3rd EACR Conference

Cancer Genomics

25 - 28 June 2017
Cambridge, UK

Scientific Programme Committee
James Brenton, Carlos Caldas (Chair)
and Yardena Samuels
Stop by Booth #6
to chat about how we can help you…

Next Gen Sequencing
Generate better next gen sequencing libraries and data

In Vitro Transcription
Improve your RNA SELEX experiments using RNase resistant RNA synthesized in vitro or produce a lot of standard RNA quickly

Competent Cells
Build or amplify more diverse GeCKO lentiviral CRISPR guide RNA libraries

And Much More!

Love science? We have a bag for you!

Lucigen
Simplifying Genomics
www.lucigen.com
Day 1 - Sunday 25 June 2017

15.00 - 17.00
REGISTRATION Main Concourse
Tea/coffee & biscuits will be available

17.00 - 17.15
CONFERENCE WELCOME Wolfson Hall
Scientific Programme Committee

17.15 - 17.45
Keynote Lecture Session Chair: Carlos Caldas
Gad Getz Broad Institute (USA)
"Coding and Non-Coding Drivers in Cancer"
Q&A: 17.45 – 18.00

18.00 - 19.00
NETWORKING RECEPTION The Buttery
Following the Keynote Lecture, there will be a drinks reception to give participants the opportunity to meet new colleagues and reconnect with friends.

19.00
WELCOME DINNER Dining Hall
All participants are invited to join this seated two course dinner served in the dining hall. The college bar will be open into the evening for discussions to be continued.

Participants at a previous edition of the Cancer Genomics conference at Churchill College.
## Day 2 - Monday 26 June 2017

### 08.30 - 09.15
**POSTER VIEWING** Main Concourse and Jock Colville Hall

**SESSION 1: Mutations and Mutational Processes in Cancer**
Session Chair: Yardena Samuels

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
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<tr>
<td>09.15</td>
<td>David Adams</td>
<td>Wellcome Trust Sanger Institute (UK)</td>
<td>&quot;Germline MC1R variants and somatic mutation burden in melanoma&quot;</td>
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<tr>
<td>09.45</td>
<td>Francesca Ciccarelli</td>
<td>The Francis Crick Institute (UK)</td>
<td>&quot;Inherited vulnerabilities and the onset of multiple colorectal cancers&quot;</td>
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### 09.15 - 10.05
**Q&A: 09.35 - 09.45**
"Germline MC1R variants and somatic mutation burden in melanoma"

### 09.45 - 10.05
**Q&A: 10.05 - 10.15**
"Inherited vulnerabilities and the onset of multiple colorectal cancers"

### 10.15 - 10.30
**EXHIBITOR INTRODUCTIONS** Wolfson Hall

### 10.30 - 11.00
**COFFEE BREAK & TRADE EXHIBITION** Main Concourse

### 11.00 - 11.10
**Proffered Paper 1:** Rajbir Nath Batra CRUK Cambridge Institute (UK)
"Decoding the regulatory role and epilonal dynamics of DNA methylation in breast cancer using sequencing-based profiling of 1482 breast tumours"

### 11.10 - 11.15
**Q&A: 11.10 - 11.15**
"Decoding the regulatory role and epilonal dynamics of DNA methylation in breast cancer using sequencing-based profiling of 1482 breast tumours"

### 11.15 - 11.25
**Proffered Paper 2:** Stefan Dentro Wellcome Trust Sanger Institute (UK)
"Pervasive intra-tumour heterogeneity and subclonal selection across cancer types"

### 11.25 - 11.30
**Q&A: 11.25 - 11.30**
"Pervasive intra-tumour heterogeneity and subclonal selection across cancer types"

### 11.30 - 11.50
**Núria Lopez-Bigas** IRB Barcelona (Spain)
"Coding and non-coding cancer mutations"

### 11.50 - 12.00
**Q&A: 11.50 - 12.00**
"Coding and non-coding cancer mutations"

### 12.00 - 12.30
**SATELLITE SYMPOSIUM 1: NanoString Technologies** Wolfson Hall

**Julia Kargl** Medical University of Graz
"A 3D Biology™ view of cancer: Simultaneous detection of somatic DNA mutations and expressed fusion transcripts plus expression profiling of phospho and total signalling proteins from lung-tumor FFPE samples"

### 12.30 - 13.30
**LUNCH** Dining Hall

### 13.30 - 15.30
**POSTER DEFENCE SESSION 1 & TRADE EXHIBITION** Main Concourse and Jock Colville Hall

*Poster Spotlight Session will take place at 15:00.*

**SESSION 2: Liquid Biopsies in Cancer**
Session Chair: James Brenton

### 15.30 - 15.50
**Caroline Dive** CRUK Manchester Institute (UK)
"Molecular analysis of circulating tumour cells in lung cancer"

### 15.50 - 16.00
**Q&A: 15.50 - 16.00**
"Molecular analysis of circulating tumour cells in lung cancer"

### 16.00 - 16.20
**Nicholas Turner** ICR (UK)
"Predicting Relapse Using Circulating Tumor DNA in Breast Cancer"

### 16.20 - 16.30
**Q&A: 16.20 - 16.30**
"Predicting Relapse Using Circulating Tumor DNA in Breast Cancer"

### 16.30 - 16.40
**Proffered Paper 3:** Jelena Belic Medical University of Graz (Austria)
"Detection and characterization of neuroendocrine prostate cancer using whole genome-sequencing in circulating tumor DNA"

### 16.40 - 16.45
**Q&A: 16.40 - 16.45**
"Detection and characterization of neuroendocrine prostate cancer using whole genome-sequencing in circulating tumor DNA"

### 16.45 - 17.05
**Michael Speicher** Medical University of Graz (Austria)
"Inferring expressed genes by whole-genome sequencing of plasma DNA"

### 17.05 - 17.15
**Q&A: 17.05 - 17.15**
"Inferring expressed genes by whole-genome sequencing of plasma DNA"

### 17.15 - 17.25
**Proffered Paper 4:** Eran Kotler Weizmann Institute of Science (Israel)
"A comprehensive library of systematically designed p53 variants elucidates patterns of evolutionary conservation and of mutations in human tumors"

### 17.25 - 17.30
**Q&A: 17.25 - 17.30**
"A comprehensive library of systematically designed p53 variants elucidates patterns of evolutionary conservation and of mutations in human tumors"

### 17.30
**HIGH TEA & DISCUSSION FORUM**
Buffet food will be served and participants will have the opportunity to converse with speakers in small groups.
Day 3 - Tuesday 27 June 2017

08.30 - 09.30
POSTER VIEWING & TRADE EXHIBITION Main Concourse and Jock Colville Hall

SESSION 3: Genomics and Immune Landscapes in Cancer I
Session Chair: James Brenton

09.30 - 09.50
Q&A: 09.50 - 10.00
Ido Amit Weizmann Institute of Science (Israel)
“The power of ONE: Immunology in the age of single cell genomics”

10.00 - 10.20
Q&A: 10.20 - 10.30
Sergio Quezada UCL (UK)
“Mapping the immune and antigenic landscape of cancer”

10.30 - 11.00
COFFEE BREAK & TRADE EXHIBITION Main Concourse

11.00 - 11.10
Q&A: 11.10 - 11.15
Proffered Paper 5: Eva Bräunlein TU Munich (Germany)
“In-depth characterization of immune responses against neoepitopes from primary melanoma tissues identified by mass spectrometry”

11.15 - 11.25
Q&A: 11.25 - 11.30
Proffered Paper 6: Marian Burr Peter MacCallum Cancer Centre (Australia)
“CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity”

11.30 - 11.50
Q&A: 11.50 - 12.00
Yardena Samuels Weizmann Institute of Science (Israel)
“Comprehensive elucidation of the antigenic and T cell melanoma landscapes reveal them to be restricted and oligoclonal”

12.00 - 12.30
SATELLITE SYMPOSIUM 2: Lucigen Wolfson Hall
Rob Brazas Lucigen Corporation
“Overcoming the Challenges of Illumina DNA Library Prep”

12.30 - 13.30
LUNCH Dining Hall

13.30 - 15.30
POSTER DEFENCE SESSION 2 & TRADE EXHIBITION Main Concourse and Jock Colville Hall
Poster Spotlight Session will take place at 15:00.

SESSION 4: Genomics and Immune Landscapes in Cancer II
Session Chair: Carlos Caldas

15.30 - 15.50
Q&A: 15.50 - 16.00
Carlos Caldas CRUK Cambridge Institute (UK)
“Clonal and cellular heterogeneity landscapes of breast cancer”

16.00 - 16.10
Q&A: 16.10 - 16.15
Proffered Paper 7: Chris de Witte UMC Utrecht (Netherlands)
“Establishment and characterization of an ovarian cancer organoid biobank”

16.15 - 16.45
Q&A: 16.45 - 17.00
Keynote Lecture
Ton Schumacher NKI (Netherlands)
“T cell recognition in human cancer”

19.30
CONFERENCE DINNER Dining Hall
All participants are invited to enjoy a three-course dinner together on the final evening of the conference. Award certificates will be presented during this event.
Day 4 - Wednesday 28 June 2017

08.30 - 09.00
POSTER VIEWING Main Concourse and Jock Colville Hall

SESSION 5: Precision Cancer Medicine
Session Chair: Carlos Caldas

09.00 - 09.20
James Brenton CRUK Cambridge Institute (UK)
“Precision medicine for high-grade serous ovarian cancer”
Q&A: 09.20 - 09.30

09.30 - 09.40
Proffered Paper 8: Caitlin A. Nichols Dana-Farber Cancer Institute (USA)
“Loss of heterozygosity of essential genes represents a novel class of cancer vulnerabilities”
Q&A: 09.40 - 09.45

09.45 - 09.55
Proffered Paper 9: Alejandra Bruna CRUK Cambridge Institute (UK)
“Interrogating the role of breast cancer functional heterogeneity in a tumour’s evolutionary trajectory upon therapeutic pressure using PDTX models”
Q&A: 09.55 - 10.00

10.00 - 10.20
René Bernards NKI (Netherlands)
“Finding vulnerabilities of drug-resistant cancer”
Q&A: 10.20 - 10.30

10.30 - 11.00
COFFEE BREAK Main Concourse

11.00 - 11.20
Charles Swanton The Francis Crick Institute (UK)
“Chromosomal Chaos and Order in Cancer Evolution: TRACERx”
Q&A: 11.20 - 11.30

11.30 - 12.00
Keynote Lecture
Olli Kallioniemi SciLifeLab (Sweden)
“Patient-derived primary cancer cells, integrative omics and functional drug testing facilitating precision cancer medicine”
Q&A: 12.00 - 12.15

12.15 - 12.30
CLOSING REMARKS
Scientific Programme Committee

12.30
LUNCH AND DEPART

See you soon!

4th EACR Conference
Cancer Genomics

23 – 26 June 2019
Cambridge • UK

EACR Meeting Bursary Award Winners

Congratulations to the winners of the EACR Meeting Bursaries. Each winner received a full registration free of charge and funds of up to 500 Euros to assist with the cost of travel.

Anja Kafka Croatia
Rebecca Poulos Australia
Camelia Quek Australia
Shobha Silva UK
Anna Supernat Poland
Interactive Activities at the Conference

An important part of the EACR Conference Series is the range of opportunities we aim to provide for participants to interact, discuss, reflect and build relationships and collaborations.

We hope you enjoy the dedicated interactive activities, which are listed below.

Networking Reception & Welcome Dinner 18.00 Sunday 25 June

Following the Keynote Lecture, there will be a drinks reception to give participants the opportunity to meet new colleagues and reconnect with friends. All participants are then invited to join a seated two course dinner served in the dining hall. The college bar will be open into the evening for discussions to be continued.

Poster Defence Sessions 13.30 – 15.30 Monday & Tuesday

There are two dedicated Poster Defence Sessions in the Programme. At these times, the presenters for that session are asked to stand by their posters to discuss their work with other participants and invited speakers.

Two EACR Poster Prizes worth €100 each will be awarded to the best poster presentations at the conference. The judging panel is comprised of speakers from the conference, and they will assess the top scoring abstracts based on the scientific content, the layout of the poster, and the verbal discussion. The winners will be announced during the Conference Dinner on Tuesday.

Poster Viewing From 08:30 each morning

Monday, Tuesday and Wednesday will begin with an optional poster viewing slot. Participants are invited to use this time for further discussion in the poster areas, but presenters are not required to be by their posters at these sessions.

Poster Spotlight Sessions 15:00 - 15:30 Monday & Tuesday

Six high-scoring abstracts have been selected to give Poster Spotlight presentations. These five minute talks, followed by a short discussion, will take place in the poster spotlight area (opposite the registration desk). All participants are invited to join in with these informal sessions.

High Tea & Discussion Forum 17.30 Monday 26 June

At the conclusion of the day’s talks, a Cambridge High Tea will be served. Participants will have the opportunity to converse with invited speakers in small groups. Following this there will be an opportunity for participants to enjoy a summer evening together in Cambridge.

Conference Dinner 19.30 Tuesday 27 June

All participants are invited to join the formal three course Conference Dinner on Tuesday evening. The evening will include the presentation of Meeting Bursary awards and EACR Poster Prizes. We hope that new friendships will be made and professional connections will continue to flourish. Tickets for the Conference Dinner are included in the registration fee.

Don’t forget to let us have your feedback about these activities in the survey we will send after the conference!

EACR Sustaining Members

The European Association for Cancer Research gratefully acknowledges the companies that support the Association as Sustaining Members. Through Sustaining Membership, companies offer ongoing support to the EACR and provide the means for the Association to develop important initiatives, including the EACR Conference Series.
The organisers wish to express their appreciation for the significant support provided by sponsors at the 3rd EACR Conference on Cancer Genomics. Their interest and enthusiasm for the conference has enabled the organisers to provide an impressive scientific programme.

Elite Sponsors

Lucigen®
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10X GENOMICS®

Scientific Grant

CANCER RESEARCH UK

Exhibitors

ANGLE

BD

Covaris

diagenode
Innovating Epigenetic Solutions

ECACC
European Collection of Authenticated Cell Cultures

Operated by Public Health England

GATC BIOTECH

QIAGEN

NEW ENGLAND Biolabs

Promega

Swift BIOSCIENCES™

Stratech

Satellite Symposia

We are pleased to announce that NanoString and Lucigen will not only be exhibiting at the conference but also inviting participants to join a Satellite Symposium. The Satellite Symposia give participants the opportunity to take part in an additional scientific session.

Monday 26 June 2017 12.00 - 12.30
Julia Kargl PhD, Medical University of Graz
“A 3D Biology™ view of cancer: Simultaneous detection of somatic DNA mutations and expressed fusion transcripts plus expression profiling of phospho and total signalling proteins from lung-tumor FFPE samples”

Tuesday 27 June 2017 12.00 - 12.30
Rob Brazas PhD, Lucigen Corporation
“Overcoming the Challenges of Illumina DNA Library Prep”
Meet the Exhibitors

The conference will give plenty of opportunities for participants to meet representatives from companies working in the same field. To find out more about our exhibitors, please visit the stands in the trade exhibition.

10x Genomics
Website: www.10xgenomics.com • Contact: scott.brouilette@10xgenomics.com
Represented at the conference by: Scott Brouilette, Justin Cooper

Describe 10x Genomics in 5 words or less
Transforming short read sequencing technologies

Tell us a little bit about 10x Genomics
10x Genomics’ Chromium™ System supports comprehensive genomics and high-throughput single cell transcriptomics. It enables researchers to discover previously inaccessible genomic information, including phased structural variants, phased single nucleotide variants, Single Cell gene expression and paired full length V(D)J repertoires —while leveraging their existing sequencing systems and workflows.

Why are you attending the conference? Who would you like to meet at the conference?
Solutions provided by 10x Genomics are directly applicable to the study of cancer and we welcome discussions with researchers looking to: resolve phased variants, study larger and more challenging structural variants, and analyse the transcriptome at high-throughput, single cell level.

ANGLE
Website: www.angleplc.com • Contact: g.abuali@angleplc.com
Represented at the conference by: Ghada Abuali, Michael O’Brien

Describe ANGLE in 5 words or less
Liquid Biopsy and CTCs Isolation

Tell us a little bit about ANGLE
ANGLE is a UK and US based medical diagnostic company. ANGLE’s Parsortix technology enables the capture and harvest of rare circulating tumour cells (CTCs) from patient blood. Genetic and protein analysis of CTCs from this ‘liquid biopsy’ has the potential to enable personalized cancer care. ANGLE is currently developing its first diagnostic product in the area of ovarian cancer.

Why are you attending the conference? Who would you like to meet at the conference?
We are attending to meet cancer researchers who are current and potential users of our technology Parsortix. We would also be interested in meeting providers of other technologies that are compatible with ours.

BD
Website: www.bd.com/genomics • Contact: Richard.henfrey@bd.com
Represented at the conference by: Winston Clarke, Richard Henfrey, Debbie Atkinson

Describe BD in 5 words or less
Global medical technology company

Tell us a little bit about BD
BD Genomics provides innovative solutions for tissue dissociation, FACS sorting of specific cell populations, followed by single cell transcriptomics from hundreds (Precise Assays) to tens of thousands (Resolve platform) of single cells.

Why are you attending the conference? Who would you like to meet at the conference?
Our goal is to share BD’s latest technology solutions with the scientific community. We are keen to meet scientists involved in cancer medicine, cancer biology, translational research and cancer immunology.
Meet the Exhibitors continued...

**Covaris**
Website: www.covarisinc.com • Contact: eucustomerservice@covarisinc.com
Represented at the conference by: Rowan Gibson, Rebecca Callard

Describe Covaris in 5 words or less
Pre-Analytical Sample Extraction

Tell us a little bit about Covaris
Covaris is an innovator in sample extraction and preparation for the diagnostic, life science and pharmaceutical markets and recognises that as applications become more sophisticated, the need for high precise, accurate sample preparation is growing.

Why are you attending the conference? Who would you like to meet at the conference?
We are attending to discuss our new range of sample collection and extraction technologies, for Whole blood, Dried Blood, FFPE and cfDNA for advanced analysis such as NGS, ddPCR and Arrays. Using Covaris TruCOLLECT or TruXTRAC can improve yield and quality of DNA and RNA.

**Diagenode**
Website: www.diagenode.com • Contact: susanna.lovell@diagenode.com
Represented at the conference by: Susanna Lovell, Philippe Cronet, Elizabeth Wright

Describe Diagenode in 5 words or less
Diagenode Innovating Epigenetics Solutions

Tell us a little bit about Diagenode
Based in Belgium and USA, our epigenetics experts develop instruments, services and reagents including the Bioruptor® for DNA and Chromatin shearing, IP-Star® for automation and MegaRuptor for long fragments. Our new RNA-seq solution and CRISPR/Cas 9 Antibodies complement our solutions for DNA methylation, and ChIP-seq.

Why are you attending the conference? Who would you like to meet at the conference?
Anyone interested in finding out more about epigenetics, new to the field or established expert, is welcome to come and try our Belgian Chocolate waffles.

**European Collection of Authenticated Cell Cultures (ECACC)**
Website: www.phe-culturecollections.org.uk/ecacc • Contact: culturecollections@phe.gov.uk; +44 (0)1980 612512
Represented at the conference by: Sharon Bahia

Describe ECACC in 5 words or less
Authenticated cell lines, quality services

Tell us a little bit about ECACC
ECACC is a premier collection of authenticated cell lines and provides cell culture services. The collection holds 40,000 cell lines representing 45 species, 50 tissue types, 300 HLA types, 450 monoclonal antibodies, 800 genetic disorders and includes > 600 healthy and disease iPS cell lines.

Why are you attending the conference? Who would you like to meet at the conference?
We are attending the conference to engage with the cancer research community in order to showcase ECACC as international provider of authenticated cell lines, research tools and services. Our aim is to help support researchers in producing reliable and reproducible results.

**GATC Biotech**
Website: www.gatc-biotech.com • Contact: customercare@gatc-biotech.com
Represented at the conference by: Laurie Newnes

Describe GATC Biotech in 5 words or less

Tell us a little bit about GATC Biotech
GATC Biotech is Europe’s leading sequencing services provider. The company offers tumour mutation profiling from FFPE samples or plasma with certified quality. GATC Biotech delivers flexible solutions for whole exome, cancer panel or single target sequencing, as well as whole genome, transcriptome and epigenome analysis.

Why are you attending the conference? Who would you like to meet at the conference?
We are attending the conference to introduce researchers and physicians to our exclusive services. We offer the unique possibility to perform plasma exome sequencing and the most comprehensive cancer panel on the market. We want to be inspired and inspire the latest cancer genomics research.
Lucigen
Website: www.lucigen.com • Contact: tradeshows@lucigen.com
Represented at the conference by: Glaselyn Miller, Rob Brazas, Katie Piercy

Describe Lucigen in 5 words or less
Simplifying and Improving Genomics Research

Tell us a little bit about Lucigen
Lucigen offers quality products and services to enable life science professionals to perform their research more efficiently and effectively. The company offers genomics research products including next gen sequencing kits, competent cells, protein expression systems, amplification reagents, nucleic acid purification kits and unique cloning vectors.

Why are you attending the conference? Who would you like to meet at the conference?
Our goal is to meet and talk to scientists using genomics as part of their cancer research in order learn about and understand the challenges they face. Based on that information, we hope to offer solutions to those challenges that make each researcher more effective and efficient every day.

NanoString
Website: www.nanostring.com • Contact: europe.sales@nanostring.com; +44 (0) 1494 582000
Represented at the conference by: Ajay Johni, Carla Simoes, Julia Kargl, Aziz Mustafa, Jim White

Describe NanoString in 5 words or less
Digital counting: DNA, RNA, Proteins

Tell us a little bit about NanoString
NanoString Technologies provides innovative products that unlock valuable and clinically actionable genomic information from small amounts of tissue. The company is committed to offering tools that enable scientists and clinicians to translate today’s leading genomic research into clinically actionable tests that improve patient care.

New England Biolabs
Website: www.neb.uk.com • Contact: millerd@neb.com; +44 (0) 7775 511711
Represented at the conference by: Davin Miller, Calin Andras

Describe New England Biolabs in 5 words or less
Excellence in Molecular Biology

Tell us a little bit about New England Biolabs
New England Biolabs (NEB) manufactures reagents for molecular biology and is a global market leader in the supply of reagents for genomics research. The cornerstones of NEB’s reputation are service excellence and products you can trust.

Promega
Website: www.promega.com • Contact: ukmarketing@promega.com
Represented at the conference by: Lucy Wheatley, Amy Timpson

Describe Promega in 5 words or less
Supporting customers, community and employees

Tell us a little bit about Promega
Promega supports customers with the highest quality products, innovative technologies, the best possible customer service, unsurpassed technical support, and many useful tools and resources. Promega consistently integrates the values of corporate responsibility and sustainable business practices into all aspects of our corporate culture and activity.

Why are you attending the conference? Who would you like to meet at the conference?
We realise that our success depends upon the connections we forge among our customers, community and employees and so at the conference, we would love to meet existing customers and new people to develop and deepen our relationships.
QIAGEN
Website: www.qiagen.com • Contact: Rahna.Ayub@qiagen.com
Represented at the conference by: Rahna Ayub, Hannah Bunten

Describe QIAGEN in 5 words or less
Molecular Biology Sample to Insight

Tell us a little bit about QIAGEN
QIAGEN is the leading global provider of Sample to Insight solutions to transform biological materials into valuable molecular insights. Our mission is to improve health, enabling our customers to achieve outstanding success and breakthroughs in life-sciences, applied testing, pharma and molecular diagnostics.

Why are you attending the conference? Who would you like to meet at the conference?
As a trusted partner in cancer research, we aim to continue providing innovative solutions and ensure our focus is aligned with current ongoing research. We have new advances in NGS, liquid biopsy and single cell research to discuss with key opinion leaders in cancer research.

Stratech
Website: www.stratech.co.uk • Contact: info@stratech.co.uk; +44 (0) 1638 782600
Represented at the conference by: David du Plessis

Describe Stratech in 5 words or less
First choice for scientific solutions

Tell us a little bit about Stratech
Stratech are not a multi-national super conglomerate biotech company, instead we are family run business dedicated to delivering exceptional product quality with unbeatable technical support. We are so confident that you will LOVE both our products and our technical support that we guarantee all our products with a full money back promise.

Why are you attending the conference? Who would you like to meet at the conference?
As one of the leading UK suppliers of life science reagents, Stratech endeavours to meet as many of their customers face-to-face as possible. Stratech have always supported EACR because it gives us the perfect opportunity to discuss the current trends in cancer research with existing and potential customers.

Swift Biosciences
Website: www.swiftbiosci.com • Contact: info@swiftbiosci.com
Represented at the conference by: Tim Harkins, Haley Fiske

Describe Swift Biosciences in 5 words or less
Transforming NGS Library Prep

Tell us a little bit about Swift Biosciences
Swift Biosciences develops novel molecular biology technologies to better characterize and understand the genome. We focus on creating tools to advance DNA sequencing sciences with products designed to help customers analyze challenging samples that are often associated with translational work, including cfDNA and FFPE samples.

Why are you attending the conference? Who would you like to meet at the conference?
Swift Biosciences is developing new genetic tools to better characterize the human genome from less input. We will discuss technical/scientific barriers attendees are facing in their cancer sequencing endeavors, finding solutions together that will help advance the fields understanding of how the genome works.
Revolutionizing Gene Expression

Making the leap from bulk RNA-seq to single cell with the Chromium™ Single Cell 3' Solution

Whether you’re working with tumor cells, stem cells, T-cells, or embryonic cells, heterogeneity is ever-present. Our elegant solution assigns expression profiles to individual cells in a highly flexible and scalable way, rapidly revolutionizing gene expression.

LEARN MORE AT GO.10XGENOMICS.COM/REVOLUTION

THE CHROMIUM™ SYSTEM: The Chromium System, powered by GemCode™ Technology, is an innovative system that transforms the capability of existing short-read sequencers. With millions of uniquely addressable partitions, the Chromium System unlocks critical genomic information.

Pictured: t-SNE projection of gene expression profiles from 1,306,127 cells from the cortex, hippocampus and subventricular zone from two E18 C57BL/6 mice. Colors indicate the closest match in a panel of sorted reference cell types.
Can you spot the imposter?

Spotting an imposter isn’t so easy when it comes to cell lines. Thousands of studies use misidentified or contaminated cell lines each year, leading to non-reproducible or invalid results, loss of time and money, and potential retraction of articles. Many high-profile journals and funding bodies now require proof of cell line authenticity.

Have confidence in your cell lines. Produce reliable results.

The European Collection of Authenticated Cell Cultures (ECACC) is the largest culture collection of Public Health England. All our cell lines are rigorously quality controlled and authenticated to ensure identity, sterility and viability.

For all your cell culture and authentication requirements, including STR profiling, contact ECACC today.

W: www.phe-culturecollections.org.uk/ecacc
E: culturecollections@phe.gov.uk
T: +44 (0)1980 612 512
Keynote Lecture

Coding and non-coding drivers in cancer

Gad Getz¹
¹ Broad Institute of MIT and Harvard, Cambridge, MA, USA

The ability to sequence and analyze large numbers of whole cancer genomes and exomes revolutionized our ability to study cancer. We can now systematically detect genes and events that drive cancer, discover and characterize mutational processes, predict order and timing of events and study the emergence and evolution of resistance to therapy. In this presentation, I will give a brief overview of our cancer genome analysis efforts and, in particular, describe recent work on analyzing non-coding drivers in breast cancer. I will also describe the international effort towards analyzing whole genomes and in particular discovering drivers across cancer. Finally, I will present a cloud-based analytical platform designed for large-scale collaborative scientific projects.

Germline MC1R status influences somatic mutation burden in melanoma

David Adams², Tim Bishop¹, Nicola Roberts², Daniela Robles²
¹ University of Leeds, Leeds, UK, ² Wellcome Trust Sanger Institute, Hinxton, UK

The role of germline variants in influencing the somatic mutation profile of normal tissues and cancers has been poorly explored. The major genetic determinants of cutaneous melanoma risk in the general population are disruptive variants (R alleles) in the melanocortin 1 receptor (MC1R) gene. These alleles are also linked to red hair, freckling, and sun sensitivity, all of which are known melanoma phenotypic risk factors. We find that in melanomas and for somatic C>T mutations, a signature linked to sun exposure, the expected single-nucleotide variant count associated with the presence of an R allele is estimated to be 42% (95% CI, 15-76%) higher than that among persons without an R allele. This figure is comparable to the expected mutational burden associated with an additional 21 years of age. We also find significant and similar enrichment of non-C>T mutation classes supporting a role for additional mutagenic processes in melanoma development in individuals carrying R alleles. I will primarily talk about melanoma but will also talk about other malignancies where we have been exploring the germlines' role in regulating the mutational landscape.
Inherited vulnerabilities and the onset of multiple colorectal cancers

Francesca D. Ciccarelli1,2
1 King’s College London, UK, 2 The Francis Crick Institute, London, UK

Synchronous colorectal cancers are physically separated tumours that develop simultaneously and account for ~2-8% of all colorectal cancer cases. The causes for the onset of simultaneous tumours are still poorly understood. Patients with hereditary disease (Lynch syndrome and familial adenomatous polyposis) have a higher occurrence of multiple cancers. Similarly, inflammatory bowel diseases (IBDs) and hyperplastic polyposis are known to predispose to synchronous tumours. These conditions, however, only account for around 10% of cases, thus suggesting that other factors contribute to the development of simultaneous tumours.

In my talk, I will present the results of our recent sequencing screening of synchronous colorectal cancers where we detected high inter- and intra-tumour heterogeneity, with the vast majority of somatic alterations being private to only one of the paired tumours. I will show how this heterogeneity is likely to impact on the response to treatment and should be considered in the selection of therapy and in the monitoring of resistance. We also investigated whether germline single nucleotide polymorphisms (SNPs) could predispose to the development of multiple cancers. Instead of testing for single gene association, we developed a mutation burden test to detect the accumulation of mutations in specific biological processes. I will show that patients with multiple colorectal cancers carry damaging SNPs in immune-related processes. Some of these genes have been previously associated with immune diseases, such as IBDs and allergic sensitisation (asthma, atopy, pathogen infection). We also observed a higher proportion of CD8+ T cells in the lamina propria and more T cells in the surface epithelium of the gut mucosa of these patients. Based on the results of this study, I will discuss our hypothesis that damaging SNPs in immune genes increase the frequency of independent cancer-initiating events. This may be mediated by the inflammatory microenvironment that is induced by genetic alterations and that favours tumorigenesis via increased genomic instability or through the production of cytokines and growth factors.

Proffered Paper 1

Decoding the regulatory role and epigenetic dynamics of DNA methylation in breast cancer using sequencing-based profiling of 1482 breast tumours

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Introduction: Breast cancer is a clinically and genomically heterogeneous disease displaying distinct therapeutic responses. While recent studies have explored the genomic and transcriptomic landscapes, the epigenetic architecture has received less attention.

Methods: An optimised Reduced Representation Bisulfite Sequencing (RRBS) protocol was performed on 1482 primary breast tumours (and 237 matched adjacent normal tissues). Copy number, gene expression, somatic mutations and extensive clinical annotation are available.

Results and discussion: We observed a noticeable erosion (both gain and loss) of homogenous DNA methylation patterns in tumours when compared to normal tissues, with markedly higher rates in late replicating genomic regions. This erosion could represent a stochastic accumulation of methylation errors approximating the underlying rate of cell division, and we found that different tumours had varied capacities for this turnover.

Next, we identified regions of methylation aberrations between different breast cancer subtypes. These alterations were compared to the tumour-specific erosion rates described above, with those deviating reflecting a biological process unrelated to cell division such as selection. Characterising these aberrations with gene expression led to the identification of subtype-specific epigenetic drivers e.g. promoter hypermethylation of SFRP1, a WNT pathway antagonist, associated with silencing exclusively in ER+ breast tumours. By integrating copy number and mutation data, we revealed that DNA methylation events are the predominant mechanism correlated with differentially expressed
genes in breast cancer. Moreover, mutually exclusive patterns between these genomic and epigenomic aberrations were identified e.g. BRCA1 promoter hypermethylation and mutations.

Finally, we analysed intra-tumoural methylation content at consecutive CpG sites to identify loci that are associated with low epigenetic polymorphism implying a selection process enhancing tumour fitness versus those with high polymorphism reflecting a stochastic process. Tumours linked with epigenetic selection had a significantly poorer prognosis compared to other tumours. This provides the first comprehensive assessment of epiclonal dynamics in epithelial tumours.

Proffered Paper 2

**Pervasive intra-tumour heterogeneity and subclonal selection across cancer types**


Big Data Institute, University of Oxford, UK, Broad Institute, Cambridge, MA, USA, CRUK Cambridge Institute, UK, European Bioinformatics Institute, Hinxton, UK, German Cancer Research Center (DKFZ), Heidelberg, GERMANY, MD Anderson Cancer Center, Houston, TX, USA, Memorial Sloan-Kettering Cancer Center, NY, USA, New York Genome Center, NY, USA, Ontario Institute for Cancer Research, Toronto, CANADA, Oregon Health and Sciences University, Portland, USA, Simon Fraser University, Burnaby, BC, CANADA, The Francis Crick Institute, London, UK, University of Chicago, IL, USA, University of Cologne, GERMANY, University of Glasgow, UK, University of Leuven, BELGIUM, University of Toronto, Ontario, CANADA, Wellcome Trust Sanger Institute, Hinxton, UK

Tumours develop through the gradual acquisition of single nucleotide variants (SNVs), small insertions and deletions (indels), copy number alterations (CNAs) and structural variants (SVs), with driver mutations giving rise to clonal expansions and intra-tumour heterogeneity (ITH).

We have characterised ITH across 2778 whole genome sequences of tumours in the International Cancer Genome Consortium (ICGC) Pan-Cancer Analysis of Whole Genomes (PCAWG) project, representing 39 distinct cancer types. We applied 6 CNA callers and 11 subclonal reconstruction algorithms and developed approaches to integrate the results in robust, high-confidence CNA calls and subclonal architectures.

The analysis reveals widespread ITH. We find subclones in nearly all (94.3%) tumours with
sufficient power to detect subclonal mutations. Distinct subgroups emerge; for example, squamous cell carcinomas have few subclonal SNVs (<20%) and higher proportions of subclonal CNAs. Hepatocellular carcinomas contain few subclones and few subclonal SNVs and indels, but a high proportion of subclonal CNAs. In contrast, pancreatic endocrine cancers contain many subclones and high numbers of subclonal SNVs, but few subclonal CNAs.

Analysis using dN/dS ratios yields clear signs of positive selection in clonal and subclonal SNVs and we find subclonal driver mutations in known driver genes. Yet only 12% of subclones contain an identified driver. Furthermore, nearly 10% of tumours contain driver mutations in genes for which inhibitors are available. These findings suggest that tumours continue to evolve up until diagnosis, that ITH could influence treatment choices and that a substantial number of late drivers have yet to be identified.

Mutational signature analysis reveals changes in signature activity. Exposures to UV light in melanomas and acid reflux in stomach and oesophageal cancers contribute more clonal mutations. While APOBEC and DNA damage repair response related signatures show increased activity in subclones.

These findings highlight distinct evolutionary narratives between and within histologically distinct tumour types.
Molecular analysis of circulating tumour cells in lung cancer

Caroline Dive¹
¹ Cancer Research UK Manchester Institute, Manchester, UK

Abstract not available at the time of printing.

BIO: Professor Caroline Dive is internationally renowned for advancing circulating biomarker research, with a strong focus on circulating tumour cells (CTCs), particularly in lung cancer. She initially trained as a pharmacist at the University of London. She then studied for her PhD in Cambridge before taking a new Blood lectureship at Aston University in Birmingham. Caroline then obtained a Lister Institute fellowship, and moved to the University of Manchester where she set up a group to study drug induced apoptosis. She became a full Professor in 2002 and moved to the CRUK Manchester Institute in 2003.

Currently, Caroline leads the Clinical and Experimental Pharmacology group at the Cancer Research UK Manchester Institute, coordinating activities of scientists, bioinformaticians and clinicians. She has validated and implemented pharmacodynamic, prognostic and predictive biomarkers in clinical trials, working in tandem with clinical researchers and the Christie NHS Foundation Trust Cancer Treatment Centre. Her team has integrated reproducible protocols for the molecular profiling of CTCs into clinical trials, enhanced sample analysis for multi-site trials, and developed methods for circulating free DNA and CTC analysis from the same blood sample.

She developed unique xenotransplantation models using CTCs enriched from small cell lung cancer patients’ blood samples, providing a fully tractable system for therapy testing and understanding drug resistance mechanisms, a landmark development in the field. Within the CRUK-funded TRACERx consortium, a pioneering study of intratumoural heterogeneity and evolution of non-small cell lung cancer, she directs the CTC analysis within the consortium and is developing the first NSCLC CTC Biobank worldwide. She is the Manchester lead of the CRUK Lung Cancer Centre of Excellence, a partnership with University College London, and the scientific-lead of the Manchester Experimental Cancer Medicine Centre.

Predicting relapse using circulating tumor DNA in breast cancer

Nicholas Turner¹
¹ ICR, London, UK

Abstract not available at the time of printing.

BIO: Dr Nicholas Turner is a Consultant Medical Oncologist who specialises in the treatment of breast cancer. Dr Turner read Natural Sciences at Cambridge University before qualifying in 1997 from the University of Oxford Medical School. After completing general medical training in London, he trained in medical oncology at Royal Free and University College Hospitals and completed a PhD at The Institute of Cancer Research in 2006. He joined the Breast Unit of The Royal Marsden as a Consultant in Medical Oncology in 2008.

He is a Team Leader in Molecular Oncology at the Breast Cancer Now Research Centre at the Institute of Cancer Research, London. He is Breast Theme Lead for The Royal Marsden NIHR Biomedical Research Centre, and a member of the NCRI Breast Cancer Clinical Studies Group. He is the Breast Domain Lead of the Genomics England Clinical Interpretation Partnerships. He sits on the organising committees of many international conferences on breast cancer, was the executive chair of the IMPAKT 2015 breast cancer conference, and is a scientific editor of the journal Cancer Discovery.

Dr Nicholas Turner is Chief Investigator of a number of national and international trials of precision therapy in breast cancer. His research interests include the development of new therapies for breast cancer and using liquid biopsies to deliver more precise treatment for breast cancer.
Proffered Paper 3

Detection and characterization of neuroendocrine prostate cancer using whole genome-sequencing in circulating tumor DNA

Jelena Belic3, Peter Ulz3, Ellen Heitzer3, Maria Smolle2, Thomas Bauernhofer2, Katja Fischereder1, Jochen B. Geigl3

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Background: Prostate adenocarcinoma treated with ADT could transdifferentiate to a more aggressive phenotype, known as neuroendocrine prostate cancer (NEPC). NEPC represents an androgen-independent, lethal form of prostate cancer with metastases in visceral organs and overall survival is below one year. In contrast to adenocarcinoma, neuroendocrine cells do not express the androgen receptor (AR) gene or secrete prostate specific antigen (PSA). There is an unmet need for further molecular characterization, cost-effective pre-screening and early diagnosis.

Methods: We applied our recently published plasma-Seq method on 95 plasma samples from 43 metastatic prostate cancer patients in order to establish genome-wide single copy number aberrations (SCNAs). Furthermore, we used a targeted resequencing approach to screen for mutations in a set of 58 cancer-associated genes.

Results: In 70% of patients we found AR gene amplifications and TMPRSS-ERG fusions in around 40% of analyzed cases. Analyzing follow-up samples, we observed the occurrence of novel copy number aberrations and clonal shifts in one-third of the patients. Three cases with sequential plasma samples showed changing copy number patterns on all autosomes and also on chromosome X. In all three cases, we observed the vanishing of the AR focal amplification, a dramatic increase of neuron-specific enolase (NSE), an additional drop in the PSA accompanied by shifts in mutant allele frequencies for TP53 and EP300 mutations. In additional four NEPC cases, we observed a good response to carboplatin/etoposide (CE) therapy, confirmed by clinical and radiological data.

Conclusion: Using plasma DNA, we were able to detect transdifferentiation from an adenocarcinoma to NEPC which could be an important implication for the therapy change in metastatic prostate cancer patients. Plasma DNA analyses may evolve to become a novel tool for the monitoring of patients with cancer and for the development of personalized medicine. CE-based chemotherapy might be a promising treatment option for NEPC.

Inferring expressed genes by whole-genome sequencing of plasma DNA

Michael Speicher1, Peter Ulz1, Jochen Geigl1, Ellen Heitzer1

1 Medical University of Graz, Graz, AUSTRIA

Our group has developed several methods for the analysis of circulating tumor DNA (ctDNA). One approach, termed “plasma-Seq” by us, employs a benchtop high-throughput platform to conduct whole-genome sequencing from plasma at a shallow sequencing depth to establish a genome-wide copy number profile of the tumor at low costs within 2 days. In addition, we sequence in parallel a panel of high-interest genes and introns with frequent fusion breakpoints with high coverage. To date, we have analyzed more than 1,000 plasma samples from patients with advanced cancer stage disease (breast, prostate, and colon carcinoma), which represents the clinical stage where most clinical studies are being conducted. These analyses allowed us to estimate the dynamics of clonal evolution of tumor genomes.

Recently we took a very different approach to whole-genome sequencing and leveraged the fact that plasma DNA is nucleosome protected DNA. Appropriate plasma whole-genome sequencing approaches followed by bioinformatics including support vector machines allowed the mapping of nucleosome positions based on the genomic sequencing coverage of plasma DNA fragments. For example, the genomic sequencing coverage of plasma DNA fragments around transcription start sites (TSSs) has a peculiar pattern allowing the identification of actively transcribed genes of cells releasing their DNA into the circulation. Indeed, the read depth was lower and had distinct coverage patterns around the TSSs of housekeeping genes and other highly expressed genes. In contrast, the sequencing coverage differed from unexpressed genes, which are densely packed by nucleosomes. Of special interest is that the expression levels of genes in the corresponding tumor were reflected by the coverage around the TSSs in plasma of patients with cancer. We will demonstrate examples how expressed cancer genes can be directly inferred from whole-genome sequencing of plasma DNA.
A comprehensive library of systematically designed p53 variants elucidates patterns of evolutionary conservation and of mutations in human tumors

Eran Kotler2, Guy Goldfeld2, Maya Lotan-Pompan2, Thomas Hopf1, Debora Marks1, Moshe Oren2, Eran Segal2
1 Harvard Medical School, Boston, MA, USA, 2 Weizmann Institute of Science, Rehovot, ISRAEL

The TP53 tumor suppressor gene, encoding the p53 transcription factor, is the most frequently mutated gene in human cancer. Unlike other tumor suppressors, the majority of cancer-associated mutations in p53 are missense mutations residing in its DNA-binding domain (DBD), leading to loss of tumor suppressive activity and possible gain of novel oncogenic functions. So far, research on p53 mutations has predominantly focused on six major “hotspot” codons which, although commonly mutated, comprise only ~30% of cancer-associated p53 mutations, leaving the remaining ~70% mostly uncharacterized. Here, we set out to systematically study the impact of a wide diversity of p53 mutations. Using a synthetically designed mutation library expressed in human cells, we measured the functional impact of ~10,000 p53 variants, including nearly all DBD mutations found in patient tumors, naturally occurring single nucleotide polymorphisms (SNPs) and novel variants. We found that the impact of mutations largely depends on their particular position within the DBD, corresponding with the protein’s major structural motifs, and that the majority of mutations dichotomize as either retaining wild-type p53 (wtp53) functionality or disrupting it. Remarkably, our experimental system faithfully reproduces the constraints that shape the DBD sequence throughout evolution. Moreover, it recapitulates and precisely predicts the prevalence of particular p53 mutations in human tumors, and thus quantitatively assesses the loss of anti-proliferative functionality that is a key selective force shaping the p53 mutational landscape. Finally, we evaluate the effects of SNPs within the DBD and show that when combined with an additional acquired mutation, the individual genetic background may modulate phenotypic outcome, and presumably tumor progression. This powerful platform illustrates design principles of p53 and their distortion in cancer.

The power of ONE: Immunology in the age of single cell genomics

Ido Amit¹
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Immune cell functional diversity is critical for the generation of the different regulator and effector responses required to safeguard the host against a broad range of threats such as pathogens and cancer, but also from attacking its own healthy cells and tissues. In multi cellular organisms, dedicated regulatory circuits control cell-type diversity and responses. The crosstalk and redundancies within these circuits and substantial cellular plasticity and heterogeneity pose a major research challenge. Over the past few years, we have developed a collection of innovative single-cell technologies, which provide unprecedented opportunities to draw a more accurate picture of the various cell types and underlying regulatory circuits, including basic mechanisms, transitions from normal to disease states and response to therapies. I will discuss some of our discoveries and how they change the current dogma in immune regulation as well novel technologies that combine single cell RNA-seq with CRISPR pooled screens and demonstrate the power of these approaches to probe and infer the wiring of mammalian circuits, fundamental to future engineering of immune cells towards desired responses, including immunotherapy.
Mapping the immune and antigenic landscape of cancer

Sergio A. Quezada
Cancer Immunology Unit, UCL Cancer Institute, University College London, UK

My laboratory is interested in defining and targeting the key pathways regulating immune recognition and destruction of cancerous cells within tumours. In recent years, a number of publications have demonstrated the essential role of the tumour microenvironment in the in vivo activity of checkpoint targeting antibodies. In this talk I will discuss novel developments in this area relating to the mechanism of action and the development of immune modulatory antibodies that promote maximal modulatory activity in different tumour microenvironments. In addition to immune regulation, another key area of research in the field of cancer immunology relates to the recognition of tumour cells by T lymphocytes. Whilst recent studies highlight the potential importance of T cell reactivity towards patient-specific mutant epitopes in the successful outcome of immune modulatory therapies, little is known about the impact of intra-tumour heterogeneity (ITH) on this relation. In the second part of this talk I will describe recent data using multi tumour region sampling to characterise the interplay between ITH, immune-reactivity and immune-regulation in non-small cell lung cancer patients.

Proffered Paper 5

In-depth characterization of immune responses against neoepitopes from primary melanoma tissues identified by mass spectrometry

Eva Bräunlein, Matteo Pecoraro, Stefan Audehm, Yinshui Chang, Jürgen Cox, Matthias Mann, Angela M. Krackhardt
German Cancer Consortium of Translational Cancer Research (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, GERMANY, Max Planck Institute of Biochemistry, Martinsried, GERMANY, Medizinische Klinik III, Klinikum rechts der Isar, Technische Universität München, München, GERMANY

Cancer immunotherapy emerged as a powerful tool in the fight against various malignancies. Overall mutational load and the recognition of tumor-specific mutations by T cells as key determinants for therapy response are currently under intensive investigation.

We recently reported on the detection of truly presented mutated peptide ligands on native melanoma tissue by combining whole exome sequencing and mass spectrometry (Bassani-Sternberg, M. et al., Nat.Commun, 2016).

Within this study, we found eleven peptides to be presented on the tissue of three different melanoma patients. MS measurement of further samples of a previously analyzed patient (Mel5) confirmed the detection of one tumor-specific mutated ligand and resulted in the identification of two additional neoepitopes supporting the high potential of the proposed approach.

Subsequent analyses of immune responses in patient Mel15 revealed polyclonal reactivities against the mutated epitope SYTL4\(^{363F}\) in peripheral blood mononuclear cells (PBMC) as well as in tumor-infiltrating lymphocytes (TIL). Cloning of peptide-stimulated and expanded PBMC resulted in the identification of two different T-cell receptors (TCR) in the peripheral blood with confirmed peptide specificity by isolated respective TCR chains. Analysis of TIL-derived SYTL4\(^{363F}\) specific T-cells revealed two different clones than those isolated from PBMC, whereas the presence of all four specific TCR in both compartments, PBMC and TIL, could be verified by real-time PCR indicating different frequencies therein. For a second epitope NCAPG2\(^{333L}\), we only observed responses within PBMC, but not in TIL. Analysis on the clonal level resulted in the isolation of one TCR directed against NCAPG2\(^{333L}\). All isolated TCR showed reactivity against the mutated ligand with different avidities but not against wildtype peptide. Moreover, recognition of endogenously processed neoantigens was confirmed.
In-depth characterization of neoantigens and neoantigen-specific T-cell responses will help to decipher the natural or therapy-associated anti-tumor immune response and therefore provides a base for further treatment optimization.

Proffered Paper 6

CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity

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¹ Cambridge Institute for Medical Research, Cambridge, UK, ² Peter MacCallum Cancer Centre, Melbourne, AUSTRALIA

Cancer cells exploit the expression of the programmed death-1 (PD-1) ligand 1 (PD-L1) to subvert T-cell mediated immunosurveillance. Consequently, therapies that disrupt PD-L1 mediated tumour tolerance have achieved substantial clinical success highlighting the need to understand the molecular regulation of PD-L1 expression. Using a genome-wide CRISPR/Cas9 deletion library screen we have identified the uncharacterized protein CMTM6 to be a key regulator of both constitutive and cytokine-induced PD-L1 expression in a broad range of cancer cells. CMTM6 is a ubiquitously expressed protein that is found in complex with PD-L1 and maintains its expression at the plasma membrane. Using a comprehensive, unbiased quantitative proteomics approach to profile plasma membrane proteins regulated by CMTM6, we find that CMTM6 displays remarkable specificity for PD-L1. Importantly, loss of CMTM6 specifically decreases PD-L1 without compromising the cell surface expression or antigen presentation capacity of MHC class I. CMTM6 depletion in patient-derived tumour cell lines alleviates the suppression of tumour specific cytotoxic T-cell activity via downregulation of cell surface PD-L1, highlighting the functional importance of CMTM6 in maintaining the PD-L1/PD-1 immune checkpoint. Together these findings provide novel insights into the biology of PD-L1 regulation, identify a new master regulator of this critical immune checkpoint and highlight a potential therapeutic target to overcome immune evasion by cancer cells.
Comprehensive elucidation of the antigenic and T cell melanoma landscapes reveal them to be restricted and oligoclonal

Yardena Samuels
Weizmann Institute of Science, Rehovot, ISRAEL

Tumor infiltrating lymphocytes (TILs) have emerged in recent decades as powerful effector cells capable of killing tumor cells, and the query for tumor shared and neo-antigens as a therapeutic approach has been the focus of cancer immunology for the past two decades. Surprisingly, to date only a handful of neo-antigen-specific TILs could be identified in patients. Here, we used HLA peptidomics to unbiasedly identify cancer/melanoma antigens and neo-antigens in 16 tumor samples, derived from seven melanoma patients, and characterize their interactions with TILs. Our study is the first to map both the antigenic and T cell landscape encompassing both the cancer/melanoma antigen and neo-antigen signatures, their immune reactivity and T cell identities – thus providing the first comprehensive analysis of the cancer cell - T cell synapse signatures. Remarkable antigenic similarities were identified between metastases derived from the same patients, as well as in their TILs signatures. TIL reactivity, tetramer staining and TCR-beta sequencing studies revealed that two major clones of neo-antigen-specific TILs comprised ~70% of the TILs population and ~80% of the TILs reactivity against its original melanoma cell line, emphasizing that a limited set of neo-antigen specific TILs mediate tumor rejection. In-vitro and in-vivo imaging studies revealed direct killing of the melanoma cell line by autologous TILs. Taken together, our data demonstrates that a limited set of neo-antigen-specific TILs mediate tumor rejection. The insights gathered through this analysis strengthens the notion that identification of few targetable antigens could guide personalized cancer immunotherapy.

Clonal and cellular heterogeneity landscapes of breast cancer

Carlos Caldas
Cancer Research UK Cambridge Institute, Cambridge, UK

Professor Caldas holds the Chair of Cancer Medicine at the University of Cambridge since 2002. He heads the Breast Cancer Functional Genomics Laboratory at the Cancer Research UK Cambridge Research Institute. He is an Honorary Consultant Medical Oncologist at Addenbrooke’s Hospital, and Director of the Breast Cancer Programme at the CRUK Cancer Centre. He is Fellow of the American College of Physicians, the Royal College of Physicians and the Royal College of Pathologists. He has been elected a Fellow of the Academy of the Medical Sciences, a Fellow of the European Academy of Cancer Sciences, and an EMBO Member. In 2016 he received the ESMO Hamilton Fairley Award.

Professor Carlos Caldas is a graduate from the University of Lisbon Medical School and trained in Internal Medicine at UT Southwestern, Dallas and Medical Oncology at Johns Hopkins Hospital, Baltimore. He then completed a research fellowship at the Institute of Cancer Research in London. In 1996 he moved to Cambridge where he has directed a research group working on the genetic alterations underlying human epithelial malignancies, with a particular focus on breast cancer.

His current research focus is in the functional genomics of breast cancer and its biological and clinical implications. His main clinical interest is in breast cancer chemotherapy and novel molecularly targeted therapies. He has published over 300 peer-reviewed papers including in New England Journal of Medicine, Nature, Cell, Nature Genetics, Nature Medicine, Cancer Cell, Science Translational Medicine, PNAS, Cancer Research, Clinical Cancer Research, Journal of Clinical Oncology, Genome Biology, PLOS Biology, PLOS Medicine, Lancet Oncology, Breast Cancer Research and Oncogene.
Proffered Paper 7

Establishment and characterization of an ovarian cancer organoid biobank

Oded Kopper¹, Chris de Witte², Jose Espejo Valle-Inclán², Kadi Lõhmussaar¹, Nizar Hami³, Petra van de Groep³, Mark van Roosmalen², Bas Ponsioen³, Ivo Renkens³, Trudy Jonges³, Paul van Roosmalen², Bas Ponsioen³, Ivo Renkens³, Wigard Kloosterman², Els Witteveen³, Ronald Zweemer³, Hans Clevers¹, Trudy Jonges³, Paul van Diest³, Hugo Snippert³, Esi Witteveen³, Ronald Zweemer³, Hans Clevers¹, Wigard Kloosterman², Els Witteveen³, Ronald Zweemer³, Hans Clevers¹, Wigard Kloosterman², Els Witteveen³, Ronald Zweemer³, Hans Clevers¹, Wigard Kloosterman²

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The majority of patients with ovarian cancer respond well to standard primary treatment, consisting of platinum-based chemotherapy and debulking surgery. However ovarian cancer recurs and become resistant to chemotherapy in nearly 75% of patients, eventually leading to death from metastasis. In order to study mechanisms of resistance and identify novel targets for treatment, an in vitro model system is required that faithfully mimics patient tumour characteristics and genetic heterogeneity. Therefore we set out to establish and thoroughly characterize an ovarian cancer organoid biobank to pave the way towards a more personalized therapy approach.

For this purpose multiple tissue samples per patient are obtained during primary debulking surgery and subsequently from the same patients at recurrence through biopsy and/or the collection of ascites or pleural fluid. Each tumour location is split to initiate 3D organoid culture, perform whole genome sequencing (WGS), RNA seq and histopathological analysis.

So far, we have established over 60 ovarian cancer organoid lines representing a broad range of histological subtypes, from high grade serous to mucinous borderline, and primary as well as recurrent disease. Features of distinct epithelial subtypes were confirmed on a morphological, histopathological and genomic level. In general organoids and corresponding tumor samples correlate based on profiles of copy number changes and driver mutations. High grade serous ovarian cancer (HGSOC) organoids harbour mutations in TP53, show resistance to Nutlin and exhibit extreme genomic instability. By applying live cell imaging, we further found that HGSOC organoids display aberrant chromosomal segregation. Finally, we have performed high throughput drug screens involving standard chemotherapeutics as well as a range of targeted drugs and we correlated the results to clinical response. This biobank provides a novel platform to study ovarian cancer and may guide choice of treatment in an individualized manner.

Keynote Lecture

T cell recognition in human cancer

Ton Schumacher¹

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Many human tumors contain large numbers of mutations, of which many hundreds can be present within expressed genes. As the resulting altered protein sequences are foreign to the immune system, immune recognition of such ‘neo-antigens’ has been proposed as a major factor in the activity of clinically used immunotherapeutics such as anti-CTLA-4 and anti-PD-1.

In support of this hypothesis, work by others and us has over the past years demonstrated that CD4 and CD8 T cell recognition of the consequences of DNA damage is a common feature in e.g. human melanoma and can be boosted by immunotherapy. These data establish ‘tumor foreignness’, as determined by neo-antigen formation, as one of the determinants of effective cancer immunotherapies.

In subsequent work we are building on these observations in a number research lines:

First, while the boosting of neo-antigen specific T cell responses appears attractive, we do not understand which tumor-specific mutations are most likely to induce T cell recognition. Through large scale screening of T cell reactivity in cancer patients we aim to address this question.

Second, while the presence of phenotypically ‘exhausted’ T cells in human tumors has led to a large clinical effort to ‘rescue’ such cells, we do not know whether all these intratumoral T cells have an intrinsic capacity to recognize autologous tumor. Through unbiased analysis of the tumor reactivity of the intratumoral TCR repertoire we aim to address whether intrinsic tumor reactivity is a common or rare property amongst the tumor-resident TCR pool in different human cancers.

Together with genetic screens to identify factors that influence tumor cell sensitivity to T cell attack, these efforts should increase our understanding of the different parameters that control tumor growth by the T cell compartment, and how to best influence cancer - immune interactions in individual patients by clinical interventions.
Precision medicine for high-grade serous ovarian cancer

James D. Brenton

1 Cancer Research UK Cambridge Institute, University of Cambridge, UK, 2 Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

Mutation in TP53 is the ubiquitous driver event in high-grade serous carcinoma (HGSOC) and is present in the earliest fallopian tube precursor lesions. The very early loss of p53 in the development of HGSOC results in endoreduplication, complex structural variants, extreme copy number abnormalities and frequent mutation of tumour suppressor genes including BRCA1 and BRCA2. Approximately 30% of patients with HGSOC have loss of homologous recombination and respond to PARP inhibitor therapy. Integrative analyses of mutational signatures have identified candidate biomarkers for loss of homologous recombination but further stratification is needed for the comprehensive development of precision medicine for women with HGSOC.

We have derived methods to deconvolve complex absolute copy-number profiles in HGSOC into individual signatures using non-negative matrix factorization analysis of shallow whole genome sequence data. These copy-number signatures have been validated on an independent cohort of 95 deep whole genome sequenced cases from TCGA and ICGC. We have identified candidate mutational processes underlying different signatures, which can be correlated with driver mutations, nucleotide signatures, and structural variation signatures. In addition, the copy number signatures predict prognosis and potential therapeutic strategies. These findings can be rapidly translated into clinical trials using cheap shallow whole genome sequencing of patient biopsies and circulating tumour DNA.

Loss of heterozygosity of essential genes represents a novel class of cancer vulnerabilities

Caitlin A. Nichols, Brenton R. Paolella, William J. Gibson, Meredith S. Brown, Laura M. Urbanski, Jack A. Kosmicki, Jeremiah Wala, Ashton Berger, Galen Gao, Andrew D. Cherniack, Rameen Beroukhim

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Despite progress in precision cancer drug discovery, few highly selective therapies exist in the clinic, indicating the need for additional therapeutic targets. We have shown that copy number alterations (CNAs) in essential genes represent novel non-driver gene vulnerabilities in cancer. Here we interrogate loss of heterozygosity (LOH) of single nucleotide polymorphisms (SNPs) located in essential genes as a novel class of candidate therapeutic targets. We hypothesized that monoallelic inactivation of the single allele of an essential gene retained in tumors can selectively kill cancer cells, while somatic cells, which retain both alleles, will tolerate allele-specific knockout. We identified a list of 1925 common missense SNPs in 1383 essential genes that undergo LOH in cancer and performed proof-of-concept allele-specific gene inactivation in two essential genes (PRIM1 and EXOSC8) using CRISPR-Cas9. We assessed the fidelity of allele-specific gene disruption and its cellular effects on gene expression, cell growth, and cell death in LOH and non-LOH genetic contexts. We determined that allele-specific knockout of PRIM1 and EXOSC8 selectively targets cells harboring only the single targeted allele of that gene. In cells retaining only the sensitive allele, we observed decreased target gene expression and cell viability that did not occur in cells retaining the resistant allele. We conclude that allele-selective inactivation of essential genes in regions of LOH (such as PRIM1 and EXOSC8) represents a novel candidate therapeutic strategy in cancer. The corresponding class of novel non-driver cancer vulnerabilities may provide a rich source of targets for future precision therapeutic development using gene editing, RNAi, or small-molecule approaches.
Proffered Paper 9

Interrogating the role of breast cancer functional heterogeneity in a tumour’s evolutionary trajectory upon therapeutic pressure using PDTX models

Alejandra Bruna1, Dimitar Georgopoulou1, Ankita Satl Batra1, Wendy Greenwood1, Abigail Shea1, John Cassidy1, Steven Kupczak1, Lisa Young1, Yi Cheng1, Alasdair Russell1, Alistair Martin1, Rajbir Batra1, Maurizio Callari1, Matthew Eldridge1, Oscar Rueda1, Carlos Caldas1

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Finding vulnerabilities of drug-resistant cancer

Rene Bernards1
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Intrinsic and acquired drug resistance represent major obstacles to effective cures for cancer. Understanding these mechanisms is instrumental for the design of strategies to counter these resistance mechanisms.

In my presentation, I will focus on an example of intrinsic drug resistance and one of acquired drug resistance. As an example of intrinsic drug resistance, I will discuss the lack of response to MEK inhibitors seen in KRAS mutant tumors of various. The consistently poor response of KRAS mutant tumors to inhibition of its downstream kinases is unexpected, as it defies the concept of “oncogene addiction”. Nevertheless, a subset of tumors responds well to MEK inhibition, but it remains unclear which biomarkers can be used to identify MEK inhibitor sensitive cancer cells. I will demonstrate that inactivating mutations in both MAP3K1 and MAP2K4 will confer strong sensitivity to MEK inhibition in cancers of any tissue origin. Given that most major cancers have a mutation rate in these two genes of around 10%, the use of these mutations may help select patient subgroups that benefit from MEK inhibitor therapy.

As an example of acquired drug resistance, I will discuss resistance of BRAF(V600E) mutant melanomas to drugs that target the BRAF and/or MEK kinases. This acquired resistance is almost always caused by reactivation of signaling through this pathway in the presence of drug. Drug withdrawal in such patients leads to a further hyper-activation of MAPK pathway, leading to a cellular state that has hallmarks of oncogene-induced senescence. I will show that drugs can be identified that maintain high levels of MAPK pathway signaling in BRAF inhibitor resistant melanomas upon cessation of BRAF inhibitor treatment. These drugs are only active in melanoma cells that have acquired resistance to BRAF inhibitors and not in parental melanoma cells, because they require MAPK hyper-activation that is induced by the gained resistance to BRAF inhibitors to be effective. In general, second line treatment of tumors that have acquired resistance to first line therapy is less effective. The unique aspect of the second line therapy identified here is that it exploits a vulnerability that is only gained upon acquisition of resistance to BRAFi therapy.
Chromosomal chaos and order in cancer evolution: TRACERx

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Increasing evidence supports complex subclonal relationships in solid tumours, manifested as intratumour heterogeneity. Parallel evolution of subclones, with distinct somatic events occurring in the same gene, signal transduction pathway or protein complex, suggests constraints to tumour evolution that might be therapeutically exploitable. Emerging data from TRACERx, a longitudinal lung cancer evolution study will be presented. Drivers of tumour heterogeneity change during the disease course and contribute to the temporally distinct origins of lung cancer driver events. APOBEC driven mutagenesis appears to be enriched in subclones in multiple tumour types. Oncogene, tumour suppressor gene and drug induced DNA replication stress are found to drive APOBEC mutagenesis. Evidence that intratumour heterogeneity and chromosomal instability is finely tuned will be presented, to create sufficient diversity for adaptation mitigating the risks of excessive genome instability resulting in cell autonomous lethality. On-going chromosomal instability, manifested as Mirrored Subclonal Allelic Imbalance (MSAI) is found to be a major driver of intratumour heterogeneity in non-small cell lung cancer, contributing to parallel evolution and selection. The finding of subclonal driver events, evidence of ongoing selection within subclones, combined with genome instability driving cell-to-cell variation is likely to limit the efficacy of targeted monotherapies, suggesting the need for new approaches to drug development and clinical trial design and integration of cancer immunotherapeutic approaches. The clonal neo-antigenic architecture may act as a tumour vulnerability, targeting multiple clonal neo-antigens present in each tumour to mitigate resistance and treatment failure.

Keynote Lecture

Patient-derived primary cancer cells, integrative omics and functional drug testing facilitating precision cancer medicine

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Making cancer care more effective, safe and individually optimized is a central aim for cancer researchers and oncologists worldwide. A common strategy to achieve this is based on sequencing tumor genomes with the aim to identify oncogenic driver mutations whose effects could be blocked by specific drugs with therapeutic outcomes. Our precision medicine strategy is based on a combination of genomic, transcriptomic and proteomic profiling as well as direct high-throughput testing of ex vivo efficacies of a panel of cancer drugs (n=460) on patient-derived cancer cells. This approach was started in acute myeloid leukemias and other hematological malignances (Pemovska et al., 2013) at FIMM/UH, and is now being expanded to solid tumors. This approach can help to reposition existing cancer drugs to new indications, prioritize emerging drugs for clinical testing in molecularly defined subgroups of patients, identify biomarkers and mechanisms of action of drugs as well as help to design tailored drugs and drug combinations for precision patient treatment in the clinic.
1 - Poster Spotlight

Understanding why prostate cancer is multifocal

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Background: Prostate cancer has been reported to be a multifocal disease in up to 80\% of cases, suggesting the presence of a field effect. In recent studies, clonal expansions were observed in morphologically normal tissue from men with prostate cancer. Interestingly, it was observed that the mutational processes driving them where the same that are at work in cancer samples. The present study aims to examine the occurrence of these two phenomena in an expanded group of morphologically normal tissue samples from men with and without prostate cancer, and determine if these changes could be driving the development of multifocal cancer in the prostate.

Methods: Whole genome sequencing was used in morphologically normal tissue samples and benign prostate hyperplasia tissue samples from patients with and without prostate cancer. Bayesian clustering methods and non-negative matrix factorization methods were used to detect clonal expansions and mutational processes respectively.

Results: Mutations were significantly fewer in samples from men without prostate cancer; among the cancer patients, the samples from tissue with benign prostate hyperplasia showed a higher number of mutations in comparison to the ones from morphologically normal tissue. Clonal expansions were detected in all samples, but a higher number of clones was found in the samples from cancer patients. Abnormal mutational processes were also found across all samples.

Conclusions: These findings are consistent with previous studies, and show that clonal expansions driven by abnormal mutational processes are occurring in morphologically normal tissue, contributing to field cancerization of the prostate.

2 - Poster Spotlight

Exploring Tumour Heterogeneity and Evolution in Non-Small Cell Lung Cancer within the TRACERx Study through Molecular Profiling of Circulating Tumour Cells (CTCs)

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Background: Tumour heterogeneity is increasingly thought to be responsible for therapeutic failure and patient relapse. Circulating tumour cells (CTCs) represent a potential tumour surrogate and they can be used to dynamically monitor tumour evolution and heterogeneity with the advantage to be a minimally invasive approach compared to traditional tissue biopsies.

Within the TRACERx study (TRAcking non-small cell lung Cancer Evolution through therapy), we are profiling CTCs from pulmonary vein collected at tumour resection and peripheral blood to establish the relationship between CTC and tumour heterogeneity using copy number alteration (CNA) and somatic mutation analysis. In the longer term, the data will also be used to compare with clinical outcomes following surgery and adjuvant therapy to determine the biomarker potential of CTC analysis.

Methods: CTCs were processed using CellSearch® and isolated by DEPArray™ and were subjected to whole genome amplification using Ampli1™ WGA kit. CTCs Next Generation Sequencing (NGS) was carried using established methods (Carter et al, 2017).

Results and Conclusions: A robust workflow was established for molecular analysis of CTCs from early stage NSCLC patients. 122 pulmonary blood samples obtained at surgery were processed and the presence of CTCs, associated with poor
prognosis (Crosbie et al, 2016), was detected in 62 samples with 1 or more CTCs. CNA analysis of 104 single CTCs from 12 patients revealed CTC heterogeneity between patients and also within individual patients. An examination of CNA and whole exome sequencing of both CTCs and matching tumour samples from the first patient identified common genetic alterations shared by CTCs and the tumour, as well as genetic changes seen only in CTCs. The combined data shows that tumour and CTC analysis can provide another layer of detail providing a valuable new perspective on tumour heterogeneity in early NSCLC.

3 - Poster Spotlight

ClinCnv: large-scale germline CNV discovery in the PCAWG cohort

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Germline copy number variants (CNV) are a common source of genomic variation involved in many genomic disorders, such as schizophrenia or cancer. Genomic microarrays, FISH, as well as many other technologies, are widely used for detection of CNVs. Whole-genome sequencing (WGS) is a well-established, highly accurate tool for the detection of point mutations and small indels. CNV detection using WGS data has been emerging as a competitive alternative for interrogating CNVs, but remains challenging.

We have developed a new method for read-depth based multi-sample germline CNV detection and analyzed a cohort of 2834 WGS samples from the Pan Cancer Analysis of Whole Genomes (PCAWG) study, 2573 of which passed QC control. We have detected 14,509, 6,038 and 833 bi-allelic deletions, duplications and mCNV events, respectively, of size greater than 3KB. FDRs for the three variant types were estimated using the IRS method and available microarray intensity data and were equal to 0.03, 0.044 and 0.035.

We analyzed a list of 128 cancer predisposition genes and found that 48 of these genes were affected by CNVs (119 deletions and 64 duplications overall). Moreover, intersection with 1660 genes potentially involved in cancer development or damage repair (custom list generated by PCAWG consortium based on literature) revealed overlap of 498 genes with 1697 deletions and 969 duplications. For each gene we performed an association test for each of the 38 cancer types comprised in PCAWG. We identified genes significantly enriched for deletions in 8 tumor types and genes significantly enriched for duplications in 7 tumor types. For instance, 16 samples with Pancreatic adenocarcinoma have a duplication of the same intronic region of PCAT19 gene, which is not found in any other cancer type. Such associations may reveal novel candidate genes related to cancer predisposition.

4 - Poster Spotlight

Amplifications and distal 6q loss are novel markers for poor survival in high-risk neuroblastoma patients

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Results: In this study, we identified two types of copy number aberrations that are associated with extremely poor outcome. (i) Distal 6q losses were detected in 5.9% of patients and were associated with a ten-year survival probability of only 5.1%. (ii) Amplifications of regions not encompassing the MYCN locus were detected in 18% of patients and were associated with a ten-year survival probability of only 7.2%.

Conclusion: Using a unique large copy number dataset of high-risk neuroblastoma cases, we identified a small subset of high-risk neuroblastoma patients with extremely low survival probability that might be eligible for inclusion in clinical trials of new therapeutics. The amplicons may also nominate alternative treatments that target the amplified genes.

5 - Poster Spotlight

Representing the cancer genome with mirror-DNA spike-in controls

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Next-generation sequencing (NGS) can be used to identify genetic mutations in tumour samples, and thereby inform diagnosis and therapy selection for cancer patients. However, the analysis of tumour samples by NGS is challenging, due to: (1) the sheer size of the human genome; (2) the diversity of mutation types that occur in cancer; (3) tumour impurity, sub-clonal heterogeneity and genomic instability; (4) the confounding influence of technical variables during library preparation, sequencing and bioinformatic analysis.

We recently developed a set of synthetic DNA sequencing spike-ins (Sequins), that act as qualitative and quantitative internal controls for NGS experiments [1]. Sequins are mirror-image representations of human DNA sequences (i.e. natural sequences arranged in reverse), and can be used to represent almost any feature of the genome, including instances of genetic variation or disease-causing mutations. Mirror-DNA sequences retain all intrinsic properties of their corresponding human sequences. However, NGS reads derived from Sequins align exclusively to a reverse-orientation copy of the human reference genome, thereby partitioning them from the accompanying sample for parallel analysis.

Here we present a set of mirror-DNA Sequin controls purpose-built for cancer genomics. This encompasses 99 recurrent and/or clinically actionable small mutations (e.g. BRAF:V600E), gene amplifications (e.g. MYCN), diagnostic microsatellite loci (e.g. BAT26), and a range genetic variants that are difficult to resolve with
NGS (e.g. structural variants, simple repeats, etc.). Cancer Sequins are assembled into quantitative ladders that emulate copy number variation and heterogeneous somatic mutations encountered in tumours. In this way, Cancer Sequins estimate the accuracy with which mutations can be detected at different allele frequencies and enable sample-specific determination of diagnostic performance. We provide Cancer Sequins as a standardised resource for the cancer genomics community.


6 - Poster Spotlight

Signatures of somatic copy number alterations in cancer genomes

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Cancer cells harbour somatic mutations that alter the function of crucial cellular machinery, leading to uncontrolled cellular division and growth. Compared to other genomic aberrations, somatic copy number alterations (SCNAs) affect a larger fraction of the genome, and have critical impacts through both general aneuploidy and changes to specific genes. Common patterns of aberration and recurrent SCNAs have been identified across cancer types, suggesting shared mechanisms behind their generation, though the driving processes remain to be elucidated.

Here, we sought to understand the universal patterns of aberration and modes of variation of SCNAs by conducting a pan-cancer analysis of 12,134 tumours belonging to 33 cancer types from The Cancer Genome Atlas (TCGA). We assessed the whole-genome allele-specific copy number profile of each tumour using Copy Aberration Regional Mapping Analysis (CARMA), which characterizes chromosome arms with indices reflecting specific aberrations, followed by signature extraction using Non-negative Matrix Factorization (NMF).

The resulting signatures describe both general patterns across cancer genomes, captured by CARMA indices of normality, gain or loss, and recurrent alterations in specific chromosome arms in the form of deletions or amplifications, that reflect driver events likely subjected to selection. Individual CARMA signature contributions can be linked to mutation generating processes such as smoking. Clustering of signatures within each cancer type identifies subgroups that are significantly correlated with features known to harbour distinct copy number profiles, such as ER and PR status in breast cancer and whole genome duplication across several tumour types. The subgroups are also significantly correlated with specific driver mutations, such as TP53 in lung adenocarcinoma.

Taken together, our results reveal both the diversity of processes underlying copy number profiles within tumour types and the shared modes of alterations across cancers, and may provide directions for the future investigation of mechanisms behind somatic copy number alterations.

7

ABCA1 genetic variation and gene expression: implications in colorectal cancer treatment and prognosis

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Nowadays, more than 20% of patients with CRC present metastasis at the moment of diagnosis and almost 50% of patients will develop distal tumors. Early diagnosis of invasiveness may be crucial for disease and treatment outcomes. We are particularly interested in how cholesterol transport alterations in tumor microenvironment influence cancer progression and prognosis. We have identified a metabolic-related signature (ColoLipidGenes) that allowed the stratification of stage II CRC with 5-fold higher risk of relapse. The signature is composed by four lipid-related “druggable” enzymes, ABCA1, ACSL1, AGPAT1 and SCD. Among them, ABCA1 protein is the main regulator of reverse cholesterol transport. By targeted re-sequencing of coding regions of ColoLipidGenes in a set of 95 stage II CRC patients we detected twelve SNVs in coding region of ABCA1 gene. Among them, D807N variant was
found in three patients. Three of twelve SNVs were predicted to be deleterious in nature by SIFT and/or Polyphen algorithms. We detected that the presence of exonic variants of ABCA1 were marginally associated to young patients (p=0.06) in the pooled dataset. Moreover, we observed several associations with prognostic clinical characteristics such differentiation grade and tumors with vascular and perineural invasion (p>0.05). We have demonstrated the involvement of this lipid-related gene in tumor relapse by increasing the levels of expression of several well-established colorectal cancer stem-cell makers such as EpCAM or LGR5, acquisition of stem-cell properties, and promotion of migration and invasion through an epithelial-mesenchymal transition (EMT) program. Importantly, by treating the cells with a new BET inhibitor we restored the proliferative and invasive rates of CRC derived cells to a level that is comparable to control situation. Thus, here we present ABCA1 as a new putative and independent marker for CRC prognosis and we propose a new treatment for ABCA1 overexpressing colorectal cancer patients.

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8

Somatic structural genomic alterations leading to altered transcription in diverse human cancers

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Tumor genomes are mosaics of somatic structural variants (SVs) that may contribute to the activation of oncogenes or inactivation of tumor suppressors, for example, by altering gene copy number amplitude. However, there are multiple other ways in which SVs can modulate transcription, but the general impact of such events on tumor transcriptional output has not been systematically determined. Here we use information on SVs, mapped using whole-genome sequencing data from 600 tumors, as well as array-based copy-number profiles from ~10,000 tumors, all made available by The Cancer Genome Atlas consortium, to investigate the relationship between SVs, copy number alterations, and mRNA expression changes. We find that 34% of copy number breakpoints can be clarified structurally and that most amplifications are due to tandem duplications. In the SV data, we observe frequent swapping of strong and weak promoters in the context of gene fusions, and find that this has a measurable global impact on mRNA levels. Copy-number profiles allowed us to identify intrachromosomal fusion events leading to gene activation through this mechanism in a larger number of samples. We also find that many SVs lead to aberrant transcription in normally non-transcribed regions. In summary, through a combination of SV, copy number, and expression data we provide insights into the structural basis of copy number changes as well as the impact of SVs on transcription pattern in tumors.

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SMAR1 mediated MHC1 regulation by modulation of calnexin expression in cancer and infection

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SMAR1 is a MAR binding protein known to repress a set specific target genes in response to various cellular stresses. We have investigated the effect of SMAR1 knockdown on the proteome of HCT116 colon carcinoma cells carrying wild-type p53 using 2D gel electrophoresis. SMAR1-dependent regulation of one the up-regulated proteins, Calnexin was further studied. To delineate the mechanism of how SMAR1 regulates calnexin gene expression, a bioinformatic analysis of calnexin promoter was performed. Interestingly, SMAR1 and GATA2 binding sites were observed proximal to each other on calnexin promoter. Chromatin immunoprecipitation confirms the binding of SMAR1, GATA2 and HDAC1 on calnexin promoter. We found that SMAR1 forms a triple complex with GATA2 and HDAC1. Recruitment of HDAC1 by SMAR1 on calnexin promoter results in deacetylation of GATA2. Under deacetylated condition, GATA2 acts as a repressor resulting in downregulation of calnexin gene. This study mechanistically highlights the co-ordinated regulation of calnexin gene by SMAR1 and GATA2. We further checked if SMAR1 can regulate MHC1 gene expression by regulating calnexin gene expression in cancer cells. Here we show that SMAR1 down regulates calnexin gene expression and also stabilizes p53 expression leading to over expression of ERAP1 and other APM machinery resulting in increased expression of MHC1.
In-vivo experiments also confirmed increased MHC1 expression and antigen presentation by tumor cells transduced with SMAR1-Adeno virus. Further, to prove our hypothesis we used influenza virus infection model which is known to increase MHC1, was also seen to induce SMAR1 and decrease calnexin expression. Our results clearly demonstrate that SMAR1 induces MHC1 expression by repressing calnexin gene expression. This study highlights the function of SMAR1 in immune surveillance during cancer and infection.

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Landscape of large mitochondrial structural alterations in human cancers
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The mitochondrion is an intracellular organelle critical for maintenance of cellular energy/metabolism and several clinical disorders are associated with mitochondrial dysfunction due to point mutations or larger deletions/duplications in mitochondrial DNA (mtDNA). Altered mitochondrial function is also a characteristic property of tumor cells, which often show increased production of ATP through non-oxidative breakdown of glucose in the cytoplasm (the Warburg effect). However, comparatively little is known about alterations in mtDNA in cancer, in particular when it comes to larger structural changes. Here, we established a computational pipeline to identify large deletions/duplications in mtDNA using high-throughput DNA and RNA sequencing data. We carefully evaluated this pipeline using simulated data as well as data from tumors sequenced by The Cancer Genome Atlas (TCGA). Presently, more than 1,500 whole genomes and 8,000 transcriptome libraries are available in TCGA, and our methodology thus provides a unique opportunity to study mtDNA deletions/duplications in a large cancer cohort, and relate them to important clinical parameters as well as other types of molecular changes in tumors. An initial analysis of ~750 tumor/normal paired whole-genome sequencing libraries revealed a subset of patients with a high frequency of deletions/duplications, which were enriched for colon adenocarcinomas and often carried somatic mutations in genes responsible for mitochondria replication and autophagy. These findings, while preliminary, point to a subset of tumors with a high degree of mtDNA instability that could be potentially vulnerable to therapeutic targeting of mitochondrial processes.

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Evidence for pervasive purifying selection on somatic synonymous mutations during evolution of cancer genomes
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An important goal of cancer genomic projects is to systematically identify cancer causing mutations. A common approach is to identify sites with high ratio of non-synonymous to synonymous mutations; however, if synonymous mutations are under purifying selection, then this methodology leads to identification of many false positive mutations. Here, using synonymous somatic mutations (SSMs) identified in over 4,000 tumours across 15 different cancer types, we sought to test this assumption by focusing on coding regions required for splicing. Exon flanks, which are enriched for sequences required for splicing fidelity, have ~17% lower SSM density compared to exonic cores, even after excluding canonical splice sites. Corroborating this result, exonic splice enhancers also have a lower density of SSMs than nonESE sequences and flanks at the 5' end of the exons have significantly lower SSM density than at the 3' end. The flank-core difference is not explained by skewed (di)nucleotide content, replication timing, nucleosome occupancy or deficiency in mismatch repair. The depletion is not seen in tumour suppressors, consistent with their role in positive tumour selection, but is otherwise observed in cancer-associated and non-cancer genes, both essential and non-essential, indicating that maintenance of splicing fidelity in non-tumour suppressors is important during somatic evolution of tumours. Thus the local synonymous mutation profile is not an unbiased reflection of the local mutational profile. These findings have implications for understanding cancer evolution, functional annotation of synonymous somatic variants and identification of cancer driving mutations.
Unravelling the role of TRIB1 in colorectal cancer – a functional molecular pathology approach

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Despite recent advances in clinical and experimental colorectal cancer (CRC) research, the optimal approach to stratifying CRC patients remains uncertain. We previously described amplification of TRIB1 and MYC in 14.5% and 7.4% of the CRC cohort (n=118), respectively, and found these amplifications significantly correlated (p = 0.0001), in a proportion of cases. Our current aims are to: (i) understand the prognostic role of TRIB1 in CRC; (ii) define the function of TRIB1 in CRC; and (iii) identify a clinical and molecular signature for this subgroup of patients that can be used for prognostication and therapeutic targeting. To this end, we are validating TRIB1 expression/amplification in 500 Maltese patients diagnosed with CRC between 2008 and 2011. A tissue microarray platform will be used to quantify gene amplification by fluorescence in situ hybridisation (FISH), gene expression by in situ Quantigene Plex Technology, and protein expression by immunofluorescence imaging. TRIB1 expression will be associated with several clinicopathological parameters including overall survival, stage, grade, location, and lymphatic and venous invasion. CRISPR/Cas9 technology will be used to create two stable TRIB1 knock-down CRC cell lines, which will be used for functional analysis using the Celigo® Imaging Cytometer to measure cell cycle progression, proliferation, tumour growth, and invasion. Western blotting will be used to interrogate several pathways including MAPK, PI3K/AKT, NF-κB, cell cycle, and apoptosis. Our data shows that Trib1 protein expression in the patient cohort is significantly positively correlated with Akt, BRCA1, Met, Erk, Stat3, MEK, Msc, Caspase 3, PTEN, Stat3 and CDK2 (p =<0.05). Finally, the relationship between co-amplification of TRIB1 and MYC and DNA damage will be assessed. These findings will fully characterise the TRIB1-positive subgroup, laying the foundation for therapeutic targeting and companion diagnostics. Correct patient stratification increases patient survival, decreases recurrences, and minimises overtreatment.

Integrative analysis of molecular and drug response data to identify pharmacogenomic associations in breast cancer

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Breast cancer is the most common cancer among women showing high clinical and molecular heterogeneity. Current clinical management causes extensive overtreatment with implications on both patients’ quality of life and healthcare costs. Moreover, intrinsic or acquired resistance to treatment leads to incurable metastatic progression in a significant proportion of patients. Consequently, there is an urgent need for better predictive biomarkers and a better understanding of mechanisms driving response to treatment.

As part of the METABRIC initiative, we fully molecularly characterized 2000 breast primary tumors, measuring gene expression, copy number aberration and somatic mutations. In addition, a biobank of breast cancer patient-derived tumor xenograft (PDTX) models (n=92) has been generated in our lab and a comprehensive molecular characterization was also obtained. An ex-vivo drug screening was performed in these models generating response data for 100 different drugs, including “best in class” PI3K, PARP and CDK4/6 inhibitors, novel biological and chemical inhibitors of HER2, ER, IGF1R and HER3, as well as standard of care agents.

In the METABRIC cohort we derived breast cancer specific molecular signatures based on the concepts of correlated gene expression and mutual exclusivity of genomic aberrations. Their association with previous breast cancer classifications, as well as their prognostic significance was studied. The predictive power of these signatures was investigated in the PDTX cohort to identify pharmacogenomics associations. We found known and novel associations between genomic/transcriptomic features and drug response. Among which, we identified metagenes (clusters of correlated genes) significantly associated with response to chemotherapy or response to PARP-inhibitors. Selected findings were validated in clinical cohorts as well as in independent PDTX models.

By integrating molecular data from large cohorts of clinical samples and PDTX we have generated a computational framework for the systematic identification of pharmacogenomics associations in breast cancer and to generate hypothesis for rational drug combinations.
Integrative analysis reveals novel subtypes of medulloblastoma subgroups

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Medulloblastoma is the most common malignant brain tumor in children. It is accepted to comprise four distinct molecular variants, and current clinical trials are stratifying patients using a combined biological and clinical risk stratification. However inside each subgroup, we observe tremendous clinical heterogeneity suggesting additional substructure. What remains unclear is the degree of biological substructure within subgroups.

We performed an integrative analysis of 763 primary medulloblastoma samples with gene expression and genome-wide DNA methylation data with the Similarity Network Fusion method (SNF) to uncover this structure. The integrative clustering faithfully recapitulated the core subgroups with a clear boundary between Group3 and 4, that is not readily apparent by either expression or methylation alone. A subsequent analysis within each subgroup revealed varying degrees of biological heterogeneity. After integration of somatic copy number alterations and clinical features, we identified twelve medulloblastoma subtypes with clear clinical and molecular characteristics; two WNT, four SHH, three Group3 and three Group4 subtypes. We discovered two infants SHH subtypes with disparate outcomes and distinct copy number profiles, a childhood subtype with poor prognosis and an adult subtype. Notably, TP53 mutation is enriched and prognostic only in the childhood SHH subtype. The worst prognosis Group3 subtype had MYC amplicons and isochromosome 17q without other focal aberrations. The other two have more favourable prognosis, one harbours similar focal copy number aberrations as the first one without high level MYC amplifications. Pathway analysis revealed subtype specific biological processes and transcriptional networks. For example, we observed an enrichment of developmental pathways in one of the two SHH infant subtypes.

As current therapies result in significant long-term neurocognitive and neuroendocrine sequelae, the identification of distinct biological processes within each subgroup allows for more refinement in biological risk stratification as well as the possible identification of novel agents for future targeted therapies.

Detection of Circulating Tumour DNA in Localised Uveal Melanoma using Droplet Digital PCR

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Background: Uveal melanoma is the commonest intraocular tumour in adults. Despite effective treatment of the primary tumour, up to 50% of patients develop metastatic disease. Outcomes for metastatic patients are poor, with median survival between 2 and 12 months from the detection of metastases. Circulating tumour DNA (ctDNA) is a non-invasive mechanism for monitoring recurrence in malignancy. Mutations in GNAQ and GNA11 are almost exclusive to uveal melanoma, and can serve as a marker for ctDNA. To date, plasma ctDNA has only been detected in uveal melanoma patients with metastatic disease.

Methods: We conducted a prospective study to test the feasibility of detecting plasma ctDNA in patients with stage I to IIIC uveal melanoma prior to radical treatment of the primary tumour. DNA was extracted from baseline plasma samples of 18 patients and 1ng DNA input was used for library preparation. Droplet digital PCR (ddPCR) was
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performed on library inputs of 12ng to detect the presence of driver mutations in GNAQ and GNA11.

Results: In 17 of 18 plasma samples from patients with stage I to IIIB uveal melanoma, total circulating free DNA (cfDNA) concentration ranged from 591 to 4146 GE/ml (median = 1787 GE/ml). ctDNA was detected in 1 patient with stage IIIB uveal melanoma (diameter 18mm, height 5mm) with a GNA11 Q209L allele fraction (AF) of 1.9%.

Conclusions: To our knowledge, this is the first study to demonstrate the presence of plasma ctDNA in a patient with uveal melanoma, in the absence of metastatic disease. In tumours with low disease burden such as uveal melanoma, ddPCR on libraries of DNA samples extracted from plasma can be used to detect ctDNA. Increasing DNA input into library preparation may improve the sensitivity of ctDNA detection further.

An apoptotic priming assay identifies signatures of apoptotic sensitivity and drug response in colorectal cancer cells

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Avoidance of apoptosis is a hallmark of cancer. The intrinsic mitochondrial pathway of apoptosis is central to chemotherapeutic response in many cancer types. It is tightly regulated by an intricate network of BCL-2 family proteins, which have altered expression in up to 70% of tumours across various cancer types. BH3 profiling assays assessing apoptotic sensitivity in tumour cells are predictive of clinical response to chemotherapy.

Despite current efforts to modulate the activity of BCL-2 family proteins in personalised therapy, relatively little is known about the varying contributions of BCL-2 family proteins to apoptosis induction and how patient-specific molecular backgrounds affect therapeutic response.

We aim to address these questions in colorectal cancer which is the fourth most common cause of cancer death worldwide. We designed and employed a high-throughput apoptotic profiling screen across a set of 45 comprehensively annotated colorectal cancer cell lines. Our screen uses a panel of different pro-apoptotic BH3 peptides to engage and assess the varying contributions of different components of the BCL-2 family network in mediating apoptosis. In addition to creating a “baseline” profile of apoptotic sensitivity for each cell line, we also employed “dynamic” apoptotic profiling to assess the effect of commonly used colorectal cancer therapeutics on the apoptotic network.

We observe variable apoptotic sensitivity across cell lines with different genomic backgrounds. To explain different sensitivity profiles, we are performing statistical analysis on several layers of characterisations of the cell lines, including genomic annotation, gene expression and proteomic data, as well as high-throughput drug screening data. Our preliminary results indicate that not only do apoptotic sensitivities correlate with gene expression signatures, but that they may also be used to predict response to anti-cancer compounds and potential drug combinations.

INQUISIT - integrated expression quantitative trait and in silico prediction of GWAS targets – predicts target genes at breast cancer risk loci and finds enrichment for breast cancer driver genes

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Genome wide association studies have been phenomenally successful at identifying cancer risk loci, but a major bottleneck in understanding the mechanisms underlying these loci is the determination of the target genes affected by the candidate causal risk variants (CCRVs). We have identified 179 loci associated with breast cancer risk but identified target genes at only 14. We therefore developed INQUISIT to rank the predicted target genes and prioritise functional analysis. We reasoned that most CCRVs act via distal or proximal gene regulation, or by impacting the protein product. We therefore applied different sets of weights for each of these scenarios. Points were awarded to each gene based on chromatin interaction data, enhancer annotations and expression quantitative trait loci (eQTL) analysis of all genes within 2 Mb, and integration of transcription factor (TF) ChIP-seq data for specific proteins in breast cells shown to be predictors of CCRVs. The intersection of CCRVs, enhancers and these TF binding sites resulted in up-weighting of the associated gene. Unexpressed genes and genes across topologically-associated domain boundaries were down-weighted. Promoter
variants were assessed for characteristic chromatin signatures, putative functional TF binding site overlap, gene expression data and eQTLs. Intragenic variants were scored using in-silico tools predicting the consequences of coding and splicing changes. INQUISIT predicts target genes at 90% of breast risk loci. Among the 689 protein coding target genes predicted, we found a strong enrichment for established breast cancer driver genes (P<10^-6), which increased with increasing INQUISIT score (P=5.7x10^-12). The enrichment was much stronger than obtained by assigning the association signal to the nearest gene. Most of the putative target genes have no known involvement in breast tumorigenesis, and will need to be validated by functional assays, but these results provide further evidence for the link between the germline and somatic genome of breast cancer.

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Pan-cancer analysis of systematic batch effects on somatic sequence variations
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Background: The Cancer Genome Atlas (TCGA) is a comprehensive database that includes multi-layered cancer genome profiles. Large-scale collection of data inevitably generates batch effects introduced by differences in processing at various stages from sample collection to data generation. However, batch effects on the sequence variation and its characteristics have not been studied extensively.

Results: We systematically evaluated batch effects on somatic sequence variations in pan-cancer TCGA data, revealing 999 somatic variants that were batch-biased with statistical significance (P<0.00001, Fisher’s exact test, false discovery rate ≤ 0.0027). Most of the batch-biased variants were associated with specific sample plates. The batch-biased variants, which had a unique mutational spectrum with frequent indel-type mutations, preferentially occurred at sites prone to sequencing errors, e.g., in long homopolymer runs. Non-indel type batch-biased variants were frequent at splicing sites with the unique consensus motif sequence ‘TTDTTTATTT’. Furthermore, some batch-biased variants occur in known cancer genes, potentially causing misinterpretation of mutation profiles.

Conclusions: Our strategy for identifying batch-biased variants and characterising sequence patterns might be useful in eliminating false variants and facilitating correct interpretation of sequence profiles.

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Mutations acquired by hepatocellular carcinoma recurrence give rise to an aggressive phenotype.
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Recurrence of hepatocellular carcinoma (HCC) even after curative resection causes dismal outcomes of patients. Here, to delineate the driver events of genomic and transcription alteration during HCC recurrence, we performed RNA-Seq profiling of the paired primary and recurrent tumors from two patients with intrahepatic HCC. By comparing the mutational and transcriptomic profiles, we identified somatic mutations acquired by HCC recurrence including novel mutants of GOLGB1 (E2721V) and SF3B3 (H804Y). By performing experimental evaluation using siRNA-mediated knockdown and overexpression constructs, we demonstrated that the mutants of GOLGB1 and SF3B3 can promote cell proliferation, colony formation, migration, and invasion of liver cancer cells. Transcriptome analysis also revealed that the recurrent HCCs reprogram their transcriptomes to acquire aggressive phenotypes. Network analysis revealed CXCL8 (IL-8) and SOX4 as common downstream targets of the mutants. In conclusion, we suggest that the mutations of GOLGB1 and SF3B3 are potential key drivers for the acquisition of an aggressive phenotype in recurrent HCC.

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Comparative analysis of GAEC1 copy number variation using real-time PCR and droplet digital PCR
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Mutations in the genetic code have been shown to lead to the activation of oncogenic properties and silencing of tumor suppression functions. A structural variation mutation such as copy number variation (CNV) plays an important role in the prediction of tumorigenesis. This study aims to compare the performance characteristics between real-time PCR (qPCR) and droplet digital PCR (ddPCR) for CNV analysis of a novel oncogene, GAEC1 (gene amplified in oesophageal carcinoma 1). Fresh-frozen colorectal cancer tissues and matched adjacent healthy tissues were collected from 20 Australian patients and
verified pathologically and its clinicopathological details were noted. A novel duplex DNA binding chemistry based ddPCR assay was then optimised for GAEC1 CNV detection. Annealing temperature, primer sets concentration, sample input concentration and amplicon sizes were manipulated to enable maximum separation of fluorescence amplitude in both 1D and 2D droplet plots of QuantaSoft software version 1.7.4 and QuantaSoftTM Analysis Pro Software 1.0.596 incorporated Poisson statistics were used to call copy number of target sequence without the need of a standard curve. Genomic DNA was then extracted before subjecting to both ddPCR and qPCR analysis. Our findings indicated that multiplexing Evagreen based assay with the use of ddPCR enhanced cost efficiency and improved overall runtime in comparison to qPCR. In addition, high 97% success in detection rate and good assay repeatability reduced sample to results time. More importantly, ddPCR was able to accurately obtain the CNV even at lower sample concentration compared to qPCR. Nonetheless, a larger sample size assay needs to be done in order to confirm this preliminary finding.

21 Ethnics and racial disparities in molecular, epidemiological and clinicopathological features of breast cancer in young and elderly women

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Age and ethnicity are important factors in breast cancer (BC) disparities and outcome. The underlying biology creating these discrepancies is not well understood. We aimed to identify ethnic/racial differences in epidemiological, clinicopathological, and genomic characteristics of breast cancer in young women (BCYW) as compared to elderly women across different populations using data from Cancer Incidence in Five Continents Volume X reports, Gulf Centre for Cancer Registration, including survival data from 11,244 Saudi/Arab women, 59,561 African-American and 511,660 non-Hispanic White women (US NCI SEER database), and using transcriptomic profile of breast tumors in young and elderly women cohorts from five different ethnic/racial origins. Our results indicated distinct biological features in BCYW as compared to the elderly patients across different populations, including age standardized rate of incidence, patient survival after diagnosis, clinicopathological features, and gene expression signatures. Comparisons of BCYW revealed common upstream aggressiveness signatures across five different populations, while there were distinct differences, notably higher immune related processes in young Arab and UK women, to a certain extent in African-American women. To our knowledge, this is the first study to examine the molecular, epidemiological, genomic signatures, and survival of women with BC patients in Arab and Western populations in age-specific cohorts. The population-specific molecular alterations and distinct clinicopathological characteristics may explain the racial and age-related discrepancies in disease outcome and may lead to more effective treatment strategies.

22 Transcriptional Regulator ZBTB33 Mediates Metastasis in Gastric Cancer

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Gastric cancer (GC), with high incidence rate among Eastern Asian population, is usually diagnosed at an advanced stage and is accompanied by malignant metastasis. Metastasis of gastric tumor cells to distant organs is the main cause of gastric cancer-related deaths. The invasion-metastasis cascade involves several crucial steps; tumor cells must experience intravasation into circulation systems, survival from attacks of immune systems, extravasation and finally seeding of metastatic colonies in distant organs. We did transcriptome sequencing for eight cases of gastric cancer samples with each case composed of adjacent non-tumor tissue, tumor tissue and lymph node metastasis, aiming to provide clues that how specific dysregulated genes contribute to the metastasis cascade.

Based on our data, Zinc Finger and BTB Domain 33 (ZBTB33) was found frequently upregulated in metastatic lymph node compared to matched non-tumor and tumor tissues. Current experiments suggested that ZBTB33 displays strong oncogenic features and overexpression of it could promote proliferation and cell motility in gastric cancer cell lines, which are essential capacities of
disseminated cancer cells to form metastatic colonies. Knockdown of ZBTB33 significantly suppresses its metastatic ability. ZBTB33 encodes a transcriptional repressor Kaiso and determination of its regulated downstream genes and pathways may raise the possibility of a better understanding GC metastasis. Clinically, ZBTB33 may serve as a prognostic indicator of disease relapse and give us insights into ZBTB33-related therapies for gastric cancer patients.

Discovery of novel mechanisms of centrosome amplification and their therapeutic value in cancer

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Introduction: Centrosomes are the major microtubule-organising centres of animal cells. Centrosome amplification (CA) – the presence of more than two centrosomes in a cell – is a common feature in cancer and was recently shown to be sufficient to drive tumorigenesis. Recent work from the Bettencourt-Dias Lab has identified a new recurrent feature of cancer cells: centriole over-elongation (COE), which also promotes CA. However, origins of those abnormalities and their therapeutic value remain poorly understood.

Methods: We have screened the NCI-60 panel of human cancer cell lines for centriole number and individual length to test their frequency and interdependence. We have thereby also generated a metric capturing each abnormality level per cell line that we then correlated with the publicly available molecular (e.g. genomic, transcriptomic and proteomic) and drug-sensitivity quantitative profiles for that panel.

Results: Our work shows lower frequency of COE compared to CA and lung and skin as the primary cancer tissues with higher centriole length heterogeneity. However, the two features are not independent, with longer centrioles being more common in cells with CA. Our results also suggest that cells do not control their overall centriolar mass but there is centriole-specific control of centriole length. In addition, our original genome-wide approach highlights putative mechanisms associated with susceptibility to both abnormalities, revealing, for instance, lower efficiency in DNA repair and higher interaction with the extracellular matrix as facilitators of COE. Correlation with drug activity highlighted some compounds as potential therapeutic options to selectively target cells with higher incidence of centrosomal abnormalities.

Conclusion: This work provides the first single-centriole-level portrait of centriole abnormalities in cancer, contributing to the understanding of their molecular origins. The results of our pioneer genome-wide analyses suggest novel diagnostic, prognostic and therapeutic targets in cancer, as well as novel molecular mechanisms in cell cycle biology.

The extent of subclonal punctuated evolutionary events across cancer types

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Cancer genomes are often highly complex, having acquired hundreds to thousands of point mutations, structural variants, and copy number changes. Through these changes, the genome tells the evolutionary history of a tumour, which often spans several decades since the fertilised egg. In contrast to a gradual accumulation of mutations, catastrophic events such as kataegis, chromoplexy and chromothripsis give rise to multiple inter-related point mutations, structural variations and copy number changes. The dynamics of these punctuated events throughout the evolutionary history of tumours is still unknown. In this study, we leveraged whole-genome sequencing data from 2,778 human cancer samples spanning 39 cancer types of the International Cancer Genome Consortium Pan-cancer Analysis of Whole Genomes project. We refined existing methods and developed novel algorithms for detecting chromoplexy, chromothripsis and kataegis and pinpoint these events to different stages of tumour development. We detected a total of 7,052 APOBEC-type kataegic foci, 5,387 of which we could pinpoint onto the evolutionary
trees: 3,849 kataegic events fall onto the trunk of the evolutionary trees, 853 of these were relatively early and 311 late during the evolution. A total of 1,538 kataegic events contributed to subclonal diversification in the cohort. In rare foci, the pattern of C>T mutations suggested that a different cytidine deaminase was involved. We also observed another type of kataegis, which could not be explained by cytidine deamination, but was characterised by substitutions at TT dinucleotides. Within our most confident chromoplexy calls, while subclonal events were detected in a broad range of cancers, the vast majority appeared to be clonal. And around one out of five detected chromothripsis events were subclonal. Altogether, our results paint a landscape of ongoing gradual as well as punctuated evolution across cancer types. We also highlight cases with genomes evidencing multiple catastrophic events of various and sometimes novel types.

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A liquid biopsy to detect Androgen Receptor Variant 7 in prostate cancer

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Background: Prostate cancer (PCa) remains the most common cancer in men. Treatment of metastatic castration resistant PCa (mCRPCa) involves targeting the androgen receptor (AR)-signaling pathways by novel hormonal therapies (NHT) such as enzalutamide or abiraterone. A high number of patients develop a resistance to NHT drugs. This resistance can be attributed to alternative AR-splicing generating AR-variants (e.g.: AR-V7). Thus, AR-V7 positive patients may benefit from taxane chemotherapy instead of NHT. Therefore, methods to detect AR-V7 in circulating tumor cells (CTCs) are of high clinical relevance and may predict resistance to NHT.

Methods: We applied in vivo and ex vivo CTC isolation devices (CellCollector and Parsortix) and combined them with a mRNA based in situ padlock probe technology. Padlock probes allow for highly specific detection and visualization of transcripts on a cellular level and can discriminate between different splice variants. We applied padlock probes for AR-V7, AR-FL (full length) and PSA (prostate specific antigen) and/or cytokeratin and CD45 immunostaining on CTCs from 32 prostate cancer patients.

Results: In total 78% (25/32) of patients were positive for CTCs. In situ analysis revealed that 69% (22/32) of patient-derived CTCs had detectable AR-V7 mRNA expression. The AR-V7 expression level ranged from 1 to 30 rolling circle products (RCPs), showing low and high AR-V7-expressing CTCs in patients.

Conclusions: Padlock Probe technology enables visualization and quantification of AR-V7, AR-FL and PSA transcripts directly in CTCs and may be a suitable diagnostic tool to identify drug resistance in prostate cancer patients.

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Recurrent promoter mutations in melanoma are defined by an extended context-specific mutational signature dependent on UV light

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Sequencing of whole tumour genomes holds the promise of revealing functional somatic regulatory mutations, such as those described in the TERT promoter. Recurrent promoter mutations have been identified in many additional genes and appear to be particularly common in melanoma, but convincing functional data has been more elusive. We have identified recurrent promoter mutations in melanoma patients using whole genome sequencing data from TCGA from 38 metastatic skin cutaneous melanoma tumours. Notably, these promoter mutations occur almost exclusively
at cytosines positioned 5' to a distinct sequence signature, 5'-TTCCGG-3'. In active, but not inactive, promoters, this signature is associated with mutation frequencies that are considerably higher than expected based on the UV trinucleotide signature, and the rate of mutation at these sites increases with total mutation load.

To determine whether these mutations arise as an artefact of UV exposure, we subjected cells to low level UVC treatment for a 10-week period and used ultra-sensitive amplicon sequencing to demonstrate that cell cultures exposed to UV light quickly develop subclonal mutations specifically in the affected positions. This exceptional position-specific vulnerability to UV light argues against positive selection, as may be suggested by the fact that the motif corresponds to the recognition sequence for ETS transcription factors. We instead propose that the binding of ETS factors increases the mutation rate in the affected sites possibly due to locally favorable conditions for UV lesion (CPD or 6,4-PP) formation, and will use UV lesion footprinting and genome-wide CPD mapping to evaluate this model.

Our findings have implications for the interpretation of somatic mutations in regulatory regions, and underscores the importance of longer sequence patterns as well as genomic context to accurately describe mutational signatures in cancer.

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Transcriptional and Mutational Analysis of Lipid Metabolism-Genes in Colorectal Carcinoma

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Over the past decade, many efforts have been performed by the scientific community, in order to find colorectal cancer (CRC) expression patterns that allow us an accurate disease stratification into different prognostic subgroups and/or in connection with response to therapies. Four transcriptomic consensus molecular subtypes (CMS) of CRC were recently redefined. Among them, CMS3 tumors exhibit a prominent metabolic activation with a clear enrichment for multiple metabolism signatures. In the context of lipid metabolism, we identified a lipid signature: ColoLipidGene (ABCA1, ACSL1, AGPAT1 and SCD genes) that predicts prognosis in CRC patients of stage II.

The recent availability of publicly large databases of patients with CRC, allow us to explore in depth, the transcriptomic and genomic landscapes of ColoLipidGenes (Genes of ColoLipidGene signature) in all stages of CRC. Thereby, we performed a transcriptomic meta-analysis on stored data at Gene Expression Omnibus (GEO) repository. In addition, since passenger mutations could also be a marker for tumor aggressiveness and response to passenger-exacerbating therapies, we explored genomic coding sequence of ColoLipidGenes in CRC patients in order to find sequence variants and/or passenger mutations that could orchestrate CRC prognosis.

Our results reinforce and broaden the prognostic value of ABCA1, ACSL1, AGPAT1 and SCD genes in CRC, leading to future precision medicine approaches for chronic diseases and “omics”-guided therapies.

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Chaotic genomic rearrangements in osteosarcoma

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The cancer genome sequencing project allowed the discovery of massively re-arranged genomes that have been detected within various types of human cancers caused by a novel mechanism called “chromothripsis”. This phenomenon causes up to thousands of clustered chromosomal rearrangements to occur as a single event in localized and confined genomic regions in one or a few chromosomes. In some tumors, such as Osteosarcoma (OS), chromothripsis is very common. To describe the various interpretations of how these fragmented and stitched chromosomes are generated, other names have been given including “chromoplexy”, “chromoanasynthesis”, “chromoanagenesis” and collectively as “chromothripsis-like” rearrangements. These type of rearrangements are thought to arise because of an atypical DNA repair. Chromothripsis may occur in 2–3% of cancers, but in OS the rate may be much higher at 33%. We performed a summary analysis of 106 OS samples based on sequencing
technology and array CGH to determine the incidence of the various classes of chaotic genomic rearrangement. Then, we performed a more detailed analysis of genomic imbalances in 10 OS tumor samples previously published by this laboratory (Hum Mol Gen 2009, Vol. 18, No. 11 doi:10.1093/hmg/ddp117). The summary analysis showed that 7 samples had chromothripsis and a further 17 had chromoplexy/chromothripsis-like rearrangements. Alterations predominantly involved chromosomes 6, 8, 9, 13 and 14. We observed in the re-analysis of our published lab samples chromothripsis-like events (i.e. > 25 copy number transitions within 25 Mb) in 4 OS samples (40%), involving the chromosomes 2, 6, 10, 12,15 and X. Chromothripsis, appears to be an important contributor to OS genomic diversity and the implications of this new mechanism should be considered when novel treatment strategies are applied to this tumour.

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Next Generation Targeted Amplicon Sequencing (NGTAS): An optimised deep sequencing approach and computational pipeline for circulating tumour DNA in cancer patients

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Circulating tumour DNA (ctDNA) has been demonstrated to have important potential applications in precise cancer management in clinic. In previous studies a limited number of mutations have been detected concurrently using various deep sequencing technologies. However, synchronous deep sequencing of large regions spanning multiple whole exons of tumour-associated genes are still technically challenging, yet particularly crucial for tumours with heterogeneous mutational profiles such as metastatic breast cancer.

We describe a method for Next Generation Targeted Amplicon Sequencing (NGTAS) to simultaneously deep sequence 20 tumour-associated genes by multiplexing 377 amplicons to cover hotspots and exons with low input ctDNA. Often the quantity of ctDNA in plasma samples obtained from cancer patients is extremely low. To address this, we describe an additional method which utilises a whole genome library to perform NGTAS to overcome the limitation. We also illustrate the detailed bioinformatics pipeline for alignment and mutation calling to achieve reproducibility and accuracy. To optimise NGTAS, we generated a serial dilution of cell free DNA using lymphocytes (NA12878 and NA11840) to create a maximum number of known SNPs with various allele frequencies. We demonstrate high sensitivity and specificity of NGTAS in liquid biopsies and tumour samples obtained from metastatic breast cancer patients. The method was used to determine mutations in ctDNA and monitor disease progression during an early phase clinical trial. This method is cheap, fast and flexible for investigation of hundreds of targets simultaneously using low input ctDNA obtained from non-invasive liquid biopsy. The highly multiplexed deep sequencing approach is particularly suitable for various cancer types with heterogeneous genetic aberrations to determine the disease mutational profile and monitor disease progression in clinic.

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Using machine-learning algorithms for classification of Medulloblastoma subgroups based on gene expression data

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Medulloblastoma (MB), the commonest malignant brain tumor of childhood, is divided into four tumor subgroups: WNT, SHH, Group 3 and Group 4, representing distinct molecular entities. The aim of the study is to identify biomarkers for clear and fast MB subtype classification. These biomarkers can be in the future accessible in clinical use for accurate diagnosis of patients’ tumor subtype that will accelerate the design of patient’s specific targeted therapies and optimize clinical decision. With this aim, machine-learning based classification was performed on public mRNA expression profiling data generated using microarray technology. Applying nearest neighbor modelling identified 5 potential biomarkers (GSG1, IMPG2, RUNX1T1, RNU6-608P, RN7SL492P), of
them three are protein-coding genes, and two are non-coding RNAs. These results lead to average sensitivity of 0.98 while preserving high specificity of 0.99. When focusing on Group 3 and Group 4, our analysis detected three potential biomarkers (ENSG00000199263, RN7SKP230, GSG1) differentiating between these two groups. Adding these three genes (two are non-coding RNAs) to the set of known 11 MB genes, and re-run the KNN analysis, increases the accuracy in 2%, reducing the false positive rate in 12.9% and 0.7% in Group 3 and Group 4 respectively. To summarize, this approach provides a promising class of genetic factors worth being deeply investigated as markers for MB diagnosis, development, prognosis, and clinical decision-making.

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Deciphering the timing of mutations in a pan-cancer analysis
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The diagnosis and sequencing of cancer cells occur in an advanced stage of tumour development with the cells carrying thousands of mutations. Copy number gains (CNG) can retain developmental information allowing to decrypt part of the tumour history. As part of the pan-cancer whole genomes analysis consortium we have systematically analysed 2,700 whole genome patients demonstrating that whole genome duplications can occur years before diagnosis and are preceded by mutations in a few number of cancer driver genes. Our analysis also detects the co-occurrence of apparently independent mutational events which demonstrate that in some cases the complex copy number events observed in several tumours has been formed in a limited number of catastrophic events.

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Copy number signatures predict survival in recurrent high-grade serous ovarian cancer
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High-grade serous ovarian carcinoma (HGSOC) is the most aggressive type of ovarian cancer. The main chemotheraphy using platinum agents is initially effective, however, the majority of patients relapse with less than 30% alive 5 years after diagnosis. HGSOC is characterised by a highly unstable genome resulting in significant copy-number alterations. To date, no clinically relevant classification of genomic alteration patterns exists. In order to address this issue, we studied the genomic profiles of primary and relapse tumour samples from the BriTROC study (British Translational Research Ovarian Cancer Collaborative). Shallow WGS (0.1x) was performed on 300 samples from 142 patients and absolute copy-number profiles were generated. Non-negative matrix factorization was used to decompose the copy-number profiles into 8 distinct copy-number signatures. The features of the signatures appear to match known patterns caused by phenomena such as chromothripsis and breakage-fusion-bridge. Survival analysis showed that two of the signatures significantly predicted worse prognosis. This observation was validated via application of the analysis to an independent cohort of 95 HGSOC samples, confirming the existence of the shorter survival groups. We found enrichment of CCNE1 amplification in one and a duplicator phenotype in the other poor prognosis group. Currently we are focusing on mechanisms that lie behind the observed copy-number signatures.
Systematic analysis of non-coding mutations in gastric cancer

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The role of mutations in the non-coding cancer genome is unknown for most human cancer types. Here we investigated the genomes of 212 gastric adenocarcinomas to identify somatic noncoding mutations that promote tumorigenesis through misregulation of gene expression. To identify positive selection in cancer genomes, it is essential to build a background mutation model that corrects for covariates that contribute to genomic mutational heterogeneity, such as local sequence context and chromatin features. To this end, we considered a large range of genomic and epigenomic features that could be correlated with somatic mutation rate, and used LASSO regression to select for the most informative epigenomic and sequence context features. Then we calculated the sample-specific background mutation probabilities by fitting a logistic regression on all data using the selected features as predictors. Finally, we used a Poisson binomial model that takes into account the variability of mutation rates among individual tumors to evaluate mutation recurrence for a given region. With this covariate-corrected mutation model, we systematically scanned the cancer genomes for mutation hotspots. We identified novel mutation hotspots that are associated with significant changes of cis gene expression. This study revealed that the disruption of regulatory sites could be a common mechanism of tumorigenesis in gastric cancer.

Oncogenic signal transduction pathway activity in glioblastoma and relation to therapy response

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Glioblastomas (GB) are the most common brain tumors in adults with a median survival of 15 months and palliative therapy options limited to surgery, radiotherapy and chemotherapy. Based on genomics analysis growth factor pathways (PI3K) are thought to play a role, however targeted drug therapy was not successful. A role for Hedgehog (HH), TGFβ and Wnt pathways has been suggested. To advance personalized pathway-targeted drug therapy, diagnostic tests are needed which reliable identify functionally active signaling pathways to predict and monitor therapy response.

We developed a novel method to identify activity of signal transduction pathways (ER, AR, HH, Wnt, TGFβ, NFKB and PI3K) in an individual tissue sample. Our method is based on measuring mRNA levels of the pathway transcription factor target genes and interpreting these expression levels using a knowledge-based Bayesian network computational model which infers the probability of activity of the respective transcription factor (Cancer Res 2014 Jun 1;74(11):2936-45). Public patient sample datasets from the GEO database were used to validate pathway models for use in brain tumors, and to investigate pathway activation in adult GB and normal brain tissue.

In contrast to normal brain, HH (60% of samples were found to have an active HH pathway), NFKB (50%) and TGFβ (20%) pathways were active in GB. The pro-neural subtype, associated with longer survival, shows no TGFβ activity while other subgroups show TGFβ activity (1/3 of cases), in agreement with known association of TGFβ activity with short survival time. In glioblastoma stem cells TGFβ pathway correlated strongly with radio-chemo resistance.

Results indicate that in addition to growth factor pathways, HH and TGFβ pathways are involved in GB, while TGFβ pathway activity may indicate resistance to radio-chemotherapy. Our pathway analysis is expected to have clinical utility, complementary to mutation analysis, in predicting GB therapy response to radio-chemotherapy and targeted drugs.
While several somatic mutation callers have been improved for cancer variant calling, the calls are often discordant due to differences in the thresholds and filters used in each caller. Hence we generated an improved method for the selection of a set of consensus somatic mutation calls with smbio.

Methods: smbio is an R package for the accurate prediction of somatic point mutations and small insertions & deletions in cancer genomes. The package leverages a random forest machine learning model and functions as a critical add-on to the community-developed bcbio-nextgen whole-genome sequencing (WGS) analysis pipeline to aid highly accurate somatic mutation calls. Two manually curated whole cancer genome datasets are used for training and benchmarking the model. Input somatic mutation calls and information are derived from four benchmarked somatic mutation callers, which provided over 70 individual features that are potentially useful in our predictive model.

Results and Conclusion: We validate and report that smbio achieved better overall accuracy over the existing individual somatic mutation callers and notably more than 20 percent increase in accuracy than selecting consensus calls from at least 3 of the 4 callers. We demonstrate the use of smbio in the analysis of 400 Hepatocellular Carcinoma whole genome samples.

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Deciphering the mutational signatures of activation-induced cytidine deaminase using experimentally-induced exposures and public databases

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Mutagens damage cellular DNA in processes that can leave specific mutational signatures discernible in developed tumors. Activation-induced cytidine deaminase (AID) is an enzyme, belonging to the APOBEC family, involved in hypermutation of immunoglobulin genes. AID overexpression on the inflammatory background has been linked to cell transformation and tumour development in human cancers and experimental models. It has been suggested to contribute to the mutational load in cancers of blood and digestive organs. Two different mutational signatures have been attributed to deregulated AID activity in human tumours. However, experimental evidence for these signatures is missing. Importantly, DNA repair proteins such as MSH2 are involved in both AID-related mutagenesis and repair of off-target lesions. MSH2 is frequently mutated in human tumours, but the effect of MSH2 deficiency on the mutational signatures of AID expression has not been explored.

We sequenced genomes of four liver tumours from transgenic mice carrying constitutively active AID either alone, or in the context of MSH2 deletion, alongside with mouse tumour samples with MSH2 deletion only. Three mutational signatures were identified in the mouse tumour-sequencing data, one of which closely matched canonical AID signature. The frequently mutated genes involved histone genes and a number of transcription factors. The putative AID signature and its genomic targets were experimentally recapitulated using genome-wide sequencing of a cell line derived from AID-expressing mouse embryonic fibroblasts.

The experimentally validated mutational signatures and candidate driver events related to AID overexpression will be presented, as well as their comparison to alterations observed in relevant human tumour types.

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The evolutionary history of 2,778 cancers

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The development of cancer is a continuous process of mutation and clonal evolution. Whole genome sequencing presents the endpoint of a cell’s journey to cancer, but the data can contain information that permits the reconstruction of events during a tumour’s evolutionary past. Here, we apply such life history analyses on an unprecedented scale, to a set of 2,778 tumours spanning 39 cancer types. Pan-cancer, these analyses show that mutations in canonical driver genes, distinctive chromosomal gains and losses, and mutational processes associated with exposure to carcinogens, are amongst the earliest events in tumour evolution. Later stages of clonal evolution, however, are characterised by an increase in genome instability, and the acceleration of mutational processes derived from defective DNA repair mechanisms. Furthermore, we extract tissue-specific patterns of somatic evolution, which include both well-established and potentially novel pathways of tumour development. For example, by making use of sports statistics models to obtain a relative ordering of mutational events in colorectal adenocarcinoma, we are able to recapitulate the typical progression of APC-KRAS-TP53 proposed by Vogelstein and Fearon. Alternatively, in glioblastoma, a quantitative approach to timing copy number gains demonstrates a novel pattern of early tumour evolution characterised by distinctive chromosomal gains of 7, 19 and 20. Using clock-like mutational signatures, we provide real time estimates for major events during tumour evolution, such as whole genome duplication and the emergence of the most recent common ancestor, and find that these events may precede diagnosis by many years.

Taken together, these data indicate that most cancers commonly have several predefined and ordered event trajectories, which might be crucial in understanding specific tumour biology, and in providing new opportunities for early detection and cancer prevention.
Identification of a gene signature as diagnostic and prognostic blood biomarker for early hepatocellular carcinoma using integrated cross-species genomic and network analyses

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Hepatocellular carcinoma (HCC) is the most common primary cancer originating in the liver and third-leading cause of cancer-related death worldwide. The disease is mostly diagnosed at advanced stages, and hence has poor prognosis. Robust biomarkers are therefore urgently needed for early diagnosis and prognostic estimation. In this study, we performed integrated genomic and network-based analysis of early HCC (eHCC) using two genome-wide gene expression profiling datasets from human eHCCs and a rat model of eHCC and identified gene signature that is conserved across species and expressed in blood. We then validated the diagnostic value of the identified gene signature on independent datasets of gene expression profiling of peripheral blood mononuclear cells (PBMC) from patients with eHCC as well as Cancer Genome Atlas (TCGA) early HCC cohort. Furthermore, we validated the prognostic potential of our gene signature on liver cancer datasets with detailed clinical data from TCGA (both microarray and RNA sequencing datasets) with samples from over 400 liver cancer patients. A prognostic model of our gene signature was developed to divide patients into high- and low-risk groups, with high expression of our gene signature were significantly associated with poor disease outcome. Moreover, we performed gene ontology enrichment, gene networks and pathway analyses and identified significantly altered pathways in eHCC. Our results indicate alterations in a number of cancer related pathways that are critical for early HCC transformation and validates our gene signature’s potential to detect the disease in early stage in patients’ biological fluids rather than using invasive procedures. We also validated the prognostic significance of the gene signature. Our results suggest that the network analysis coupled with cross-species genomic analysis may provide a robust methodology to identify key biological programs associated with early HCC and may lead to improved diagnosis, prognosis, and therapeutic options.

A likely novel molecular marker of pilomyxoid astrocytoma

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Pilocytic Astrocytoma (PA) is the most common pediatric brain tumors and auspiciously, they are not aggressive, typically slow growing brain tumors. Patients with PA have much higher survival rates and only a small percentage of the patients demonstrate a variable clinical course with higher mortality rates. Recently, a unique group of tumors previously diagnosed as PA were identified and named as pilomyxoid astrocytomas (PMA). The PMAs usually show unique histological characteristics and have more aggressive clinical course. PMAs mostly appear in the optic-chiasmatic and hypothalamic region but sometime they are reported to be found in the posterior fossa, temporal lobe, and in the spinal cord. The occurrence and location of these tumors are helpful features for differential diagnosis as compared to PA. Although PA and PMA are histologically well-characterized and similarities and differences are well known between PA and PMA, there are not many genetic studies done on PMA. Our study focuses on one of the largest PMA sample collections to understand the genetic and cytogenetic makeup of this solid tumor type. Hence, we performed a molecular cytogenetic study on formalin-fixed paraffin embedded archived clinical samples of PMAs using OncoScan arrays from Affymetrix Inc. (Santa Cruz, CA, USA) as well as targeted mutations screening using Sanger Sequencing. Our analyses yielded a duplication (KIAA1549-BRAF Fusion) that was found among most of the tested samples (n=14). Beside the fusion gene, three samples had additional cytogenetic abnormalities. Moreover, one sample lacking any gross abnormality including the fusion gene had a small hemizygous deletion of chromosome 6q22.3. Our study may help better diagnosis and characterization of PMA.
Regulation of HIF-1α gene expression through an hnRNP family

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Hypoxia induces HIF-1α expression, leading to the malignant cell transformation. Despite the numerous reports concerning the cancer-associated function of HIF-1α, little is known about the factors involved in nuclear expression of HIF-1α. Previously, one of the heterogenous ribonucleoprotein (hnRNP) family was suggested to be crucial for HIF-1α expression or stability. We exploited the molecular mechanism of HIF-1α gene expression using a moracin O analogue (MOA) compound, which recently was reported to inhibit HIF-1α expression under hypoxic condition. MOA was found to strongly reduce the level of HIF-1α in HeLa cells either in response to hypoxia-mimetic CoCl₂ treatment or under hypoxic condition.

Identification of binding proteins using agarose-bead conjugated MOA (AC-685) combined with subsequent MS data revealed several proteins. AC-685 co-localized with a nuclear hnRNPX protein in CoCl₂ treated HeLa cells. Amongst several cytoplasmic or nuclear proteins, hnRNPX was only found to be responsible for CoCl₂-induced HIF-1α expression as supported by siRNA knockdown of the HIF-1α gene. Subsequent studies showed that hnRNPX regulates HIF-1α expression through elf2α phosphorylation and translational attenuation of HIF-1α gene, but not affecting its transcriptional control. Furthermore, formation of stress granules was implied to be mediating HIF-1α expression. Hence, all the data suggest that hnRNPX regulates HIF-1α gene expression through elf2α-mediated translational attenuation and stress granule formation, and that MOA regulation of hnRNPX expression would be a novel approach to cancer treatment in hypoxic environment.

Genomic differences in oral squamous cell carcinoma are found to correspond to clinical subgroups

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Introduction: Oral cavity squamous cell carcinoma (OSCC) contributes to significant mortality and morbidity, and is strongly correlated to tobacco and alcohol use. However, non-smoking non-drinking (NSND) patients also develop OSCC and have worse survival than patients who smoke or drink (SD). We have conducted a large single centre study to elucidate genomic differences corresponding to this subgroup.

Methods: Two cohorts of patients – prospective and retrospective - were recruited from an Australian tertiary head and neck cancer centre. Fresh frozen samples were collected for the prospective cohort, and archival paraffin blocks were retrieved from storage for the retrospective cohort. Samples from both cohorts of patients were fed into a next-generation sequencing workflow, targeting a custom designed panel of 69 genes and 4 human papilloma virus (HPV) subtypes. Bioinformatic techniques for correction of artefact and removal of somatic mutations in paraffin samples were developed.

Results: Tumours from a total of 178 patients were sequenced, with 61 patients (34%) belonging to the NSND group. No statistically significant between group differences were found in TNM stage, perineural invasion, lymphovascular invasion or extracapsular nodal disease. There was however statistically worse survival in the NSND group. Statistically significant differences in mutation rates of 5 genes, including the key tumour suppressor CDKN2A was noted between the NSND and SD group. Other clinical-genomic correlations will also be discussed.

Discussion: Genomic differences have been uncovered between the NSND and SD groups, suggesting molecular differences resulting from differing aetiologies. HPV does not appear to play a significant role in OSCC and the NSND group. These data would recommend the development...
of clinical protocols to personalise treatment for NSND patients. Follow up studies to identify the oncogenic mechanisms in the NSND group are also required. Bioinformatic techniques used for paraffin sample processing will also be presented.

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Impact of BRAF kinase inhibitors on exomes, transcriptomes, and miRNomes of melanoma cell lines

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Metastatic melanoma is an aggressive malignancy with poor prognosis and increasing incidence worldwide. The development of BRAF kinase inhibitors (e.g. vemurafenib/PLX4032, dabrafenib/GSK2118436), as targeted treatment options for patients with BRAF-mutant tumours contributed profoundly to an increased overall survival of patients with metastatic melanoma. Despite these promising results, the emergence of resistance to BRAF kinase inhibitors remains a serious clinical issue.

To investigate the impact of BRAF inhibitors on exomes, miRNomes, and transcriptomes, we used in vitro melanoma models consisting of BRAF inhibitor-sensitive and -resistant cell lines generated in our laboratory. First, we performed whole exome sequencing to analyse variants in the coding part of the genome that might be responsible for the onset of resistance. Secondly, a microarray analysis was implemented with the aim to describe changes in the transcriptome and the miRNome that might play a role in resistance.

Our data demonstrates that BRAF inhibition might be bypassed by activating mutations (e.g. NRAS Q61K or G13K), however, those are rare events that only appear in a fraction of resistant cell lines. Additionally, we show that the resistant cell lines display an increased amount of copy number variants (CNVs) as compared to the drug-sensitive cell lines, suggesting that CNVs are involved in development of resistance to targeted therapy in melanoma. Besides, we show that the responses to BRAF inhibition mainly occur in a cell line-specific manner, as the resistant cell lines were more similar to their parental sensitive lines than to other resistant cell lines. In addition, several resistance-specific genes and miRNAs were identified, which might be considered as prognostic and/or diagnostic biomarkers in melanoma drug resistance.

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The impact of specific single nucleotide polymorphisms on the phosphorylation and function of the GRHL3 transcription factor

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The Grainyhead-like 3 (GRHL3) transcription factor is expressed primarily in epithelial tissues. It regulates the expression of genes involved in epithelial development and serves a protective role against squamous cell carcinoma (SCC) of the skin. We performed targeted next generation sequencing of GRHL genes isolated from samples from patients with skin cancer. The results showed that frequencies of some of the single nucleotide polymorphisms (SNPs) in the patient population were significantly higher than those reported for the European population by the 1000 Genomes Project, including rs141193530 (p.Pro455Ala), a missense variant in GRHL3. It is known that this variant and rs41268753 (p.Thr454Met) also increase the risk of nonsyndromic cleft palate and that rs41268753 negatively affects GRHL3 transcriptional activity.

These two SNPs are located in adjacent codons of the GRHL3 gene, and the occurrence of either SNP abolishes the putative ThrPro phosphorylation site Thr454 in the encoded protein. Using immunoprecipitation coupled to mass spectrometry, we showed that in the HaCaT cell line GRHL3 is phosphorylated at several residues, including Thr454. We performed in vitro phosphorylation assays and discovered that GRHL3 is phosphorylated at Thr454 by ERK and p38 MAPK. Mutation of Pro455 to alanine decreases its phosphorylation level, indicating that this residue is important for Thr454 phosphorylation. We evaluated whether abolishing Thr454 phosphorylation site by substitution of this residue with methionine influences GRHL3 function in HaCaT cells. This modification does not impact on GRHL3 subcellular localization. However, this substitution decreases the ability of GRHL3 to activate reporter gene and endogenous target gene transcription. Taken together, our results show that Thr454 in GRHL3 is phosphorylated, probably by MAPK. Missense variants rs41268753 and rs141193530 abolish this modification, which is normally essential for the full activity of GRHL3.

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Serum Carcinoembryonic Antigen Levels and the Risk of Whole-body Metastatic Potential in Advanced Non-small Cell Lung Cancer

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Kallikrein-related peptidase 6 induces chemotherapeutic resistance by attenuating auranofin-induced cell death through activation of autophagy in gastric cancer

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Kallikrein-related peptidase 6 (KLK6) is a biomarker of gastric cancer associated with poor prognosis. Mechanisms by which KLK6 could be exploited for chemotherapeutic use are unclear. We evaluated auranofin (AF), a compound with cytotoxic effects, in KLK6-deficient cells, and we investigated whether KLK6 expression induces autophagy and acquisition of drug resistance in gastric cancer. Using cultured human cells and a mouse xenograft model, we investigated how KLK6 affects antitumor-reagent-induced cell death and autophagy. Expression levels of KLK6, p53, and autophagy marker LC3B were determined in gastric cancer tissues. We analyzed the effects of knockdown/overexpression of KLK6, LC3B, and p53 on AF-induced cell death in cancer cells. Increased KLK6 expression in human gastric cancer tissues and cells inhibited AF-induced cell motility due to increased autophagy and p53 levels. p53 dependent induction of KLK6 expression increased autophagy and drug resistance, whereas KLK6 silencing decreased the autophagy level and increased drug sensitivity. During AF-induced cell death, KLK6 and LC3B colocalized to autophagosomes, associated with p53, and were then trafficked to the cytosol. In the xenograft model of gastric cancer, KLK6 expression decreased AF-induced cell death and KLK6-induced autophagy increased AF resistance. Taken together, the data suggest that the induction of autophagic processes through KLK6 expression may increase acquisition of resistance to AF. Our findings may contribute to a new paradigm for tumor therapeutics.

An increase in BAG-1 by PD-L1 confers resistance to tyrosine kinase inhibitor in non-small cell lung cancer via persistent activation of ERK signaling

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High PD-L1 expression in tumors was associated with poor outcomes in non-small cell lung cancer (NSCLC) due to evasion of tumor immune surveillance. However, the role of PD-L1 in tumor invasion and resistance to tyrosine kinase inhibitor (TKI) treatments are not fully understood. We provide evidence to support the involvement of PD-L1 expression in the invasiveness and TKI resistance in NSCLC cells by increased Bcl-2-associated athanogene-1 (BAG-1) expression. The upregulation of BAG-1 transcription by PD-L1 was verified by constructing the BAG-1 promoters using the polymerase chain reaction (PCR) and deletion mutations for luciferase reporter assays. The results indicated that C/EBPβ phosphorylation by ERK signaling was responsible for PD-L1-mediated BAG-1 transcription. Mechanistically, the PD-L1-induced BAG-1 expression reciprocally increased PD-L1 expression due to persistent activation of ERK signaling and it consequently conferred TKI resistance in NSCLC cells. The mechanistic action of this cell model was further confirmed by an animal model, affirming that PD-L1 conferred tumor invasiveness and TKI resistance via persistent activation of ERK signaling by the PD-L1/BAG-1 axis. We therefore suggest a combination of an ERK inhibitor with a TKI as a potential strategy for conquering PD-L1-mediated tumor invasion and TKI resistance in NSCLC patients whose tumors harbor high PD-L1/high BAG-1 expression.

Oncogenic deregulation of the methyltransferase EZH2 in hepatocellular carcinoma

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Background: Epigenetic mechanisms underlying nuclear chromatin remodeling are increasingly being recognized as crucial factors in hepatocellular carcinoma (HCC). Polycomb group member Ezh2 is a key epigenetic regulator of embryonic stem cell identity; however, its role in HCC is poorly understood.
Methods: To investigate the roles of EZH2 and H3K27me3 in HCC, we conducted a review of patients who received surgical resection for HCC from 2009 to 2013. We analyzed EZH2 expression and H3K27me3 methylation status in 67 human HCC samples, and the global RNA expression by RNA-sequencing (RNA-seq) based on different EZH2 expression. Additionally, we used the EZH2, H3K4me3, and H3K27me3 chromatin immunoprecipitation-sequencing (ChIP-seq) data in ENCODE HepG2 for interpreting our RNA-seq results.

Results: In contrast to our previous results of combined Ezh1 and Ezh2 loss in mouse experimental data, H3K27me3 expression was significantly related with EZH2 expression in 67 human HCC samples (P=0.01). High EZH2 expression was related with short overall survival (P=0.05), but H3K27me3 expression was not related with prognosis. Transcriptome analysis of HCC samples with high EZH2 expression by RNA-seq showed up-regulation of genes related to the cell cycle and DNA replication and down-regulation of estrogen response related genes. ChIP-seq for EZH2, H3K4me3, and H3K27me3 showed that estrogen receptor 1 (ESR1) and early growth response gene-1 (EGR-1) were regulated by EZH2 through methyltransferase function.

Conclusions: This study shows that high EZH2 expression is related with poor prognosis in HCC. The EZH2 gene functions as a tumor oncogene by suppressing ESR1 and EGR-1 through methyltransferase function in human HCC.

Identification of novel GAEC1 (gene amplified in esophageal cancer 1) mutations in colorectal cancer

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Aim: Transforming oncogene GAEC1 (gene amplified in esophageal cancer 1) showed frequent amplifications in cancer tissues compared to non-neoplastic tissues, indicating its pathogenic potential in colorectal cancer (CRC). Even so, mutation profile for GAEC1 remains unknown. This study aimed to identify mutations within the sequence of GAEC1 and analysing its association with GAEC1 copy number variation (CNV) in patients with colorectal cancer (CRC).

Methodology: Fresh-frozen CRC tissues and matched adjacent non-neoplastic tissues near the surgical resection margin were collected from 80 Australian patients. Each tissue was pathologically verified and the clinicopathological details of the patient were noted. Genomic DNA was extracted from these samples and subjected to droplet digital (dd) PCR analysis. Mutation within the sequence of GAEC1 was identified by Sanger sequencing. The analysis was also done to identify the association of GAEC1 mutation and CNV with the clinicopathological data.

Results: Polymorphisms rs803097 and rs2242581 were identified, with homozygosity for C allele in rs2242581 significantly associated with advanced T-stages of CRC (P=0.043). A total of 20 mutations were found in 13 patients with CRC. These mutations were significantly associated with advanced T-stages of CRC (P=0.011). Conversely, higher GAEC1 CNV (CNV ≥2) was significantly associated with earlier pathological stage and advanced T stages (P<0.05), indicating the role of GAEC1 in tumour invasion but not in metastasis. Higher GAEC1 CNV was also significantly associated with tumours at the proximal colon (P=0.046).

Conclusion: To conclude, GAEC1 gene alternations are common in CRC. The results suggest GAEC1 is important in the pathogenesis of non-metastasizing CRC.

Inhibition of miR-186-5p suppress colon cancer growth: an anti-miRNA mediated upregulation of tumour suppressor FAM134B

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Objectives: The role and underlying mechanism of miR-186-5p in colorectal cancer remain unknown. The present study aims to examine the various cellular effects of miR-186-5p in the carcinogenesis of colorectal cancer. Also, the interacting targets and association of clinicopathological factors with miR-186-5p expression in patients with colorectal
cancer were analyzed.

Methods: The miR-186-5p expression levels in colorectal cancer tissues (n=126) and colon cancer cell lines (n=3) were analyzed by real-time PCR. Matched non-neoplastic colorectal tissues and a non-neoplastic colonic epithelial cell line was used as controls. Various in vitro assays such as cell proliferation, wound healing and colony formation assays were performed to examine the miR-186-5p specific cellular effects. Western blots and immunohistochemistry analysis were performed to examine the modulation of FAM134B, PARP9, and KLF7 proteins expression.

Results: Significant high expression of miR-186-5p was noted in cancer tissues (p< 0.001) and cell lines (p<0.05) when compared to control tissues and cells. The majority of the patients with colorectal cancer (88/126) had shown overexpression of miR-186-5p. This miR-186-5p overexpression was predominantly noted within cancer with distant metastasis (p=0.001), lymphovascular permeation (p=0.037), microsatellite instability (MSI) stable (p=0.015), in distal colorectum (p=0.043) and with associated adenomas (p=0.047). Overexpression of miR-186-5p resulted in increased cell proliferation, colony formation, wound healing capacities and induced alteration of cell cycle kinetics in colon cancer cells. On the other hand, inhibition of endogenous miR-186-5p reduced the cancer growth properties. miR-186-5p overexpression reduced FAM134B expression significantly in the cancer cells (p<0.01). Also, FAM134B and miR-186-5p expressions are inversely correlated in colorectal cancer tissues and cells.

Conclusion The miR-186-5p expression promotes colorectal cancer pathogenesis by regulating tumour suppressor FAM134B. Reduced cancer cells growth followed by inhibition of miR-186-5p highlights the potential of miR-186-5p inhibitor as a novel strategy for targeting colorectal cancer initiation and progression.
the activity of neutrophils to create a pro-tumoral microenvironment. This effect on the microenvironment would conceivably aggravate its known neoplastic effect on mammary epithelial cells during mammary involution and increase the risk of parity-associated breast cancer.

53 Identification of microRNA Signatures for Survival Prognosis in Head and Neck Cancer Using the Cancer Genome Atlas

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Head and neck cancer (HNC) accounts for more than 550,000 cases and 380,000 deaths worldwide each year. To develop biomarkers and identify high-and low-risk patients is critical for improving individualized patient care. Identification of novel prognostic biomarkers typically requires a large dataset which provides sufficient statistical power for discovery research. We obtained data from The Cancer Genome Atlas (TCGA) to identify a set of prognostic biomarkers in HNC. The miRNA-seq data were used to identify prognostic biomarkers for HNC. The average expression level of all miRNA, 500, was used for cutoff value. Our study identified that high expression of mir-9-1, mir-9-2, mir-9-3, and mir-150 had significantly better overall survival, and high expression of mir-1-1, mir-1-2, mir-127, mir-133a-1, mir-152, mir-206, mir-379 had significantly poorer overall survival in HNC patients (p < 0.05). This result demonstrates the power of using TCGA as a potential resource to develop prognostic tools. Further studies to validate the finds in vitro and in vivo are warranted.

54 Probabilistic modeling of extended mutational signatures in cancer

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Trinucleotide-based mutational signatures, which describe the sequence properties of specific mutational processes, have emerged as an important tool in cancer genomics. Such signatures are useful to estimate expected mutation frequencies at specific genomic positions, which can help in separating recurrent driver mutations from non-functional passengers. However, recent studies have revealed longer genomic sequence elements that are associated with strong elevations in local mutation rates, and these phenomena cannot be adequately modelled using trinucleotide patterns. Here we propose a probabilistic mutational signature model that incorporates longer sequence patterns, and applied this method to somatic mutation data from diverse human tumors. In addition to known trinucleotide signatures, this revealed an extended signature present specifically in melanoma, which corresponds to an element recently described by our group to mediate a strong local elevation in susceptibility to UV mutagenesis. Segmentation of the mutation data according to genomic region also allowed us to describe associations between signatures and specific epigenomic contexts. In summary, our results show that trinucleotide-based models are severely limited in describing mutational heterogeneity in tumors, and suggest that a model that takes genomic context as well as longer sequence patterns into account can help to improve detection of driver mutations in cancer.

55 Alternation of CHSY1 is associated with poor disease free survival and promotes drug resistance in hepatocellular carcinoma

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The high mortality of hepatocellular carcinoma (HCC) mainly attributes to late diagnosis and limited treatment for advanced HCC. Currently, sorafenib is the first and the only approved treatment for advanced HCC, but the outcome of the treatment is still disappointing. It is an urgent need to development new therapeutic strategies for HCC. Chondroitin sulfate (CS) is one type of glycosaminoglycans (GAGs). Accumulating evidence indicated that CS chains participate in cancer progression by interacted with proteases, growth factors, and adhesion molecules. In addition, CS chains are reported increased in human HCC, and the altered sulfation status were associated with poorly histological grade. One recent study reported that a distinct modification of CS chains is highly expressed on HCC, which can be used as a marker for cancer diagnosis or target therapy.
Although the expression of CS chains in HCC has been studied, the biological functions of CS chains in HCC remain unknown. Here, we reported that chondroitin sulfate synthase 1 (CHSY1) is the main chondroitin sulfate synthase in HCC cells. Analyzing TCGA data base by cBioPortal, results indicated that 6% of HCC patient in this data base is CHSY1 amplification and/or mRNA up-regulation. In addition, genomic alteration of CHSY1 is significantly associated with worse disease free survival. In vitro experiments revealed that expression levels of CHSY1 modulated sorafenib's effectiveness in HCC cells. Although the underlying mechanisms need further investigation, our results indicated that genomic alteration of CHSY1 could be a risk factor of human HCC, and the expression level of CHSY1 could predict resistance of sorafenib treatment.

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Somatic mutations in the TP53 tumor suppressor gene in Sri Lankan patients with Head and Neck cancer

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Most commonly used chemotherapeutic drugs in treating cancer patients are widely based on DNA damaging combination therapy (DDCT) and microtubule stabilizing therapy (MST). DDCT responds to patients with wild type TP53 whereas MST responds to TP53 mutants. Thus, knowledge on TP53 mutation status is important in deciding treatment options for a patient. Somatic TP53 gene mutation analysis is better detection method over the Immunohistochemical method. However it is not affordable to screen the entire TP53 gene, but identification of hotspots regions in Sri Lankan context, will be feasible to screen only those identified regions prior to administration of therapy. There is only one previous study investigating somatic TP53 gene mutations in Sri Lankan oral cancer patients and this was limited to exons 5 to 8. Thus one of the specific objectives of the current research is to establish the TP53 mutation spectrum in Sri Lankan Head and Neck cancer patients as it is the most common cancer in developing countries as well as most prevalent cancer in Sri Lankan male. DNA was extracted from fresh tissues of patient samples (N=25). PCR amplified exonic, splicing site regions of TP53 were sequenced using 3500Dx Genetic Analyzer. Sequence variants were analyzed by BioEdit and Mutation Surveyor®. Results showed nine pathogenic variants: one novel deletion in exon 4 (c.298het_delC), five reported missense variants (c.844C>T, c.493C>T, c.422G>T, c.467G>A, c.524G>A) and one reported deletion (c.383delC) in exon 5, two reported missense variants in Exon 6 (c.583A>T, c.578A>G). Likely pathogenic missense variant in Exon 6 (c.576G>C) and several benign variants (intron 2: two mutations, intron 3: two mutations, intron 6: one mutation, intron 7: two mutations) were detected. Further studies are in progress to study a larger number of patients to establish somatic TP53 mutation spectrum in Sri Lankan cancer patients.

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Evolution of Barrett’s Esophagus through space and time at single-crypt and whole-biopsy levels

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Barrett’s Oesophagus (BO) is a metaplastic lesion in which individual crypts replace the squamous lining of the oesophagus. BO is a precursor of oesophageal adenocarcinoma (OAC) but patients rarely progress to OAC, creating a need for improved biomarkers comprehending the genetic evolution towards malignancy. We performed copy-number alteration (CNA) analysis of individual crypts to characterise clonal evolution over space and time at unprecedented resolution. In 4 non-progressors, 3 biopsies from each of two time-points were analysed. In 4 progressors, 3 biopsies from an initial endoscopy and 8 biopsies from later surgical resections were analysed. 8 individual crypts were microdissected from each biopsy. Each crypt and remaining epithelium from the biopsy were profiled using SNP-arrays (612 samples total), which allowed us to characterize genetic
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Engineered cancer-stomal interfaces to probe cancer associated fibroblast and tumor cell interaction mechanisms

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It is well recognized that cancer progression occurs in the context of a cellular community of tumour and other cell types within a dynamic tumour microenvironment (TME). In the TME, cancer-associated fibroblasts (CAFs) have been shown to enhance disease progression. Interactions between CAFs and tumour cells are bi-directional, dynamic, and dependent on the microenvironment and the molecular mechanisms driving these interactions are not well understood. Current 3D co-culture models provide only limited control over the organization and microenvironment of the two cell types. We have developed a novel tumour model platform called the Tissue Roll for the Analysis of Cellular Environment and Response (TRACER). In TRACER, cells are seeded within a hydrogel into a thin cellulose scaffold that is subsequently rolled onto a cylindrical core to generate a stacked configuration in which each layer of the scaffold rol experiences differential oxygen and nutrient levels mimicking the variation observed in 3D tissues. The design of the TRACER system enables the creation of highly tunable 3D co-cultures with different cell populations in specific layers to create a tumour section with distinct tissue regions. Furthermore, TRACER cultures can be disassembled back to a thin strip format for analysis by simply unrolling. Cell behaviours and gene expression from specific regions within the stack can then be analyzed and mapped back to specific 3D spatial location within the culture. Here we generate co-culture TRACERs from head and neck squamous cell carcinoma (HNSCC) patient-derived CAF and HNSCC tumour cell populations and use various construct configurations to probe how tumour-CAF interactions change tumour cell phenotype. We find that CAFs have limited impact on tumor cell proliferation and response to radiation therapy but significantly enhance invasiveness. Currently we are exploiting the ability of our systems to separate and analyze invasive tumor cells from non-invasive tumor cells within the culture to probe the molecular mechanisms driving tumor cell invasion at the CAF-tumor interface.

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Coexistence of multiple tumor-immune microenvironments in an ovarian cancer patient

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The majority of women with ovarian cancer (OC) have advanced disease at the time of diagnosis
which is associated with 5-year survival rates < 20%. Women with stage 1 disease at diagnosis have significantly better 5-year survival rates > 90%. Currently symptomatic women are investigated with serum CA125 and transvaginal ultrasound scan (TVUS). Recently large screening studies using CA125 and TVUS have failed to show an improvement in mortality. There is therefore a pressing need for new diagnostic biomarkers in OC.

Somatic TP53 mutations are ubiquitous in high-grade serous OC (HGSOC), the most common subtype, and tumour specific assays for TP53 have been utilized to detect circulating tumour DNA (ctDNA) in plasma samples in > 80% of women with relapsed HGSOC. Levels of pre-treatment ctDNA are strongly correlated with disease volume and dynamic changes in ctDNA can be used to predict clinical response in a relapse setting.

The objective of this study is to assess the proportion of women with detectable ctDNA in pre-treatment plasma samples in a neo-adjuvant setting.

Without the need for tumour specific assays targeted sequencing for TP53 will be performed on plasma samples collected pre neo-adjuvant chemotherapy from 130 women with HGSOC. Somatic mutation calling will be performed blinded to tumour specific mutations already identified from FFPE samples. Plasma TP53 mutant allele fraction (MAF) will be correlated with pre-treatment disease volume calculated by 3-D CT reconstruction. Serial plasma samples were collected before every subsequent cycle of neo-adjuvant chemotherapy and at the time of relapse if relevant. We will assess whetherctDNA can be used to predict clinical response in a neo-adjuvant setting by comparing the change in TP53 MAF between baseline and pre cycle 3 neo-adjuvant chemotherapy with clinical response measured by RECIST, CA125 and volume of disease pre interval debulking surgery.


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Genome-wide allelic imbalance analysis followed by functional assays identifies new candidate breast cancer risk genes

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GWAS have identified ~170 breast cancer risk loci with the risk-associated SNPs mostly being within non-coding regions and especially enriched in regulatory elements suggesting they act by modulating expression of nearby genes. Analysis of allelic imbalance in gene expression is a robust method to identify such cis-regulatory effects. The OncoArray Consortium has recently developed a custom-designed array and genotyped in over 200,000 breast cancer cases and controls resulted in successful fine-mapping of 150 breast cancer GWAS loci. We analysed the candidate causal SNPs from the high quality pre-publication fine-mapping data (~7500 SNPs) for allelic imbalance of genes within 1Mb up- and downstream using RNA-seq data and imputed genotypes from breast datasets including 1095 TCGA tumor, 111 TCGA normal, and 92 GTEx normal samples. For each target gene, allelic imbalances for heterozygous coding SNPs were computed and averaged across that gene. For each risk-associated SNP, genes with significantly higher allelic imbalance in heterozygous compared to homozygous samples were identified (~300 genes). Of them, 50 genes passed multiple testing correction including BARX2, LGR6, NTN4, and RAD23B which were selected for functional validation. The cis-regulatory effects of the risk-associated SNPs on these 4 genes were confirmed by chromosome conformation capture (3C) which showed a significant interaction between each gene’s promoter and the corresponding risk-associated SNP. As further validation, allele-specific 3C showed an allelic difference in chromatin looping between NTN4 and the risk-associated SNP. DNA repair and cell death/survival were the top networks identified by pathway analysis of the significant genes suggesting possible mechanisms for cancer development. Our study provides the first genome-wide allelic imbalance profiling of genes neighbouring breast cancer risk-associated SNPs identified by the OncoArray project. These findings highlight the importance of non-coding regions in cancer research and improve understanding of breast cancer pathogenesis by introducing and validating new candidate risk genes.
Telomere length alterations in microsatellite stable colon cancer are associated with the immune response

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Background: Telomeres are repetitive sequences (TTAGGG) located at the end of chromosomes. Telomeres progressively shorten with each cell replication cycle, ultimately leading to chromosomal instability and loss of cell viability. Telomere length anomaly appears to be one of the earliest and most prevalent genetic alterations in malignant transformation. Here we aim at estimating telomere length from whole-exome sequencing data in colorectal tumors and normal colonic mucosa, and at analyzing the potential association of telomere length with clinical factors and gene expression in colorectal cancer.

Methods: Whole-exome sequencing was performed in genomic DNA from a set of 42 adjacent normal-tumor paired samples. From the raw sequences of each exome sequencing sample (fastq files), reads containing at least five repetitions of the telomere sequence (TTAGGG) were extracted. The number of reads obtained in the tumor sample was normalized to build the Tumor Telomere Length Ratio (TTLR), considered as an estimation of telomere length. We evaluated the associations between TTLR, and clinical factors, gene expression and copy number (CN) alterations. Finally, we carried out a correlation study between TTLR and the level of gene expression using the ranked list of p-values for functional enrichment analysis.

Results: Colon tumors showed significantly shorter telomeres than their paired normal samples. Partial correlation analysis between gene expression and TTLR, adjusting for CN, depicted several genes, none of them closely related with telomeres. A functional gene set enrichment analysis showed that the most significantly related pathways were associated with the immune response.

Conclusions: Our study demonstrates that a relative measure of telomere length may be extracted from whole-exome sequencing data. As expected, telomere shortening occurs in colorectal tumors, and we observe that this alteration is associated with expression changes in genes related to immune response and inflammation.
PROMO: A new tool for analyzing large high-throughput genomic cancer datasets

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Modern genomic datasets may include hundreds of samples, each measured by several high-throughput technologies and described by extensive clinical information. Analysis of such large and versatile datasets poses significant challenges not easily met by existing bioinformatic tools.

PROMO (Profiler of Multi-Omics data) is an interactive Matlab-based tool, designed to meet these challenges. It enables importing multi-label datasets from various file formats, visualizing and exploring the data, applying unsupervised analysis on both samples and features and utilizing various popular statistical tests including survival analysis. Special features that are specific to multi-omic datasets include dataset integration and joint multi-omic clustering.

We describe PROMO’s main capabilities and show how it was used in our recent study [1] to identify clinically significant novel breast cancer subtypes by analyzing both expression and methylation data from The Cancer Genome Atlas (TCGA) project.


Yes-associated protein expression is correlated to the differentiation of prostate adenocarcinoma

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Background: Yes-associated protein (YAP) in the Hippo signaling pathway is a growth control pathway that regulates cell proliferation and stem cell functions. Abnormal regulation of YAP was reported in human cancers including liver, lung, breast, skin, colon and ovarian cancer. However, the function of YAP is not known in prostate adenocarcinoma. The purpose of this study is to investigate the role of YAP in tumorigenesis, differentiation of the carcinoma, and prognosis of prostate adenocarcinoma.

Methods: The nuclear and cytoplasmic expression of YAP was examined in 188 cases of prostate adenocarcinoma using immunohistochemistry. YAP expression levels were evaluated in the nucleus and cytoplasm of the prostate adenocarcinoma cells and the adjacent normal prostate tissue. The presence of immunopositive tumor cells was evaluated and interpreted in comparison with the patients’ clinicopathologic data. Results: YAP expression levels were not significantly different between normal epithelial cells and prostate adenocarcinoma. However, YAP expression level was significantly higher in carcinomas with a high Gleason grade (8-10) than in carcinomas with a low Gleason grade (6-7)(p<.01). There was no statistical correlation between YAP expression with stage, age, PSA level or tumor volume.

Biochemical recurrence (BCR) free survival was significantly lower in patients with high YAP expressing cancers (p=.02). However high YAP expression was not an independent prognostic factors for BCR in the Cox proportional hazards model. Conclusions: The results suggested that YAP was not associated with prostate adenocarcinoma development, but it may be associated with the differentiation stages of the adenocarcinoma. YAP was not associated with BCR.

GTPase RRAD as a therapeutic target in glioblastoma

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Glioblastoma is characterized by frequent overexpression and activation of EGFR. We explored the molecular mechanisms of EGFR activation in glioblastoma. We have found that GTP-binding protein RRAD (RAS-related associated with diabetes) physically associates with EGFR, enhancing the stability and nuclear translocation of EGFR. Functionally, RRAD contributes to the EGFR-mediated activation of STAT3 and expression of the stem cell factors, thereby enhancing tumor stemness, EMT, and in vivo tumorigenesis. To identify small molecules which have potential activity against RRAD, we have screened inhibitor libraries. The identified potential inhibitory molecule effectively suppressed EGFR translocation to nucleus and target gene expression. Inhibitory molecule treatment resulted
in the decrease of proliferation and tumor sphere formation. These results provide preclinical evidence to continue the development of the inhibitory molecule as a potent therapeutic agent against RRAD positive glioblastoma.

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**survivALL: an R package for assessing the robustness of quantitative biomarkers across or within datasets**

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In routine practice, biomarker performance is calculated by splitting a patient cohort at some arbitrary level, often by median gene expression. The logic behind this is to divide patients into “high” or “low” expression groups that in turn correlate with either good or poor prognosis. However, this median-split approach assumes that the dataset composition adheres to a strict 1:1 proportion of high vs. low expression, that for every one “low” there is an equivalent “high”. In reality, datasets derived from primary patient tissues are molecularly heterogeneous in their composition - i.e. this 1:1 relationship is unlikely to exist and the true relationship unknown. Given this limitation, it remains difficult to determine where the most significant separation should be made. For example, estrogen receptor (ER) status determined by IHC is standard practice in predicting hormone therapy response, where ER is found in an ~1:3 ratio (-:+) in the population. We would expect therefore, upon dividing patients by ER expression, 25% to be classified “low” and 75% “high”, and an otherwise 50-50 split to misclassify 25% of our patient cohort, rendering our survival estimate underpowered. We therefore propose a data-driven approach to calculate the relative survival estimates for all possible points of separation - i.e. at all possible ratios of “high” vs. “low” - allowing a biomarker’s prognostic capacity to be more reliably determined and quantified. We see this as a solution to a flaw in common research practice, namely the failure of a true biomarker as part of a meta-analysis. Using a combination of 5 publicly available 100% ER+ datasets (GSE2034, GSE2990, GSE6532, GSE7390, GSE12093), and the more heterogeneous METABRIC dataset, we demonstrate that our method allows patients to be stratified based on a robustly determined level of expression that is consistent in meta-analysis and validation cohorts.

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**Somatic mutation landscape in cancer analyzed with sparse dictionary learning methods**

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We use the TCGA dataset to provide a description of the somatic mutation landscape of cancer. We approach the problem by using the somatic mutations data. For each sample we build a new representation based on the base substitutions and incorporating information on the bases immediately 5’ and 3’ to each mutated base. Overall, considering the 6 possible classes of mutations and their neighborhood, an “alphabet” of 96 distinct mutations can be identified. The new representation is a frequency distribution, defined by counting how many times each of the 96 mutations has occurred.

From this starting point, our project aims at finding mutational signatures characterizing different cancer types by means of sparse coding techniques and regularization methods, similar to the approach described by Alexandrov et al, Nature 2013. The peculiarity of our pipeline is the use of penalty terms that enforce sparsity and/or incorporate prior knowledge on the problem (Salzo et al., Neural computation 2014). The method aims at using the data matrix as input signal and finding a linear combination of patterns that best approximates the data, assuming that each input used just some patterns, hence enforcing sparsity on the coefficient matrix. This procedure provides as output a set of patterns (i.e., the dictionary) and a matrix of coefficients: the patterns correspond to the mutational signatures, whereas the coefficients indicate which pattern characterizes a specific set of samples, possibly from the same cancer type. Following this analysis, we look for associations between the signature and its biological cause (e.g., smoking, UV exposure, age) and to validate our findings with current state-of-the-art.
Cytosine methylation analysis reveals distinct patterns of genome-wide CpG mutation variation across cancer types

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Methylated cytosines (5mCs) are frequently mutated in the genome, typically attributed to the propensity for 5mC to undergo spontaneous deamination. However, no studies have yet comprehensively analysed the mutation-methylation associations across cancer types using whole-genome sequencing datasets. Here we analyse 918 cancer genomes, together with tissue type-specific methylation and replication-timing data. Using multivariable regression models, we identify distinct mutation-methylation associations across 15 cancer types and subtypes, and we associate these with specific mutational and repair processes. We focus our analyses on colorectal cancer subtypes and describe strong mutation-methylation associations in samples with Polymerase epsilon (POLE) exonuclease domain mutations or microsatellite instability (MSI). By analysing mutation strand asymmetry around origins of replication, we propose a novel mutator phenotype in MSI-mutant cancers specifically at methylated cytosines within a TCG context. Highlighting the importance of this finding for oncogenesis, we show that certain coding mutation hotspots in POLE-mutant cancers occur at highly methylated CpG dinucleotides in cancer driver genes. In our analysis of MSI colorectal cancers, we implicate mismatch repair (MMR) in the correction of 5mC deamination events. MMR has been shown to underlie mutation rate variation across the genome. We demonstrate that the involvement of MMR in the correction of methylation-induced mismatches contributes to the extent of regional mutation rate variation loss in MSI cancer genomes. Investigating other cancer types, we describe both nucleotide excision repair- and AID/APOBEC-induced processes to be responsible for reducing mutation frequencies at highly methylated cytosines in skin and breast cancer genomes, respectively. In summary, we find differential associations between mutation rate and methylation across cancer types, and we show these to be vital for accurately predicting expected mutation loads. Our findings reveal novel links between methylation and common mutation and repair processes, with these processes significantly contributing to the mutational landscape of cancer genomes.

Target capture-sequencing of regulatory regions in 96 colorectal cancers and an assessment of sequencing methodology

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Whole-genome sequencing (WGS) of cancer samples has led to the identification of a small number of somatic regulatory mutations present at high rates of recurrence in cancer genomes. However, to identify infrequent driver mutations requires the sequencing of large cancer cohorts. Here we describe the target capture-sequencing of regulatory regions and certain coding exons across 96 colorectal cancer (CRC) samples and matched-normals, randomly-selected from an existing biobank. Encompassing ~35 million nucleotides per genome, we report sequencing depth in tumour and matched-normal samples averaging 170x and 80x respectively. We find a total of 172,354 somatic mutations, but find no recurrently mutated nucleotides in regulatory regions (>3 samples, with >2/63 mutated samples in a CRC TCGA cohort), with the exception of nucleotides in polyadenine tracts in MSI samples. Within our cohort, we find two samples with somatic mutations in the polymerase epsilon (POLE) exonuclease domain and, via mutational signature analysis, two samples with suspected impaired POLE-function in the absence of POLE somatic or germline coding variants. Interestingly, by analysing germline polymorphisms, we also find one patient with undiagnosed Lynch Syndrome and three patients with potentially CRC-predisposing variants in APC. Additionally, we find one patient to harbour a germline heterozygous non-synonymous mutation in MUTYH, but no evidence of bi-allelic MUTYH impairment despite a strong correlation with signature 18 – a signature which indicates loss of base-excision repair. Finally, we also perform WGS (tumour: 60x, normal: 13x) on one target capture-sequenced sample. We find that 52% of mutations are identified by target capture-sequencing only, with these mutations present at lower variant allele frequencies compared to mutations found in both sequencing experiments (0.17 versus 0.27). To our knowledge, our study is the first to report high-depth target capture-sequencing of regulatory regions in a CRC cohort, and we highlight the benefits and challenges of this experimental approach.
The relationship between cancer related inflammation and genomic instability in patients undergoing surgery for colorectal cancer

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Introduction: Cancer related inflammation (CRI) is considered the 7th hallmark of cancer and is associated with poorer survival in patients undergoing surgery for colorectal (CRC). CRC is attributed to two genomic instability pathways; microsatellite instability (MSI) and microsatellite stability (MSS). This study examined the relationships between CRI, genomic instability, clinicopathological factors and survival.

Methods: 654 patients who underwent resection for CRC between 2000-2008 were studied. Clinicopathological factors were recorded and blood markers of systemic inflammation were available pre-operatively. DNA was extracted from formalin fixed paraffin embedded tumour tissue and the presence of MSI was assessed at 5 loci NR21, BAT26, BAT25, NR24 and MONO27 by PCR. MSI phenotype was defined as MSI at 2 or more of these loci.

Results: MSI was associated with female gender (P=0.031), proximal location (P<0.001), poor differentiation (P<0.001), mucin production (P=0.005), low LNR (P=0.035), raised CRP (P=0.018), high mGPS (P=0.011) and anaemia (P=0.002).

Mean follow up was 137 months with 118 cancer deaths. On univariate analysis, higher T-stage (P<0.001), higher LNR (P<0.001), poor differentiation (P=0.001), mucin production (P=0.002), raised CRP (P=0.002), high NLR (P=0.021), high mGPS (P<0.001), poor Klintrup-Makinen score (P<0.001) and MSS (P=0.016) were associated with poorer cancer-specific survival and this association with outcome was potentiated in the MSS group and non-significant in the MSI group.

Conclusion: The MSI phenotype is associated with cancer inflammation and the prognostic power of the mGPS, NLR, Klintrup-Makinen score and pathological factors relates to MSS phenotype. This study suggests that MSI analysis may aid adjuvant treatment planning.

Making sense of spatial and temporal intratumor heterogeneity in a patient with chronic lymphocytic leukemia

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Several studies have revealed extensive genomic differences among malignant cells conforming a tumor. Such diversity has important clinical implications because renders single tumor samples not representative of the spectrum of somatic cancer mutations present in a patient. Here, we are analyzing WES and WGS data from a patient with chronic lymphocytic leukemia, the most common leukemia in adults, in order to describe the clone structure and the evolutionary relationships among such clones present in two different tumor samples - peripheral blood and bone marrow - before and after treatment. We will use a saliva as healthy tissue and T-lymphocytes and hematopoietic stem cells to dive into the origin of the disease.
**Case study of circulating tumour DNA-based monitoring of bladder cancer using PI3KCA E542K and TP53 Y163C mutations**

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Bladder Cancer (BC) has the highest recurrence rate amongst cancer types and the risk of progression to invasive disease is a major concern. Regular monitoring by cystoscopy is a burden to patients and health services. Circulating tumour DNA (ctDNA) is a biomarker with potential clinical applications. Liquid biopsy is less invasive and ctDNA may enable earlier detection of recurrences, if reliable assays can be established. The aim of this study was to monitor ctDNA in plasma and urine from patients with BC using digital droplet PCR (ddPCR). The objectives were; 1) to identify assayable tumour mutations using SNaPshot and Sanger sequencing in tumour biopsy, 2) to monitor the disease status of two BC cases, using pre-validated, commercially available ddPCR assays.

A PI3KCA E542K and TP53 Y163C mutation were identified in tumours by SNaPshot and Sanger sequencing, respectively.

Case 1. The presence of the PIK3CA E542K was confirmed in the tumour by ddPCR. The mutation was detected in plasma collected prior to cystectomy. In samples collected at 45 months post-cystectomy, the mutation was not detected in plasma, however positive signals were detected in urine, indicating that these results require further validation.

Case 2. The presence of the TP53 Y163C was confirmed in the tumour by ddPCR. The mutation was not detected in plasma collected at the initial time point. At 36 months after the initial sampling, the mutation was detected in plasma. A similar result was observed in urine.

Taken together, our results showed that, using pre-validated, commercially available ddPCR assays, tumour-specific mutations could be detected and monitored in both plasma and urine from BC patients. In order for ctDNA markers to be employed clinically, it is timely to undertake a further validation study using samples collected from more BC patients and at multiple time points during follow up.

**Looking for optimal coverage in RNA sequencing**

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Next-Generation Sequencing (NGS) technologies have revolutionised the way we study disease. However, experimental design is often overlooked resulting in suboptimal statistical power and higher financial costs. In an NGS experiment the coverage, the average number of times that a base of a genome or transcriptome is sequenced, and the number of samples are fundamental factors affecting the quality of an experiment. The choice of coverage is especially critical in cancer genomics where normal tissue is mixed with tumour and some cancer mutations appear with a very low frequency. Power calculations to identify the optimal experimental design are not trivial.

The trigger behind our study was to plan the sequencing design of a set of RNA samples in the Australasian Leukaemia and Lymphoma Group’s cohort of patients with core binding factor acute myeloid leukaemia (CBF-AML).

We reassessed published work that used transcriptome profiling for mutation detection in leukaemia and implemented an algorithm to inform our experimental design. We used forty-six publicly available RNA-seq samples from CBF-AMLs, produced by the Leucegene group. This data was sequenced at a very high coverage and their analyses have been recently published (Lavallée et al. 2016). Using their results as a benchmark, we studied how much information was lost by randomly and sequentially reducing the coverage of the original data. This helped us to design a cost-efficient and powerful study; this approach may help inform the experimental design for other genomic studies.
Gene expression profiling to predict responsiveness and resistance to anti-PD-1 immunotherapy in metastatic melanoma

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Background: In advanced melanoma, immune checkpoint inhibitors such as pembrolizumab and nivolumab (anti-PD-1 inhibitors) are the standard therapeutic strategy. Significant clinical responses occur in 30-50% of patients, which are durable in the majority. However, it is currently unclear what biological and molecular properties determine clinical response. The present study aimed to profile gene expression between responders (CR/PR) and non-responders (SD/PD) to anti-PD-1 in patients with metastatic melanoma.

Methodology: FFPE (Formalin-Fixed Paraffin-Embedded) melanoma samples obtained from patients treated with anti-PD-1 immunotherapy were subjected to RNA sequencing. These samples included 20 responders and 15 non-responders at pre-treatment, and 9 paired pre-treatment and early during treatment (EDT). Multiplex immunofluorescent staining, Vectra, was also performed on each FFPE tissue. Each sample was stained for a panel of immune-associated gene markers including CD8, PD-1, FOXP3, EOMES, SOX10, and Granzyme B. Gene set enrichment was performed using a non-parametric unsupervised method to identify dysfunctional pathways related to treatment response.

Results: Two unique gene clusters associated with CD8+ T cells (CD27, CD8, and FasL) and PD-1 signalling (Lck, PD-1, PD-L1, PI3K, PKC, TBX21, and ZAP70) were identified in different responders to immunotherapy. Gene clusters associated with CD8+ T cells and PD-1 signalling were down-regulated in non-responders in PD-1 immunotherapy, indicating a strong correlation of increased fractions of PD-1 and activated T cells with response to therapy. Additionally, multi-spectral immunofluorescent imaging further quantified the expression profiles of targetable genes, highlighting activated T-cell gene signatures and the tumour subpopulations were highly significantly higher in the EDT samples of good responders (p<0.05).

Conclusion: The collective data from immune gene expression profiling suggest that the relative abundance of CD8+ T cells predicts response to anti-PD-1 immunotherapy. These gene signatures are useful biomarkers for selecting patients with clinical response to PD-1 signalling pathway.

Export of oncogenes as cargo of extracellular vesicles (exosomes) – implications for liquid biopsy and targeting inter-cellular communication in cancer

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Oncogenic driver events affecting cancer cell genome and epigenome profoundly impact both cell-autonomous and non-cell-autonomous aspects of cancer progression, including pathways of intercellular communication. Those are epitomized by intercellular trafficking of extracellular vesicles (EVs), and their subsets (exosomes). These membrane structures export/deport multiple molecular entities (proteins, RNA, DNA) from their parental (donor) cells, and mediate their selective transmission (local and systemic) to multiple cellular targets (recipients). We describe evidence that RAS, EGFR and other oncogenes may impact several aspects of EV-mediated cell-cell communication in cancer, including: (i) rate of EV release and their protein content; (ii) molecular composition of tumour-derived EVs; (iii) inclusion of oncogenic and mutant macromolecules, and their effectors in the tumour EV (oncosome) cargo; (iv) pathways leading to EV-mediated release of genomic DNA; (v) deregulation of core mechanisms responsible for EV biogenesis (vesiculome), impacting the properties/profiles of EVs being released; (vi) mechanisms of deregulated EV uptake by cancer cells. EV-mediated intercellular transfer of mutant and oncogenic molecules between cancer cells and their indolent counterparts may exert profound (and targetable) biological effects, but several biological barriers curtail a permanent horizontal transformation of normal cells through this mechanism. Importantly, cancer-related EVs can be collected from biofluids and serve as a liquid biopsy platform for detection of driver mutations and molecular tumour evolution and in real time.
Short Tandem Repeat stutter model inferred from direct measurement of in vitro stutter noise

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Short tandem repeats (STRs) are polymorphic genomic loci valuable for various applications such as research, diagnostics and forensics. However, their polymorphic nature acts as a double-edged sword, as during in vitro amplification STRs undergo mutational processes that cause stutter noise, especially in the shorter, more mutable, repeat types. Although it is possible to overcome stutter noise by using amplification-free library preparation, such protocols are presently incompatible with single cell analysis and with known targeted-enrichment protocols. To address this challenge, we have designed a method for direct measurement of in vitro noise. Using a synthetic STR sequencing library, we have calibrated a proposed Markov model for the prediction of stutter patterns at any amplification cycle. By employing this model, we have managed to genotype accurately even cases of severe amplification noise, where as little as 3% of the reads accurately reflect the original STR size.

Novel Neuroblastoma Biomarkers of Clinical Relevance

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Neuroblastoma arises from the developing sympathetic nervous system, and is the most common solid tumor in infants. The disease has a diverse clinical heterogeneity ranging from tendency of spontaneous regression to frequent formation of metastases. Earlier studies have identified several recurrent genetic alterations, including mutations in genes such as ALK and ATRX, as well as chromosomal rearrangements such as MYCN amplifications, 11q deletion and 17q gain, which are common in the most malignant cases.

Despite recent progress in the treatment of high-risk neuroblastoma, patients in this subgroup still have a poor prognosis, underscoring the need to identify new targetable alterations. Here whole genome sequencing of 28 tumor/normal pairs of 26 neuroblastoma patients was performed. For each sample, the mutational landscape comprising single nucleotide and structural variants, copy number changes, mutational signatures, and telomerase content was investigated. The overall aim of this study is to stratify these patients based on the underlying genomic alterations, and to identify new drug targets suitable for the treatment of high-risk patients.

Characterising the effect of neoadjuvant treatment on tumour clonal composition and immune microenvironment in patients with early breast cancer

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Introduction: Neoadjuvant (pre-surgical) chemotherapy has become standard practice in patients with high-risk early breast cancer as it improves rates of breast conservation surgery and enables prediction of local recurrence and survival by using tumour response to treatment as a surrogate. Our aim was to identify the change in tumour clonal dynamics as well as tumour microenvironment in treatment naïve early breast cancer while subjected to the selection pressures of chemotherapy and targeted therapies.

Materials and methods: Sequential tumour biopsies were obtained from 74 patients with Stage I-III breast cancer undergoing neo-adjuvant chemotherapy. Genomic and transcriptomic characterisation of the tumour samples was performed using whole exome, shallow whole genome and RNA sequencing, allowing the detection of somatic variants, copy number alterations as well as transcriptomic aberrations.
Using Bayesian clustering methods, the change in clonal composition of tumours during the duration of chemotherapy was characterised. Tumour microenvironment components were deconvoluted using RNA expression data.

Results and discussion: Somatic TP53 mutations heavily dominated the mutational landscape, followed closely by mutations within PIK3CA and GATA3. Three different patterns of genomic response were evident during treatment, with distinct shifts in tumour clonal dynamics correlating with response to chemotherapy. Resistance to treatment was associated with maintained copy number alterations and minimal changes in the prevalence of mutations in breast cancer driver genes. The chemotherapy agent switch done midway during treatment to improve response helped eliminate resistant clones maintained in the first half of the treatment regimen. Additionally, the change in immune infiltration within the tumour played a great role in determining response to treatment, with increased immune infiltration strongly correlating with better response.

Conclusion: We have shown that cytotoxic and biological therapies can cause a significant shift in both tumour clonal landscape as well as the composition of the surrounding microenvironment, which can be predictive of response to treatment.

80 An analysis of Herceptin® resistance public gene expression datasets
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The possible uses of publicly available gene expression raw data are various and include preliminary discovery of potential biomarkers for disease screening and diagnosis, prognosis, monitoring and prediction of response to targeted therapies. The Gene Expression Omnibus (GEO) Curated Dataset Browser (www.ncbi.nlm.nih.gov/geo) was used to mine microarray-based data resulting from pre-clinical and clinical studies involving trastuzumab treatment and response assessment. Following a highly inclusive search using only the search terms “trastuzumab” or “Herceptin” (yielding the same results), all studies involving measures of trastuzumab resistance and having available data tables were manually selected. The classification of samples as trastuzumab-sensitive (controls) or trastuzumab-resistant (cases), followed by subsequent statistical analyses, were carried out by GEO2R. A list of genes differentially expressed between the cases and controls (adj. p-value <0.05) was generated for each analysis. This was input into Ingenuity Pathway Analysis (IPA) software to find top canonical pathways enriched by each gene signature and their upstream regulators/downstream effectors. Genes of interest were also searched for in The Cancer Genome Atlas (TCGA) database for correlation with patient survival. Selection of relevant, patient sample-derived studies narrowed down the number of datasets to ten, with only four giving differentially expressed genes between the two patient groups. Interestingly, none of the genes overlapped between the different clinical studies. One of the reasons may be differences in study design and the use of different microarray platforms; the ability of high-throughput platforms to generate predictive models in a reproducible way is an important consideration. IPA analysis showed the enrichment of several pathways already implicated in tumourigenesis, trastuzumab resistance and worse prognosis in HER2-positive patients, as well as multiple immune-related pathways. This study also highlighted the need for more data sharing with consistent study designs and definitions of clinical response criteria, for combination into large, multicentre meta-analyses.

81 Circulating cell-free DNA copy-number profiles as a biomarker of relapse in melanoma
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Rationale: Melanoma is the most aggressive form of skin cancer. By detecting relapses sooner, we could initiate treatments earlier, potentially improving outcomes. Fragments of tumour-DNA (circular cell-free DNA, ccfDNA) can be detected in blood, and genetic-profiles of ccfDNA can potentially be utilised as easily accessible biomarkers.

Methods: CcfDNA was extracted from plasma collected from 83 melanoma patients. Low-coverage genome-wide copy-number profiles were generated using next-generation sequencing (Illumina Hi-Seq®). Matched genomic/FFPE-DNA was similarly sequenced. Read-profiles for each ccfDNA sample were normalised against
the corresponding genomic-DNA and corrected for GC-content, to generate copy-number ratios. Log-transformed Z-scores for 1Mb-windows were standardised to mean copy-number ratios from a cohort of 10 healthy controls. A “copy-number aberration score” (CNAS) was then calculated by summing the square of standardized Z-scores across the genome.

Results: Of the 83 patients, 44 had active melanoma, and 39 had recently-excised disease. CcfDNA samples were sequenced using DNA inputs ranging from 2.55ng–108ng. On average, 89% of reads aligned to the reference-genome GRCh38, with a mean genome-coverage of 0.4X. The CNAS for cases with active disease were higher than those with recently-excised disease (p=0.0012). The CNAS was a good predictor of the presence of active disease (area under ROC curve = 0.8998). Mortality was higher among those with scores above the 75th percentile, compared to those with lower scores (HR (95%CI)1.08, 1.001-1.166, p=0.047), after adjusting for stage of disease, disease status (active/resected), BRAF status, mitotic-rate and ccfDNA levels (ng/ml).

Conclusion: We have successfully created copy-number profiles using low quantity ccfDNA inputs, and have established a model based on CNAS, which is a good discriminator of active disease. We are testing this model on a longitudinal sample-set to examine its validity in reliably detecting relapse in this cohort.

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Analysis of tumour derived mutant DNA in plasma and urine of bladder cancer patients reveals an association with clinical outcomes and on-therapy clonal evolution

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Muscle Invasive Bladder Cancer (MIBC) is an aggressive disease with a significant risk of metastatic progression. Whilst patients can achieve a 6% improvement in overall survival with Neo-Adjuvant Chemotherapy (NAC), many don’t respond. Presently there is no accurate and prompt predictor of treatment response.

Body fluid mutant DNA (mutDNA) may allow non-invasive identification of treatment failure. To test this hypothesis we collected 248 liquid biopsy samples including plasma (PLS), cell pellet (UCP) and supernatant (USN) from spun urine, along with matched initial endoscopic resection (TUR) anduffy coat from 17 patients undergoing NAC. We assessed single nucleotide variants (SNV) and copy number alterations (CNA) in TUR and mutDNA using Tagged-Amplicon- and shallow Whole Genome- Sequencing respectively.

We detected SNV and CNA in 75% and 100% of the 16 available TUR samples respectively. Considering SNV and CNA together, MutDNA was detected in 35.3%, 47.1% and 52.9% of pre-NAC PLS, UCP and USN samples respectively. Whilst we found that urine samples (UCP and USN) contained higher levels of mutDNA as compared to PLS (p<0.0001), no single peripheral sample type captured all mutations.

Analysis of longitudinal mutDNA demonstrated tumour evolution under the selective pressure of NAC e.g. in one case, urine analysis tracked at least two distinct clones with contrasting treatment sensitivity. In an effort to better characterise NAC resistance, whole exome sequencing of this patient’s peripheral samples is underway and preliminary data will be presented.

The majority of mutDNA was detected in peripheral samples of patients that recurred (local or systemic) after definitive therapy. Of note, persistence of mutDNA in samples taken at the first on-treatment time-point (2nd NAC cycle) predicted disease recurrence (83% sensitivity, 100% specificity, p=0.003), emphasising its potential as an early biomarker for chemotherapy response.
**In silico screening of proteins targeting circulating miRNAs for improved diagnosis of Multiple Myeloma**

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Multiple Myeloma is a B-cell malignancy, which is characterized by the expansion of clonal plasma cells in the bone marrow, thereby leading to abnormal accumulation of monoclonal antibodies in circulation. The condition arises from an asymptomatic multiplication of plasma cells, called MGUS (Monoclonal gammopathy of undetermined significance) which eventually progresses to Myeloma. Till date, there are no explicit assays that can discriminate between the premalignant and malignant stages. Circulating miRNAs are deregulated in MM cells and bone marrow. Their differential expression profiles in various body fluids can be quantified and used for the diagnosis of MM. The study focuses on identification of such a protein which would show exclusive affinity for a selected panel of circulating miRNAs reported to be deregulated in MM. The panel comprised of three miRNAs ;miR-720 and miR-1246 found to be upregulated, whereas miR-1308 was found to be downregulated in MM patients. Human RNA binding proteins were selected based on their RNA binding domains and their interacting probabilities with the panel of miRNAs. The 3D structure of miRNAs and proteins were predicted. Five RNA binding proteins TROVE, CUGBP2, DHX8, PUM2 and DKC1 were used for Molecular Docking using AutoDock Vina. Out of these selected proteins, DKC1 showed significant hydrogen bonding as well as remarkable binding affinity values of -17.4 kcal/mol with miR-720 (2 H-bonds), -16 kcal/mol with miR-1246 (1 H-bond) and -16.9 kcal/mol with miR-1308 (3 H-bonds). miR-26 was used as an internal control for docking as it is a circulating miRNA without any significant relation to MM. This protein-miRNA interaction could be used as an economical and reliable probe/ELISA based method for the improved diagnosis of Multiple Myeloma patients.

**A simple Q-PCR test to evaluate various molecular subtypes in CRC for better prognosis and treatment**

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Colorectal cancer develops from benign intra epithelial lesions known as adenomas to malignant carcinoma. Broadly, the genesis of CRC may be classified into Chromosomal Instability (CIN), Microsatellite Instability (MSI) and CpG Island Methylator Phenotype (CIMP). Alteration in key signalling pathways define this molecular heterogeneity. These sub types decides the clinicopathological features as well as their response to therapy. Presence and absence of mutation and methylation in certain key genes can distinctly classify the molecular subtypes. Moreover, various studies have shown a strong correlation with subtypes and response to anti EGFR, anti VGFR, adjuvant therapies and define disease pronosis. Here in this study, we are attempting to develop a realtime multiplex SYBR green PCR system in which, in a single reaction will establish mutant status of KRAS, BRAF gene and methylation status of MLH1 gene, which eventually be able to distinguish between major molecular subtypes. SYBR green PCR reaction was performed with normal and bisulphite converted genomic DNA to check the mutation status using primers for codon 12 and 13 in KRAS, V600E in BRAF gene and methylation status of MLH1 gene. Reaction was performed in a single tube. Care was taken to design primers in a way so that the amplicons size of these candidate genes were different. Individual amplicons will have different melt curve and Tm. Melt curve analysis of reaction revealed a Tm of 78.8 ºC (216 bp) and 82.5 ºC (297 bp) for BRAF and KRAS gene. Tm of MLH1 methylated/ unmethylated promoter ranged between 71-74 ºC (344 bp: Bisulphite converted amplicon). Tumor samples are being tested for mutation and methylation in candidate genes using this one step method. This study perhaps would help in distinguishing between major molecular subtypes of CRC sample that will help in selecting the drug regime for the patient.
Differential oncogenic capacity of gene fusions in colon cancer

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Genomic rearrangements can give rise to gene fusions which contribute to tumorigenesis and may be potential targets for treatment. In a recent study we systematically analyzed the prevalence of oncogenic gene fusions in a cohort of primary colon cancer patients. We showed that 2.5% of patients had a fusion in relevant genes such as BRAF, NTRK3, RET and ERAS, which may represent functional drivers and provide potential novel leads for personalized therapies.

In order to evaluate the contribution and oncogenic capacity of an individual gene fusion to colorectal cancer (CRC) development and to assess response to targeted therapy, we utilized the recently developed 3-D organoid culture system. As a first step we stably introduced the identified gene fusions in genetically characterized patient-derived CRC organoids (PDO) as well as healthy colon organoids with CRISPR-Cas9 engineered APC and TP53 mutations.

We investigated the effects of gene fusion expression on growth rate, response to targeted treatment and MAPK/PI3K signaling cascades. We observed strong differences in the proliferation potential of organoids depending on the expressed gene fusion. In particular, the USP9X-ERAS gene fusion consistently resulted in a two-fold increase in growth rate in all organoid lines tested. Importantly, all gene fusion expressing organoid lines responded to MEK-Inhibition, which is explained by the gene fusion-induced activation of the MAPK and PI3K signaling cascades. Further ongoing efforts are aimed at expanding the repertoire of characterized gene fusions and the inclusion of PDOs which harbor an endogenous gene fusion. Moreover, genomic instability analysis upon gene fusion expression will be performed.

Our results clearly demonstrate strong differences in cellular and molecular effects of gene fusion expression and their potential for targeted treatment approaches.

Single nucleotide editing within a genome regulatory element to map germline variant’s effect on early prostate cancer genomic events

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Prostate cancer (PCa), the second most common cancer among men, is a highly heritable molecularly and clinically heterogeneous disease. In this work we focused on the study of germline variants as putative trigger for early somatic events in PCa. Our previous in silico analysis identified a non-coding polymorphic regulatory element at the 7p14.3 locus associated with DNA repair and hormone regulated transcript levels and with an early recurrent prostate cancer specific somatic mutation in the Speckle-Type POZ protein (SPOP) gene (OR=5.54, P=1.22e-08). In vitro studies demonstrated that androgen receptor (AR) and CCAAT/Enhancer Binding Protein (C/EBP) beta (CEBPB) bind to the locus and that their transcriptional activity is allele-specific.

To further investigate the enhancer activity of the 7p14.3 locus we deleted 731bp of this region in PC-3 cells with CRISPR-Cas9 engineered APC and TP53 mutations. We investigated the effects of gene fusion expression on growth rate, response to targeted treatment and MAPK/PI3K signaling cascades. We observed strong differences in the proliferation potential of organoids depending on the expressed gene fusion. In particular, the USP9X-ERAS gene fusion consistently resulted in a two-fold increase in growth rate in all organoid lines tested. Importantly, all gene fusion expressing organoid lines responded to MEK-Inhibition, which is explained by the gene fusion-induced activation of the MAPK and PI3K signaling cascades. Further ongoing efforts are aimed at expanding the repertoire of characterized gene fusions and the inclusion of PDOs which harbor an endogenous gene fusion. Moreover, genomic instability analysis upon gene fusion expression will be performed.

Our results clearly demonstrate strong differences in cellular and molecular effects of gene fusion expression and their potential for targeted treatment approaches.

This work is a proof of concept of germline predisposition to molecularly distinct cancer subclasses and has the potential to nominate new mechanisms of cancer development.
Multiple myeloma is protected from endoplasmic reticulum stress and associated apoptosis by the activation of NRF2

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Background: The proteasome inhibitor bortezomib (Bz) is the mainstream chemotherapy for multiple myeloma (MM), which is a cancer of post-germinal centre B cell. The mechanism of action of Bz is to increase reactive oxygen species (ROS) which triggers endoplasmic reticulum (ER) stress associated apoptosis. However, MM still remains incurable as resistance to Bz is common. 

We hypothesize that the transcription factor NRF2, whose normal role is to protect cells from ROS via the regulation of drug metabolism and antioxidant genes, protects MM cells from Bz induced apoptosis.

Aim: to determine if Bz induced NRF2 signalling orchestrates survival of MM cells by influencing ER stress associated apoptosis.

Methods: Protein and gene expression were examined by western blotting and qRT-PCR. NRF2 activity was measured using nuclear expression profiling and promoter assays. CellTiter-Glo and PI/Annexin V was used to determine cell viability and apoptosis. ROS was measured using DCF staining and ER stress was assayed using ER-Tracker Dyes. The pharmacological inhibition and lentiviral mediated shRNA knockdown (KD) of NRF2 in MM cells were also performed.

Results: Bortezomib induces the activation of NRF2 in various MM cell lines and 11 primary MM cells as measured by nuclear NRF2 and NRF2 promoter assays. Pharmacological or genetic inhibition of NRF2 in bortezomib treated cells increased ER stress through the regulation of CHOP, ATF3 and ATF4. Moreover, inhibition of NRF2 in combination with Bz significantly increased apoptosis in MM cells.

Summary: NRF2 cytoprotective responses are activated in MM cells by Bz. The inhibition of NRF2 in MM cells increased ER stress associated apoptosis in MM cells. This highlights the importance of NRF2 in regulating chemotherapy resistance in MM.

Exosome microRNAs (miRNAs) present in peripheral blood of colon cancer patients undergoing surgical resection

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Background: Circulating exosome miRNAs have been recently described as promising cancer biomarkers. Literature suggests that the accuracy of these short RNAs could outperform currently used prognostic markers (TNM staging, residual tumor classification and histological indictors). Our aim was to study pre- and post-surgery expression of exosome miRNAs in colon cancer patients.

Samples and methods: Exosome miRNAs were extracted from peripheral blood of 23 subjects with operable colon cancer (pre- and post surgery), 6 healthy controls and supernatants of 6 primary colon cancer cell cultures. Exosome RNA profiles were measured using RNA-Seq and aligned to the most recent GENCODE human reference. 48 miRNA transcripts were verified by reverse transcription quantitative PCR (RT-qPCR) in TaqMan Low Density Array setting.

Results: RNA-Seq analysis yielded 15 miRNAs present in peripheral blood of cancer samples and absent in controls (miR-31, miR-33b, miR-99a, miR-138, miR-149, miR-152, miR-196a, miR-197, miR-200c, miR-301b, miR-324, miR-561, miR-577, miR-118). RT-qPCR analysis confirmed that 7 miRNAs were present in cancer patient serum samples and absent in control serum samples: miR-26a, miR-92a, miR-181b, miR-196a, miR-425, miR-552 and miR-30a. Expression of miR-26a, miR-92a and miR-552 was also detected in all studied supernatant samples. Out of the aforementioned miRNAs, the levels of miR-26a, miR-181b, miR-425 and miR-30a altered in a dynamic manner in four cases for whom more than
two time points were analyzed. Additionally, levels of miR-191 and miR-223 decreased significantly after surgery (14 versus 6 samples with elevated expression, p=0.02; and 16 versus 7 samples with elevated expression, p=0.01, respectively).

Conclusions: Our results indicate that several exosomal miRNAs are detectable in colon cancer patients and that their levels fluctuate during treatment. These dynamic biomarkers could potentially be used in personalized therapy of colon cancer patients. Prognostic and predictive value of these assays warrants further investigation and is underway.

Identification of non-neutral tumour evolution across human cancer types

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Clusters of subclonal mutations attributed to subclonal expansions have been observed across most cancer types. To date it is not clear what fraction of these expansions can be ascribed to selective sweeps or genetic drifts. The widely held theory states that tumour expansion arises from a series of selective sweeps. However, Williams et al. (Nat. Genet. 48:238-224, 2016) recently claimed to have identified neutral tumour evolution in one third of 904 samples from The Cancer Genome Atlas (TCGA). Here, we have assessed the reproducibility and validity of their method and the extent of positive selection in subclonal mutations across cancer types. First, using the same equations we simulated data of tumour undergoing selective sweeps. Application of the method led to over-calling of neutrality. Second, we simulated data under more realistic models, in which the test was unable to distinguish neutrality from non-neutrality (area under the curve \(\approx 50\%\)). Finally, we analysed the mutations from \(\approx 1,600\) exomes and \(\approx 1,500\) whole genomes of real tumours from TCGA and the International Cancer Genome Consortium, respectively. We grouped them into neutral and non-neutral tumours according to the rationale described by Williams et al. We then applied dN/dS, an orthogonal widely used approach to detect selection in non-synonymous mutations of coding regions. This revealed significant and widespread positive selection in clonal and subclonal mutations within both neutral and non-neutral groups. Altogether, our results point to the lack of evidence for neutral tumour evolution and uncovered strong positive selection within subclonal mutations across cancers.

Molecular analysis of endometrial cancer to stratify patients and guide therapy

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Background: Endometrial cancer (EC) is now the 5th most common malignancy in women1. Advances in molecular classification by TCGA have defined molecular profiling, however, use of these advances to direct therapy are lacking. Furthermore, tumour heterogeneity and current biopsy methods harbour therapeutic misguidance. Circulating tumour DNA (ctDNA) as a liquid biopsy may provide a more comprehensive tumour profile compared to the standard biopsy. Previously, ctDNA detection by targeted next-generation sequencing in breast cancer has demonstrated its potential to detect treatment altering mutations2. To date, no attempt has been made to incorporate both tumour profiling and ctDNA to guide therapy in EC.

Methods: We developed a targeted 170 amplicon next-generation sequencing (NGS) panel for detection of hotspot mutations in 10 genes commonly mutated in EC and applied this to 32 EC patient FFPE tissues. Plasma was available for 3 patients, for which we used a validated 30 amplicon panel3 for sequencing. Two genes amplified in EC and suggested to affect overall patient survival were analysed by qPCR. Assay efficiency was tested using TaqMan qPCR with standard curves, validated with EC cell lines Ishikawa and HEC-1A for gene amplification, with breast cancer MCF-7 as positive control. (Positive
amplification = CN>4.2, normalised to RPPH1, GAPDH & CNTNAP1.)

Results: NGS revealed 91% (29/32) of patients had a common EC mutation. CtdNA was detected in 2/3 available patient plasmas. Fifty-one percent (16/32) of patients had common CNV markers, 2 of which were amplified (CN>4.2). ESR1 p.D538G mutations were detected in patient tumours. Novel cell line CNV assays successfully demonstrated detection of ctdNA aberrations against genomic DNA. Assay sensitivity was achieved <2% concentration against background cell-free DNA.

Conclusion: Detection of molecular aberrations in ctdNA can present a profile mimicked in tumour DNA at low concentration, potentially providing stratification of EC patients using a liquid biopsy.

Iron Deprivation-Induced Alternative Splicing in Hela Cells

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Iron is required for the surviving of organisms by contributing to multiple biological functions, such as the transport of oxygen, oxidation-reduction reaction, cellular transport, energy production, immune system, nervous system and enzyme cofactor. Iron deprivation can mimic hypoxia by inhibiting prolyl-hydroxylases (PHD) enzyme that hydroxylates hypoxia-inducible factors (HIF) and its degradation. We are interested in discovering how hypoxia-mimic situation can influence the alternative splicing of gene expression. Hela cells (a cervical cancer cell line) were treated in 100 micro-molar Desferoxamine (DFO)-containing medium and subject to transcriptome analysis (RNA-seq). We found 121 genes are up-regulated and 84 genes down-regulated, respectively. Furthermore, analysis of alternative splicing elucidates 16 genes of which isoforms showed significant difference of gene expression. Interestingly, the down-regulation of an intron-retention form of erythropoietin receptor (EPOR) in DFO-treated Hela cells was validated by qPCR.

The identification and characterisation of oncogene PDE7A in gastric cancer metastasis.

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Gastric cancer is one of the most common and lethal malignancies worldwide with a particular burden in eastern Asia. Symptoms often develop in the advanced stage of the disease when the cancer has already metastasised,
resulting in a poor prognosis. Improving our current understanding of the genetics of gastric cancer development, progression and metastasis is important for the development of improved therapeutics and better prognosis for these patients. We have identified a novel oncogene likely involved in gastric cancer metastasis. PDE7A is a 3'-5' cyclic adenosine monophosphate (cAMP)-specific phosphodiesterase (PDE) involved in the regulation of cAMP signalling via degrading intracellular cAMP. Decreased cAMP signalling due to PDE overexpression has been observed in other cancers and the stimulation of cAMP signalling in these cancer cells was shown to induce apoptosis. Our RNA-sequencing data of Chinese gastric cancer patients demonstrated significant PDE7A upregulation in the metastatic lymph node and primary tumour tissue when compared to the paired normal tissue. We sought to determine whether PDE7A upregulation has functional relevance in the progression and metastasis of gastric cancer. We observed a strong oncogenic capability of PDE7A-overexpressed gastric cancer cells, displaying increased migrative and invasive capabilities as well as an advantage of growth. The elucidation of the molecular networks resulting from PDE7A upregulation will provide insight into the oncogenesis and metastasis of gastric cancer as well as provide grounds for the development of new therapies and improved prognosis.

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PTEN loss associates with STAT1/3 expression and tumour-infiltrating CD8+ T lymphocyte density in prostate cancer

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PTEN gene deletion with protein loss occurs in 20-30% of prostate cancer (PCa) cases and is associated with a poor outcome. PTEN has been demonstrated to regulate cellular interferon (IFN) responses. Given that inflammation could potentially promote PCa progression, it is important to establish IFN response markers in PCa tumours in the context of PTEN status for new therapies design. The aim of this study is to determine if PTEN loss is associated with IFN response in the PCa tumour microenvironment mediated by STAT1 and phosphorylated STAT3 expression. An in silico analysis of genomic and corresponding transcriptomic profiles of PCa tumours (n=493) from the Genomic Data Commons (GDC) cohort was performed. The proteomic expression of PTEN, STAT1, and pSTAT3 and their correlation with CD8+ tumour-infiltrating lymphocyte (TIL) density and clinical parameters was evaluated in a representative cohort of 286 PCa samples from the Centre hospitalier de l’Université de Montréal (CHUM) between 1993 and 2006. In silico data analysis revealed significant alterations in innate immune response pathways between PTEN deleted and PTEN intact tumours. Evaluation of 286 tumours confirmed significant correlations between PTEN with STAT1 and pSTAT3 expression in addition to CD8+ TIL density. A total of 124 (28%) differentially expressed immune response genes between tumours with PTEN loss and PTEN intact status were identified in the GDC cohort. In the 286 tumours, PTEN loss associated with reduced expression of stromal STAT1 and stromal and epithelial pSTAT3, together with low intra-epithelial CD8+ TIL density, which further associated with clinical features of aggressive disease. Our findings from large independent PCa cohorts suggest that PCa with PTEN loss exhibits dysregulated Type I IFN response that potentially permits progression to an aggressive disease phenotype. Further investigation is needed to define the mechanisms underlying these correlations to facilitate the design for improved therapies.
Conserved mutational patterns of mismatch repair deficiency in *C. elegans* and MMR defective cancers

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The analysis of cancer genomes over the past years has revealed profound differences of mutation patterns between individual tumors and across tumor types. Mutational signatures - the unique base substitution patterns with respect to local sequence context - became a very useful tool of cancer investigation in the last years. However, the signatures identified so far mostly represent complex conglomerates of the action of different mutational processes. For many signatures, the link with the underlying mutational processes is still unclear.

Here we use *C. elegans* whole genome sequencing to analyze mutation accumulation in mh-1 and pms-2 DNA mismatch repair (MMR) mutants alone and in conjunction with pole-4, a non-essential subunit of the leading-strand DNA polymerase POLE. MMR mends DNA replication errors and defects are associated with inherited colorectal cancer. Base substitution analysis revealed a robust mutational pattern indicative of *C. elegans* MMR deficiency. Adjusted to the base composition of the human genome, we observed limited overlap across previously computed human MMR related signatures. De novo signature extraction from from an existing cohort of 215 colorectal and 289 gastric adenocarcinomas revealed 4 MMR associated signatures one of which is conserved with the 'humanized' *C. elegans* MMR pattern. We conclude this conserved pattern reflect on DNA replication mistakes recognized by the MMR system. Of the remaining cancer MMR signatures one likely reflects the failure to mend NCG C>T changes due to methylcytosine deamination which does not occurring in *C. elegans*. 2 MMR signatures likely reflect the interplay between mutants affecting replication efficiency and or genome stability in conjunction with MMR deficiency. Consistent with this we find that mutational patterns are altered when *C. elegans* MMR double mutants are combined with non essential subunit of polymerase Epsilon. Thus, combining information from genetically defined models with cancer samples allows for better aligning mutational signatures to mutagenic processes.

Tailored patient-specific analysis improves detection of circulating tumour DNA in plasma and urine

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Introduction: Circulating tumour DNA (ctDNA) analysis provides a snapshot of tumour burden. In some cancer patients, particularly with low disease burden, ctDNA concentration in plasma can be low, making detection challenging. Urine may also be used to detect mutations, although total cell-free DNA (cfDNA) levels are lower than in plasma. Sampling error for rare molecules hampers detection of ctDNA, particularly when single-locus assays are used.

We present a TAilored PAatient-Specific analysis (‘TAPAS’) workflow for detection of ctDNA in samples with low mutant concentrations using multiple mutations per patient to increase sensitivity. We applied this method to longitudinal matched plasma and urine samples from 10 metastatic melanoma patients receiving therapy.

Methods: Exome sequencing was performed on metastatic tumour samples at baseline, whose identified mutations were used to design a tailored patient-specific panel, which targeted a median of 789 mutations per patient. We developed error-suppression methods for sequencing data both with and without molecular barcodes. Detection of low levels of ctDNA was achieved by leveraging signal across multiple mutations.

Results: By targeting multiple mutations at high depth, we were able to demonstrate detection of low-volume residual disease, and show significant detection of 1 mutant molecule in 1x10⁶. Mutant DNA was detected in cfDNA from small volumes of urine supernatant (< 3mL). Plasma ctDNA allele fraction correlated with mutant DNA allele fraction in urine; the estimated allele fraction of ctDNA in urine was approximately 30-50x lower than in plasma. Rising plasma ctDNA preceded rising serum lactate dehydrogenase (a standard measure of melanoma disease burden) concentrations by a median of 70 days.

Conclusion: Targeting multiple mutations in plasma and urine cfDNA samples with a novel ‘TAPAS’ workflow improves mutation detection. This method has potential utility for improving detection of ctDNA in low-volume or early stage disease, and/or when sample material is limiting.
Characterization of a stemness-related gene RALYL in the development and progression of hepatocellular carcinoma

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Recent discovery of tumor biology postulates that tumor development is driven by a small population of stem-like cells. Poorly differentiated tumors which usually preserving phenotype of their ancestral precursor cells, are commonly associated with high recurrence and poor prognosis. Here, we identified a gene, named as RALY RNA binding protein-like (RALYL), specifically expressed in liver progenitor and progenitor-like cells, which were acquired in a vitro hepatic differentiation model from embryonic stem (ES) cells. Interestingly, RALYL was rarely expressed in mature liver and the expression of RALYL was associated with poor differentiation in hepatocellular carcinoma (HCC) clinical samples. Functional assays showed that RALYL could promote tumor cell proliferation, foci formation, colony formation in soft agar and sphere formation and tumor formation in nude mice. Furthermore, higher RALYL expression was observed in CD133+ HCC cells. RALYL overexpression could increase the population of CD133+ cells, which was decreased when RALYL was silenced with short hairpin RNA (shRNA). qRT-PCR results indicated that RALYL could promote stemness related gene expression such as CD133, CD44 and lgr5 in human immortalized liver cell line as well as HCC cell lines. Mechanically, RALYL is a RNA binding protein and expected to function in RNA splicing. RNP Immunoprecipitation (RIP) sequencing demonstrated that RNA splicing involved in stemness related pathways were regulated by RALYL. In conclusion, our study has identified and characterized RALYL as a novel stemness-related gene in HCC which might facilitate the treatment of HCC.

Genomic consequences of aberrant DNA repair mechanisms stratify ovarian cancer histotypes

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Background: Aetiologic diversity, genomic instability profiles and distinct treatment responses differentiate each ovarian cancer histotype. However, the relative patterns of both mutational and structural variation have not yet been studied to determine whether the properties of somatic genomes can further subdivide the histotypes.

Methods: We performed whole genome sequencing on 133 ovarian tumours, including 123 carcinomas (59 high-grade serous (HGSC), 35 clear cell (CCOC), 29 endometrioid (ENOC)) and 10 granulosa cell tumours (GCT). Profiles of mutations, copy number aberrations, loss of heterozygosity (LOH) and structural variations (SVs) were assessed. We then computed mutational signatures and genomic structural characteristics reflective of specific DNA repair processes for each patient.

Results: Integrative clustering based on mutation and SV signatures identified seven distinct subgroups of patients both between and within histotypes. LOH and the homologous recombination deficiency signature mainly distinguished HGSC cases from non-serous histotypes. HGSC cases were further stratified into two prognostically significant subgroups (n=24, 41% and n=31, 53%) on the basis of SV profiles. The subgroup enriched in foldback inversions was associated with inferior overall and progression-free survival (logrank p-values=0.0053 and 0.0232). Moreover, co-localization of foldback inversion and focal high-level amplifications was observed, particularly in MECOM, MYC, KRAS and CCNE1. The findings were recapitulated in ICGC (n=82) and TCGA (n=435) HGSC cohorts,
transcending BRCA mutation status and gene expression molecular subgroups. Endometriosis-associated cancers were primarily stratified on the basis of mutational signatures. CCOC cases were divided by APOBEC deamination (n=9, 26%) and age-related mutational signatures (n=14, 40%). A distinct mismatch repair defect signature identified a group of microsatellite instable ENOC cases (n=8, 28%). A signature related to breast cancers uniquely identified GCT cases.

Conclusion: Our results suggest that mutational and structural variation signatures constitute new and defining features of ovarian carcinoma that identify new biological strata reflective of different DNA repair processes.

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Resistance to MEK Inhibition in KRAS-mutant Colorectal Cancer is Associated with Inflammatory Gene Expression

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MEK inhibitors (MEKi) such as trametinib have demonstrated clinical activity in BRAF-mutant melanoma. However, few clinical responses have been observed in KRAS-mutant colorectal and pancreatic cancers in Phase I trials. Understanding the mechanisms through which these cancers manifest either acquired or intrinsic resistance to MEKi may guide us to better deploy these agents in patients with KRAS-mutant disease.

We compared gene expression signatures of MEKi-sensitive versus MEKi-resistant cell lines to identify candidate mechanisms of intrinsic resistance towards MEK inhibition in KRAS-mutant colorectal cancer. We identified enrichment of inflammation-related gene expression signatures in cell lines intrinsically resistant to PD0325901 and in those exhibiting acquired resistance to trametinib. Moreover, high expression of some of these genes was associated with significantly reduced disease-free survival in colorectal cancer patients (TCGA).

In parallel, we screened a library of 488 FDA-approved drugs and tool compounds for synergy with trametinib in resistant cells. We identified inhibitors that sensitised to MEK inhibition and induced apoptosis, including the BRD4 inhibitor JQ1. The synergistic effect of combined trametinib and JQ1 translated to a panel of organoid cultures derived from KRAS-mutant colorectal cancer patients, which displayed high expression of inflammatory genes and were also resistant to MEKi. Strikingly, our combinatorial strategy also led to an attenuation of inflammatory signalling and gene expression in the resistant cell lines or organoids; possibly through a combination of transcriptional inhibition but also through preventing NFκB nuclear translocation and target gene expression.

Taken together, our findings provide a potential explanation for the limited clinical activity of MEK inhibitors in KRAS-mutant colorectal cancer, known for its inflammatory nature. Furthermore, we identified a gene expression signature that has potential to stratify patients for response to MEK inhibition. Excitingly, we have discovered a novel therapeutic approach to overcome intrinsic and acquired resistance to MEK inhibition in colorectal cancer.

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A Comprehensive Next-Generation Sequencing Platform for Identification of Mutations Associated with Oral Squamous Cell Carcinoma induced by Betel Nuts Consumption

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Betel leaf and areca nut consumption is a tradition dating back thousands of years in areas from South Asia to Southeast Asia. Long-term consumption is associated with oral submucosal fibrosis, pre-cancerous lesions in the form of leukoplakia and squamous cell carcinoma of the oral cavity and esophagus. Several known carcinogens are found in betel nuts, including hydroxychavicol, safrole and arecoline. Arecoline, the primary psychoactive ingredient with addictive properties, contributes to histological changes in the oral mucosa. Arecoline acts by impairing the PADPR-inducing DNA repair system, which results in the accumulation of genomic DNA damage. Our goal is to design an NGS-based analytical system capable of capturing a variety of somatic mutational events with high specificity and sensitivity. Specifically, genomic DNA extracted from FFPE tumor samples will be analyzed for single nucleotide variants and copy number variants of somatic origin. In addition,
RNA extracted from FFPE tumor samples with be analyzed for transcript expression levels as well as potential chromosomal breakpoints. To ascertain the relevant gene contents in the NGS analytical system, we start by conducting a systematic literature review and targeted pathway analysis. Using the KEGG pathway as well as literature searches, we identified 4 overlapping biochemical pathways including cell cycle, MAP kinase, notch and TP53. Together, these result in 275 non-overlapping genes. In addition, we also analyzed a variety of publicly available chromosomal aberration data, including cBioPortal, Mitelman Database of Chromosome Aberrations and Gene Fusions and Atlas of Genetics and Cytogenetics to identify potential chromosome breakage enriched in oral squamous cell carcinoma. 27 potential gene fusion transcripts resulting from the fusion events are included in gene panels. Using this target platform, we will test a set of seven oral squamous cell carcinoma tissues and their associated normal controls from paraffin embedded tissue blocks. We will be analyzed for transcript expression levels as well as potential chromosomal breakpoints.

Molecular Signaling in Multiple Myeloma: Association of RAS/RAF Mutations and MEK/ERK Pathway Activation

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Multiple myeloma (MM) is a genetically highly heterogeneous malignancy of plasma cells. Most frequent mutations are found in RAS/RAF genes, highlighting the potential significance of RAF/MEK/ERK pathway as a therapeutic target. However, the clinical relevance of this finding is unclear as clinical responses to MEK inhibition in RAS mutant MM have been mixed. This suggests a variable degree of dependency on MEK/ERK signaling in RAS/RAF mutant MM. We therefore address this issue by assessing RAS/RAF mutations status and MEK/ERK pathway activation by both targeted sequencing and phospho-ERK immunohistochemistry (IHC) in primary biopsies from 103 newly diagnosed pts and 77 relapsed/refractory pts.

We found KRAS and NRAS were both mutated in about 1/4 of the pts, BRAF was mutated in 8% of the pts. Overall RAS/RAF mutations were significantly enriched in rMM (p = 0.010), mainly driven by an increase in NRAS mutations (p = 0.017). The top nine recurrently detected individual mutations were KRASG12D (n = 11), NRASG121R (11), NRASG12V (10), KRASG12D/G12V (6 each), BRAFV600E (6), NRASG13D (5) and NRASG13R/Q61H (4 each). KRAS but not NRAS mutations were associated with ERK activation compared to RAS/RAFwt (p = 0.003). When each recurrent mutation was tested against all RAS/RAFwt samples, only KRASG12D and BRAFV600E were consistently associated with ERK activation (p < 0.001 each), indicating that ERK activation is dependent on specific type of mutation.

In summary, our results demonstrate for the first time that BRAF/RAS mutation status alone is not generally associated with MEK/ERK pathway activation, and therefore may not be predictive for therapeutic response to MEK inhibition in MM. Our data indicate that pathway-specific IHC should be considered to assess pathway activation with the potential to inform future clinical trials of targeted therapies. Further validation of this concept should be performed in the context of prospective clinical trials.

Landscape of immuno-genomic alterations in high-grade serous ovarian cancer from exceptional long-and short-term survivors

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Despite poor overall survival outcomes for patients diagnosed with high-grade serous ovarian cancer (HGSOC), a subset of patients experience sustained response to chemotherapy, resulting in long-term survival (≥ 10 years, versus 1.7 years median overall survival). In this study, we compared clinical, genomic, and transcriptomic characteristics of 41 treatment-naive tumour specimens from HGSOC long-term survivors (LT, >10 years, n=20) and exceptionally short-term (ST, < 2 years) survivors (n=21). From exome and transcriptome analysis of LT versus
ST HGSOC, we observed increased somatic mutation burden (median 3 vs. 2.5 mutations/million base-pairs of coding genome, p=0.03), and enrichment of BRCA1/2 loss of function mutations. Through single sample gene-set enrichment analysis, we found increased representation of activated CD4+ T-cells, activated CD8+ T-cells, and effector memory CD4+ T cells in the tumor microenvironment from LT survivors. Molecular characteristics of tumors from the ST group included focal copy number gain of CCNE1 with corresponding over-expression, a lack of BRCA associated mutation signature, and low homologous recombination deficiency score. Together, LT survival in HGSOC is determined by combination of factors involving BRCA1/2 mutations, mutation burden, and tumor immune infiltration.

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The role of Th17-interleukin-17 axis in human cancer

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Th17 cells are a subset of T helper cells secreting interleukin-17 (IL-17A and IL-17F). IL-17A has been demonstrated to promote development of prostate cancer, colon cancer, skin cancer, breast cancer, lung cancer, and pancreas cancer. We have systematically investigated the role of Th17-IL-17 axis in prostate cancer. We found that IL-17 receptor C (IL-17RC) expression was up-regulated in human prostate intraepithelial neoplasia (PIN), hormone naïve prostate cancer, and castration-resistant prostate cancer. Using an Il-17rc;Pten (Phosphatase and tensin homolog) double knockout mouse model, we found that IL-17 promoted development of hormone-naïve and castration-resistant prostate cancer through multiple mechanisms, including: 1) directly stimulating expression of C-C motif ligand 2 (CCL2), CCL20, C-X-C motif ligand 1 (CXCL1), IL-6, matrix metalloproteinase 7 (MMP7), cyclooxygenase-2 (COX-2), and vascular endothelial growth factor A (VEGFA); 2) directly inducing inflammatory cell infiltration; 3) increasing the ratio of immunosuppressive immune cells such as myeloid-derived suppressor cells (MDSCs) and M2 macrophages; 4) increasing angiogenesis; 5) enhancing cellular proliferation; and 6) inhibiting cellular apoptosis. Using an Mmp7;Pten double knockout mouse model, we found that MMP7 promoted prostate adenocarcinoma through induction of epithelial-to-mesenchymal transition (EMT). MMP7 disrupted E-cadherin/β-catenin complex to up-regulate EMT transcription factors in mouse prostate tumors. IL-17 induced MMP7 and EMT in human prostate cancer LNCaP, C4-2B, and PC-3 cell lines, while siRNA knockdown of MMP7 inhibited IL-17-induced EMT. Selective inhibitor of MMP7, inhibitor of Th17 cell differentiation, and anti-IL-17A neutralizing antibodies were able to partially inhibit prostate cancer formation in the Pten knockout mice. These findings demonstrate that Th17-IL-17 axis plays an important role in prostate cancer development, indicating Th17-IL-17 axis as a potential target for developing new strategies in the prevention and treatment of prostate cancer.

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Identify the contribution of Rho GTPases regulator to gastric cancer metastasis

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Gastric cancer is an aggressive disease that continues to have a daunting impact on global health. Gastric cancer is usually diagnosed at an advanced metastasis stage and the patients with metastasis continue to have a dismal outcome. In our study, we aim to investigate key regulators that drive the metastasis process and molecular pathways underlying the metastasis process. In our work, we did the RNA-seq of 8 gastric cancer patient cases (each case included the matched adjacent non-tumor tissue, primary tumor and lymph node metastasis). After data analysis, we found that the most significant pathway altered in metastasis was the Rho GTPase signaling pathway and most of differential expressed genes were regulators of Rho GTPases, such as ARHGAP15. In vitro cell invasion and migration assays showed that ARHGAP15 could effectively affect cell invasion and motility. The metastatic process is highly inefficient, which depends on the ability of disseminated single cancer cells to survive through the circulatory system and further colonize in a foreign site. The single cancer cell survival ability is required to establish lethal metastatic lesions at distant sites. When gastric cancer cells were plated in low-density plating conditions where single cells had to expand into colonies, ARHGAP15 overexpression increased the number of colonies than the control cells. Our data indicate that the ARHGAP15 could contribute to the single cell survival ability. All these preliminary findings strongly suggest that the
Somatic DNA copy number variants in the peripheral blood of patients with solid tumors

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Cancer is increasingly recognized as a systemic disease that extends beyond the tumor itself. We report here a previously unknown type of genomic aberration in the blood cells of patients with solid tumors. Through a high-resolution genome-wide comparison analysis of DNA copy number variants (CNVs) in the blood, tumor and tumor-adjacent tissues of 8,870 patients with 28 types of cancers, we identified genomic hotspots that harbor recurrent focal CNVs in the blood. Detected in more than 13% of the patients, these CNVs represent unique somatic DNA aberrations that were absent or under-presented in the corresponding solid tumors or tumor-adjacent tissues. The occurrence of the blood-specific CNVs was correlated with older patient age, shorter progression-free survival, and, based on integrative pathway analyses, elevated immune activities within several types of primary tumors. Therefore, novel tumor-extrinsic systemic elements may influence the development and clinical progression of solid tumors by way of altering anti-tumor immune responses.

Keywords: cancer genome, copy number variation, copy number alteration, copy number variant, somatic mutation, cancer immune response, cancer blood, blood abnormality, genomic aberration, genomic instability, TCGA.

Potential biomarkers for the individualized cancer therapy in elderly patients of pancreatic ductal adenocarcinoma

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An increasing proportion of cancer care will be delivered to elderly patients. There is a wide range of health status among the elderly, and with the “chronological age” which often substantially differ from “biological age”. New screened Biomarkers of aging can be used to better determine a patient’s biological age and would have potential clinical implications for the treatment of elderly patients with cancer. In particular, incidence of pancreatic cancer is highly correlated with aging. In 2030 the rising ratio will dramatically increase in pancreatic cancer (PDAC), as compared to other types of cancer. Therefore, in our study, we will mainly focus on searching of geriatric-oncological biomarkers (ONCO-AGING biomarkers) for optimizing individualized therapeutic strategy for elderly PDAC patients. 50 young (<65 year-old) and 50 old (>65 year-old) pancreatic ductal adenocarcinoma patients are planned to be recruited into the study with liquid biopsies and tissue materials of resectable patients. Serologic markers including IL-6, IL-18, IL1RA, TNF-a, Ferritin, CRP, Endothelin-1 were analysed. Telomerase activity and length will also be detected for a biological age estimation. RNA sequencing was performed in 10 pairs of primary tumor and corresponding non-tumor normal tissues with duplicate of the same tissues from the treatment naïve patients for screening. For advanced unresectable metastatic PDAC patients, we collected the peripheral blood before and after non-surgical interventions for further PBMC and circulating tumor cell isolation and approve for immunological
profile and EMT potential evaluation respectively. Our aim is to integrate a signature independent of chronological age, to some extent, revealing the cancer progression prognosis as well as therapy response against different treatment strategies for pancreatic cancer.
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