EW
Appl Immunohistochem Mol Morphol, 18(6):512-517, 12 Apr 2010

Cited by: 4 articles | PMID: 20661134

63. ALL AZOOSPERMIC MALES SHOULD BE SCREENED FOR CYSTIC FIBROSIS MUTATIONS BEFORE INTRACYTOPLASMIC SPERM INJECTION.

Mocanu E, Shattock R, Barton D, Rogers M, Conroy R, Sheils O, Collins C, Martin C, Harrison R, O'Leary J
Fertil Steril, 94(6):2448-2450, 09 Apr 2010

Cited by: 9 articles | PMID: 20381036

64. REGULATION OF MICRORNA BIOSYNTHESIS AND EXPRESSION IN 2102EP EMBRYONAL CARCINOMA STEM CELLS IS MIRRORED IN OVARIAN SEROUS ADENOCARCINOMA PATIENTS.

Gallagher MF, Flavin RJ, Elbaruni SA, McInerney JK, Smyth PC, Salley YM, Vencken SF, O'Toole SA, Laios A, Lee MY, Denning K, Li J, Aherne ST, Lao KQ, Martin CM, Sheils OM, O'Leary JJ
J Ovarian Res, 2:19, 16 Dec 2009

Cited by: 13 articles | PMID: 20015364 | PMCID: PMC2805659

65. GENE EXPRESSION ANALYSIS OF DIAGNOSTIC BIOPSIES PREDICTS PATHOLOGICAL RESPONSE TO NEOADJUVANT CHEMORADIOTHERAPY OF ESOPHAGEAL CANCER.

Maher SG, Gillham CM, Duggan SP, Smyth PC, Miller N, Muldoon C, O'Byrne KJ, Sheils OM, Hollywood D, Reynolds JV
Ann Surg, 250(5):729-737, 01 Nov 2009

Cited by: 49 articles | PMID: 1980192

66. FOXA1 IS A POTENTIAL ONCOGENE IN ANAPLASTIC THYROID CARCINOMA.

Nucera C, Eeckhoute J, Finn S, Carroll JS, Ligon AH, Priolo C, Fadda G, Toner M, Sheils O, Attard M, Pontecorvi A, Nose V, Loda M, Brown M
Clin Cancer Res, 15(11):3680-3689, 26 May 2009

Cited by: 46 articles | PMID: 19470727

67. MIR-29B EXPRESSION IS ASSOCIATED WITH DISEASE-FREE SURVIVAL IN PATIENTS WITH OVARIAN SEROUS CARCINOMA.

Flavin R, Smyth P, Barrett C, Russell S, Wen H, Wei J, Laios A, O'Toole S, Ring M, Denning K, Li J, Aherne S, Sammarae D, Aziz NA, Alhadi A, Finn SP, Loda M, B S, Sheils O, O'Leary JJ
Int J Gynecol Cancer, 19(4):641-647, 01 May 2009

Cited by: 37 articles | PMID: 19509563

68. RET/PTC REARRANGEMENT OCCURRING IN PRIMARY PERITONEAL CARCINOMA.

Flavin R, Jackl G, Finn S, Smyth P, Ring M, O'Regan E, Cahill S, Unger K, Denning K, Jinghuan Li, Aherne S, Tallini G, Gaffney E, O'Leary JJ, Zitzelsberger H, Sheils O
Int J Surg Pathol, 17(3):187-197, 14 Jan 2009

Cited by: 6 articles | PMID: 19147513

69. GENE EXPRESSION PROFILING IN CERVICAL CANCER: IDENTIFICATION OF NOVEL MARKERS FOR DISEASE DIAGNOSIS AND THERAPY.

Martin CM, Astbury K, McEvoy L, O'Toole S, Sheils O, O'Leary JJ
Methods Mol Biol, 511:333-359, 01 Jan 2009

Cited by: 27 articles | PMID: 19347305

70. GEOGRAPHICAL MAPPING OF A MULTIFOCAL THYROID TUMOUR USING GENETIC ALTERATION ANALYSIS & MIRNA PROFILING.

Aherne ST, Smyth PC, Flavin RJ, Russell SM, Denning KM, Li JH, Guenther SM, O'Leary JJ, Sheils OM
Mol Cancer, 7:89, 04 Dec 2008

Cited by: 15 articles | PMID: 19055826 | PMCID: PMC2612696

71. LACK OF ASSOCIATION BETWEEN MEASLES VIRUS VACCINE AND AUTISM WITH ENTEROPATHY: A CASE-CONTROL STUDY.

Hornig M, Briese T, Buie T, Bauman ML, Lauwers G, Siemetzki U, Hummel K, Rota PA, Bellini WJ, O'Leary JJ, Sheils O, Alden E, Pickering L, Lipkin WI
PLoS One, 3(9):e3140, 04 Sep 2008

Cited by: 42 articles | PMID: 18769550 | PMCID: PMC2526159

72. POTENTIALLY IMPORTANT MICRORNA CLUSTER ON CHROMOSOME 17P13.1 IN PRIMARY PERITONEAL CARCINOMA.

Flavin RJ, Smyth PC, Laios A, O'Toole SA, Barrett C, Finn SP, Russell S, Ring M, Denning KM, Li J, Aherne ST, Sammarae DA, Aziz NA, Alhadi A, Sheppard BL, Lao K, Sheils OM, O'Leary JJ
Mod Pathol, 22(2):197-205, 01 Aug 2008

Cited by: 44 articles | PMID: 18677302

73. RET/PTC-1 EXPRESSION ALTERS THE IMMUNOPROFILE OF THYROID FOLLICULAR CELLS.

Denning K, Smyth P, Cahill S, Li J, Flavin R, Aherne S, O' Leary JJ, Sheils O
Mol Cancer, 7:44, 27 May 2008

Cited by: 3 articles | PMID: 18505566 | PMCID: PMC2423371

74. POTENTIAL ROLE OF MIR-9 AND MIR-223 IN RECURRENT OVARIAN CANCER.

Laios A, O'Toole S, Flavin R, Martin C, Kelly L, Ring M, Finn SP, Barrett C, Loda M, Gleeson N, D'Arcy T, McGuinness E, Sheils O, Sheppard B, O' Leary J
Mol Cancer, 7:35, 28 Apr 2008

Cited by: 204 articles | PMID: 18442408 | PMCID: PMC2383925

75. ALTERED EIF6 AND DICER EXPRESSION IS ASSOCIATED WITH CLINICOPATHOLOGICAL FEATURES IN OVARIAN SEROUS CARCINOMA PATIENTS.

Flavin RJ, Smyth PC, Finn SP, Laios A, O'Toole SA, Barrett C, Ring M, Denning KM, Li J, Aherne ST, Aziz NA, Alhadi A, Sheppard BL, Loda M, Martin C, Sheils OM, O'Leary JJ
Mod Pathol, 21(6):676-684, 07 Mar 2008

Cited by: 63 articles | PMID: 18327211

76. P16(INK4A) GENETIC AND EPIGENETIC PROFILES DIFFER IN RELATION TO AGE AND SITE IN HEAD AND NECK SQUAMOUS CELL CARCINOMAS.

O'Regan EM, Toner ME, Finn SP, Fan CY, Ring M, Hagmar B, Timon C, Smyth P, Cahill S, Flavin R, Sheils OM, O'Leary JJ
Hum Pathol, 39(3):452-458, 01 Mar 2008

Cited by: 44 articles | PMID: 18261630

77. FUTURE MOLECULAR ASPECTS OF CERVICAL CYTOLOGY.

Astbury,K;Martin,CM;Ring,M;Pilkington,L;Bolger,N;Sheils,OM;O'Leary,JJ;

Current Diagnostic Pathology, 2006;12:2,104- 113

78. IDENTIFICATIONOFCELLCYCLEREGULATORY MOLECULES AS BIOMARKERS IN CERVICAL CANCER BY GENE EXPRESSION PROFILING:

Astbury,K;Martin,CM;Sheils,OM;O'Leary,JJ;
International Journal of Gynecological Cancer, 2006;16:726

79. IMPROVED RNA QUALITY AND TAQMAN PRE-AMPLIFICATION METHOD (PREAMP) TO ENHANCE EXPRESSION ANALYSIS FROM FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) MATERIALS.

Li J, Smyth P, Cahill S, Denning K, Flavin R, Aherne S, Pirotta M, Guenther SM, O'Leary JJ, Sheils O
BMC Biotechnol, 8:10, 06 Feb 2008

Cited by: 53 articles | PMID: 18254955 | PMCID: PMC2259333

80. AN INTEGRATIVE MODEL FOR RECURRENCE IN OVARIAN CANCER.

Laios A, O'Toole SA, Flavin R, Martin C, Ring M, Gleeson N, D'Arcy T, McGuinness EP, Sheils O, Sheppard BL, O' Leary JJ
Mol Cancer, 7:8, 22 Jan 2008

Cited by: 25 articles | PMID: 18211683 | PMCID: PMC2248209

81. A MOLECULAR EXPRESSION SIGNATURE DISTINGUISHING FOLLICULAR LESIONS IN THYROID CARCINOMA USING PREAMPLIFICATION RT-PCR IN ARCHIVAL SAMPLES.

Denning KM, Smyth PC, Cahill SF, Finn SP, Conlon E, Li J, Flavin RJ, Aherne ST, Guenther SM, Ferlinz A, O'Leary JJ, Sheils OM
Mod Pathol, 20(10):1095-1102, 27 Jul 2007

Cited by: 24 articles | PMID: 17660800

82. COMPARISON OF MIRNA EXPRESSION PATTERNS USING TOTAL RNA EXTRACTED FROM MATCHED SAMPLES OF FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) CELLS AND SNAP FROZEN CELLS.

Li J, Smyth P, Flavin R, Cahill S, Denning K, Aherne S, Guenther SM, O'Leary JJ, Sheils O
BMC Biotechnol, 7:36, 29 Jun 2007

Cited by: 206 articles | PMID: 17603869 | PMCID: PMC1914054

83. GLOBAL MRNA ANALYSIS TO DETERMINE A TRANSCRIPTOME PROFILE OF CANCER STEMNESS IN A MOUSE MODEL.

Heffron CC, Gallagher MF, Guenther S, Sherlock J, Henfrey R, Martin C, Sheils O, O'Leary JJ
Anticancer Res, 27(3a):1319-1324, 01 May 2007

Cited by: 2 articles | PMID: 17593625

84. BRAF T1799A MUTATION OCCURRING IN A CASE OF MALIGNANT STRUMA OVARII.

Flavin R, Smyth P, Crotty P, Finn S, Cahill S, Denning K, Jinghuan Li, O'Regan E, O'Leary J, Sheils O
Int J Surg Pathol, 15(2):116-120, 01 Apr 2007

Cited by: 18 articles | PMID: 17478764

85. EFFECT OF BRAFV600E MUTATION ON TRANSCRIPTION AND POST-TRANSCRIPTIONAL REGULATION IN A PAPILLARY THYROID CARCINOMA MODEL.

Cahill S, Smyth P, Denning K, Flavin R, Li J, Potratz A, Guenther SM, Henfrey R, O'Leary JJ, Sheils O
Mol Cancer, 6:21, 13 Mar 2007

Cited by: 34 articles | PMID: 17355635 | PMCID: PMC1831483

86. LOW-LEVEL TOP2A AMPLIFICATION IN PROSTATE CANCER IS ASSOCIATED WITH HER2 DUPLICATION, ANDROGEN RESISTANCE, AND DECREASED SURVIVAL.

Murphy AJ, Hughes CA, Barrett C, Magee H, Loftus B, O'Leary JJ, Sheils O
Cancer Res, 67(6):2893-2898, 01 Mar 2007

Cited by: 24 articles | PMID: 17363613

87. FREE FETAL DNA IS NOT INCREASED BEFORE 20 WEEKS IN INTRAUTERINE GROWTH RESTRICTION OR PRE-ECLAMPSIA.

Crowley A, Martin C, Fitzpatrick P, Sheils O, O'Herlihy C, O'Leary JJ, Byrne BM
Prenat Diagn, 27(2):174-179, 01 Feb 2007

Cited by: 24 articles | PMID: 17191257

88. EXPRESSION MICROARRAY ANALYSIS OF PAPILLARY THYROID CARCINOMA AND BENIGN THYROID TISSUE: EMPHASIS ON THE FOLLICULAR VARIANT AND POTENTIAL MARKERS OF MALIGNANCY.

Finn SP, Smyth P, Cahill S, Streck C, O'Regan EM, Flavin R, Sherlock J, Howells D, Henfrey R, Cullen M, Toner M, Timon C, O'Leary JJ, Sheils OM
Virchows Arch, 450(3):249-260, 25 Jan 2007

Cited by: 25 articles | PMID: 17252232 | PMCID: PMC1888716

89. GENE EXPRESSION PROFILING MAY PREDICT RESPONSE TO NEOADJUVANT RADIOCHEMOTHERAPY (RCT)

McDowell,DT;Smith,F; Gilham, C; Pidgeon,G; Smyth,P, Sheils, O; Muldoon, C; Mehigan, B; Stephens, R; Kennedy, M J;

Journal of the American College of Surgeons, 2007;205:3,89-90

90. LOW-LEVEL GENOMIC INSTABILITY IS A FEATURE OF PAPILLARY THYROID CARCINOMA: AN ARRAY COMPARATIVE GENOMIC HYBRIDIZATION STUDY OF LASER CAPTURE MICRODISSECTED PAPILLARY THYROID CARCINOMA TUMORS AND CLONAL CELL LINES.

Finn S, Smyth P, O'Regan E, Cahill S, Toner M, Timon C, Flavin R, O'Leary J, Sheils O
Arch Pathol Lab Med, 131(1):65-73, 01 Jan 2007

Cited by: 14 articles | PMID: 17227125

91. HETEROGENEOUS EXPRESSION OF ALPHA-METHYLACYL-COA RACEMASE IN PROSTATIC CANCER CORRELATES WITH GLEASON SCORE.

Murphy AJ, Hughes CA, Lannigan G, Sheils O, O'Leary J, Loftus B
Histopathology, 50(2):243-251, 01 Jan 2007

Cited by: 15 articles | PMID: 17222253

92. EFFECT OF RET/PTC 1 REARRANGEMENT ON TRANSCRIPTION AND POST-TRANSCRIPTIONAL REGULATION IN A PAPILLARY THYROID CARCINOMA MODEL.

Cahill S, Smyth P, Finn SP, Denning K, Flavin R, O'Regan EM, Li J, Potratz A, Guenther SM, Henfrey R, O'Leary JJ, Sheils O
Mol Cancer, 5:70, 11 Dec 2006

Cited by: 39 articles | PMID: 17156473 | PMCID: PMC1713250

93. DISTINCT ARRAY COMPARATIVE GENOMIC HYBRIDIZATION PROFILES IN ORAL SQUAMOUS CELL CARCINOMA OCCURRING IN YOUNG PATIENTS.

O'Regan EM, Toner ME, Smyth PC, Finn SP, Timon C, Cahill S, Flavin R, O'Leary JJ, Sheils O
Head Neck, 28(4):330-338, 01 Apr 2006

Cited by: 27 articles | PMID: 16470878

94. TOPOISOMERASE II-ALPHA EXPRESSION INCREASES WITH INCREASING GLEASON SCORE AND WITH HORMONE INSENSITIVITY IN PROSTATE CARCINOMA.

Hughes C, Murphy A, Martin C, Fox E, Ring M, Sheils O, Loftus B, O'Leary J
J Clin Pathol, 59(7):721-724, 23 Mar 2006

Cited by: 16 articles | PMID: 16556661 | PMCID: PMC1860426

95. GENOME-WIDE ANALYSIS OF DEOXYRIBONUCLEIC ACID IN ENDOMETRIAL CANCER USING COMPARATIVE GENOMIC HYBRIDIZATION MICROARRAYS.

O'Toole SA, Dunn E, Sheppard BL, Klocker H, Bektic J, Smyth P, Martin C, Sheils O, O'Leary JJ
Int J Gynecol Cancer, 16(2):834-842, 01 Mar 2006

Cited by: 10 articles | PMID: 16681770

96. MOLECULAR CLASSIFICATION AND BIOMARKER DISCOVERY IN PAPILLARY THYROID CARCINOMA.

Sheils O
Expert Rev Mol Diagn, 5(6):927-946, 01 Nov 2005

Cited by: 20 articles | PMID: 16255634

97. SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK IN YOUNG IRISH ADULTS.

O'Regan EM, Timon C, Sheils O, Codd M, O'Leary JJ, Toner M
Br J Oral Maxillofac Surg, 44(3):203-206, 07 Jul 2005

Cited by: 19 articles | PMID: 16005553

98. MOLECULAR PATHOLOGY OF PROSTATE CANCER.

Hughes C, Murphy A, Martin C, Sheils O, O'Leary J
J Clin Pathol, 58(7):673-684, 01 Jul 2005

Cited by: 70 articles | PMID: 15976331 | PMCID: PMC1770715

99. OESTROGEN REGULATED GENE EXPRESSION IN NORMAL AND MALIGNANT ENDOMETRIAL TISSUE.

O'Toole SA, Dunn E, Sheppard BL, Sheils O, O'Leary JJ, Wuttke W, Seidlova-Wuttke D
Maturitas, 51(2):187-198, 01 Jun 2005

Cited by: 14 articles | PMID: 15917160

100. QUANTITATION OF CDC6 AND MCM5 MRNA IN CERVICAL INTRAEPITHELIAL NEOPLASIA AND INVASIVE SQUAMOUS CELL CARCINOMA OF THE CERVIX.

Murphy N, Ring M, Heffron CC, Martin CM, McGuinness E, Sheils O, O'Leary JJ
Mod Pathol, 18(6):844-849, 01 Jun 2005

Cited by: 26 articles | PMID: 15696126

101. ANALYSIS OF DNA IN ENDOMETRIAL CANCER CELLS TREATED WITH PHYTO-ESTROGENIC COMPOUNDS USING COMPARATIVE GENOMIC HYBRIDISATION MICROARRAYS.

O'Toole SA, Sheppard BL, Sheils O, O'Leary JJ, Spengler B, Christoffel V
Planta Med, 71(5):435-439, 01 May 2005

Cited by: 4 articles | PMID: 15931582

102. P16INK4A, CDC6, AND MCM5: PREDICTIVE BIOMARKERS IN CERVICAL PREINVASIVE NEOPLASIA AND CERVICAL CANCER.

Murphy N, Ring M, Heffron CC, King B, Killalea AG, Hughes C, Martin CM, McGuinness E, Sheils O, O'Leary JJ
J Clin Pathol, 58(5):525-534, 01 May 2005

Cited by: 115 articles | PMID: 15858126 | PMCID: PMC1770660

103. DETECTION OF A TYROSINE PHOSPHATASE LAR ON INTESTINAL EPITHELIAL CELLS AND INTRAEPITHELIAL LYMPHOCYTES IN THE HUMAN DUODENUM.

Murphy AM, Sheils OM, McDonald GS, Kelleher DP
Mediators Inflamm, 2005(1):23-30, 01 Feb 2005

Cited by: 3 articles | PMID: 15770063 | PMCID: PMC1513056

104. RET/PTC AND BRAF ACT AS DISTINCT MOLECULAR, TIME-DEPENDANT TRIGGERS IN A SPORADIC IRISH COHORT OF PAPILLARY THYROID CARCINOMA.

Smyth P, Finn S, Cahill S, O'Regan E, Flavin R, O'Leary JJ, Sheils O
Int J Surg Pathol, 13(1):1-8, 01 Jan 2005

Cited by: 27 articles | PMID: 15735849

105. P16INK4A POSITIVITY IN BENIGN, PREMALIGNANT AND MALIGNANT CERVICAL GLANDULAR LESIONS: A POTENTIAL DIAGNOSTIC PROBLEM.

Murphy N, Heffron CC, King B, Ganuguapati UG, Ring M, McGuinness E, Sheils O, O'Leary JJ
Virchows Arch, 445(6):610-615, 18 Sep 2004

Cited by: 28 articles | PMID: 15378361

106. ARRAY COMPARATIVE GENOMIC HYBRIDISATION ANALYSIS OF GAMMA-IRRADIATED HUMAN THYROCYTES.

Finn SP, Smyth P, O'regan E, Cahill S, Flavin R, O'leary J, Sheils O
Virchows Arch, 445(4):396-404, 17 Jul 2004

Cited by: 10 articles | PMID: 15258756

107. REAL-TIME ANALYSIS OF BETA- AND GAMMA-CATENIN MRNA EXPRESSION IN RET/PTC-1 ACTIVATED AND NONACTIVATED THYROID TISSUES.

Smyth P, Finn S, O'Leary J, Sheils O
Diagn Mol Pathol, 12(1):44-49, 01 Mar 2003

Cited by: 3 articles | PMID: 12605035

108. RET/PTC CHIMERIC TRANSCRIPTS IN AN IRISH COHORT OF SPORADIC PAPILLARY THYROID CARCINOMA.

Finn SP, Smyth P, O'Leary J, Sweeney EC, Sheils O
J Clin Endocrinol Metab, 88(2):938-941, 01 Feb 2003

Cited by: 15 articles | PMID: 12574236

109. P16INK4A AS A MARKER FOR CERVICAL DYSKARYOSIS: CIN AND CGIN IN CERVICAL BIOPSIES AND THINPREP SMEARS.

Murphy N, Ring M, Killalea AG, Uhlmann V, O'Donovan M, Mulcahy F, Turner M, McGuinness E, Griffin M, Martin C, Sheils O, O'Leary JJ
J Clin Pathol, 56(1):56-63, 01 Jan 2003

Cited by: 121 articles | PMID: 12499437 | PMCID: PMC1769860

110. RET/PTC REARRANGEMENTS IN HASHIMOTO'S THYROIDITIS.

Sheils O, Smyth P, Finn S, Sweeney EC, O'Leary JJ
Int J Surg Pathol, 10(2):167-8; author reply 168-9, 01 Apr 2002

Cited by: 7 articles | PMID: 12075413

111. POTENTIAL VIRAL PATHOGENIC MECHANISM FOR NEW VARIANT INFLAMMATORY BOWEL DISEASE.

Uhlmann V, Martin CM, Sheils O, Pilkington L, Silva I, Killalea A, Murch SB, Walker-Smith J, Thomson M, Wakefield AJ, O'Leary JJ
Mol Pathol, 55(2):84-90, 01 Apr 2002

Cited by: 64 articles | PMID: 11950955 | PMCID: PMC1187154

112. DETECTION OF MEASLES VIRUS IN CHILDREN WITH ILEO-COLONIC LYMPHOID NODULAR HYPERPLASIA, ENTEROCOLITIS AND DEVELOPMENTAL DISORDER.

Martin CM, Uhlmann V, Killalea A, Sheils O, O'Leary JJ
Mol Psychiatry, 7 Suppl 2:S47-8, 01 Jan 2002

Cited by: 9 articles | PMID: 12142949

113. RET/PTC REARRANGEMENTS IN HASHIMOTO'S THYROIDITIS.

Sheils O, Smyth P, Finn S, Sweeney EC, O'Leary JJ.

Int J Surg Pathol. 2002 Apr;10(2):167-8; author reply 168---9. PubMed PMID: 12075413.

114. QUALITY-CONTROL ISSUES FOR PCR-BASED ASSAYS IN THE MOLECULAR LABORATORY.

Sheils, OM; Finn, S; O'Leary,JJ;

Current Diagnostic Pathology, 2003;9:3,165-172

115. NUCLEIC ACID MICROARRAYS: AN OVERVIEW.

Sheils,O;Finn,S;O'Leary,J;

2003,Current Diagnostic Pathology;9:3,155-158

116. REAL-TIME QUANTITATIVE ANALYSIS OF E-CADHERIN EXPRESSION IN RET/PTC-1-ACTIVATED THYROID NEOPLASMS.

Smyth P, Sheils O, Finn S, Martin C, O'Leary J, Sweeney EC
Int J Surg Pathol, 9(4):265-272, 01 Oct 2001

Cited by: 11 articles | PMID: 12574841

117. EXPRESSION PROFILE OF HUMAN HERPESVIRUS 8 (HHV-8) IN PYOTHORAX ASSOCIATED LYMPHOMA AND IN EFFUSION LYMPHOMA.

O'Donovan M, Silva I, Uhlmann V, Bermingham N, Luttich K, Martin C, Sheils O, Killalea A, Kenny C, Pileri S, O'Leary JJ
Mol Pathol, 54(2):80-85, 01 Apr 2001

Cited by: 9 articles | PMID: 11322168 | PMCID: PMC1187007

118. ASSESSMENT OF RET/PTC-1 REARRANGEMENTS IN NEOPLASTIC THYROID TISSUE USING TAQMAN RT-PCR.

Sheils OM, O'Leary JJ, Sweeney EC
J Pathol, 192(1):32-36, 01 Sep 2000

Cited by: 28 articles | PMID: 10951397

119. RET/PTC-1 ACTIVATION IN HASHIMOTO THYROIDITIS.

Sheils OM, O'eary JJ, Uhlmann V, Lättich K, Sweeney EC
Int J Surg Pathol, 8(3):185-189, 01 Jul 2000

Cited by: 80 articles | PMID: 11493988

120. CELLULAR LOCALISATION OF HHV-8 IN CASTLEMAN'S DISEASE: IS THERE A LINK WITH LYMPH NODE VASCULARITY?

O'Leary J, Kennedy M, Howells D, Silva I, Uhlmann V, Luttich K, Biddolph S, Lucas S, Russell J, Bermingham N, O'Donovan M, Ring M, Kenny C, Sweeney M, Sheils O, Martin C, Picton S, Gatter K
Mol Pathol, 53(2):69-76, 01 Apr 2000

Cited by: 11 articles | PMID: 10889905 | PMCID: PMC1186908

121. IMPROVED IN SITU DETECTION METHOD FOR TELOMERIC TANDEM REPEATS IN METAPHASE SPREADS AND INTERPHASE NUCLEI.

Uhlmann V, Prasad M, Silva I, Luettich K, Grande L, Alonso L, Thisted M, Pluzek KJ, Gorst J, Ring M, Sweeney M, Kenny C, Martin C, Russell J, Bermingham N, O'Donovan M, Sheils O, O'Leary JJ
Mol Pathol, 53(1):48-50, 01 Feb 2000

Cited by: 4 articles | PMID: 10884922 | PMCID: PMC1186902

122. LOCALISATION OF HHV-8 IN AIDS RELATED LYMPHADENOPATHY.

O'Leary JJ, Kennedy M, Luttich K, Uhlmann V, Silva I, Russell J, Sheils O, Ring M, Sweeney M, Kenny C, Bermingham N, Martin C, O'Donovan M, Howells D, Picton S, Lucas SB
Mol Pathol, 53(1):43-47, 01 Feb 2000

Cited by: 3 articles | PMID: 10884921 | PMCID: PMC1186901

123. TSH RECEPTOR STATUS OF THYROID NEOPLASMS--TAQMAN RT-PCR ANALYSIS OF ARCHIVAL MATERIAL.

Sheils OM, Sweeney EC
J Pathol, 188(1):87-92, 01 May 1999

Cited by: 38 articles | PMID: 1039814

124. SORTING OUT MIX-UPS. THE PROVENANCE OF TISSUE SECTIONS MAY BE CONFIRMED BY PCR USING MICROSATELLITE MARKERS.

O'Briain DS, Sheils O, McElwaine S, McCann SR, Lawler M
Am J Clin Pathol, 106(6):758-764, 01 Dec 1996

Cited by: 9 articles | PMID: 8980351

125. COLOCALISATION OF HUMAN IMMUNODEFICIENCY VIRUS AND HUMAN CYTOMEGALOVIRUS INFECTION IN BRAIN AUTOPSY TISSUE FROM AIDS PATIENTS.

Balluz IM, Farrell MA, Kay E, Staunton MJ, Keating JN, Sheils O, Cosby SL, Mabruk MJ, Sheahan BJ, Atkins GJ
Ir J Med Sci, 165(2):133-138, 01 Apr 1996

Cited by: 8 articles | PMID: 8698561

126. DETECTION OF EPSTEIN-BARR VIRUS DNA IN TONGUE TISSUES FROM AIDS AUTOPSIES WITHOUT CLINICAL EVIDENCE OF ORAL HAIRY LEUKOPLAKIA.

Mabruk MJ, Flint SR, Toner M, Leonard N, Sheils O, Coleman DC, Atkins GJ
J Oral Pathol Med, 24(3):109-112, 01 Mar 1995

Cited by: 9 articles | PMID: 7776261

127. TYROSINE PHOSPHORYLATION IN THE HUMAN DUODENUM.

Kelleher D, Murphy A, Sheils O, Long A, McDevitt J
Gut, 36(1):34-38, 01 Jan 1995

Cited by: 3 articles | PMID: 7534252 | PMCID: PMC1382349

128. ADENOVIRUS 12 E1A GENE DETECTION BY POLYMERASE CHAIN REACTION IN BOTH THE NORMAL AND COELIAC DUODENUM.

Lawler M, Humphries P, O'Farrelly C, Hoey H, Sheils O, Jeffers M, O'Briain DS, Kelleher D
Gut, 35(9):1226-1232, 01 Sep 1994

Cited by: 15 articles | PMID: 7959228 | PMCID: PMC1375698

129. EFFECT OF CYCLOSPORIN ON IMMUNE COMPLEX DEPOSITION IN MURINE GLOMERULONEPHRITIS.

Quinn DG, Fennell JS, Sheils O, Gaffney EF, Feighery CF
Immunology, 72(4):550-554, 01 Apr 1991

Cited by: 3 articles | PMID: 1828056

130. COMPLEMENT ACTIVATION WITHIN THE COELIAC SMALL INTESTINE IS LOCALISED TO BRUNNER'S GLANDS.

Gallagher RB, Kelly CP, Neville S, Sheils O, Weir DG, Feighery CF
Gut, 30(11):1568-1573, 01 Nov 1989

Cited by: 11 articles | PMID: 2599443 | PMCID: PMC1434320

# FULL PUBLICATION LIST

## (INC CONFERENCE PROCEEDINGS – SOURCE GOOGLE SCHOLAR)

| Title | Citations | Year |
|---|---|---|
| hsa_circ_0001275 Is One of a Number of circRNAs Dysregulated in Enzalutamide Resistant Prostate Cancer and Confers Enzalutamide Resistance In Vitro<br>MCJ Lim, AM Baird, J Greene, C McNevin, K Ronan, P Podlesniy, ...<br>Cancers 13 (24), 6383 | | 2021 |
| The induction of a mesenchymal phenotype by platelet cloaking of cancer cells is a universal phenomenon<br>CD Spillane, NM Cooke, MP Ward, D Kenny, G Blackshields, T Kelly, ...<br>Translational oncology 14 (12), 101229 | | 2021 |
| Pathogenic *BRCA* Variants as Biomarkers for Risk in Prostate Cancer<br>CS McNevin, K Cadoo, AM Baird, P Murchan, O Sheils, R McDermott, ...<br>Cancers 13 (22), 5697 | 2 | 2021 |
| Circulating Tumour Cell Numbers Correlate with Platelet Count and Circulating Lymphocyte Subsets in Men with Advanced Prostate Cancer: Data from the ExPeCT Clinical Trial … <br>B Hayes, L Brady, G Sheill, AM Baird, E Guinan, B Stanfill, J Dunne, ...<br>Cancers 13 (18), 4690 | 2 | 2021 |
| Deep learning of histopathological features for the prediction of tumour molecular genetics<br>P Murchan, C Ó'Brien, S O'Connell, CS McNevin, AM Baird, O Sheils, ...<br>Diagnostics 11 (8), 1406 | 6 | 2021 |
| The role of human papilloma virus in dictating outcomes in head and neck squamous cell carcinoma<br>S Brennan, AM Baird, E O'Regan, O Sheils | 4 | 2021 |

| | | |
|---|---|---|
| Frontiers in Molecular Biosciences 8, 468 | | |
| Differential circRNA expression signatures may serve as potential novel biomarkers in prostate cancer<br><br>J Greene, AM Baird, M Lim, J Flynn, C McNevin, L Brady, O Sheils, ...<br><br>Frontiers in cell and developmental biology 9, 605686 | 4 | 2021 |
| The role of cancer stem cells in drug resistance in gastroesophageal junction adenocarcinoma<br><br>K Dinneen, AM Baird, C Ryan, O Sheils<br><br>Frontiers in Molecular Biosciences 8, 600373 | 2 | 2021 |
| Marker and Target as a Diagnostic Variable and Target for Therapy of Metastatic Cancer<br><br>J O'leary, O Sheils, C Spillane, S O'toole, C Cluxton, C Gardiner<br><br>US Patent App. 16/332,549 | | 2021 |
| Correlation of integrated ERG/PTEN assessment with biochemical recurrence in prostate cancer<br><br>L Brady, J Carlsson, AM Baird, O Casey, T Vlajnic, P Murchan, ...<br><br>Cancer treatment and research communications 29, 100451 | 2 | 2021 |
| Epigenetic Modifier UHRF1 May Be a Potential Target in Malignant Pleural Mesothelioma<br><br>AM Baird, SP Finn, SG Gray, O Sheils<br><br>Journal of Thoracic Oncology 16 (1), 14-16 | | 2021 |
| Platelet cloaking of circulating tumour cells in patients with metastatic prostate cancer: Results from ExPeCT, a randomised controlled trial<br><br>L Brady, B Hayes, G Sheill, AM Baird, E Guinan, B Stanfill, T Vlajnic, ...<br><br>PloS one 15 (12), e0243928 | 6 | 2020 |
| The role of cancer stem cells in gastrooesophageal junction adenocarcinoma<br><br>K Dinneen, C Ryan, A Baird, B Hernandez, S Brennan, P Smyth, ...<br><br>VIRCHOWS ARCHIV 477, S257-S257 | | 2020 |

| | | |
|---|---|---|
| Influence of platelets and neutrophils on circulating tumour cells<br>MP Ward, BM Mohamed, L Kane, M Bates, J Berghoff, CL Spillane, ...<br>Cancer Research 80 (16_Supplement), 764-764 | | 2020 |
| Prostate cancer-derived holoclones: a novel and effective model for evaluating cancer stemness<br>L Flynn, MP Barr, AM Baird, P Smyth, OM Casey, G Blackshields, ...<br>Scientific reports 10 (1), 1-17 | 6 | 2020 |
| LUNG CANCER WORLDWIDE<br>AM Baird, SP Finn, SG Gray, O Sheils | | 2020 |
| Anal cancer in people living with HIV: A case series<br>C Sadlier, A Lynam, C Kerr, O Sheils, C Bergin<br>International journal of STD & AIDS 31 (1), 82-84 | | 2020 |
| A randomized trial of exercise on quality of life in men with metastatic prostate cancer: The ExPeCT Trial.<br>G Sheill, L Brady, EM Guinan, JM Hussey, B Hayes, AM Baird, B Stanfill, ...<br>Journal of Clinical Oncology 37 (31_suppl), 97-97 | 1 | 2019 |
| Suppression of Natural Killer cell NKG2D and CD226 anti-tumour cascades by platelet cloaked cancer cells: Implications for the metastatic cascade<br>CD Cluxton, C Spillane, SA O'Toole, O Sheils, CM Gardiner, JJ O'Leary<br>PloS one 14 (3), e0211538 | 32 | 2019 |
| The effect of a structured exercise intervention on CTCs and platelet cloaking in patients with metastatic prostate cancer.<br>L Brady, B Hayes, G Sheill, AM Baird, EM Guinan, B Stanfill, T Vlajnic, ...<br>Journal of Clinical Oncology 37 (7_suppl), 243-243 | 2 | 2019 |

| | | |
|---|---|---|
| The feasibility of implementing an exercise programme for deconditioned cancer survivors in a national cancer centre: FIXCAS Study<br><br>K Devenney, N Murphy, R Ryan, C Grant, J Kennedy, RP Manecksha, ...<br><br>HRB Open Research 2 | | 2019 |
| Simultaneous detection of lung fusions using a multiplex RT-PCR next generation sequencing-based approach: a multi-institutional research study<br><br>CP Vaughn, JL Costa, HE Feilotter, R Petraroli, V Bagai, AM Rachiglio, ...<br><br>BMC cancer 18 (1), 1-8 | 21 | 2018 |
| Diagnosis, monitoring and prevention of exposure-related non-communicable diseases in the living and working environment: DiMoPEx-project is designed to determine the impacts … <br><br>LT Budnik, B Adam, M Albin, B Banelli, X Baur, F Belpoggi, C Bolognesi, ...<br><br>Journal of Occupational Medicine and Toxicology 13 (1), 1-22 | 38 | 2018 |
| Multi institutional evaluation of a new NGS assay for mutation detection from cfDNA<br><br>J Costa, R Weren, A Rachiglio, A Mafficini, H Kurth, A Reiman, D Lecorre, ...<br><br>EUROPEAN JOURNAL OF HUMAN GENETICS 26, 681-682 | | 2018 |
| Integrating biomarkers across omic platforms: an approach to improve stratification of patients with indolent and aggressive prostate cancer<br><br>K Murphy, BT Murphy, S Boyce, L Flynn, S Gilgunn, CJ O'Rourke, ...<br><br>Molecular oncology 12 (9), 1513-1525 | 34 | 2018 |
| Abstract A057: Examining the link between obesity, inflammation, and exercise in patients with metastatic prostate cancer—An interim analysis from the ExPeCT trial<br><br>L Brady, G Sheill, AM Baird, EH Allott, T Vlajnic, J Greene, O Casey, ...<br><br>Cancer Research 78 (16_Supplement), A057-A057 | | 2018 |

| | | |
|---|---|---|
| A novel role for the macrophage galactose-type lectin receptor in mediating von Willebrand factor clearance<br><br>SE Ward, JM O'Sullivan, C Drakeford, S Aguila, CN Jondle, J Sharma, ...<br><br>Blood, The Journal of the American Society of Hematology 131 (8), 911-916 | 49 | 2018 |
| Potentially important miRNAs in enteropathy-associated T-cell lymphoma pathogenesis: A pilot study<br><br>L Clarke, RSR Adduri, P Smyth, F Quinn, M Jeffers, B Dunne, J O'Leary, ...<br><br>Leukemia research reports 10, 52 | 1 | 2018 |
| miR-223 potentially targets SWI/SNF complex protein SMARCD1 in atypical proliferative serous tumor and high-grade ovarian serous carcinoma<br><br>FA Arts, L Keogh, P Smyth, S O'Toole, R Ta, N Gleeson, JJ O'Leary, ...<br><br>Human Pathology 70, 98-104 | 11 | 2017 |
| The evidence base for circulating tumour DNA blood-based biomarkers for the early detection of cancer: a systematic mapping review<br><br>IA Cree, L Uttley, H Buckley Woods, H Kikuchi, A Reiman, S Harnan, ...<br><br>BMC cancer 17 (1), 1-17 | 93 | 2017 |
| Peripheral blood microRNA and *VEGFA* mRNA changes following electroconvulsive therapy: implications for psychotic depression<br><br>E Kolshus, KM Ryan, G Blackshields, P Smyth, O Sheils, DM McLoughlin<br><br>Acta Psychiatrica Scandinavica 136 (6), 594-606 | 28 | 2017 |
| The ExPeCT (Examining Exercise, Prostate Cancer and Circulating Tumour Cells) trial: study protocol for a randomised controlled trial<br><br>G Sheill, L Brady, E Guinan, B Hayes, O Casey, J Greene, T Vlajnic, ...<br><br>Trials 18 (1), 1-12 | 6 | 2017 |
| Multi institutional evaluation of a new NGS assay for mutation detection from cfDNA in lung cancer<br><br>JL Costa, R Weren, AM Rachiglio, A Mafficini, H Kurth, A Reiman, ... | 1 | 2017 |

| | | |
|---|---|---|
| Cancer Research 77 (13_Supplement), 5694-5694 | | |
| Multi institutional evaluation of a high sensitive NGS assay for liquid biopsy mutation detection in lung cancer<br><br>C Vollbrecht, JL Costa, R Weren, AM Rachiglio, A Mafficini, H Kurth, ...<br><br>JOURNAL OF MOLECULAR DIAGNOSTICS 19 (2), S51-S52 | | 2017 |
| A Digital Pathology Method to Identify All Forms of CTC<br><br>C Spillane, B Ffrench, A Cooney, C Ruttle, A Bogdanska, N Gleeson, ...<br><br>LABORATORY INVESTIGATION 97, 463A-463A | | 2017 |
| Platelet Cloaked Tumour Cells Educate M2 Macrophage Differentiation<br><br>C Cluxton, C Spillane, M Ward, CM Martin, O Sheils, F Sheedy, S O'Toole, ...<br><br>LABORATORY INVESTIGATION 97, 455A-455A | | 2017 |
| N-linked glycan truncation causes enhanced clearance of plasma-derived von Willebrand factor<br><br>JM O'sullivan, S Aguila, E McRae, SE Ward, O Rawley, PG Fallon, ...<br><br>Journal of Thrombosis and Haemostasis 14 (12), 2446-2457 | 33 | 2016 |
| A NOVEL DIGITIAL PATHOLOGY APPROACH TO DEFINITIVELY IDENTIFY OVARIAN CANCER CTCS<br><br>C Spillane, B Ffrench, A Cooney, C Ruttle, A Bogdanska, N Gleeson, ...<br><br>INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER 26, 796-796 | | 2016 |
| MicroRNA expression in enteropathy associated T-cell lymphoma type 1<br><br>L Clarke, P Smyth, G Blackshields, M Jeffers, F Quinn, J O'Leary, ...<br><br>VIRCHOWS ARCHIV 469 (SUPPL 1), S35-S35 | | 2016 |
| N-linked glycan sites within the A2 domain of von Willebrand factor modulate macrophage-mediated clearance.<br><br>ODJS Chion A, O'Sullivan JM, Drakeford C, Bergsson G, Dalton N, Aguila S ...<br><br>Blood 4 (709436) | 32<br><br>* | 2016 |

| | | |
|---|---|---|
| Altered expression of mir-222 and mir-25 influences diverse gene expression changes in transformed normal and anaplastic thyroid cells, and impacts on MEK and TRAIL protein …<br><br>ST Aherne, P Smyth, M Freeley, L Smith, C Spillane, J O'leary, O Sheils<br><br>International Journal of Molecular Medicine 38 (2), 433-445 | 12 | 2016 |
| Clinical research results for a NGS-based kit for targeted detection of clinically relevant gene rearrangements in lung tumor samples<br><br>JJ Schageman, JL Costa, O Sheils, JE Glassco, D Chi, J Sherlock, ...<br><br>Cancer Research 76 (14_Supplement), 1386-1386 | | 2016 |
| HPV Infection and Prevention of HPV Infection in Men Who Have Sex with Men (MSM)<br><br>C Sadlier, O Sheils, C Bergin<br><br>Human Papillomavirus-Research in a Global Perspective | | 2016 |
| HPV vaccine acceptability in HIV-infected and HIV negative men who have sex with men (MSM) in Ireland<br><br>C Sadlier, A Lynam, S O'Dea, S Delamere, M Quinlan, S Clarke, O Sheils, ...<br><br>Human vaccines & immunotherapeutics 12 (6), 1536-1541 | 17 | 2016 |
| Next-Generation Sequencing of the Rat Hippocampal MiRNome Following Chronic Electroconvulsive Stimulation<br><br>KM Ryan, P Smyth, G Blackshields, O Sheils, DM McLoughlin<br><br>BIOLOGICAL PSYCHIATRY 79 (9), 326S-327S | | 2016 |
| Prevalence, type and factors associated with HPV infection at multiple sites in young HIV--positive MSM<br><br>C Sadlier, S O'Dea, S Delamere, P Smyth, N Myers, G Blackshields, ...<br><br>HIV MEDICINE 17, 5-5 | | 2016 |
| Response to letter: limitations of human papillomavirus DNA testing in measuring previous exposure and vaccine protection.<br><br>C Sadlier, O Sheils, C Bergin<br><br>HIV medicine 17 (7), 557-558 | | 2016 |

| | | |
|---|---|---|
| Platelet Cloaking Maintains Migratory and Invasive Characteristics of CTCs<br><br>CD Spillane, S Skehan, CM Martin, S O'Toole, O Sheils, J O'Leary<br><br>LABORATORY INVESTIGATION 96, 460A-460A | | 2016 |
| Digital Multi-Colour CTC Detection<br><br>A Cooney, B Ffrench, CD Spillane, C Ruttle, N Gleeson, C O'Riain, ...<br><br>LABORATORY INVESTIGATION 96, 511A-512A | | 2016 |
| Pluripotency markers are differentially induced by MEK inhibition in thyroid and melanoma BRAFV600E cell lines.<br><br>SO Dorris ER, Blackshields G, Sommerville G, Alhashemi M, Dias A, McEneaney ...<br><br>Cancer Biol Ther. | 7 | 2016 |
| Psychosis is different– microRNA and VEGFA changes in depression treated with ECT<br><br>E Kolshus, KM Ryan, G Blackshields, P Smyth, O Sheils, DM McLoughlin<br><br>European Neuropsychopharmacology 2 (26), S316 | 1 | 2016 |
| Chronic electroconvulsive stimulation in rats induces alterations in the miRNome that are hippocampal specific<br><br>KM Ryan, P Smyth, G Blackshields, O Sheils, DM McLoughlin<br><br>European Neuropsychopharmacology 2 (26), S450 | | 2016 |
| Interleukin-15 is associated with disease severity in viral bronchiolitis<br><br>TR Leahy, R McManus, DG Doherty, R Grealy, T Coulter, P Smyth, ...<br><br>European Respiratory Journal 47 (1), 212-222 | 17 | 2016 |
| Aspirin and P2Y12 inhibition attenuate platelet-induced ovarian cancer cell invasion<br><br>NM Cooke, CD Spillane, O Sheils, J O'Leary, D Kenny<br><br>BMC cancer 15 (1), 1-10 | 58 | 2015 |
| Identifying novel hypoxia-associated markers of chemoresistance in ovarian cancer | 52 | 2015 |

| | | |
|---|---|---|
| LM McEvoy, SA O'Toole, CD Spillane, CM Martin, MF Gallagher, ...<br><br>BMC cancer 15 (1), 1-13 | | |
| Development and Validation of an Ion AmpliSeq RNA Fusion Lung Cancer Research Panel through a Global Collaborative Research Study with the Onconetwork Consortium<br><br>V Bagal, K Bramlett, R Petraroli, C Vaughn, O Sheils, B Tops, D Corre, ...<br><br>JOURNAL OF MOLECULAR DIAGNOSTICS 17 (6), 831-831 | | 2015 |
| PLATLETS DRIVE A MESTATIC PROGRAMME IN OVARIAN CANCER CELLS<br><br>C Spillane, NM Cooke, S O'Toole, D Kenny, O Sheils, JJ O'Leary<br><br>INTERNATIONAL JOURNAL OF GYNECOLOGICAL CANCER 25 (9), 1501-1501 | | 2015 |
| Human papillomavirus DNA and mRNA prevalence and association with cervical cytological abnormalities in the Irish HIV population<br><br>A Loy, J McInerney, L Pilkington, H Keegan, S Delamere, CM Martin, ...<br><br>International journal of STD & AIDS 26 (11), 789-795 | 1 | 2015 |
| Comparative Assessment of HPV Detection Assays in the Management of Women Referred to Colposcopy with Minor Abnormalities<br><br>PPT Tewari, CW White, LK Kelly, PK Kearney, LK Pilkington, TD D'Arcy, ...<br><br>JOURNAL OF PATHOLOGY 237, S28-S28 | | 2015 |
| Platelets Drive Metastatic Changes in Ovarian Cancer Cells<br><br>CD Spillane, NM Cooke, S O'Toole, D Kenny, O Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 237, S16-S16 | | 2015 |
| Platelet Cloaked Tumour Cells Suppress NK Cell Immune Surveillance<br><br>CD Cluxton, CD Spillane, A Glaviano, S O'Toole, CM Martin, O Sheils, ...<br><br>JOURNAL OF PATHOLOGY 237, S16-S16 | | 2015 |

| | | |
|---|---|---|
| A Platform for Single Cell Manipulation and Analysis (PASCA)<br><br>V McEneaney, H Keegan, M Gallagher, O Sheils, A Gross, J Schoendube, ...<br><br>JOURNAL OF PATHOLOGY 237, S52-S52 | | 2015 |
| Platelet Cloaking of Cancer Cells is Universal and Drives EMT<br><br>CD Spillane, NM Cooke, S O'Toole, D Kenny, O Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 237, S28-S28 | | 2015 |
| Novel Hypoxia-Associated Markers of Chemoresistance in High Grade Serous Ovarian Cancer<br><br>L McEvoy, PSA O'Toole, CD Spillane, B Stordal, M Gallagher, CM Martin, ...<br><br>JOURNAL OF PATHOLOGY 237, S13-S13 | | 2015 |
| Enhanced regulation of cell cycle and suppression of osteoblast differentiation molecular signatures by prostate cancer stem-like holoclones<br><br>MF Gallagher, Y Salley, CD Spillane, B Ffrench, S El Baruni, ...<br><br>Journal of Clinical Pathology 68 (9), 692-702 | 7 | 2015 |
| Comprehensive genetic profiling of chromosomal translocations in lung cancer tumors: development and validation of a next-generation sequencing panel in an international …<br><br>P Laurent-Puig, JL Costa, O Sheils, B Tops, A Mafficini, D Le Corre, ...<br><br>Cancer Research 75 (15_Supplement), 4891-4891 | | 2015 |
| Rapid and fully automated bacterial pathogen detection on a centrifugal-microfluidic LabDisk using highly sensitive nested PCR with integrated sample preparation.<br><br>MD Czilwik G, Messinger T, Strohmeier O, Wadle S, von Stetten F, Paust N ...<br><br>Lab on a Chip | 134 | 2015 |
| Ligation of TLR7 on $CD19^{+}CD1d^{hi}$ B cells suppresses allergic lung inflammation via regulatory T cells<br><br>AR Khan, S Amu, SP Saunders, E Hams, G Blackshields, MO Leonard, ... | 40 | 2015 |

| | | |
|---|---|---|
| European Journal of Immunology 45 (6), 1842-1854 | | |
| An Association between MicroRNA-21 Expression and Vitamin D Deficiency in Coronary Artery Disease.<br><br>CG Sheane BJ, Smyth P, Scott K, Aziz R, Buckley M, Lodge E, Kiely N ...<br><br>Microrna | 26 | 2015 |
| Platelet Cloaking of Cancer Cells Inhibits Immune Surveillance<br><br>C Cluxton, C Spillane, A Glaviano, S O'Toole, C Martin, O Sheils, ...<br><br>LABORATORY INVESTIGATION 95, 456A-456A | | 2015 |
| Fluorescent Sorting and Printing of Single Cells in Cancer Applications<br><br>V McEneaney, H Keegan, M Gallagher, A Gross, C Martin, O Sheils, ...<br><br>LABORATORY INVESTIGATION 95, 520A-520A | | 2015 |
| Clinicopathological and Molecular Features of an Irish Cohort of Enteropathy-Associated T-Cell Lymphoma<br><br>L Clarke, F Quinn, P Smyth, J Nolan, O Sheils, J O'Leary, M Jeffers, ...<br><br>LABORATORY INVESTIGATION 95, 340A-340A | | 2015 |
| Development of a semi-conductor sequencing-based panel for genotyping of colon and lung cancer by the Onconetwork consortium.<br><br>LPP Tops B, Normanno N, Kurth H, Amato E, Mafficini A, Rieber N, Le Corre D ...<br><br>BMC Cancer 15 (26) | 54 | 2015 |
| Noninvasive Early Biomarkers in Ovarian Cancer<br><br>SA O'Toole, E Ancuta, R Langhe, DJ Cahill, M Murphy, C Martin, ...<br><br>Noninvasive Molecular Markers in Gynecologic Cancers, 326-359 | | 2015 |
| Ligation of TLR7 on CD19 (+) CD1d (hi) B cells suppresses allergic lung inflammation via regulatory T cells.<br><br>P FALLON, O SHEILS, E HAMS | | 2015 |
| P. 2. a. 010 Analysis of the rat hippocampal microRNA expression profile following chronic electroconvulsive stimulation | | 2015 |

| | | |
|---|---|---|
| KM Ryan, PS Smyth, G Blackshields, O Sheils, DM McLoughlin<br><br>European Neuropsychopharmacology, S380-S381 | | |
| An integrated analysis of the SOX2 microRNA response program in human pluripotent and nullipotent stem cell lines<br><br>SF Vencken, P Sethupathy, G Blackshields, C Spillane, S Elbaruni, ...<br><br>BMC genomics 15 (1), 1-16 | 23 | 2014 |
| Journal of Translational Medicine reviewer acknowledgement 2013<br><br>F Marincola<br><br>Journal of Translational Medicine 12 (1), 1-17 | | 2014 |
| Reviewer acknowledgement 2013<br><br>I Pala<br><br>BMC Genomics 15 (1), 1-43 | | 2014 |
| Human papillomavirus (HPV) and the usefulness of the HPV vaccine for men who have sex with men<br><br>C Sadlier, D Rowley, D Higgins, O Sheils, C Bergin<br><br>The Journal of infectious diseases 210 (10), 1679-1679 | 7 | 2014 |
| Platelet cloaking of CTCs is a universal phenomenon, which drives metastasis<br><br>CD Spillane, NM Cooke, K Egan, S O'Toole, S Finn, D Kenny, O Sheils, ...<br><br>Cancer Research 74 (19_Supplement), 4951-4951 | | 2014 |
| The OncoNetwork Consortium: A global collaborative research study on the development and verification of an Ion AmpliSeq RNA gene lung fusion panel<br><br>SM Magdaleno, A Cheng, R Petraroli, O Sheils, B Tops, DL Corre, H Kurth, ...<br><br>Cancer Research 74 (19_Supplement), 3575-3575 | 5 | 2014 |
| Prevalence of human papillomavirus in men who have sex with men in the era of an effective vaccine; a call to act | 35 | 2014 |

| | | |
|---|---|---|
| C Sadlier, D Rowley, D Morley, S Surah, S O'dea, S Delamere, J O'Leary, ...<br><br>HIV medicine 15 (8), 499-504 | | |
| The MyD88+ phenotype is an adverse prognostic factor in epithelial ovarian cancer<br><br>CJ d'Adhemar, CD Spillane, MF Gallagher, M Bates, KM Costello, ...<br><br>PLoS One 9 (6), e100816 | 44 | 2014 |
| Identification of Stem Cell Hierarchies in a Prostate Cancer Cell Line<br><br>LJ Flynn, O Sheils, C Spillane, C O'Brien, J O'Leary, S Finn<br><br>LABORATORY INVESTIGATION 94, 228A-228A | | 2014 |
| Platelets Enhance the Invasive Capabilities of Metastatic Ovarian Cancer In Vitro<br><br>CD Spillane, N Cooke, O Sheils, D Kenny, JJ O'Leary<br><br>LABORATORY INVESTIGATION 94, 467A-467A | | 2014 |
| Platelet Cloaking of Cancer Cells Is a Universal Phenomenon<br><br>CD Spillane, NM Cooke, D Kenny, O Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 94, 467A-467A | | 2014 |
| Aldehyde Dehydrogenase Positive Cancer Stem Cells Are at the Center of an Ovarian Cancer Stemness Hierarchy<br><br>B Ffrench, C Gasch, L Kehoe, B Doyle, G Blackshields, S O'Toole, ...<br><br>LABORATORY INVESTIGATION 94, 283A-283A | | 2014 |
| Platelet-Modulation of Natural Killer Cell Reactivity on Cancer Cells<br><br>N Glaviano, CD Spillane, NM Cooke, B Ffrench, S O'Toole, D Kenny, ...<br><br>LABORATORY INVESTIGATION 94, 460A-461A | | 2014 |
| Comparing ion torrent with pyrosequencing and Sanger sequencing for the detection of TP53 mutations in colorectal cancer<br><br>B Doyle, CC Lee, TT Harkins, R Petraroli, P Smyth, K Sheahan, JJ O'leary, ...<br><br>EPMA Journal 5 (1), 1-1 | | 2014 |

| | | |
|---|---|---|
| Noninvasive Biomarkers in Ovarian Cancer<br>SA O'Toole, E Ancuta, R Langhe, DJ Cahill, M Murphy, C Martin, ...<br>Cancer Biomarkers, 639-666 | | 2014 |
| The MyD88+ Phenotype Is an Adverse Prognostic Factor in Epithelial Ovarian Cancer.<br>S O'TOOLE, L O'NEILL, N GLEESON, KEN O'BYRNE, LAJ O'NEILL, ... | | 2014 |
| P. 2. e. 004 Analysis of the electroconvulsive stimulation hippocampal miRNome using a deep sequencing approach<br>KM Ryan, P Smyth, G Blackshields, O Sheils, DM McLoughlin<br>European Neuropsychopharmacology, S449 | | 2014 |
| Comparison of Ion Torrent and Pyrosequencing in the Detection of TP53 Mutations<br>B Doyle, CC Lee, TT Harkins, R Petraroli, P Smyth, K Sheahan, ...<br>JOURNAL OF PATHOLOGY 231, 24-24 | | 2013 |
| Activated eosinophils in association with enteric nerves in inflammatory bowel disease<br>CM Smyth, N Akasheh, S Woods, E Kay, RK Morgan, MA Thornton, ...<br>PLoS One 8 (5), e64216 | 40 | 2013 |
| The OncoNetwork Consortium: A European Collaborative Research study on the development of an Ion AmpliSeq gene panel targeting hot spots in colon and lung cancers.<br>N Normanno, R Petraroli, A Rico, H Kurth, B Tops, E Amato, A Mafficini, ...<br>Cancer Research 73 (8_Supplement), 36-36 | 9 | 2013 |
| First evidence of prevalence of human papillomavirus infection in men who have sex with men in Ireland: a stimulus for vaccine review<br>C Sadlier, D Rowley, D Morley, S Surah, S Delamere, P Smyth, S Clarke, ...<br>HIV MEDICINE 14, 76-76 | 1 | 2013 |

| | | |
|---|---|---|
| Novel strategies in the management of anaplastic thyroid carcinoma<br><br>A Dias, M Alhashemi, ER Dorris, P Smyth, M Flanagan, V McEneaney, ...<br><br>IRISH JOURNAL OF MEDICAL SCIENCE 182, S39-S40 | | 2013 |
| Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe<br><br>WA Tjalma, A Fiander, O Reich, N Powell, AM Nowakowski, B Kirschner, ...<br><br>International journal of cancer 132 (4), 854-867 | 222 | 2013 |
| miRNA Signatures of Indolent and Aggressive Prostate Cancer<br><br>L Flynn, O Sheils, JJ O'Leary, SP Finn, W Watson, M Loda, L Mucci<br><br>LABORATORY INVESTIGATION 93, 211A-211A | | 2013 |
| Interaction of Platelets with Ovarian Carcinoma Cells Leads to Induction of an Epithelial-Mesenchymal-Transition (EMT)-Associated Gene Expression Profile in Carcinoma Cells<br><br>N Conlon, C Spillane, N Cooke, S O'Toole, C Martin, O Sheils, D Kenny, ...<br><br>LABORATORY INVESTIGATION 93, 270A-270A | | 2013 |
| Mechanisms of Expression and Regulation of SOX2 and Its Targets in Two Embryonal Carcinoma Cell Lines<br><br>S Vencken, MF Gallagher, G Blacksheilds, C Martin, OM Sheils, ...<br><br>LABORATORY INVESTIGATION 93, 297A-297A | | 2013 |
| Upregulation of miR-141 Potentially Limits Expression of JAG1 in Epithelial Ovarian Neoplasia<br><br>L Keogh, P Smyth, J O'Leary, O Sheils, R Flavin<br><br>LABORATORY INVESTIGATION 93, 283A-283A | | 2013 |
| MEK Inhibition Induces Differential Mechanisms of Resistance in Thyroid and Melanoma BRAF (V600E) Cell Lines<br><br>ER Dorris, M Alhashemi, R Dunne, P Smyth, JJ O'Leary, O Sheils<br><br>LABORATORY INVESTIGATION 93, 434A-434A | | 2013 |

| | | |
|---|---|---|
| Novel Hypoxia-Associated Markers of Chemoresistance in Ovarian Cancer<br><br>L McEvoy, S O'Toole, C Spillane, B Stordal, M Gallagher, C Martin, ...<br><br>The 102nd USCAP Annual Meeting | | 2013 |
| Isolation and Interrogation of Ovarian Cancer Stem Cells<br><br>B Ffrench, M Gallagher, A Cooke, B Stordal, S O'Toole, C Martin, O Sheils, ...<br><br>LABORATORY INVESTIGATION 93, 274A-275A | | 2013 |
| MyD88 Is Central to the Process of Differentiation in Cancer Stem Cells Which May Explain Its Role in Chemoresistance of Ovarian Cancer<br><br>C D'Adhemar, C Spillane, M Gallagher, S O'Toole, C Martin, B Stordal, ...<br><br>LABORATORY INVESTIGATION 93, 270A-270A | | 2013 |
| A Pragmatic Approach to Assay Selection: Comparison of In-House Validated Assays Versus CE-IVD Assays for the Analysis of High-Throughput Molecular Theranostic Assays<br><br>C O'Brien, E Logan, J Gibbons, O Sheils, SE Langabeer, SP Finn<br><br>Laboratory Investigation 93, 480A-480A | | 2013 |
| Pre-treatment of platinum resistant ovarian cancer cells with an MMP-9/MMP-2 inhibitor prior to cisplatin enhances cytotoxicity as determined by high content screening<br><br>A Laios, BM Mohamed, L Kelly, R Flavin, S Finn, L McEvoy, M Gallagher, ...<br><br>International journal of molecular sciences 14 (1), 2085-2103 | 28 | 2013 |
| HERACLES/SCALE Study Group: Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe<br><br>WA Tjalma, A Fiander, O Reich, N Powell, AM Nowakowski, B Kirschner, ...<br><br>Int J Cancer 132 (4), 854-67 | 16 | 2013 |

| | | |
|---|---|---|
| MicroRNA-31 modulates tumour sensitivity to radiation in oesophageal adenocarcinoma<br><br>N Lynam-Lennon, JV Reynolds, L Marignol, OM Sheils, GP Pidgeon, ...<br><br>Journal of molecular medicine 90 (12), 1449-1458 | 122 | 2012 |
| Centrifugo-magnetophoretic particle separation<br><br>D Kirby, J Siegrist, G Kijanka, L Zavattoni, O Sheils, J O'Leary, R Burger, ...<br><br>Microfluidics and nanofluidics 13 (6), 899-908 | 70 | 2012 |
| Suppression of cancer stemness p21-regulating mRNA and microRNA signatures in recurrent ovarian cancer patient samples<br><br>MF Gallagher, CCBB Heffron, A Laios, SA O'Toole, B Ffrench, PC Smyth, ...<br><br>Journal of Ovarian Research 5 (1), 1-11 | 15 | 2012 |
| RET protein expression in papillary renal cell carcinoma<br><br>R Flavin, SP Finn, TK Choueiri, H Ingoldsby, M Ring, C Barrett, M Rogers, ...<br><br>Urologic Oncology: Seminars and Original Investigations 30 (6), 900-905 | 17 | 2012 |
| *MIR141* Expression Differentiates Hashimoto Thyroiditis from PTC and Benign Thyrocytes in Irish Archival Thyroid Tissues<br><br>ER Dorris, P Smyth, JJ O'Leary, O Sheils<br><br>Frontiers in Endocrinology 3, 102 | 22 | 2012 |
| Novel Strategies in the Management of Anaplastic Thyroid Carcinoma<br><br>A Dias, M Alhashemi, ER Dorris, P Smyth, M Flanagan, V McEneaney, ...<br><br>IRISH JOURNAL OF MEDICAL SCIENCE 181, S164-S164 | | 2012 |
| Clinical correlation and molecular evaluation confirm that the MLH1 p. Arg182Gly (c. 544A> G) mutation is pathogenic and causes Lynch syndrome<br><br>MP Farrell, DJ Hughes, IR Berry, DJ Gallagher, EA Glogowski, SJ Payne, ... | 4 | 2012 |

| | | |
|---|---|---|
| Familial cancer 11 (3), 509-518 | | |
| Epitope presentation is an important determinant of the utility of antigens identified from protein arrays in the development of autoantibody diagnostic assays<br><br>MA Murphy, DJ O'Connell, SL O'Kane, JK O'Brien, S O'Toole, C Martin, ...<br><br>Journal of proteomics 75 (15), 4668-4675 | 28 | 2012 |
| Clinical utility of using C-reactive protein and procalcitonin as biomarkers for a novel neonatal sepsis diagnostic platform (ASCMicroPlat)<br><br>K McAllister, M Sheridan-Pereira, N O'Sullivan, R O'Kelly, D Mark, ...<br><br>Critical Care 16 (3), 1-58 | 6 | 2012 |
| Low MAD2 expression levels associate with reduced progression-free survival in patients with high-grade serous epithelial ovarian cancer<br><br>F Furlong, P Fitzpatrick, S O'Toole, S Phelan, B McGrogan, A Maguire, ...<br><br>The Journal of pathology 226 (5), 746-755 | 77 | 2012 |
| Downregulation of Genes Contributes to Chemoresistance Induced by Hypoxia<br><br>LM McEvoy, SA O'Toole, CD Spillane, CM Martin, O Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 92, 454A-454A | | 2012 |
| Stemness Gene Expression Profiles in Cancer Stem Cell Progenies Derived from a Cell Line Panel plus/-BRAF Mutation<br><br>G Sommerville, P Smyth, JJ O'Leary, O Sheils<br><br>LABORATORY INVESTIGATION 92, 456A-457A | | 2012 |
| Stemness Gene Expression Profiles in Cancer Stem Cell Progenies Derived from a Cell Line Panel plus/-BRAF Mutation<br><br>G Sommerville, P Smyth, JJ O'Leary, O Sheils<br><br>LABORATORY INVESTIGATION 92, 456A-457A | | 2012 |
| Isolation and Interrogation of Ovarian Cancer Stem Cells<br><br>B Ffrench, M Gallagher, A Cooke, B Stordal, S O'Toole, O Sheils, ... | | 2012 |

| | | |
|---|---|---|
| LABORATORY INVESTIGATION 92, 270A-270A | | |
| Genome Expression Profiling of Holoclones Derived from Prostate Cancer<br><br>Y Salley, M Gallagher, S Elbaruni, P Smyth, C Spillane, CM Martin, ...<br><br>LABORATORY INVESTIGATION 92, 239A-239A | | 2012 |
| Identification of Circulating Autoantibodies as Novel Ovarian Cancer Biomarkers<br><br>MA Murphy, DJ O'Connell, JK O'Brien, S O'Toole, SL O'Kane, C Martin, ...<br><br>LABORATORY INVESTIGATION 92, 455A-455A | | 2012 |
| M1R-335 Is Upregulated upon Retinoic Acid-Induced Differentiation of NTera-2 Human Embryonal Carcinoma Cell Line and Can Induce Differentiation<br><br>S Vencken, M Gallagher, S Elbaruni, C Martin, O Sheils, J O'Leary<br><br>LABORATORY INVESTIGATION 92, 458A-458A | | 2012 |
| Immunomagnetic purification of cancer cells from whole blood on a centrifugal microfluidic platform<br><br>D Kirby, G Kijanka, J Siegrist, R Burger, O Sheils, J O'Leary, J Ducrée<br><br>Proc. µTAS, 1126-1128 | 3 | 2012 |
| LASER-BASED MANIPULATION AND FLUORESCENT DETECTION OF INDIVIDUAL, CENTRIFUGALLY ARRAYED BIOPARTICLES<br><br>R Burger, D Kurzbuch, R Gorkin, O Sheils, J O'Leary, M Glynn, G Kijanka, ...<br><br>Society for Chemistry and Micro-Nano Systems) Proc. The 16th International … | 1 | 2012 |
| 809 Treatment With MEK Inhibitors Promotes Emergence of a Stem-like Population in Resistant Melanoma Cells<br><br>ER Dorris, P Smyth, R Dunne, JJ O'Leary, O Sheils<br><br>european journal of cancer, S193 | | 2012 |
| 816 K-Ras Mutation Detection in Colorectal Cancer–a Combined Approach Using TaqMan® Mutation Detection Assays (TMDA) and Digital PCR | | 2012 |

| | | |
|---|---|---|
| O Sheils, P Smyth, J O'Leary, E Grigorenko, D Dewolf, R Petraroli<br><br>european journal of cancer, S195 | | |
| The role of secreted frizzled-related protein 2 expression in prostate cancer<br><br>G O'Hurley, AS Perry, A O'Grady, B Loftus, P Smyth, JJ O'Leary, O Sheils, ...<br><br>Histopathology 59 (6), 1240-1248 | 24 | 2011 |
| Abstract B135: Treatment with the MEK inhibitor PD0325901 modulates expression of key members of the MEK pathway in $BRAF^{V600E}$ but not $BRAF^{WT}$ thyroid …<br><br>ER Dorris, P Smyth, JJ O'Leary, O Sheils<br><br>Molecular Cancer Therapeutics 10 (11_Supplement), B135-B135 | | 2011 |
| Platelet adhesion and degranulation induce pro-survival and pro-angiogenic signalling in ovarian cancer cells<br><br>K Egan, D Crowley, P Smyth, S O'Toole, C Spillane, C Martin, ...<br><br>PloS one 6 (10), e26125 | 176 | 2011 |
| Arrayed capture, assaying and binary counting of cells in a stopped-flow sedimentation mode<br><br>R Burger, G Kijanka, O Sheils, J O'Leary, J Ducrée<br><br>15th International Conference on Miniaturized Systems for Chemistry and Life … | 5 | 2011 |
| MiR-31 modulates tumour sensitivity to radiation in oesophageal cancer<br><br>NL Lennon, JV Reynolds, L Marignol, OM Sheils, GP Pidgeon, SG Maher<br><br>IRISH JOURNAL OF MEDICAL SCIENCE 180, S276-S276 | | 2011 |
| miR-31 modulates tumor sensitivity to radiation in esophageal cancer by regulation of DNA repair genes<br><br>N Lynam-Lennon, JV Reynolds, L Marignol, OM Sheils, GP Pidgeon, ...<br><br>Cancer Research 71 (8_Supplement), 3991-3991 | | 2011 |
| $BRAF^{V600E}$: Implications for Carcinogenesis and Molecular Therapy | 490 | 2011 |

| | | |
|---|---|---|
| ER Cantwell-Dorris, JJ O'Leary, OM Sheils<br><br>Molecular cancer therapeutics 10 (3), 385-394 | | |
| TLR4 and MyD88 Expression in Cancer Stem Cells.<br><br>AA Cooke, MF Gallagher, SA O'Toole, CM Martin, OM Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 91, 441A-441A | | 2011 |
| Platelet Cloaking of Cancer Cells Alters Apoptotic Potential: Implications for Metastasis.<br><br>DJ Crowley, PA Smyth, CD Spillane, SA O'Toole, K Egan, D Kenny, ...<br><br>LABORATORY INVESTIGATION 91, 442A-442A | | 2011 |
| The NSAID Sulindac Inhibits Proliferation and Induces Apoptosis in Cervical Cancer Cell Lines<br><br>CD Spillane, C White, L Kehoe, O Sheils, CM Martin, JJ O'Leary<br><br>LABORATORY INVESTIGATION 91, 267A-267A | | 2011 |
| The Epithelial Mesenchymal Transition (EMT) Profile of Platelet Cloaked Cancer Cells.<br><br>DJ Crowley, PA Smyth, SA O'Toole, K Egan, D Kenny, OM Sheils, ...<br><br>Laboratory Investigation 91, 441A-441A | 2 | 2011 |
| Hypoxia Potentiates the Inverse Relationship between Resistance to Cisplatin and Paclitaxel in Ovarian Cancer Cell Lines.<br><br>LM McEvoy, SA O'Toole, CD Spillane, CM Martin, O Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 91, 259A-259A | | 2011 |
| Platelet Cloaking of Cancer Cells: Platelet Adhesiveness, Effect of Vascular Shear and Access of Chemotherapeutic Agents.<br><br>DJ Crowley, K Egan, E Dunne, SA O'Toole, C Martin, PA Smyth, D Kenny, ...<br><br>LABORATORY INVESTIGATION 91, 241A-242A | | 2011 |
| Expression Profiling of Holoclone and Non-Holoclone Derived Cell Lineage in Prostate Cancer<br><br>YM Salley, MF Gallagher, PC Smyth, CM Martin, OM Sheils, JJ O'Leary | | 2011 |

| | | |
|---|---|---|
| LABORATORY INVESTIGATION 91, 446A-446A | | |
| Alterations in MyD88 and microRNA Expression Are Associated with Chemoresistance in Epithelial Ovarian Cancer.<br><br>C D'Adhemar, SA O'Toole, B Stordal, CM Martin, L McEvoy, DJ Crowley, ...<br><br>LABORATORY INVESTIGATION 91, 242A-242A | 2 | 2011 |
| Platelet Adhesion and Degranulation Induce Pro-Survival and Pro-Angiogenic Signalling in Ovarian Cancer Cells<br><br>BK STORDAL, S O'TOOLE, V MC ENEANEY, C SPILLANE, H KEEGAN, ...<br><br>PLoS | | 2011 |
| MYBL2 (B-MYB) in cervical cancer: putative biomarker<br><br>K Astbury, L McEvoy, H Brian, C Spillane, O Sheils, C Martin, JJ O'Leary<br><br>International Journal of Gynecologic Cancer 21 (2) | 25 | 2011 |
| Inkjet-like printing of single-cells<br><br>A Yusof, H Keegan, CD Spillane, OM Sheils, CM Martin, JJ O'Leary, ...<br><br>Lab on a Chip 11 (14), 2447-2454 | 153 | 2011 |
| Evaluation of Zinc-α-2-glycoprotein and proteasome subunit β-type 6 expression in prostate cancer using tissue microarray technology<br><br>G O'Hurley, A O'Grady, P Smyth, J Byrne, JJ O'Leary, O Sheils, ...<br><br>Applied Immunohistochemistry & Molecular Morphology 18 (6), 512-517 | 11 | 2010 |
| All azoospermic males should be screened for cystic fibrosis mutations before intracytoplasmic sperm injection<br><br>E Mocanu, R Shattock, D Barton, M Rogers, R Conroy, O Sheils, C Collins, ...<br><br>Fertility and sterility 94 (6), 2448-2450 | 20 | 2010 |
| Integration and persistence of oncogenic, human papilloma virus mRNA in HIV-positive women<br><br>A Loy, J McInerney, S Delemere, H Keegan, L Pilkington, O Sheils, ...<br><br>HIV MEDICINE 11, 39-39 | | 2010 |

| | | |
|---|---|---|
| Micro RNA" recurrent metastatic" predicts progression free survival in ovarian cancer<br><br>A Laios, SA O'Toole, B Sheppard, N Gleeson, T D'Arcy, M Ring, O Sheils, ...<br><br>IRISH JOURNAL OF MEDICAL SCIENCE 179, S56-S56 | | 2010 |
| Sensitization of ovarian cancer cell lines to cisplatin by MMP-9 inhibitor: a hormetic effect<br><br>A Laios, SA O'Toole, M Bashir, R Flavin, L Kelly, T D'Arcy, N Gleeson, ...<br><br>IRISH JOURNAL OF MEDICAL SCIENCE 179, S55-S55 | | 2010 |
| Examining the Relationship between Expression of Selected Tumour Associated Genes, Platelet Count and Survival in Ovarian Cancer<br><br>DJ Crowley, RJ Flavin, SA O'Toole, ST Aherne, PC Smyth, K Egan, ...<br><br>LABORATORY INVESTIGATION 90, 238A-239A | | 2010 |
| Human Papillomavirus DNA and mRNA Prevalence and Persistence in a Cohort of Human Immunodeficency Virus Positive Women in Ireland<br><br>J Mc Inerney, H Keegan, L Pilkington, O Sheils, CM Martin, A Loy, ...<br><br>LABORATORY INVESTIGATION 90, 100A-100A | | 2010 |
| Mechanistic Investigation of the p16 [INK4A] Pathway<br><br>L Kehoe, CD Spillane, M Gallagher, O Sheils, C Martin, JJ O'Leary<br><br>LABORATORY INVESTIGATION 90, 387A-388A | | 2010 |
| Differential Expression of Cell Cycle Regulated and Tumor Suppressor Gene Proteins in Localised and Advanced Prostatic Adenocarcinoma<br><br>C Barrett, R Flavin, A Murphy, B Loftus, O Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 90, 177A-177A | | 2010 |
| miRNA Profiling in Cervical Cancer<br><br>L Kehoe, CD Spillane, M Gallagher, O Sheils, C Martin, JJ O'Leary<br><br>LABORATORY INVESTIGATION 90, 249A-249A | | 2010 |
| Holoclone and Non-Holoclone Derived Cell Lineage Characterisation Analysis in Prostate Cancer | | 2010 |

| | | |
|---|---|---|
| YM Salley, MF Gallagher, PC Smyth, CM Martin, OM Sheils, JJ O'Leary<br>LABORATORY INVESTIGATION 90, 391A-391A | | |
| Silencing of HPV Viral Oncogenes E6 and E7 in Cervical Cancer<br>CD Spillane, L Kehoe, O Sheils, CM Marin, JJ O'Leary<br>LABORATORY INVESTIGATION 90, 429A-429A | | 2010 |
| Differential Expression of Cell Cycle Regulated and Tumor Suppressor Gene Proteins in Localised and Advanced Prostatic Adenocarcinoma<br>C Barrett, R Flavin, A Murphy, B Loftus, O Sheils, JJ O'Leary<br>LABORATORY INVESTIGATION 90, 177A-177A | | 2010 |
| Functional Expression Analysis of TLR-4 and MYD88 in Epithelial Ovarian Neoplasia<br>CJ d'Adhemar, MF Gallagher, S O'Toole, C Murphy, PC Smyth, C Martin, ...<br>LABORATORY INVESTIGATION 90, 239A-239A | 1 | 2010 |
| Castleman's Disease<br>R Flavin, CM Martin, O Sheils, JJ O'Leary<br>Molecular Pathology of Hematolymphoid Diseases, 541-544 | | 2010 |
| Papillary Thyroid Carcinoma: Detection of Copy Gain of Platelet Derived Growth Factor B Using Array Comparative Genomic Hybridization in Combination with Laser Capture …<br>SP Finn, JJ O'Leary, OM Sheils<br>Methods of Cancer Diagnosis, Therapy, and Prognosis, 387-398 | | 2010 |
| Regulation of microRNA biosynthesis and expression in 2102Ep embryonal carcinoma stem cells is mirrored in ovarian serous adenocarcinoma patients<br>MF Gallagher, RJ Flavin, SA Elbaruni, JK McInerney, PC Smyth, ...<br>Journal of ovarian research 2 (1), 1-16 | 28 | 2009 |
| Gene expression analysis of diagnostic biopsies predicts pathological response to neoadjuvant chemoradiotherapy of esophageal cancer<br>SG Maher, CM Gillham, SP Duggan, PC Smyth, N Miller, C Muldoon, ... | 84 | 2009 |

| | | |
|---|---|---|
| Annals of surgery 250 (5), 729-737 | | |
| Head and neck squamous cell carcinoma in the young: a spectrum or a distinct group? Part 1<br><br>M Toner, EM O'Regan<br><br>Head and neck pathology 3 (3), 246-248 | 93 | 2009 |
| RET/PTC rearrangement occurring in primary peritoneal carcinoma<br><br>R Flavin, G Jackl, S Finn, P Smyth, M Ring, E O'Regan, S Cahill, K Unger, ...<br><br>International journal of surgical pathology 17 (3), 187-197 | 13 | 2009 |
| FOXA1 is a potential oncogene in anaplastic thyroid carcinoma<br><br>C Nucera, J Eeckhoute, S Finn, JS Carroll, AH Ligon, C Priolo, G Fadda, ...<br><br>Clinical Cancer Research 15 (11), 3680-3689 | 96 | 2009 |
| Methods of cancer diagnosis, therapy, and prognosis: liver cancer<br><br>MA Hayat<br><br>Springer Science & Business Media | 37<br><br>* | 2009 |
| miR-29b expression is associated with disease-free survival in patients with ovarian serous carcinoma<br><br>R Flavin, P Smyth, C Barrett, S Russell, H Wen, J Wei, A Laios, S O'Toole, ...<br><br>International Journal of Gynecologic Cancer 19 (4) | 73 | 2009 |
| Potentially important microRNA cluster on chromosome 17p13. 1 in primary peritoneal carcinoma<br><br>RJ Flavin, PC Smyth, A Laios, SA O'toole, C Barrett, SP Finn, S Russell, ...<br><br>Modern Pathology 22 (2), 197-205 | 105 | 2009 |
| Analysis of microRNA let-7a and mir-140 Expression Levels Using Laser Capture Microdissected (LCM) Samples of Thyroid Formalin Fixed Paraffin Embedded (FFPE) Tissues<br><br>J Li, P Smyth, K Denning, R Flavin, S Aherne, S Guenther, J O'Leary, ... | | 2009 |

| | | |
|---|---|---|
| Laboratory Investigation 89 | | |
| Gene Expression Profiles in Archival Thyroid Carcinoma Using Pre-amplification RT-PCR, Immunohistochemistry and MicroRNA Expression Analysis<br><br>K Denning, P Smyth, R Flavin, S Finn, S Russell, J Li, S Aherne, E Conlon, ...<br><br>LABORATORY INVESTIGATION 89 | | 2009 |
| Geographical Mapping of a Multifocal Thyroid Tumour Using Genetic Alteration Analysis and MiRNA Profiling<br><br>ST Aherne, PC Smyth, RJ Flavin, SM Russell, KM Denning, J Li, ...<br><br>MODERN PATHOLOGY 22 | | 2009 |
| FOXA1 is a potential oncogene in anaplastic thyroid carcinoma<br><br>ME TONER, O SHEILS | | 2009 |
| Downregulation of miRNAs in Pluripotent Cancer Stem Cells Is Mirrored in Advanced Ovarian Serous Carcinoma<br><br>RJ Flavin, M Gallagher, S Elbaruni, CC Heffron, S O'Toole, P Smyth, ...<br><br>LABORATORY INVESTIGATION 89, 213A-213A | | 2009 |
| Holoclone and Non-Holoclone Derived Cell Lineage miRNA Analysis in Prostate Cancer<br><br>YM Salley, P Smyth, CM Martin, O Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 89, 342A-342A | | 2009 |
| Regulation of Toll-Like Receptors in Cancer Stemness: A Pro-Inflammatory Switch Model<br><br>M Gallagher, S Elbaruni, R Flavin, C Martin, A Laios, S O'Toole, O Sheils, ...<br><br>LABORATORY INVESTIGATION 89, 214A-214A | | 2009 |
| Co-Analysis of HPV DNA, mRNA E6/E7 HR-HPV Expression and mcm2 and TOPIIa (ProEx C) in Cervical Pre-Cancer<br><br>M Barrett, C Hannigan, J McInerney, R Flavin, L Pilkington, CM Martin, ... | | 2009 |

| | | |
|---|---|---|
| LABORATORY INVESTIGATION 89, 82A-82A | | |
| Characterization of Gleason Grade 3 Tumor in Localized and Advanced Prostatic Adenocarcinoma Using a Panel of Functional Gene Expression Markers<br><br>C Barrett, P Smyth, B Loftus, O Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 89, 159A-159A | | 2009 |
| Gene expression profiling in cervical cancer: identification of novel markers for disease diagnosis and therapy<br><br>CM Martin, K Astbury, L McEvoy, S O'Toole, O Sheils, JJ O'Leary<br><br>Inflammation and Cancer, 333-359 | 96 | 2009 |
| Potential role of miR-9 and miR-223 in recurrent ovarian cancer<br><br>A Laios, S O'Toole, R Flavin, C Martin, L Kelly, M Ring, SP Finn, C Barrett, ...<br><br>Molecular cancer 7 (1), 1-14 | 367 | 2008 |
| ret/PTC-1 expression alters the immunoprofile of thyroid follicular cells<br><br>K Denning, P Smyth, S Cahill, J Li, R Flavin, S Aherne, JJ O'Leary, ...<br><br>Molecular cancer 7 (1), 1-10 | 6 | 2008 |
| An integrative model for recurrence in ovarian cancer<br><br>A Laios, SA O'Toole, R Flavin, C Martin, M Ring, N Gleeson, T D'Arcy, ...<br><br>Molecular Cancer 7 (1), 1-11 | 34 | 2008 |
| Geographical mapping of a multifocal thyroid tumour using genetic alteration analysis & miRNA profiling<br><br>ST Aherne, PC Smyth, RJ Flavin, SM Russell, KM Denning, JH Li, ...<br><br>Molecular cancer 7 (1), 1-13 | 36 | 2008 |
| Improved RNA quality and TaqMan® Pre-amplification method (PreAmp) to enhance expression analysis from formalin fixed paraffin embedded (FFPE) materials<br><br>J Li, P Smyth, S Cahill, K Denning, R Flavin, S Aherne, M Pirotta, ... | 105 | 2008 |

| | | |
|---|---|---|
| Bmc Biotechnology 8 (1), 1-11 | | |
| Geographical mapping of a multifocal thyroid tumor using genetic alteration analysis and miRNA profiling<br><br>S Aherne, P Smyth, R Flavin, S Russell, K Denning, J Li, S Guenther, ... | | 2008 |
| MIR-29B expression is associated with disease-free survival in ovarian serous carcinoma patients<br><br>R Flavin, P Smyth, C Barrett, S Russell, H Wen, J Wei, A Laios, S OToole, ... | | 2008 |
| RET/PTC rearrangement in non-thyroid papillary tumors<br><br>R Flavin, G Jackl, M Ring, P Smyth, S Finn, E ORegan, S Cahill, ... | | 2008 |
| Lack of association between measles virus vaccine and autism with enteropathy: a case-control study<br><br>M Hornig, T Briese, T Buie, ML Bauman, G Lauwers, U Siemetzki, ...<br><br>PloS one 3 (9), e3140 | 203 | 2008 |
| Altered eIF6 and Dicer expression is associated with clinicopathological features in ovarian serous carcinoma patients<br><br>RJ Flavin, PC Smyth, SP Finn, A Laios, SA O'toole, C Barrett, M Ring, ...<br><br>Modern Pathology 21 (6), 676-684 | 102 | 2008 |
| p16INK4A genetic and epigenetic profiles differ in relation to age and site in head and neck squamous cell carcinomas<br><br>EM O'Regan, ME Toner, SP Finn, CY Fan, M Ring, B Hagmar, C Timon, ...<br><br>Human pathology 39 (3), 452-458 | 88 | 2008 |
| Differential microrna expression in primary peritoneal serous carcinoma versus ovarian serous carcinoma<br><br>JJ O'Leary, P Smyth, A Laios, S O'Toole, S Russell, J Li, K Denning, ... | | 2008 |

| | | |
|---|---|---|
| Down-regulation of MIR-195 and MIR-497 from the MicroRNA cluster at chromosome 17p13. 1 in papillary serous carcinoma of the peritoneum<br><br>RJ Flavin, P Smyth, M Ring, S Russell, A Laios, SO Toole, J Li, K Denning, ... | | 2008 |
| Women's knowledge and attitudes regarding cervical screening and human papillomavirus (HPV): focus group findings from Ireland<br><br>J Murphy, L Sharp, C Martin, L Pilkington, O Sheils, J O'Leary<br><br>Cytopathology 19 (Suppl 2), 12 | 2 | 2008 |
| Human papillomavirus prevalence and genotype distribution in the Irish cervical screening population<br><br>J McInerney, L Pilkington, H Keegan, M Ring, N Bolger, O Sheils, ...<br><br>J Cytopathol 19 (Suppl 2), 13 | 2 | 2008 |
| How to Learn<br><br>C Hughes, M Tight<br><br>Open University Press | | 2008 |
| Differential regulation of key stemness genes during early differentiation of teratocarcinoma stem cells<br><br>MF Gallagher, S Elbaruni, C Heffron, SM Guenther, R Henfrey, C Martin, ...<br><br>LABORATORY INVESTIGATION 88, 327A-327A | | 2008 |
| Characlerisation of early stemness regulation in teratocarcinoma stem cells<br><br>MF Gallagher, S Elbaruni, C Heffron, SM Guenther, R Henfrey, C Martin, ...<br><br>LABORATORY INVESTIGATION 88, 327A-327A | | 2008 |
| Effect of $BRAF^{V600E}$ mutation on transcription and post-transcriptional regulation in a papillary thyroid carcinoma model<br><br>S Cahill, P Smyth, K Denning, R Flavin, J Li, A Potratz, SM Guenther, ...<br><br>Molecular Cancer 6 (1), 21 | 77 | 2007 |

| | | |
|---|---|---|
| Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells<br>J Li, P Smyth, R Flavin, S Cahill, K Denning, S Aherne, SM Guenther, ...<br>BMC biotechnology 7 (1), 1-6 | 434 | 2007 |
| A molecular expression signature distinguishing follicular lesions in thyroid carcinoma using preamplification RT-PCR in archival samples<br>KM Denning, PC Smyth, SF Cahill, SP Finn, E Conlon, JH Li, RJ Flavin, ...<br>Modern Pathology 20 (10), 1095-1102 | 47 | 2007 |
| Lymph node status prediction after multi-modal therapy (RCT) in rectal cancer by genome microarray analysis<br>DT McDowell, F Smith, C Gilham, G Pidgeon, P Smith, O Sheils, ...<br>Journal of the American College of Surgeons 205 (3), S89 | | 2007 |
| Gene expression profiling may predict response to neoadjuvant radiochemotherapy (RCT)<br>DT McDowell, F Smith, C Gilham, G Pidgeon, P Smith, O Sheils, ...<br>Journal of the American College of Surgeons 205 (3), S89-S90 | | 2007 |
| Global mRNA analysis to determine a transcriptome profile of cancer stemness in a mouse model<br>CCBB Heffron, MF Gallagher, S Guenther, J Sherlock, R Henfrey, ...<br>Anticancer research 27 (3A), 1319-1324 | 11 | 2007 |
| BRAF T1799A mutation occurring in a case of malignant struma ovarii<br>R Flavin, P Smyth, P Crotty, S Finn, S Cahill, K Denning, J Li, E O'Regan, ...<br>International journal of surgical pathology 15 (2), 116-120 | 34 | 2007 |
| Low-level TOP2A amplification in prostate cancer is associated with HER2 duplication, androgen resistance, and decreased survival<br>AJ Murphy, CA Hughes, C Barrett, H Magee, B Loftus, JJ O'Leary, ...<br>Cancer research 67 (6), 2893-2898 | 44 | 2007 |

| | | |
|---|---|---|
| Extraction and amplification methods to enhance expression analysis from formalin fixed paraffin embedded (FFPE) tissues<br><br>J Li, P Smyth, S Cahill, K Denning, R Flavin, S Guenther, J O'Leary, ... | | 2007 |
| Molecular markers of follicular variant papillary thyroid carcinoma (FVPTC) and follicular adenoma<br><br>K Denning, P Smyth, S Cahill, S Finn, E Conlon, JH Li, R Flavin, ... | | 2007 |
| MicroRNA gene expression profiling in human ovarian and primary peritoneal serous carcinomas<br><br>RJ Flavin, S O'Toole, P Smyth, A Laois, S Cahill, J Li, K Denning, C Martin, ... | 29 | 2007 |
| miRNA signatures in primary versus recurrent ovarian cancers<br><br>SA O'Toole, A Laios, B Sheppard, N Gleeson, T D'Arcy, E McGuinness, ...<br><br>LABORATORY INVESTIGATION 87, 209A-209A | | 2007 |
| Significant dysregulation of DNA replication license and replisome proteins in villoglandular carcinomas of the cervix indicates possible aggressive biological behaviour<br><br>M Ring, S Crowther, C Martin, K Astbury, O Sheils, M Griffin, E Gaffney, ...<br><br>LABORATORY INVESTIGATION 87, 212A-212A | | 2007 |
| MicroRNA signatures in cervical cancer: Suggests hierarchical miRNA control of gene expression<br><br>CM Martin, K Astbury, C Becker, R Henfrey, J Sherlock, S Guenther, ...<br><br>LABORATORY INVESTIGATION 87, 207A-207A | | 2007 |
| Recurrence in ovarian cancer; A transcriptomic approach<br><br>A Laios, SA O'Toole, B Sheppard, N Gleeson, T D'Arcy, E McGuinness, ...<br><br>LABORATORY INVESTIGATION 87, 204A-204A | | 2007 |

| | | |
|---|---|---|
| Regulation of cancer stemness: Understanding self-renewal and early differentiation in cancer stem cells<br><br>C Heffron, MF Gallagher, S Guenther, R Henfrey, J Sherlock, O Sheils, ...<br><br>LABORATORY INVESTIGATION 87, 307A-307A | | 2007 |
| Expression microarray analysis of papillary thyroid carcinoma and benign thyroid tissue: emphasis on the follicular variant and potential markers of malignancy<br><br>SP Finn, P Smyth, S Cahill, C Streck, EM O'regan, R Flavin, J Sherlock, ...<br><br>Virchows Archiv 450 (3), 249-260 | 54 | 2007 |
| Potential predictors of chemotherapy response in ovarian cancer—how do we define chemosensitivity?<br><br>SA O'toole, BL Sheppard, A Laios, JJ O'Leary, EPJ McGuinness, T D'Arcy, ...<br><br>Gynecologic oncology 104 (2), 345-351 | 33 | 2007 |
| Free fetal DNA is not increased before 20 weeks in intrauterine growth restriction or pre-eclampsia<br><br>A Crowley, C Martin, P Fitzpatrick, O Sheils, C O'Herlihy, JJ O'Leary, ...<br><br>Prenatal Diagnosis: Published in Affiliation With the International Society … | 57 | 2007 |
| Reflective Teaching Portfolios for Continuous Professional Development at Trinity College Dublin.<br><br>O Hanratty, C O'Farrell<br><br>Centre for Academic Practice and Student Learning (CAPSL), Trinity College … | 2 | 2007 |
| 5008 ORAL miRNA signatures in recurrent ovarian cancer<br><br>A Laios, SA O'Toole, B Sheppard, NC Gleeson, T D'Arcy, ...<br><br>EJC Supplements 4 (5), 313 | | 2007 |
| Heterogeneous expression of α-methylacyl-CoA racemase in prostatic cancer correlates with Gleason score<br><br>AJ Murphy, CA Hughes, G Lannigan, O Sheils, J O'Leary, B Loftus | 31 | 2007 |

| | | |
|---|---|---|
| Histopathology 50 (2), 243-251 | | |
| Low-level genomic instability is a feature of papillary thyroid carcinoma: an array comparative genomic hybridization study of laser capture microdissected papillary thyroid …<br><br>S Finn, P Smyth, E O'Regan, S Cahill, M Toner, C Timon, R Flavin, ...<br><br>Archives of pathology & laboratory medicine 131 (1), 65-73 | 37 | 2007 |
| Effect of ret/PTC 1 rearrangement on transcription and post-transcriptional regulation in a papillary thyroid carcinoma model<br><br>S Cahill, P Smyth, SP Finn, K Denning, R Flavin, EM O'Regan, J Li, ...<br><br>Molecular Cancer 5 (1), 70 | 89 | 2006 |
| Activated eosinophils in association with enteric nerves in inflammatory bowel disease. Mechanism of recruitment and correlation with disease activity<br><br>CM Smyth, S Woods, M Thornton, A O'Grady, R Morgan, R Cummins, ...<br><br>JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY 21, A262-A262 | | 2006 |
| 0447: Identification of Cell Cycle Regulatory Molecules As Biomarkers in Cervical Cancer By Gene Expression Profiling<br><br>K Astbury, CM Martin, OM Sheils, JJ O'leary<br><br>International Journal of Gynecologic Cancer 16 (Suppl 3) | | 2006 |
| Expanding applications of diagnostic tools-Isolating papillary thyroid cancer biomarkers<br><br>O Sheils<br><br>GENETIC ENGINEERING NEWS 26 (16), 50-51 | | 2006 |
| BRAF (T1799A) mutations are not expressed in non-thyroid papillary tumours<br><br>RJ Flavin, P Smyth, SP Finn, S Cahill, EM O'Regan, M Ring, J O'Leary, ...<br><br>JOURNAL OF PATHOLOGY 210, 4-4 | | 2006 |
| Recurrent ovarian cancer cDNA signatures | | 2006 |

| | | |
|---|---|---|
| A Laios, SA O'Toole, BL Sheppard, N Gleeson, T D'Arcy, EPJ McGuinness, ...<br><br>JOURNAL OF PATHOLOGY 210, 2-2 | | |
| Genome wide expression microarray analysis of papillary thyroid carcinoma and benign thyroid tissue: emphasis on the follicular variant and potential biomarkers of malignancy.<br><br>P Smyth, S Finn, S Cahill, C Streck, E O'Regan, M Toner, R Henfrey, ...<br><br>JOURNAL OF PATHOLOGY 210, 43-43 | | 2006 |
| TOP2A amplification in prostate cancer is associated with HER-2 amplification, androgen resistance & decreased survival<br><br>AJ Murphy, CA Hughes, C Barrett, H Magee, B Loftus, J O'Leary, O Sheils<br><br>JOURNAL OF PATHOLOGY 210, 60-60 | | 2006 |
| MicroRNA profiling of papillary thyroid carcinoma cell lines.<br><br>S Cahill, P Smyth, K Denning, R Flavin, JH Lee, SP Finn, EM Oregan, ...<br><br>JOURNAL OF PATHOLOGY 210, 43-43 | | 2006 |
| Expression microarray analysis of thyroid carcinoma cell lines harboring BRAF V600E mutations<br><br>S Cahill, P Smyth, K Denning, JH Lee, SP Finn, E O'Regan, M Gallagher, ...<br><br>JOURNAL OF PATHOLOGY 210, 43-43 | | 2006 |
| The NOD2/CARD15 3020insC polymorphism is not associated with gastrointestinal disease in autistic patients.<br><br>A Lynch, M Ring, O Sheils, CM Martin, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 210, 13-13 | | 2006 |
| Topoisomerase II-α expression increases with increasing Gleason score and with hormone insensitivity in prostate carcinoma<br><br>C Hughes, A Murphy, C Martin, E Fox, M Ring, O Sheils, B Loftus, ...<br><br>Journal of clinical pathology 59 (7), 721-724 | 34 | 2006 |
| Squamous cell carcinoma of the head and neck in young Irish adults | 49 | 2006 |

| | | |
|---|---|---|
| EM O'regan, C Timon, O Sheils, M Codd, JJ O'leary, M Toner<br>British Journal of Oral and Maxillofacial Surgery 44 (3), 203-206 | | |
| Future molecular aspects of cervical cytology<br>K Astbury, CM Martin, M Ring, L Pilkington, N Bolger, OM Sheils, ...<br>Current Diagnostic Pathology 12 (2), 104-113 | 6 | 2006 |
| Distinct array comparative genomic hybridization profiles in oral squamous cell carcinoma occurring in young patients<br>EM O'Regan, ME Toner, PC Smyth, SP Finn, C Timon, S Cahill, R Flavin, ...<br>Head & neck 28 (4), 330-338 | 51 | 2006 |
| Molecular profiling of cervical neoplasia<br>CM Martin, K Astbury, JJ O'Leary<br>Expert review of molecular diagnostics 6 (2), 217-229 | 61 | 2006 |
| Genome-wide analysis of deoxyribonucleic acid in endometrial cancer using comparative genomic hybridization microarrays<br>SA O'toole, E Dunn, BL Sheppard, H Klocker, J Bektic, P Smyth, C Martin, ...<br>International Journal of Gynecologic Cancer 16 (2) | 16 | 2006 |
| cervical dyskaryosis<br>N Murphy, M Ring, O Sheils, J O'Leary<br>The Health Professional's HPV Handbook: Human Papillomavirus and Cervical … | | 2006 |
| Aberrant expression of VEGF-C is related to grade of cervical intraepithelial neoplasia (CIN) and high risk HPV, but does not predict virus clearance after treatment of CIN or …<br>M Branca, C Giorgi, D Santini, L Di Bonito, M Ciotti, A Benedetto, P Paba, ...<br>Journal of clinical pathology 59 (1), 40-47 | 64 | 2006 |
| cDNA microarray analysis of thyroid carcinoma cell lines harboring ret/PTC oncogenes and BRAFV600E mutations | | 2006 |

| | | |
|---|---|---|
| P Smyth, SP Finn, S Cahill, E O'Regan, M Toner, C Streck, R Henfrey, ...<br><br>LABORATORY INVESTIGATION 86, 98A-98A | | |
| Narrow repertoire of gene expression including aberrant expression of MHC type 2 molecules distinguishes FVPTC from classic morphology PTC and benign thyroid tissue<br><br>SP Finn, PC Smyth, S Cahill, C Streck, EM O'Regan, ME Toner, C Timon, ...<br><br>LABORATORY INVESTIGATION 86, 93A-93A | | 2006 |
| Coexpression of HER2 and TOP2A in prostate cancer correlates with Gleason score and HER2 amplification<br><br>A Murphy, C Hughes, C Barrett, H Magee, O Sheils, J O'Leary, B Loftus<br><br>LABORATORY INVESTIGATION 86, 151A-151A | | 2006 |
| Genetic and epigenetic alteration profiles for p16 in head and neck squamous cell carcinoma in young adults<br><br>EM O'Regan, CY Fan, ME Toner, M Ring, SP Finn, C Timon, P Smyth, ...<br><br>LABORATORY INVESTIGATION 86, 210A-210A | 1 | 2006 |
| miRNA expression signatures in cervical cancer<br><br>CM Martin, K Astbury, R Henfrey, O Sheils, M Gallagher, C Heffron, ...<br><br>LABORATORY INVESTIGATION 86, 189A-189A | 2 | 2006 |
| Molecular profiling of primary versus recurrent ovarian serous papillary adenocarcinomas<br><br>A Laios, SA O'Toole, BL Sheppard, NC Gleeson, T D'Arcy, ...<br><br>Laboratory Investigation 86, 185A-185A | 1 | 2006 |
| The science of laboratory diagnosis<br><br>J Crocker, D Burnett<br><br>John Wiley & Sons | ~~24~~ | 2005 |
| The science of laboratory diagnosis<br><br>J Crocker, D Burnett<br><br>John Wiley & Sons | 24 | 2005 |

| | | |
|---|---|---|
| Molecular classification and biomarker discovery in papillary thyroid carcinoma<br>O Sheils<br>Expert Review of Molecular Diagnostics 5 (6), 927-946 | 36 | 2005 |
| Angiogenesis markers in ovarian cancer act as prognostic markers but not indicators of chemotherapy response<br>S O'Toole, BL Sheppard, JJ O'Leary, O Sheils, T D'arcy, N Gleeson, ...<br>JOURNAL OF PATHOLOGY 207, 1-1 | | 2005 |
| A novel combination of biomarkers for cervical glandular neoplasia<br>C Heffron, N Murphy, M Ring, C Martin, O Sheils, JJ O'Leary<br>JOURNAL OF PATHOLOGY 207, 23-23 | | 2005 |
| Topoisomerase II-Alpha expression correlates with Gleason Score in prostate carcinoma<br>C Hughes, A Murphy, C Martin, O Sheils, B Loftus, JJ O'Leary<br>JOURNAL OF PATHOLOGY 207, 14-14 | | 2005 |
| Molecular pathology of prostate cancer<br>C Hughes, A Murphy, C Martin, O Sheils, J O'leary<br>Journal of clinical pathology 58 (7), 673-684 | 212 | 2005 |
| Oestrogen regulated gene expression in normal and malignant endometrial tissue<br>SA O'Toole, E Dunn, BL Sheppard, O Sheils, JJ O'Leary, W Wuttke, ...<br>Maturitas 51 (2), 187-198 | 39 | 2005 |
| Multiple signaling pathways converge on β-catenin in thyroid cancer<br>PH Abbosh, KP Nephew<br>Thyroid 15 (6), 551-561 | 80 | 2005 |
| Quantitation of CDC6 and MCM5 mRNA in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix<br>N Murphy, M Ring, CCBB Heffron, CM Martin, E McGuinness, O Sheils, ... | 68 | 2005 |

| | | |
|---|---|---|
| Modern pathology 18 (6), 844-849 | | |
| Analysis of DNA in endometrial cancer cells treated with phyto-estrogenic compounds using comparative genomic hybridisation microarrays<br><br>SA O'Toole, BL Sheppard, O Sheils, JJ O'Leary, B Spengler, V Christoffel<br><br>Planta medica 71 (05), 435-439 | 12 | 2005 |
| p16INK4A, CDC6, and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer<br><br>N Murphy, M Ring, C Heffron, B King, AG Killalea, C Hughes, CM Martin, ...<br><br>Journal of clinical pathology 58 (5), 525-534 | 301 | 2005 |
| Detection of a tyrosine phosphatase LAR on intestinal epithelial cells and intraepithelial lymphocytes in the human duodenum<br><br>AM Murphy, OM Sheils, GSA McDonald, DP Kelleher<br><br>Mediators of inflammation 2005 (1), 23-30 | 4 | 2005 |
| High-density SNP and cDNA array analysis<br><br>O Sheils, CM Martin, P Smyth, J Sherlock, S Finn, E O'Regan, S Picton, ...<br><br>THE SCIENCE OF, 515 | | 2005 |
| CGH array analysis of human tissues<br><br>E O'Regan, P Smyth, S Finn, CM Martin, O Sheils, J O'Leary<br><br>THE SCIENCE OF, 523 | | 2005 |
| Gene expression profile of PTC: Comparison of Follicular Variant and classic morphology PTC with benign follicular patterned lesions<br><br>SP Finn, PC Smyth, EM O'Regan, S Cahill, M Toner, C Timon, RJ Flavin, ...<br><br>LABORATORY INVESTIGATION 85, 90A-90A | | 2005 |
| Absence of p16 gene (CDKN2A) deletions in HNSCC occurring in young adults<br><br>EM O'Regan, OM Sheils, NC Murphy, M Ring, ME Toner, SP Finn, ... | | 2005 |

| | | |
|---|---|---|
| LABORATORY INVESTIGATION 85, 216A-216A | | |
| Variable expression of alpha-Methylacyl-CoA racemase in benign prostatic hyperplasia & prostate cancer<br><br>AJ Murphy, CA Hughes, O Sheils, JJ O'Leary, B Loftus<br><br>LABORATORY INVESTIGATION 85, 156A-156A | | 2005 |
| Detection of a Tyrosine Phosphatase LAR on Intestinal Epithelial Cells and Intraepithelial Lymphocytes in the Human Duodenum.<br><br>DP KELLEHER, A MURPHY, O SHEILS<br><br>Hindawi | | 2005 |
| ret/PTC and BRAF act as distinct molecular, time-dependant triggers in a sporadic Irish cohort of papillary thyroid carcinoma<br><br>P Smyth, S Finn, S Cahill, E O'Regan, R Flavin, JJ O'Leary, O Sheils<br><br>International Journal of Surgical Pathology 13 (1), 1-8 | 48 | 2005 |
| p16INK4A positivity in benign, premalignant and malignant cervical glandular lesions: a potential diagnostic problem<br><br>N Murphy, C Heffron, B King, UG Ganuguapati, M Ring, E McGuinness, ...<br><br>Virchows Archiv 445 (6), 610-615 | 96 | 2004 |
| Array comparative genomic hybridisation analysis of gamma-irradiated human thyrocytes<br><br>SP Finn, P Smyth, E O'Regan, S Cahill, R Flavin, J O'Leary, O Sheils<br><br>Virchows Archiv 445 (4), 396-404 | 28 | 2004 |
| Fetal DNA in the maternal plasma, in early pregnancy. Its use as a marker of abnormal pregnancy.<br><br>A Crowley, O Sheils, C Martin, P Fitzpatrick, C O'Herlihy, J O'Leary, ...<br><br>JOURNAL OF PATHOLOGY 204, 42A-42A | | 2004 |
| Array CGH profiling of laser capture microdissected Papillary Thyroid Carcinoma tumours and clonal cell lines.<br><br>SP Finn, PA Smyth, EM O'Regan, C Timon, S Cahill, M Toner, R Flavin, ... | | 2004 |

| | | |
|---|---|---|
| JOURNAL OF PATHOLOGY 204, 27A-27A | | |
| Cytokine mRNA expression profiles in children with lymphonodular hyperplasia and autistic spectrum disorders.<br><br>CM Martin, L Pilkington, O Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 204, 32A-32A | | 2004 |
| Serous carcinoma of the ovary: A link between radiation exposure and RET/PTC-1 activation?<br><br>RJ Flavin, SP Finn, M Ring, EM O'Regan, S Cahill, E Gaffney, JJ O'Leary, ...<br><br>JOURNAL OF PATHOLOGY 204, 20A-20A | | 2004 |
| Human papillomavirus DNA vs mRNA: Clinical indicators for the development of cervical neoplasia?<br><br>N Murphy, H Skomedal, MO Oye, P Gronn, IM Falang, O Sheils, ...<br><br>JOURNAL OF PATHOLOGY 204, 20A-20A | | 2004 |
| P16 (INK4A), MCM5, CDC6 and high-risk HPV status: A screening algorithm for cervical disease<br><br>N Murphy, M Ring, M Griffin, O Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 204, 19A-19A | | 2004 |
| Infertile males-Who should be screened for cystic fibrosis mutations<br><br>EV Mocanu, R Shattock, C Martin, D Barton, M Rogers, O Sheils, ...<br><br>JOURNAL OF PATHOLOGY 204, 34A-34A | | 2004 |
| Analysis of head and neck squamous cell carcinoma in young patients using microarray comparative genomic hybridisation<br><br>EM O'Regan, P Smyth, SP Finn, S Cahill, R Flavin, C Timon, M Toner, ...<br><br>JOURNAL OF PATHOLOGY 204, 9A-9A | | 2004 |
| Temporal trends in BRAF T1796A and ret/PTC oncogene detection in Irish thyroid neoplasms<br><br>P Smyth, S Finn, R Flavin, S Cahill, E O'Regan, JJ O'Leary, O Sheils<br><br>JOURNAL OF PATHOLOGY 204, 27A-27A | | 2004 |
| 5. Molecular markers in cervical dyskaryosis | 1 | 2004 |

| | | |
|---|---|---|
| N Murphy, M Ring, O Sheils, J O'Leary<br><br>HPV HANDBOOK, 73 | | |
| 3 Blots, dots, amplification, and sequencing<br><br>JJ O'Leary, C Martin, O Sheils<br><br>Molecular Biology and Pathology of Paediatric Cancer, 56 | | 2003 |
| Gene expression profiling of inflammatory bowel disease<br><br>CM Martin, O Sheils, AJ Wakefield, S Murch, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 201, 14A-14A | | 2003 |
| Fetal trafficking in the first trimester of pregnancy-A marker of intrauterine growth restriction?<br><br>A Crowley, C O'Herlihy, O Sheils, JJ O'Leary, B Byrne<br><br>JOURNAL OF PATHOLOGY 201, 40A-40A | | 2003 |
| Common endothelial cell genetic events occur during HHV 8 transformation: Evidence from primary transformed endothelial and co-hybrid transformed culture model systems<br><br>I Silva, P Smyth, C Martin, O Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 201, 8A-8A | | 2003 |
| Osteoclastoma like anaplastic thyroid carcinoma: molecular evidence of its histogenesis<br><br>EM O'Regan, P Smyth, SP Finn, O Sheils, O O'Leary, M Toner<br><br>JOURNAL OF PATHOLOGY 201, 51A-51A | | 2003 |
| Absolute quantitation of CDC6 and MCM5 mRNA in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix<br><br>N Murphy, C Long, F Mulcahy, E McGuinness, M Griffin, CM Martin, ...<br><br>JOURNAL OF PATHOLOGY 201, 11A-11A | | 2003 |
| The dynamics of fetal trafficking in pregnancy<br><br>A Crowley, C Martin, O Sheils, C O'Herlihy, JJ O'Leary, B Byrne<br><br>JOURNAL OF PATHOLOGY 201, 9A-9A | | 2003 |

| | | |
|---|---|---|
| Cytokine gene expression analysis in autistic enterocolitis<br>C Martin, R Hickey, O Sheils, AJ Wakefield, S Murch, JJ O'Leary<br>JOURNAL OF PATHOLOGY 201, 14A-14A | 2 | 2003 |
| Molecular biology in cellular pathology<br>J Crocker, PG Murray<br>John Wiley & Sons | 22 | 2003 |
| Quality-control issues for PCR-based assays in the molecular laboratory<br>OM Sheils, S Finn, JJ O'Leary<br>Current diagnostic pathology 9 (3), 165-172 | 3 | 2003 |
| Nucleic acid microarrays: an overview<br>O Sheils, S Finn, J O'Leary<br>Current Diagnostic Pathology 9 (3), 155-158 | 23 | 2003 |
| The In-situ Polymerase Chain Reaction<br>JJ O'Leary, C Martin, O Sheils<br>Molecular Biology in Cellular Pathology, 233 | | 2003 |
| TaqMan Technology and Real-Time Polymerase Chain Reaction<br>JJ O'Leary, O Sheils, C Martin, A Crowley<br>Molecular biology in cellular pathology, 251 | 4 | 2003 |
| Real-Time Analysis of β-and γ-Catenin mRNA Expression in ret/PTC-1 Activated and Nonactivated Thyroid Tissues<br>P Smyth, S Finn, J O'Leary, O Sheils<br>Diagnostic Molecular Pathology 12 (1), 44-49 | 6 | 2003 |
| Ret/PTC chimeric transcripts in an Irish cohort of sporadic papillary thyroid carcinoma<br>SP Finn, P Smyth, J O'Leary, EC Sweeney, O Sheils<br>The Journal of Clinical Endocrinology & Metabolism 88 (2), 938-941 | 27 | 2003 |

| | | |
|---|---|---|
| p16INK4A as a marker for cervical dyskaryosis: CIN and cGIN in cervical biopsies and ThinPrep™ smears<br><br>N Murphy, M Ring, AG Killalea, V Uhlmann, M O'Donovan, F Mulcahy, ...<br><br>Journal of Clinical Pathology 56 (1), 56-63 | 367 | 2003 |
| Increased maternal fetal cell trafficking and Rh" D" gene immunization in pre-eclampsia<br><br>A Cotter, CM Martin, O Sheils, SF Daly, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 198, 2A-2A | | 2002 |
| Ret/PTC-3 correlates with follicular/solid morphology in" sporadic" papillary thyroid carcinoma<br><br>SP Finn, P Smyth, EC Sweeney, JJ O'Leary, OM Sheils<br><br>JOURNAL OF PATHOLOGY 198, 50A-50A | | 2002 |
| Molecular Analysis of Primary Effusion Lymphoma using mFISH and CGH analysis: demonstrates common molecular-genetic pathways<br><br>M O'Donovan, I Silva, C Martin, OM Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 198, 13A-13A | | 2002 |
| Quantification of Fetal DNA in the peripheral blood of women with pre-eclampsia in South Africa and Ireland-A useful marker of disease severity?<br><br>A Crowley, JJ O'Leary, O Sheils, C Martin, F Taulo, J Anthony, C O'Herlihy, ...<br><br>JOURNAL OF PATHOLOGY 198, 22A-22A | | 2002 |
| Cytokine profile analysis in multiple sclerosis<br><br>D Murphy, V Uhlmann, C Martin, O Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 198, 52A-52A | | 2002 |
| Development of a detailed molecular profile for prostate cancer<br><br>C Hughes, C Martin, O Sheils, B Loftus, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 198, 21A-21A | | 2002 |

| | | |
|---|---|---|
| E-cadherin expression is reduced in reVPTC-1 but not reVPTC-3 activated thyroid neoplasms<br><br>P Smyth, S Finn, JJ O'Leary, OM Sheils<br><br>JOURNAL OF PATHOLOGY 198, 11A-11A | | 2002 |
| Analysis of beta-catenin expression in thyroid neoplasia<br><br>P Smyth, S Finn, JJ O'Leary, O Sheils<br><br>JOURNAL OF PATHOLOGY 198, 49A-49A | | 2002 |
| Increased maternal fetal cell trafficking in early pregancy is associated with an increased risk of pre-eclampsia<br><br>A Cotter, CM Martin, O Sheils, SF Daly, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 198, 8A-8A | | 2002 |
| p16INK4A, CDC6, MCM5 and HPV status: predictive biomarkers of cervical pre-neoplasia?<br><br>N Murphy, M Ring, AG Killalea, F Mulcahy, E McGuinness, M Griffin, ...<br><br>JOURNAL OF PATHOLOGY 198, 16A-16A | | 2002 |
| Potential viral pathogenic mechanism for now variant inflammatory bowel disease<br><br>CM Martin, V Uhlmann, O Sheils, L Pilkington, I Silva, A Killalea, JW Smith, ...<br><br>JOURNAL OF PATHOLOGY 198, 37A-37A | | 2002 |
| Development of an'allelic discrimination'type assay to differentiate between the strain origins of measles virus detected in intestinal tissue of children with ileocolonic …<br><br>O Sheils, P Smyth, C Martin, JJ O'Leary<br><br>Journal of Pathology 198, 5A-5A | 12 | 2002 |
| Detection of measles virus in children with ileo-colonic lymphoid nodular hyperplasia, enterocolitis and developmental disorder<br><br>CM Martin, V Uhlmann, A Killalea, O Sheils, JJ O'Leary<br><br>Molecular psychiatry 7 (2), S47-S48 | 28 | 2002 |

| | | |
|---|---|---|
| RET/PTC rearrangements in Hashimoto's thyroiditis<br>O Sheils, P Smyth, S Finn, EC Sweeney, JJ O'Leary<br>International journal of surgical pathology 10 (2), 167-168 | 17 | 2002 |
| RET/PTC rearrangements in Hashimoto's thyroiditis/Reply<br>O Shiels, P Smyth, S Finn, EC Sweeney<br>International Journal of Surgical Pathology 10 (2), 167 | | 2002 |
| Potential viral pathogenic mechanism for new variant inflammatory bowel disease<br>V Uhlmann, CM Martin, O Sheils, L Pilkington, I Silva, A Killalea, ...<br>Molecular Pathology 55 (2), 84 | 265 | 2002 |
| p16 (INK4A) as a marker of cervical dyskaryosis in cytopathology<br>OM Sheils, N Murphy, A Killalea, JJ O'Leary<br>LABORATORY INVESTIGATION 82 (1), 86A-86A | | 2002 |
| In-vitro and in-vivo evidence of an association between ret/PTC expression and cell adhesiveness and morphology in thyroid neoplasia<br>OM Sheils, P Smyth, S Finn, C Martin, JJ O'Leary<br>LABORATORY INVESTIGATION 82 (1), 120A-120A | | 2002 |
| Genome wide evaluation of a HHV 8 virally transformed co-hybrid cell culture model system<br>I Silva, M O'Donovan, C Martin, N Murphy, O Sheils, SB Lucas, JJ O'Leary<br>Journal of Pathology 198, 27A-27A | | 2002 |
| Expression Profiling of Kaposi's Sarcoma reveals dysregulation of immunomodulatory, pro-inflammatory, connective tissue and vascular/haematological related genes<br>M O'Donovan, I Silva, C Martin, SB Lucas, OM Sheils, JJ O'Leary<br>Journal of Pathology 198, 10A-10A | | 2002 |
| Expression profiling of Kaposi's Sarcoma<br>M O'Donovan, I Silva, OM Sheils, JJ O'Leary | | 2002 |

| | | |
|---|---|---|
| LABORATORY INVESTIGATION 82 (1), 307A-307A | | |
| Molecular analysis of primary effusion lymphoma<br><br>M O'Donovan, I Silva, OM Sheils, JJ O'Leary<br><br>LABORATORY INVESTIGATION 82 (1), 257A-257A | 1 | 2002 |
| Real-time quantitative analysis of E-cadherin expression in ret/PTC-1-activated thyroid neoplasms<br><br>P Smyth, O Sheils, S Finn, C Martin, J O'leary, EC Sweeney<br><br>International Journal of Surgical Pathology 9 (4), 265-272 | 24 | 2001 |
| E-cadherin expression in ret/PTC-1 activated thyroid neoplasms, detected by TaqMan RT-PCR.<br><br>P Smyth, S Finn, C Martin, EC Sweeney, O Sheils, JJ O'Leary<br><br>JOURNAL OF PATHOLOGY 195, 37A-37A | | 2001 |
| Immunocytochemical analysis of p16 protein expression in cervical cancer and cervical intraepithelial neoplasia.<br><br>N Murphy, N Bermingham, M Ring, M O'Donovan, K Luttich, F Mulcahy, ...<br><br>JOURNAL OF PATHOLOGY 195, 2A-2A | 8 | 2001 |
| Expression profile of human herpesvirus 8 (HHV-8) in pyothorax associated lymphoma and in effusion lymphoma<br><br>M O'Donovan, I Silva, V Uhlmann, N Bermingham, K Luttich, C Martin, ...<br><br>Molecular Pathology 54 (2), 80 | 28 | 2001 |
| The use of laser capture microdissection to produce homogenous cell populations from archival material for RT-TaqMan analysis.<br><br>OM Sheils, V Uhlmann, B Smyth, C Martin, N Bermingham, M O'Donovan, ...<br><br>JOURNAL OF PATHOLOGY 193, 48A-48A | | 2001 |
| Novel methodology for the analysis of foetal DNA in maternal venous blood and detection of abnormalities in chromosomes 13, 16, 18 and 21 in first trimester miscarriage. | | 2001 |

| | | |
|---|---|---|
| J Skinner, K Luttich, M Ring, V Uhlmann, C Martin, O Sheils, JJ O'Leary, ...<br><br>JOURNAL OF PATHOLOGY 193, 49A-49A | | |
| Persistent messles virus (MV) infection of lleal lymphoid tissue in children with lise-colonic lymphoid nodular hyperplasia, entero-colitis and developmental disorder<br><br>V Uhlmann, C Martin, O Shiels, A Anthony, M Fogarty, S Murch, ...<br><br>Gastroenterology 5 (120), A512 | | 2001 |
| Assessment of *ret*/PTC-1 rearrangements in neoplastic thyroid tissue using TaqMan RT-PCR<br><br>OM Sheils, JJ O'Leary, EC Sweeney<br><br>The Journal of pathology 192 (1), 32-36 | 39 | 2000 |
| ret/PTC-1 activation in Hashimoto thyroiditis<br><br>OM Sheils, JJ O'Leary, V Uhlmann, K Lüttich, EC Sweeney<br><br>International Journal of Surgical Pathology 8 (3), 185-189 | ~~153~~ | 2000 |
| ret/PTC-1 activation in Hashimoto thyroiditis<br><br>OM Sheils, JJ O'Leary, V Uhlmann, K Lüttich, EC Sweeney<br><br>International Journal of Surgical Pathology 8 (3), 185-189 | 153 | 2000 |
| Cellular localisation of HHV-8 in Castleman's disease: is there a link with lymph node vascularity?<br><br>J O'leary, M Kennedy, D Howells, I Silva, V Uhlmann, K Luttich, ...<br><br>Molecular Pathology 53 (2), 69 | 33 | 2000 |
| Assessment of ret/PTC-l expression in papillary thyroid carcinoma<br><br>O Sheils, V Uhlmann, K Luttich, M O'Donovan, N Bermingham, D Howell, ...<br><br>JOURNAL OF PATHOLOGY 190, 7A-7A | | 2000 |
| Genome wide evaluation of Kaposi's sarcoma derived primary spindle cell cultures and HHV 8 transformed endothelial cells.<br><br>M O'Donovan, N Bermingham, I Silva, K Luttich, D Howells, S Picton, ... | | 2000 |

| | | |
|---|---|---|
| JOURNAL OF PATHOLOGY 190, 7A-7A | | |
| HHV8 viral sequences and transcripts in CD34+ cells and dendritic cells isolated from myeloma patients<br><br>N Bermingham, K Luttich, M O'Donovan, M Ring, M Sweeney, V Uhlmann, ...<br><br>JOURNAL OF PATHOLOGY 190, 33A-33A | | 2000 |
| Identification of human herpes virus 8 (HHV8) characterisation of a novel polymorphism in affected women with CIN<br><br>JJ O'Leary, K Luttich, N Bermingham, M O'Donovan, I Silva, M Ring, ...<br><br>JOURNAL OF PATHOLOGY 190, 29A-29A | 5 | 2000 |
| Novel in-cell amplification for the detection of DNA and RNA in tissue sections<br><br>V Uhlmann, K Luttich, M O'Donovan, N Bermingham, I Silva, C Kenny, ...<br><br>JOURNAL OF PATHOLOGY 190, 34A-34A | | 2000 |
| Demonstration of ret/PTC-1 chimeric transcripts in a papillary thyroid cancer cell line using in-situ RT-TaqMan PCR<br><br>O Sheils, V Uhlmann, K Luttich, M O'Donovan, N Bermingham, D Howell, ...<br><br>JOURNAL OF PATHOLOGY 190, 49A-49A | | 2000 |
| Measles virus (MV) in reactive lympho-nodular hyperplasia and ileo-colitis of children<br><br>V Uhlmann, C Martin, O Sheils, M O'Donovan, N Bermingham, M Ring, ...<br><br>Journal of Pathology 190, 33A-33A | 2 | 2000 |
| Improved in situ detection method for telomeric tandem repeats in metaphase spreads and interphase nuclei<br><br>V Uhlmann, M Prasad, I Silva, K Luettich, L Grande, L Alonso, M Thisted, ...<br><br>Molecular Pathology 53 (1), 48 | 14 | 2000 |
| Localisation of HHV-8 in AIDS related lymphadenopathy | 14 | 2000 |

| | | |
|---|---|---|
| JJ O'Leary, M Kennedy, K Luttich, V Uhlmann, I Silva, J Russell, O Sheils, ...<br>Molecular Pathology 53 (1), 43 | | |
| Detection of ret/PTC-1 activation in Hashimoto thyroiditis using laser capture microdissection and TaqMan RT-PCR.<br>OM Sheils, V Uhlmann, JJ O'Leary, EC Sweeney<br>JOURNAL OF MOLECULAR MEDICINE-JMM 78 (7), B33-B33 | | 2000 |
| Prognostic Markers in Thyroid Neoplasia<br>O Sheils<br>Trinity College Dublin | | 2000 |
| TSH receptor status of thyroid neoplasms—TaqMan RT-PCR analysis of archival material<br>OM Sheils, EC Sweeney<br>The Journal of Pathology 188 (1), 87-92 | 82 | 1999 |
| Differential expression of TSH receptor in archival thyroid carcinomas using 5'nuclease assay (TaqMan)<br>OM Sheils, EC Sweeney<br>BRITISH JOURNAL OF CANCER 78, 60-60 | | 1998 |
| Sorting out mix-ups: the provenance of tissue sections may be confirmed by PCR using microsatellite markers<br>D Sean O'Briain, O Sheils, S McElwaine, SR McCann, M Lawler<br>American journal of clinical pathology 106 (6), 758-764 | 19 | 1996 |
| Colocalisation of human immunodeficiency virus and human cytomegalovirus infection in brain autopsy tissue from AIDS patients<br>IM Balluz, MA Farrell, E Kay, MJ Staunton, JN Keating, O Sheils, ...<br>Irish journal of medical science 165 (2), 133-138 | 12 | 1996 |
| High grade enteropathy associated T cell lymphoma (EATL), a distinct form of T cell lymphoma<br>DS OBriain, M McDermott, D Kelleher, O Sheils, E Delahanty, PA Daly<br>LABORATORY INVESTIGATION 74 (1), 691-691 | | 1996 |

| | | |
|---|---|---|
| A rapid microwave-in situ hybridization method for the definitive diagnosis of oral hairy leukoplakia: comparison with immunohistochemistry.<br><br>AGJ Mabruk MJ, Flint SR, Coleman DC, Shiels O, Toner M<br><br>J Oral Path 4 (Apr;25), 170-6. | 22<br><br>* | 1996 |
| CELL KINETICS AND p53 EXPRESSION IN ANAPLASTIC THYROID CARCINOMA.<br><br>CM Kilgallen, OM Sheils, C Sweeney<br><br>Irish Journal of Medical Science 165, 7 | | 1996 |
| LOSS OF HETEROZYGOSITY AND p53 MUTATIONS IN FEMALE PATIENTS WITH MULTIPLE CANCERS.<br><br>R McManus, JN McMahon, JF Stephens, O Sheils, NA Parfrey, EF Gaffney, ...<br><br>Irish Journal of Medical Science 165, 21 | | 1996 |
| Irish Association for Cancer Research<br><br>M Lawler, A Locasciulli, A Bacigalupo, P Humphries, P Ljungman, ...<br><br>Irish Journal of Medical Science 164 (3), 219-244 | | 1995 |
| Malignant lymphoma of the epididymis. A case report of bilateral involvement by a follicular large cell lymphoma<br><br>MB McDermott, D O'Briain, OM Shiels, PA Daly<br><br>Cancer 75 (8), 2174-2179 | 35 | 1995 |
| Detection of Epstein-Barr virus DNA in tongue tissues from AIDS autopsies without clinical evidence of oral hairy leukoplakia<br><br>MR Mabruk, SR Flint, M Toner, N Leonard, O Shells, DC Coleman, ...<br><br>Journal of oral pathology & medicine 24 (3), 109-112 | 24 | 1995 |
| THE PROVENANCE OF TISSUE-SECTIONS MAY BE CONFIRMED BY PCR USING MICROSATELLITE MARKERS<br><br>DS OBRIAIN, O SHEILS, M LAWLER<br><br>LABORATORY INVESTIGATION 72 (1), A167-A167 | | 1995 |

| | | |
|---|---|---|
| EPSTEIN-BARR-VIRUS IS INFREQUENTLY ASSOCIATED WITH NON-HODGKINS-LYMPHOMAS ARISING IN WALDEYERS RING<br>M MCDERMOTT, R MCDERMOTT, O SHEILS, PA DALY, DS OBRIAIN<br>LABORATORY INVESTIGATION 72 (1), A103-A103 | | 1995 |
| Tyrosine phosphorylation in the human duodenum.<br>D Kelleher, A Murphy, O Sheils, A Long, J McDevitt<br>Gut 36 (1), 34-38 | 5 | 1995 |
| Adenovirus 12 E1A gene detection by polymerase chain reaction in both the normal and coeliac duodenum.<br>M Lawler, P Humphries, C O'Farrelly, H Hoey, O Sheils, M Jeffers, ...<br>Gut 35 (9), 1226-1232 | 43 | 1994 |
| Irish association for cancer research<br>F Gannon, RC Forde, PV Nester, P Webb, J McCaffrey, JJ Fennelly, ...<br>Irish Journal of Medical Science 163 (7), 341-354 | | 1994 |
| ALTERED CELL-PROLIFERATION IN LEIOMYOSARCOMA IS NOT ASSOCIATED WITH MUTATIONS IN P53 EXONS 5-8<br>JN MCMAHON, JF STEPHENS, NA PARFREY, O SHEILS, EF GAFFNEY<br>LABORATORY INVESTIGATION 70 (1), A8-A8 | | 1994 |
| Adenovirus 12 E1A gene detection by polymerase chain reaction in both the normal and coeliac duodenum.<br>DP KELLEHER, M LAWLESS, O SHEILS<br>BMJ | | 1994 |
| Coeliac disease with histological features of peptic duodenitis: value of assessment of intraepithelial lymphocytes.<br>MD Jeffers, DO Hourihane<br>Journal of clinical pathology 46 (5), 420-424 | 24 | 1993 |
| LARGE GROWTH FRACTION IN SYNOVIAL SARCOMA IS NOT ASSOCIATED WITH ACCUMULATION OF MUTANT P53-PROTEIN<br>JN MCMAHON, EF GAFFNEY, D GREHAN, O SHEILS, M MCCABE, ... | | 1993 |

| | | |
|---|---|---|
| LABORATORY INVESTIGATION 68 (1), A8-A8 | | |
| Irish society of gastroenterology<br>LA Cotter, M Healy, M Buckley, C O'Morain, C Keane, RR O'Moore, ...<br>Irish Journal of Medical Science 161 (3), 81-97 | | 1992 |
| Effect of cyclosporin on immune complex deposition in murine glomerulonephritis.<br>DG Quinn, JS Fennell, O Sheils, EF Gaffney, CF Feighery<br>Immunology 72 (4), 550 | 5 | 1991 |
| Complement activation within the coeliac small intestine is localised to Brunner's glands.<br>RB Gallagher, CP Kelly, S Neville, O Sheils, DG Weir, CF Feighery<br>Gut 30 (11), 1568-1573 | 21 | 1989 |
| IMMUNOLOGICAL INVOLVEMENT OF BRUNNER GLANDS IN CELIAC-DISEASE<br>RB GALLAGHER, O SHEILS, DG WEIR<br>GUT 30 (10), A1451-A1452 | | 1989 |
| Diagnosis, Monitoring and Prevention of Exposure Related Non-Communicable Diseases: DiMoPEx project follow up<br>LT Budnik, B Adam, M Albin, B Banelli, X Baur, F Belpoggi, C Bolognesi, ... | | |
| N-linked glycan sites within the A2 domain of von Willebrand factor modulate macrophage-mediated clearance<br>A Chion, JM O'Sullivan, C Drakeford, G Bergsson, N Dalton, S Aguila, ...<br>American Society of Hematology | | |
| FIXCAS Study [version 1; peer review: awaiting peer review] | | |

| | | |
|---|---|---|
| K Devenney, N Murphy, R Ryan, C Grant, J Kennedy, RP Manecksha, ... | | |
| ADDITIONAL FILE<br><br>TG Gustavsson, A Fischer, D Jarosinska, F Manservisi, R O'Kennedy, ... | | |
| Clinical validation of a NGS based in vitro diagnostic (CE-IVD) kit for targeted detection of actionable gene rearrangements in lung cancer specimens<br><br>JJ Schageman, RP Petraroli, JL Costa, O Sheils, JE Glassco, D Chi, ... | | |
| Flavin r, O'Leary J, Sheils O (2007) Low-level genomic instability is a feature of papillary thyroid carcinoma: an array comparative genomic hybridization study of laser …<br><br>S Finn, P Smyth, E O'regan, S Cahill, M Toner, C Timon<br><br>Arch Pathol Lab Med 131, 65-73 | | |
| Gene Expression Profiling and MicroRNA Discovery in a Carcinoma Model<br><br>PCR Real-Time | | |
| Scientific Contributors<br><br>A Ferlinz, J Skaf, I Russell, S Guenther | | |
| The relationship between Circulating Tumour Cells (CTCs), platelets and Epithelial-Mesenchymal-Transition (EMT)<br><br>OM Sheils, JJ O'Leary | | |
| Lack of association between measles virus vaccine and autism with enteropathy: a case-control study: Appendix S2<br><br>M Hornig, T Briese, T Buie, ML Bauman, G Lauwers, U Siemetzki, ... | | |