**Poster 1**

**AKT and EZH2 inhibitors kill TNBCs by hijacking mechanisms of involution**

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Triple negative breast cancer (TNBC) is the most aggressive breast cancer subtype and has the highest rate of recurrence. The predominant standard of care for advanced TNBC is systemic chemotherapy with or without immunotherapy, however responses are typically short-lived. Thus, there is an urgent need to develop more effective treatments. PI3K pathway components represent plausible therapeutic targets, as approximately 40% of TNBCs have PIK3CA/AKT1/PTEN alterations. However, unlike hormone receptor-positive tumors, it is still unclear if or how PI3K pathway inhibitors will be effective in triple-negative disease. Here we identify a promising AKT inhibitor-based therapeutic combination for TNBC. Specifically, we show that AKT inhibitors potently synergize with agents that suppress the histone methyltransferase, EZH2, and promote robust tumor regression in multiple TNBC models in vivo. AKT and EZH2 inhibitors exert these effects by first cooperatively driving basal-like TNBC cells into a more differentiated, luminal-like state, which cannot be effectively induced by either agent alone. More importantly, once differentiated, these agents kill TNBCs by hijacking signals that normally drive mammary gland involution.

Together these findings identify a promising therapeutic strategy for this highly aggressive tumor type and illustrate how deregulated epigenetic enzymes can insulate tumors from oncogenic vulnerabilities. These studies also reveal how developmental tissue-specific cell death pathways may be co-opted for therapeutic benefit.

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**Poster 2**

**Team: STORMing Cancer**

Challenge: Determine the mechanisms that cause cancer without known mutagenesis, such as obesity, in order to devise novel interventions

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**Poster 3**

**Elucidating the invasive potential of Fusobacterium and its role in colorectal cancer**

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The microbiome has long been an alluring target to study and recent advancements in microbial detection and omics-technologies has further revolutionized our view of how human diseases are impacted by the microbiome. A member of the human microbiome that has garnered such attention is Fusobacterium nucleatum, a Gram-negative, anaerobic bacterium, that normally inhabits the human oral cavity. F. nucleatum is a heterogenous species consisting of four subspecies, animalis, nucleatum, polymorphum, and vincentii. Interestingly, F. nucleatum is highly invasive into surrounding cells and tissues of the periodontal pocket and capable of disseminating throughout the entire body. Because of this, F. nucleatum is associated with a wide variety of diseases, most recently and strikingly, colorectal cancer. Despite the pathogenic potential of F. nucleatum, there is limited knowledge about the molecular mechanisms contributing to the invasive nature and role of this oral bacterium in the pathogenesis of human cancer.

Herein we characterize the invasion and survival capabilities of colon tumor isolated Fusobacterium nucleatum strains encompassing the four subspecies by confocal microscopy, flow cytometry, and classical antibiotic protection assays. Using human colorectal cell lines, we performed cocultures with our panel of clinical isolates and observed that F. nucleatum subspecies have varying invasive potentials. To investigate mechanisms by which F. nucleatum may drive CRC, we assessed the effect of these strains on the proliferation of several CRC cell lines. We found that invasive CRC isolated F. nucleatum does not stimulate proliferation of any of the CRC cell lines examined. Additionally, to understand the cascade of events that occur during F. nucleatum invasion, we took a transcriptomic approach using RNA-Seq analysis on human CRC colonocytes during F. nucleatum infection. Exposure to F. nucleatum results in the upregulation of host inflammatory responses most notably CXCL1, CXCL2, CXCL3, and CCL20.

**Chris Bailey, PhD Student, eDyNAmiC, Francis Crick Institute, UK**

**Poster 4**

Focal gene amplifications are found across multiple tumour types, drive tumour growth and evolution and are frequently the target for therapeutics. It has been shown that ecDNA drives extreme oncogenic focal amplification formation with gene copy number counts that reach 250, however detailed description of non-oncogenic ecDNA, the mutational processes that govern ecDNA formation and replication, and the impact of ecDNA on survival in specific tumour types are yet to be described. Moreover, ecDNA species are highly heterogeneous; large datasets provide an opportunity to characterise where and how ecDNA forms across the genome to inform therapeutic approaches in different tumour types.

We present an analysis of a cohort of pan-cancer whole genome sequencing to date, comprising 14,757 patients, including primary and metastatic samples in 16 tumour types. These tumour types include lung adenocarcinoma, lung squamous cell carcinoma, breast, pancreatic carcinoma, colorectal carcinoma, endometrial carcinoma, glioblastoma, prostate adenocarcinoma, bladder carcinoma, sarcoma, clear cell renal carcinoma, ovarian carcinoma, melanoma and haematological malignancies such as ALL, myeloma and AML.

Firstly, we find that the most recurrent focal gene amplifications occur in known oncogenes such as MDM2 in sarcoma, EGFR in glioblastoma and ERBB2 in breast cancer. When amplified, we discover that oncogenes such as MDM2 and EGFR tend to form ecDNA, whereas FGFR1 and KRAS are more likely to have formed as a consequence of breakage-fusion-bridge cycling, and SRC and IRF4 amplifications are almost never form ecDNA.

Secondly, we discover multiple species of ecDNA that do not contain oncogenes. These include ecDNA that contain regulatory elements only. We also identify a specific, previously undescribed ecDNA that is highly enriched in luminal breast cancer that contains no previously described oncogenes but contains a complex of 32 genes including RSF1, AQP11 and KCTD14.

Thirdly, by mapping mutations on ecDNA and determining if they have occurred pre- or post ecDNA formation, we provide evidence of ecDNA formation that has occurred after treatment.

Finally, we find that ecDNA is enriched in metastasis and results in poorer survival across the cohort.

**Claire Mulvey, postdoctoral researcher, IMAXT, CRUK Cambridge Institute, University of Cambridge, UK**

**Poster 5**

Integrated whole-organ imaging and spatial molecular profiling highlight functional heterogeneity at the metastatic site in a mouse model of triple-negative breast cancer

Claire Mulvey1*, Marta Paez-Ribes1*, Atfeh Fatemi1, Tristan Whitmarsh2, Eduardo González-Solares2, Alireza Molaeinezhad2, Ali Dariush2, CRUK IMAXT Grand Challenge Team, Dario Bressan1 and Greg Hannon1.

1 CRUK Cambridge Institute, Li Ka Shing Centre, University of Cambridge, Cambridge, UK.
Metastasis is responsible for the majority of cancer-related deaths. However, the mechanisms that define and control metastatic dissemination, invasion and organ tropism remain poorly understood. This is partly due to the difficulties associated with studying very early metastatic events, such as identifying micro-metastases and disseminated tumour cells (DTCs) within an entire organ or at a secondary site.

The IMAXT Grand Challenge consortium has developed a platform that enables identification and characterisation of early metastatic lesions using a combination of volumetric fluorescent imaging, spatial proteomics and spatial transcriptomics. The consortium has also developed a bespoke analysis pipeline for combining these technologies to ultimately provide annotated 3D volumes of tumours. We present here a showcase dataset, where we have combined fluorescent 3D volumetric imaging using serial two photon tomography (STPT) with imaging mass cytometry (IMC), to identify and characterise early metastatic lesions of the 4T1 mouse model of triple negative breast cancer (TNBC). We have a particular interest in investigating how metastatic cells interact with and are influenced by their immediate tumour microenvironment (TME).

TdTomato-expressing 4T1 TNBC cells were orthotopically implanted into the mammary fat pad of NSG-GFP+ mice and allowed to develop for several days, at which point the primary tumours were resected. Metastatic disease was allowed to progress, and mice were culled at relevant time-points from day of resection (Day 0) through to advanced metastatic disease (Day 21 post-resection). Lungs were inflated with agarose and other organs were also collected, fixed and embedded for further analysis. STPT imaging was performed for all time-points and 200 x 15um sections were acquired for all lung samples. During STPT, a series of 2D mosaic images are stitched to reconstruct the tissue volume and all corresponding sections are collected onto histological slides. Slides containing individual metastatic lesions of interest were processed for IMC, a multiplexed immunohistochemistry method for simultaneous analysis of up to 40 metal-conjugated antibodies, where the panel of antibodies can represent a variety of cell types and cell states.

Using this approach, we have identified very small metastatic lesions in the lung as early as five days after primary tumour resection. Comparative IMC analysis of the time-points is currently being used to characterise spatially distributed markers for the different stages of the metastatic process. We have also identified metastatic lesions in lungs, liver, kidney and lymph node of a single animal, allowing us to compare phenotypic differences in the microenvironment at various metastatic sites. Finally, we have demonstrated distinct metastatic signatures between different lesions within the same tissue section, suggesting functional heterogeneity in metastases located in close proximity to each other.

The IMAXT workflow has enabled the molecular annotation of early metastatic events in a model of TNBC, revealing a complex spatial distribution of tumour and TME markers. A greater understanding of these processes will play a role in guiding new treatment targets with potential to improve survival and quality of life of the patient.

**Design of a Fap2-encoding mRNA LNP vaccine against the oncomicrobe F. nucleatum**

**Cody Despins, graduate student, OPTIMISTICC, BC Cancer Research Institute, CA**

**Poster 6**

Over 15% of the global cancer burden is attributable to cancer-associated pathogens (oncomicrobes). Fusobacterium nucleatum, an emerging colorectal cancer (CRC) oncomicrobe, is an invasive, anaerobic, Gram-negative bacterium normally found in the healthy oral cavity. F. nucleatum can spread beyond its oral niche through the bloodstream to colonize tumor sites. Clinically, high F. nucleatum tumor burden is associated with poor patient outcomes, chemoresistance, and increased risk of metastasis. Fap2 is a surface protein and key virulence factor of F. nucleatum. Fap2 mediates F. nucleatum tumor enrichment by binding GalGalNAc, which is overexpressed by many tumor types including CRC. Generating anti-Fap2 immunity with an mRNA-LNP vaccine may induce Fap2-specific neutralizing antibodies and evoke CD8+ T cells responses, blocking tumor enrichment and eliminating F. nucleatum invaded host cells, respectively. Towards developing a Fap2-encoding mRNA-LNP vaccine against F. nucleatum, we designed and validated mRNA LNPs that lead to high expression, secretion, and surface-display of antigens of interest using RFP as a prototype antigen. Next, we created mRNA LNPs that encode secreted truncations of the Fap2 passenger domain, and we evaluated their immunogenicity in vivo using a prime-boost vaccination regime. We have observed robust antibody responses to our Fap2 truncation candidates. We are currently assessing alternative designs of these Fap2 candidates (multimeric secreted and surface displayed) and other candidate antigens for improved
immunogenicity. Next, we will test the efficacy of our lead Fap2 candidate vaccines in mitigating F. nucleatum infection in vivo, using HLA-humanized mouse models. An effective F. nucleatum vaccine will be a useful research tool and may have utility in circumventing the unfavourable outcomes associated with F. nucleatum positive CRC.

**Dave Chutter, patient advocate, eDyNAmiC Poster 7**
Team: eDyNAmiC
Challenge: Extrachromosomal DNA: Understand the biology of ecDNA generation and action, and develop approaches to target these mechanisms in cancer.

**Debarati Ghosh, research scientist, IMAXT, Massachusetts Institute of Technology, US Poster 8**

**Expansion sequencing to spatially map early metastatic cancer at nanoscale resolution**

Debarati Ghosh1*, Brett Pryor1, Ruihan Zhang2, Yi Cui1,2, Shahar Alon1,2,3, Daniel Goodwin1,2, Anubhav Sinha1,4, Asmamaw T. Wassie1,2,5,6, Fei Chen1,7, Edward S Boyden1,2,5,6,8,9

McGovern Institute, MIT1, Media Arts and Sciences, MIT2, Faculty of Engineering, Bar-Ilan University3, Program in Health Sciences and Technology, MIT4, Department of Biological Engineering, MIT5, Department of Brain and Cognitive Science, MIT6; Broad Institute of MIT and Harvard7, Howard Hughes Medical Institute8; Koch Institute for Integrative Cancer Research, MIT9

* Presenting author

Metastatic breast cancers cause overwhelming numbers of deaths. During metastasis, disseminated tumor cells arrive at secondary sites where their fates are determined by cell intrinsic features, as well as the microenvironment. Understanding such early stages of the metastatic process is key to managing disease. However, a high-resolution spatial mapping method is a prerequisite for mapping the relationship between a disseminated tumor cell and its neighboring cells.

Next-generation sequencing of RNA has transformed the exploration of molecular components including different cancers. However, available methods either homogenize tissue, or dissociate cells, before sequencing, thus loses the spatial information. In situ mapping of RNA preserves spatial information, but earlier multiplexed methods for spatial mapping of RNA do not achieve such high resolution to understand molecular characteristics at single cell or sub-cellular resolution.

To this end, we have adapted expansion microscopy, which physically magnifies tissues through a chemical process, and combined it with targeted in situ RNA sequencing. It is called targeted expansion sequencing (Targeted ExSeq). Our method can detect a pre-defined set of genes to map cell types, cell states, and their spatial relationship, in situ. Moreover, it has spatial and axial resolution to resolve nanoscale morphological features in cells and tissues.

Targeted ExSeq was implemented to sequence human metastatic breast cancer infiltrates to the liver. Sequencing of 297 genes of interest led to 771,904 mappable reads in 2395 DAPI-segmented nuclei. Our data furthermore showed sub-cellular nanoscale mapping of 516 RNAs inside nuclear structures <1um in size which are otherwise challenging to detect using any prior methods. Expression clustering revealed spatial relations between tumor cells and immune cells which may point to potential influences on each other.

We have improved our method beyond the initial published state, using a new epoxy-based multifunctional anchoring chemistry which allows mapping of multiple types of molecules including RNA and protein at high resolution. This reduces cost by 50-fold over our earlier version. Using a new chemistry, we have sequenced spatial transcriptomes of patient-derived xerograph (PDX) cancer tissues. With a panel of 87 genes, and 3,339 cells, we can spatially classify, and map, tumor cells according to their expressed gene functions and pathways.

Finally, we are developing targeted ExSeq to map single cells at high resolution within an entire organ. Our technology will enable detection of early events of metastasis, when a cell arriving at a distant site decides whether to initiate proliferation to enter metastasis, to remain indolent, or potentially is recognized and cleared by the immune system.
### Poster 9

**Human organoid models to understand the progression towards liver cancer**

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Human organoids provide a unique platform to study the effect of a specific gene mutation in a wild type context, allowing to address the early events of tumorigenesis as well as gene function. This has been accomplished using different CRISPR-Cas9 genome engineering strategies, including the generation of gene knock-outs, but also recreating exact point mutations using next-generation tools, such as base-editing or prime editing. Our lab has shown the feasibility of such approaches in the context of modelling a variety of cancers, including colon cancer and elucidating the roles of specific cancer genes, such as BAP1 in liver cancer. In our recent work, we have generated a set of novel human organoid models to study a metabolic disease that confers high susceptibility to liver cancer: non-alcoholic fatty liver disease (NAFLD). Using human hepatocyte organoids conjugated to the use of dietary challenges and diverse CRISPR engineering, we modelled the first stage of NAFLD, steatosis. These novel models reflect different steatosis triggers: free fatty acid loading, inter-individual genetic variability (PNPLA3 I148M), and monogenic lipid disorders (APOB and MTTP mutations). Screening of drug candidates revealed compounds effective at resolving steatosis. Mechanistic evaluation of drug action uncovered repression of de novo lipogenesis as the convergent molecular pathway. Exploiting the APOB- and MTTP-mutant organoids, we developed a CRISPR screening platform to identify steatosis modulators and putative targets. From a screen targeting 35 genes implicated in lipid metabolism and/or NAFLD risk, fatty-acid desaturase 2 (FADS2) emerged as an important determinant of hepatic steatosis. Increasing FADS2 activity increases polyunsaturated fatty acid abundancy, which in turn reduces de novo lipogenesis. In our current and future work, we are invested to explore these models to understand the progression from early NAFLD to late-stage NAFLD, including non-alcoholic steatohepatitis (NASH) and liver cancer. In particular, we are studying the biology underlying genetic predisposition in NAFLD and the associated increased susceptibility of these individuals to develop clinical liver cancer. We aim to do so by studying mutational interactions under dietary challenges in our novel organoid models, as well as by creating complex co-culture organoid models to study niche interactions. Ultimately, a better understanding of liver disease progression may lead to the identification of novel targets to halt and/or reverse disease progression to prevent liver cancer development.

### Poster 10

**High Throughput Data and Analysis Infrastructure**

Eduardo A. González-Solares (1)*, Ali Darius (1), Alireza Molaeinezhad (1), Mohammad Al Sa’d (1), Leigh Smith (1), Tristan Whitmarsh (1), Neil Millar (1), Dario Bressan (2), Nicholas A. Walton (1)

1. Institute of Astronomy, University of Cambridge, Cambridge, UK  
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Challenges of the increasing amount of data produced by current and future instrumentation include the need of available pipelines that can process these data routinely in a timely fashion and data access mechanisms that make working with large datasets achievable.

With the aim of producing a 3D representation of tumours, IMAXT uses a large variety of modalities in order to acquire tumour samples and produce a map of every cell in the tumour and its host environment. With the large volume and variety of data produced in the project, we developed automatic data workflows and analysis pipelines.

We also introduce a research methodology where scientists connect to a cloud environment to perform analysis close to where data are located, instead of bringing data to their local computers.

Here we present the data and analysis infrastructure, discuss the unique computational challenges and describe the analysis chains developed and deployed to generate molecularly annotated tumour models. Registration is achieved by use of a novel technique involving spherical fiducial marks that are visible in all imaging modalities used within IMAXT. The
The identification of the cancer cell of origin (COO) in which the first initiating mutation is acquired, leading to clonal outgrowth and malignant progression, is a fundamental question in cancer biology. Here, we address this question using a mouse skin carcinogenesis model in which tumours are inducible knockdown of Lgr6 results in significant inhibition of skin tumour development in vivo, and longer mouse survival. Lgr6+ SCs initiated in vivo by Dimethylbenzanthracene (DMBA) show the characteristic mutational signature induced by this carcinogen, and respond to tumour promoter

**Erin Runbeck, research associate, NexTGen, Children’s Hospital of Philadelphia, US**

**Poster 11**

Erin Runbeck1*, Nick Hartnett1, Keelan O’Reilly1, Quinlin F. Marshall1, Ben R. Kiefel2, Matt Beasley2, Mark Yarmarkovich1, John M. Maris1,3.

1Center for Childhood Cancer Research, Division of Oncology, Children’s Hospital of Philadelphia, Philadelphia, PA, USA
2Myrio Tx, Melbourne, Victoria, Australia
3Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Background: The 5-year survival rate of high-risk neuroblastoma remains below 50%. Recently, the Maris laboratory has demonstrated the targeting of aberrantly expressed nonmutated peptides derived from intracellular oncoproteins and complexed with Human Leukocyte Antigen (HLA) in neuroblastoma with peptide-centric CAR (PC-CAR) T cells. The restriction of Preferentially Expressed Antigen in MElanoma (PRAME) to tumor tissue has been shown across multiple cancer types, including neuroblastoma, and thus would be an appropriate target for PC-CAR therapy. Here, we aimed to show proof-of-concept for PC-CAR T cells targeting the PRAME-derived peptide SLLQHLIGL presented by HLA A*02.

Methods: Candidate scFvs were developed through the ReD platform at Myrio Tx and screened for specificity and activity as second-generation CARs. The second-generation CAR included a CD3z signaling domain and CD28 hinge/transmembrane and costimulatory domains. This construct was expressed in human primary T cells using lentiviral transduction and tested for specific binding to the target using flow cytometry. Cytotoxicity of the PC-CAR T cells was determined by analyzing loss of GFP signal from stably expressing target cells on the Incucyte platform in a panel of 7 HLA A*02 cell lines.

Results: Eight PRAME-directed scFvs were screened and the clone PR2294 was prioritized. PR2294 showed specific binding to the PRAME-HLA*A02+ colorectal carcinoma cell line SW620 pulsed with target peptide and not to SW620 pulsed with irrelevant peptide. Additionally, primary T cells expressing the PC-CAR construct PR2294-28z exhibited binding to an SLLQHLIGL-A*02 dextramer, but not an A*02 dextramer loaded with an irrelevant peptide. PR2294-28z showed potent cytotoxicity against exogenously and endogenously PRAME-expressing A*02+ cell lines across effector to target (E:T) ratios of 10:1 to 1:1. Cytotoxicity was accompanied by IFNγ release. Two neuroblastoma cell lines were resistant to PRAME PC-CAR mediated killing. It was hypothesized that this was due to lower levels of MHCI and PRAME expression. Pre-treatment with the demethylator decitabine increased MHCI and PRAME expression and sensitized these cells to PR2294-28z T cells.

Conclusion: The PC-CAR construct PR2294-28z is effective at specifically targeting the pan-cancer antigen PRAME in A*02+PRAME+ neuroblastoma, glioblastoma and melanoma cell lines. Ongoing preclinical trials of this PC-CAR in murine models will be presented.

**Eve Kandyba, post doctorate, PROMINENT, University of California, San Francisco, US**

**Poster 12**

Chemically-induced skin tumours arise from long-lived stem cells of the upper hair follicle

Eve Kandyba*, Yun Rose Li, Arnaud Jabouille, Matvei Khoroshkin, Hani Goodarzi, David Quigley, Diana Cristea, Quan Tran, Di Wu, Reyno Del Rosario, Jesse Salk and Allan Balmain

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The identification of the cancer cell of origin (COO) in which the first initiating mutation is acquired, leading to clonal outgrowth and malignant progression, is a fundamental question in cancer biology. Here, we address this question using a mouse skin carcinogenesis model in which tumours are induced by sequential exposure to mutagens and tumour promoting factors. In contrast to tumours from genetically engineered mice, which have very few point mutations, this model more closely replicates human cancer etiology and clonal selection by exposure to environmental carcinogens. We employed fluorescent lineage tracing of progeny cells derived from several independent skin stem cell (SC) populations, together with single cell transcriptomics and Duplex sequencing, to identify the COO of chemically induced skin tumours in vivo. We demonstrate that skin tumours arise predominantly from Lgr6+ and/or Lrig1+ SCs of the upper hair follicle, but only very rarely from Lgr5+ and Krt19+ hair follicle bulge SCs. Lgr6 is not only a COO marker for these tumours, but is essential for their clonal outgrowth, as inducible knockdown of Lgr6 results in significant inhibition of skin tumour development in vivo, and longer mouse survival. Lgr6+ SCs initiated in vivo by Dimethylbenzanthracene (DMBA) show the characteristic mutational signature induced by this carcinogen, and respond to tumour promoter
treatment resulting in clonal expansion of initiated cells preferentially carrying the canonical Hras Q61L mutation. These Lgr6+ initiated SCs can remain dormant in mouse skin for 6–12 months, and are still capable of forming tumours after exposure to tumour promoters, thus fulfilling the criteria established decades ago for the initiation of carcinogenesis. Collectively, our data identify upper hair follicle SCs as functional targets for chemical initiation and promotion of skin carcinogenesis.

Evi Karali, senior research scientist, Rosetta, The Institute of Cancer Research, UK

Poster 13
Deconstructing inter- and intra-tumour metabolic heterogeneity in breast cancer using a multi-modal imaging approach.

Evdokia Karali*1, Emine Kazanc1,2, Paolo Inglese2, Manas Kohli1, Chelsea J. Nikula3, Alan M. Race3,5, Weiwei Zhou3, Shreya Sharma4, Najah Islam4, Nikolaos Koundouros1, Aurelien Tripp1, Athanasios Tsalikis1, John Marshall4, Josephine Bunch3, Zoltan Takats2, George Poulougiannis1


Molecular heterogeneity is a predominant challenge in cancer medicine and is a common phenomenon within breast tumours. Tumour heterogeneity frequently impacts prognosis and drug response. As our knowledge of cancer metabolism has increased, it has become apparent that cancer's metabolic processes are extremely heterogeneous too. Metabolic reprogramming is a hallmark of malignancy that has been widely acknowledged in recent literature. The causes behind this heterogeneity include genetic diversity, the existence of multiple and redundant metabolic pathways and altered microenvironment conditions. Among human tumours, metabolic heterogeneity poses a challenge to developing effective targeted therapies. Consequently, it is crucial to approach this with an unlabelled yet spatially specific read-out of metabolic and genetic information.

Hence, in this study, we aim to map the inter-and intra-tumour metabolic heterogeneity in breast cancer by integrating a multimodal Mass Spectrometry Imaging (MSI) based mapping strategy, incorporating Desorption Electrospray Ionization (DESI) and Matrix Assisted Laser Desorption Ionisation Mass Spectrometry (MALDI), with Imaging Mass Cytometry (IMC) analysis of the tumour sections, to provide explicit metabolic, structural and molecular pathway marker information. Successive to this deep tissue metabolic cartography, high-throughput genetic characterisation employing whole exome next generation sequencing and transcriptomics was used to reveal potential key genetic drivers of metabolically discrete subtypes.

The multimodal analysis workflow was initially performed using consecutive breast cancer primary tumour sections, as well as wild-type and mutant PIK3CA Patient-Derived Xenograft (PDX) models. Following MSI acquisition, data from spatial segmentation of tissues revealed the presence of intra- and inter-tumour metabolic heterogeneity within and between samples, respectively. The degree of this was assessed by the existence of discrete ion clusters within cancerous regions. These clusters facilitate the segmentation of tumour areas into metabolically distinct sub-regions that were processed for genomic profiling or transcriptomic analysis.

Felipe Galvez Cancino, post doctorate, NexTGen, University College London, UK

Poster 14
Although at a small frequency, effector CD8 and CD4 T cells are present in GBM tumours, displaying an exhausted phenotype while regulatory CD4 T cells (Tregs) express high levels of the IL2 receptor, CD25, suggesting that these cells are actively suppressing the immune response against GBM. EGFRvIII is the most common rearrangement of amplified EGFR genes, located in the cell surface of tumour cells which makes it a good candidate for the development of therapeutic antibodies. As EGFRvIII is expressed in up to a 60% of all GBMs, these tumours could be therapeutically targeted with antibodies against Tregs and/or EGFRvIII. Our laboratory has developed the new anti-CD25NIB that depletes Tregs via antibody dependent cell cytotoxicity (ADCC) by engaging activating Fc receptors on macrophages and NK cells, whilst preserving IL2 signalling on effector CD4 and CD8 T cells. The anti-CD25NIB in mouse models of GBM has shown that one dose promotes effector CD8 and CD4 T cell activation that results in a 60% survival. This effect is accompanied by the recruitment of monocytes derived macrophages expressing high levels of activating Fc receptors which can be concomitantly engaged with another ADCC optimized antibody. Coadministration of anti-CD25NIB together with an Fc-optimized anti-EGFRvIII antibody led to a synergistic effect with complete elimination of all GBM tumours in the mice. Finally, using a newly developed human GBM explant system we have shown that the human version of our anti-CD25NIB can efficiently deplete Tregs whilst promoting CD8 T cell activation. These results suggest that Treg depletion can be used as a strategy to increase the overall density of FcγRs in the tumor, allowing for the development of novel combination therapies.
Frank McKeon, investigator, STORMing Cancer, University of Houston, US  
**Poster 15**  
**Targeting Clonogenic Cells of the Barrett’s-Dysplasia-Cancer Axis**  
Wa Xian*, Jaffer Ajani, Frank McKeon  
1 University of Houston, 2 MD Anderson Cancer Center  
The concepts surrounding the cancer stem cell and its role in tumor progression and chemotherapy resistance remain in flux. Here we employ novel single-cell technology to clone patient-matched stem cells of Barrett’s, dysplasia, and esophageal adenocarcinoma (EAC). These clones meet all stem cell criteria and respectively differentiate to intestinal metaplasia, low- and high-dysplasia, and cancer. Genomic analysis of these clones readily details the phylogenetics underlying the evolution of precursors to the index EAC, as well as clades that did not. Unexpectedly, dysplasia and EAC stem cell clones display a near absolute genomic stability throughout cloning, expansion, and months of tumor formation in xenografts, arguing for a prior “Big Bang” source of intratumor diversity and against genomic instability in the evasion of chemotherapeutics. Finally, we leverage Barrett’s and normal mucosal stem cells in parallel drug screens for prospective preemptive therapeutics, and show these same drugs simultaneously and effectively eliminate stem cells of dysplasia and EAC.

Hilary Stobart, patient advocate, PRECISION  
**Poster 16**  
Team: PRECISION  
Challenge: Distinguish between lethal cancers that need treating, and non-lethal cancers that don’t.

Ignacio Vázquez-García, research fellow, IMAXT, Memorial Sloan Kettering Cancer Center, US  
**Poster 17**  
**Ovarian cancer mutational processes drive site-specific immune evasion**  
Ignacio Vázquez-García*†, Florian Uhlitz*†, Nicholas Ceglia, Jamie L.P. Lim, Michelle Wu, Neeman Mohibullah, Juliana Niyazov, Arvin Eric B. Ruiz, MSK SPECTRUM Consortium, Andrew McPherson, Britta Weigelt, Dmitriy Zamarin‡, Sohrab P. Shah‡  
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‡Senior authors  
Genomic instability is a hallmark of human cancer, with fundamental relevance to cancer etiology and evolution, anti-tumor immunity and therapeutic response. High-grade serous ovarian cancer (HGSOC) is an archetypal cancer of genomic instability defined by distinct mutational processes, intraperitoneal spread and tumor heterogeneity. As immunotherapies have thus far proven ineffective in this disease, we sought to establish the determinants of immune recognition and evasion in its natural disease history. We studied the impact of mutational processes and of spatial heterogeneity on cellular phenotypes in the tumor microenvironment (TME), using genome-based stratification of homologous recombination proficient (HRP) and deficient (HRD) disease subtypes, profiling single cell phenotypes from ~1 million cells including cancer cells, T cells, myeloid cells and fibroblasts derived by single cell RNA sequencing, and in situ multiplexed immunofluorescence of cancer cell, T cell and macrophage states of 160 tumor sites obtained from 42 treatment-naive patients.  
Mutational processes in HRD-Dup (BRCA1 mutant-like) tumors were associated with cancer cell-intrinsic JAK/STAT signaling and predominance of dysfunctional CD8+ T cells in the TME; HRD-Del (BRCA2 mutant-like) tumors were linked with expansion of M2-type macrophages; and foldback inversion (FBI, HRP) tumors were associated with cancer cell-intrinsic TGFβ signaling and immune exclusion, with predominantly naive T cells. HLA loss of heterozygosity (LOH) was a common mechanism of immune evasion in the HRD, but not FBI tumors, connecting evolutionary selection with immune states.
Multi-region sampling revealed substantial spatial variation at the anatomical scale, highlighting site-specific properties of the ovary and fallopian tube as putative "immune-privileged" sites, and suggesting that local microenvironments of organ sites can direct immune pruning in patients with widespread disease. At the cellular level, spatial topology was a major determinant of tumor-immune interactions based on in situ spatial profiling, which revealed site-specific PD1-PDL1 interactions in HRD tumors. This profound intra-patient spatial variation in CD8+ T cell states directly impacted malignant clone diversity, indicating both active immune pruning and acquired immune evasion capacity.

Together, our findings yield mechanistic insights for how distinct mutational processes in HGSOC lead to diverse patterns of intrinsic factors associated with altered body composition and CAC; including bulk tumour genomic and transcriptomic analysis, as well as plasma proteomics. Primary NSCLC tumours from patients in the CAC group were characterised by enrichment of inflammatory signalling and epithelial-mesenchymal transitional pathways, with differentially expressed genes including the opossum LBP and matrix metalloproteases, such as ADAMTS3. In an exploratory analysis of circulating factors associated with CAC, proteomic evaluation of 256 plasma samples revealed a significant association between a putative pro-cachetic mediator – Growth Differentiation Factor 15 (GDF15) – and loss of body weight, SKM, SAT and VAT at relapse. GDF15 is a highly conserved member of the Transforming Growth Factor β (TGF-β) superfamily, which has been shown to be elevated in mouse models of cachexia. Moreover, there is pre-clinical evidence that animal cachexia-like phenotypes can be reversed with administration of anti-GDF15 pathway targeted monoclonal antibodies. Orthogonal validation of our proteome results with an ELISA-based serological assay demonstrated that GDF15 levels are differentially elevated in the CAC group at both baseline and relapse – suggesting that serum GDF15 may have relevance as a clinically-applicable measurement in human cachexia.

Overall, our findings infer a key role for altered body composition in determining lung cancer prognosis and add to the wider literature in providing a rationale supporting GDF15 as a promising therapeutic target in the management of cancer-associated cachexia.
Dozens of unique mutational signatures have been identified in human genomes, from both normal tissues and cancer. Some mutations are caused by endogenous processes within cells (e.g., spontaneous deamination of cytosine) and some are caused by exposure to DNA-damaging chemicals or radiation, such as tobacco smoke or UV light. The origins of many signatures are clear, yet the aetiology of a large number remains unknown. As part of the Mutographs of Cancer team, our research group aims to better understand some of the mutational signatures observed in people by investigating the experimentally-derived mutation patterns of genotoxic environmental carcinogens and chemotherapeutics in cell-based models. We previously extracted whole-genome mutational signatures from human induced pluripotent stem cells treated with 79 agents and identified 41 characteristic single base substitution (SBS) signatures, some of which were highly similar to signatures found in human tumours. More recently, we have been using organoid cultures derived from normal human tissues to expand upon that work. These three-dimensional cultures are self-renewing stem cell populations, as well as many tissue-specific, differentiated cell types, and therefore serve as models that more closely mimic normal tissue. In combination with a new, genome-wide, duplex sequencing technology termed nanorate sequencing (NanoSeq), we have examined mutations caused by a panel of environmental and chemotherapeutic agents in organoids. NanoSeq enables highly sensitive, error-free detection of subclonal mutations, eliminating the laborious and time-consuming step of single cell cloning in experimental mutation assays. After treating organoids derived from 5 tissues – stomach, colon, kidney, pancreas and liver – with several environmental carcinogens (aristolochic acid I [AAI], benzo(a)pyrene, aflatoxin B1 and 2-amino-1-methyl-6-phenylimidazo[4-5-b]pyridine [PhIP]), we found that each organoid model accumulated mutations as detected by NanoSeq. Using SigProfiler we extracted carcinogen-specific mutational signatures consistent with those previously identified by conventional whole-genome sequencing. Further, applying this approach to stomach organoids treated with 30 different chemotherapeutics, we identified an SBS signature for temozolomide that matches a signature found in human tumours associated with temozolomide treatment (COSMIC SBS11), as well as SBS signatures for mitomycin C and nitrogen mustard alkylating agents (e.g. chlorambucil), among others. Overall, this work shows that organoids are a very useful tool in the study of environmental carcinogens and genetic toxicology and that NanoSeq is a key advance in mutation detection from experimental model systems.

| Kim Rhoads, patient advocate, PROMINENT |
| Poster 20 |
| Team: PROMINENT |
| Normal Phenotypes: Understand how cells and tissues maintain "normal" phenotypes whilst harbouring oncogenic mutations and how they transition to become a tumour. |

| Kelsey Thompson, research associate, OPTIMISTIC C, Harvard T.H. Chan School of Public Health, US |
| Poster 21 |
| Identifying strain-specific associations in colorectal cancer |

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Colorectal cancer (CRC) is the second most commonly diagnosed malignancy in women and the third in men, and accounts for around 10% of all deaths related to cancer. The progression from healthy intestinal cells, to benign tumors (adenomas), and then to more malignant forms has profound impacts on the composition of the intestinal microbiota. Additionally, the factors influencing this progression are idiopathic but likely involve a combination of genetics, local tumor environment, and extrinsic factors such as diet. Here, we focus on further elucidating the role of the gut microbiome, a large component of the tumor microenvironment, in cancer initiation and progression by considerably expanding on the current largest meta-analysis to include a total of 3,395 samples from 17 public and private studies. Through expanded sample size, increased resolution of the computational tools, and bioinformatic advances we have improved the understanding of the gut ecosystem in CRC. We found several taxa from the Solobacterium group and Clostridia groups enriched in CRC, and several unknown taxa enriched in healthy individuals. However, as has recently been observed with pks+ E. coli, while species-level identification does provide valuable insights; strain-level resolution can often provide the most actionable downstream targets and help to further elucidate the basic biology of the system. Thus, we developed ANPAN, a collection of statistical methods for microbial strain analysis that can identify associations between several different types of microbial genetic variation and host health outcomes (particularly CRC). This collection of methods includes gene-level, phylogenetic, and pathway-level models. When applied to our dataset, the gene model identified 26 transposases or transposable elements associated with CRC status across 16 species, potentially indicating a role for these genes in helping the microbes acquire other genetic elements.
necessary to adapt to the inflammatory microenvironment of the CRC gut. Meanwhile, the phylogenetic model identifies strong species-wide phylogenetic signals in several species, including species’ typically found to be CRC-associated: Ruminococcus gnavus, Clostridium leptum, Bacteroides fragilis, which could indicate these species have clades with variable CRC risk.

Khanh Dinh, postdoctoral researcher, IMAXT, Columbia University, US
Poster 22
Modeling and simulation of cancer evolution in single cells
Khanh N. Dinh*1, Rhea Malhotra2, Ignacio Vázquez-Garcia1,3, Simon Tavare1
1 Columbia University
2 Stanford University
3 Memorial Sloan Kettering Cancer Center

It has been apparent during the DNA sequencing era that cancer is characterized both by small point mutations and indels affecting driver genes and large-scale copy number aberrations (CNAs) and structural variants. We have created a mathematical framework for modeling cancer evolution as driven by creation and selection of both of these mechanisms. The framework allows for adaptive cancer-specific population dynamics through negative feedback, and implementation of distinct selection models. Moreover, we have developed an algorithm that can simulate realistically large cell populations, yet retain statistical accuracy in the sampled cells’ phylogeny, while maintaining runtime to the level that is practical for simulation-based parameter inference. We used the model to explore the interplay between selection and heterogeneity, and between Whole Genome Duplication and Copy Number Aberrations (CNAs) of smaller scale. The model has been fitted to PCAWG data and provided a mechanical link between frequencies of gains and losses of chromosome arms and their distributions of Tumor Suppressor Genes (TSGs) and oncogenes, which has been validated with experimental pan-cancer measures. Finally, we develop a fitting mechanism to infer selection forces and CNA rates from single-cell DNA-seq data (particularly DLP+).

Lluvia del Rio, patient advocate, PROMINENT
Poster 23
Team: PROMINENT

Normal Phenotypes: Understand how cells and tissues maintain "normal" phenotypes whilst harbouring oncogenic mutations and how they transition to become a tumour.

Maria Caterina Rotiroti, post doctorate, NexTGen, Stanford University School of Medicine, US
Poster 24
Recruiting proximal signaling molecules to enhance CAR T cell activity
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Chimeric antigen receptor (CAR) T cells have demonstrated remarkable clinical activity in children with relapsed/refractory B cell acute lymphoblastic leukemia (B-ALL). However, approximately 50% of B-ALL patients relapse after CAR T cell treatment, and additional curative treatment options are nearly non-existent. Antigen escape with loss or downregulation of the target antigen has emerged as a major mechanism of resistance. As they are currently designed, CAR T cells do not sufficiently activate in response to tumor cells expressing low levels of their target antigen because they are inefficient at recruiting proximal signaling molecules to the immune synapse. Novel designs are required to target cancers with heterogeneous antigen expression.
We hypothesized that overexpression of proximal signaling molecules could improve the performance of CAR T cells and amplify the response to low antigen density tumor cells. We compared overexpression of LCK, LAT, and SLP-76 and found that SLP-76 overexpression significantly improved CAR T cell functionality; this effect was further enhanced when we engineered a novel membrane tethered version of SLP-76 (MT-SLP-76). MT-SLP-76 overexpression resulted in substantially enhanced cytokine production in response to tumor cells expressing high, moderate, and low antigen densities. In vivo, in a model of CD22 low leukemia, CD22 CAR T cells overexpressing MT-SLP-76 mediated tumor eradication, while wild-type CD22 CAR T cells failed. Early mechanistic studies demonstrate that the observed enhancement requires recruitment of the downstream signaling molecules ITK and PLC-y. Additional experiments will better elucidate how MT-SLP-76 cooperates with these and other native T cell signaling molecules to compensate for impaired CAR signaling. In conclusion, we have identified that overexpression of MT-SLP-76 can endow CAR T cells with the capacity to target low antigen density expressing tumor cells. These results provide a valuable resource to overcome resistance through antigen downregulation and to expand the use of the CAR technology to solid tumors, which manifest substantial heterogeneity in target antigen expression.

Mark Taylor, post doctorate, PROMENT, University of California, San Francisco, US
Poster 25
Adult mammalian stem cells play critical roles in normal tissue homeostasis, as well as in tumour development, by contributing to cell heterogeneity, plasticity, and development of drug resistance. The relationship between different types of normal and cancer stem cells is highly controversial and poorly understood. Single-cell expression profiles provide insight into how stem cells and their progeny evolve, but relatively few independent single-cell samples can be assayed with current methods. However, hundreds of bulk-tissue tumour samples have been assayed which each represent independent evolutionary outcomes. Here, we summarized these outcomes with gene expression networks of normal and tumour samples from genetically heterogeneous mice to create network metagenes. We then visualized stem-cell metagenes, rather than individual stem-cell markers, at the single-cell level during multistage carcinogenesis. We combined this approach with lineage tracing and single-cell RNAseq of stem cells and their progeny, identifying a previously unrecognised hierarchy in which Lgr6+ stem cells from tumours generate progeny that express a range of other stem-cell markers including Sox2, Pithx1, Foxa1, Klf5, and Cd44. Lgr6 is a stem-cell marker that is expressed by the cell of origin that responds to initiators and promoters, and it is necessary for wound healing and tumor promotion. Our data identify a convergence of multiple stem-cell and tumour-suppressor pathways in benign tumour cells expressing markers of lineage plasticity and oxidative stress. This same single-cell population expresses network metagenes corresponding to markers of cancer drug resistance in human tumours of the skin, lung and prostate. Our data have allowed us to create a simplified model of multistage carcinogenesis that identifies distinct stem-cell states at different stages of tumour progression, thereby identifying networks involved in lineage plasticity, drug resistance, and immune surveillance, providing a rich source of potential targets for cancer therapy.

Max White, PhD student, OPTIMSTICC, Johns Hopkins University, US
Poster 26
A procarcinogenic bacterial metalloprotease binds claudin-4 to mediate toxicity on the colonic epithelium

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Colorectal cancer (CRC) is the second most common cause of cancer-related deaths worldwide and is increasing in young adults. In recent years, select members of the gut microbiota have been recognized as putative CRC risk factors that may drive a subset of sporadic CRCs. One such microbe, enterotoxigenic Bacteroides fragilis (ETBF), triggers colonic inflammation and tumorigenesis through the action of a single metalloprotease, the Bacteroides fragilis toxin (BFT). BFT was previously shown to function by binding to an unknown colonic epithelial cell (CEC) receptor and triggering cleavage of the adherens junction protein E-cadherin, leading to epithelial barrier disruption and activation of the procarcinogenic Wnt/β-catenin signaling pathway. However, BFT’s receptor and the mechanism by which BFT triggers E-cadherin cleavage remain unknown. To identify the BFT receptor, we performed a CRISPR-knockout screen in the colon carcinoma cell line HT29 for genes critical to BFT-mediated E-cadherin cleavage, revealing the tight junction protein claudin-4 as the top hit. Subsequent knockout of claudin-4, but not the highly homologous claudin-3, in HT29 cells both conferred substantial BFT resistance and ablated BFT binding to the cell surface, leading to the hypothesis that claudin-4 is a BFT receptor critical to its toxicity. BFT was subsequently shown to co-immunoprecipitate with claudin-4 in toxin-treated HT29 cells, and ectopic expression of claudin-4 in claudin-negative HEK293-T cells conferred the ability to bind BFT, supporting the conclusion that claudin-4 is a BFT receptor. While BFT was unable to cleave E-cadherin ectopically expressed in HEK293-T cells, co-expression of claudin-4 enabled cleavage. Based on these data, we propose a
model in which binding to claudin-4 induces conformational changes in BFT that enable recognition and cleavage of E-cadherin. These results pave the way for the development of claudin-4-based anti-BFT therapeutics for the treatment and/or prevention of ETBF-mediated diseases including acute diarrhea and potentially a subset of CRCs.

Michael Olanipekun, postdoctoral researcher, Mutographs and PROMINENT, International Agency for Research on Cancer, FR

Poster 27

Understanding the Influence of Mutational Signatures on the Metabolome of Renal Cell Cancer

Michael Olanipekun*, Aida Ferreiro, Thomas Cattiaux and Paul Brennan
Genomic Epidemiology (GEM), International Agency for Research on Cancer (IARC), World Health Organisation, Lyon, France

Renal Cell Cancer (RCC) is a leading cause of cancer death in Europe. RCC has high incidence rates in specific European regions and is linked to various risk factors including obesity, tobacco smoking and hypertension. These risk factors, however, do not explain the incidence rates seen in these geographical regions, suggesting other exogenous and endogenous factors could be responsible.

To explore this, 962 patient samples were collected from 11 countries (Czech Republic, Russia, United Kingdom, Brazil, Canada, Serbia, Romania, Japan, Lithuania, Poland, and Thailand) and subjected to whole genome sequencing (WGS), from which somatic mutational signatures were extracted. In addition, untargeted metabolic profiling was performed on 901 plasma samples from these patients, yielding 2,392 features (post-QC and filtering).

These analyses identified a mutational signature (SBS40b) to associate with increasing RCC incidence and was found in individuals from each country. Furthermore, enrichment analysis revealed tryptophan and vitamin E (tocopherol) metabolic pathways to associate with some of the key mutational signatures identified. This suggests a role of tryptophan metabolite serotonin and tocopherol metabolite 3'-carboxy-alpha-chromanol as linked to the mutational landscape of RCC and these networks could propose novel aetiologies for mutational signatures where their causes are still unknown. Importantly, a blood-based biomarker of renal function was additionally identified and associated with SBS40b. These results taken add to the growing body of evidence implicating nephrotoxicity as a cause of RCC.

Through characterising the mutational and metabolic signatures of RCC the endogenous and exogenous causes of RCC were explored in a major step towards exposing the mechanisms underlying RCC onset.

Nicholas Neill, staff scientist, SPECIFICANCER, Baylor College of Medicine, US

Poster 28

Integrative proteogenomics and forward genetics reveals a novel mitotic vulnerability in triple-negative breast cancer

Nicholas J. Neill*(1,2), Shankha Satpathy(3), Karsten Krug(3), Jitendra K. Meena(1), Lacey E. Dobrolecki(4), Alaina N. Lewis(4), Christina Sallas(4), Meenakshi Anurag(4,5,6), Beom-Jun Kim(4), Sufeng Mao(4), Heyuan Li1(1), Amrittha Nair(1,2), Tingting Sun(1), Hsiang-Ching Chung(1), Timothy D. Martin (14, 15), Filip Mundt(3), D.R. Mani(3), Michael A. Gillette(3), Mei-Yin Polley(11), Susan G. Hilsenbeck(4,5), C. Kent Osborne(5,10), Michael T. Lewis(4,12,13), Matthew J. Ellis(4,5), Steven A. Carr(3), Teresa Davoli(8), Stephen J. Elledge(14,15), Thomas F. Westbrook(1,2,5,7,8,12)

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Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with a poorly understood molecular etiology and no effective targeted therapies. Taxanes and other mitosis-stabilizing agents are frontline chemotherapies for TNBC and their potency is attributed to the inherent mitotic instability of TNBC, a hallmark of the disease. However, the molecular pathways that cause TNBC mitotic instability are largely unknown, thus preventing the rational selection of taxane-responsive patients and the development of new therapeutic strategies that selectively target this vulnerability of TNBC. In this study, we searched for tumor-selective vulnerabilities in TNBCs with dysfunctional PTPN12, a commonly inactivated tumor suppressor in TNBC. By integrating proteogenomic characterization of PDX models and functional genetic screening, we discovered that inactivation of PTPN12 drives defects in cell cycle progression and mitosis through dysregulation of the anaphase promoting complex (APC). PTPN12-deficient TNBCs exhibit decreased expression and activity of multiple APC substrates that are critical for proper mitotic progression. Moreover, inhibition of the APC restores the expression of these substrates and rescues the mitotic defects observed in PTPN12-deficient cells. Consistent with their compromised mitotic fidelity, primary TNBC PDX models and human TNBC patients harboring germline or post-translational loss of PTPN12 exhibit heightened sensitivity to taxane chemotherapy that exacerbates these mitotic defects. Collectively, these data suggest that dysregulation of the PTPN12-APC axis is a common driver of mitotic instability and taxane sensitivity in TNBC and may represent a new therapeutic entry point to exploit mitotic instability in cancer.

Nivetha Ramesh Babu, research assistant, Baylor College of Medicine, US
Poster 29

Tissue-of-origin drives the oncogenic mechanisms and synthetic vulnerabilities of the tumor suppressor PTPN12

Nivetha Ramesh Babu* (1), Nicholas J. Nell(1,2), Nuray Gunduz (3), Alexander P. Raven (3), Timothy D. Martin (9, 10), Jitendra K. Meena(1), Sufeng Mao(5), Amritha Nair(1,2), Tingting Sun(1), Susan G. Hilsenbeck(5,6), George Miles(5), Teresa Davoli(7), Owen J. Sansom (3,4), Stephen J. Elledge(9,10), Thomas F. Westbrook(1,2,5,6,8)

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It has long been recognized that cancer driver genes exhibit tissue specificity in their mutation profiles, and that the impact of a driver mutation on tumorigenesis can vary widely between tissues. However, our understanding of the mechanisms that underlie these phenomena remains poor. Therefore, in the current study we integrated comprehensive molecular profiling and genome-wide synthetic lethality screens to uncover the mechanisms underlying the tissue tropism of the tumor suppressor PTPN12, which is commonly inactivated in breast and colorectal cancer but not other tissues. Transcriptomic, proteomic, and phospho-proteomic profiling in both permissive (breast and colorectal) and non-permissive (pancreas and lung) cell types revealed that PTPN12 inactivation results in hyperactivation of RTK signaling pathways exclusively in permissive cell types, potentially explaining the specificity of PTPN12 alterations to those tissues in human cancer. Furthermore, this profiling also revealed that while activation of downstream markers of cell proliferation, such as CCND1 transcription, was observed in both permissive cell types, surprising differences were seen in the upstream components of the pathway. This finding suggests that therapeutic strategies targeting PTPN12-deficient tumors may need to be tailored to each specific tissue-of-origin. To address this problem and identify additional selective vulnerabilities, we performed in vitro synthetic lethal screens, using both RNAi and CRISPR libraries, in isogenic cell pairs derived from both permissive and non-permissive cell types. From these screens, we discovered that permissive cell types share a common vulnerability to perturbation of mitotic regulators, such as PLK1, which may represent a promising therapeutic strategy to target PTPN12-deficient tumors specifically in permissive cell types. Overall, our work demonstrates the robustness of this approach for identifying novel therapeutic strategies for difficult-to-target cancer drivers and dissecting the mechanisms that underlie the tissue specificity of cancer driver alterations.
### Olesja Popow, postdoctoral research fellow, SPECIFICANCER, Dana-Farber Cancer Institute, US

**Poster 30**

**Dissecting The Tissue-Specific Oncogenic Activity Of K-RasG12D**

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KRAS is the most commonly mutated oncogene in human cancers, but its mutational pattern is restricted to tumors originating in a subset of tissues. Notably, K-Ras expression levels do not correlate with mutation frequency in a given cancer type. Overall the biological mechanisms underlying the tissue-specific mutational pattern of KRAS are not understood.

We hypothesize that while some tissues are inherently sensitive to the oncogenic activity of mutant K-Ras others are non-permissive. Elucidating the molecular properties that distinguish these tissues will provide us with important therapeutic insights.

Utilizing a variety of GEMMs carrying the Cre-dependent KrasLSL-G12D allele allowed us to force expression of activated K-Ras in the entire animal and study the response at the phenotypic and molecular level focusing on ten tissues (pancreas, spleen, colon, small intestine, kidney, liver, lung, heart, skeletal muscle, brain). We used EdU incorporation in combination with fluorescent imaging to measure tissue-wide changes in proliferation. Concurrently, we designed a hierarchical generalized linear model to simultaneously estimate the effects of the experimental conditions and stochastic variation on the number of proliferating cells. We acquired TMT-based (phospho-) proteomic measurements from wild-type mouse tissues to identify patterns between the baseline cell circuitry and changes in proliferation in response to K-RasG12D expression. In addition, we generated a mass spectrometry-based targeted proteomics assay for 180 members of the murine Ras pathway. This tool enabled us to obtain precise measurements for the relative abundance of these partly very low abundant proteins in a small amount of starting material (<100 µg) in less than three hours of MS instrument time.

Our results showed that expression of active K-RasG12D resulted in an increase of ERK-1/2 phosphorylation in most assayed tissues. Consistent with our hypothesis, only a subset of tissues displayed histological changes in response to oncogenic K-Ras expression (colon, spleen, lung, pancreas) whereas the others appeared unaffected. Strikingly, histological changes did not always correlate with changes in proliferation and vice versa. While K-RasG12D expression expectedly increased proliferation in some tissues (colon, lung, spleen) it reduced proliferation others (skeletal muscle, brain, kidney, pancreas), while still others were not affected (liver, small intestine). In the heart expression of K-RasG12D enhanced proliferation in the short- but not in the long-term. Notably, many tissues displayed sex-dependent proliferation responses.

Lastly, utilizing our proteomics data sets we identified cases of strong correlation between the expression of nodes in the Ras signaling pathway and a tissue’s proliferation response to K-RasG12D expression.

### Patrick Loi, PhD student, SPECIFICANCER, Harvard Medical School, US

**Poster 31**

**Polycomb Repressive Complex 2 (PRC2) is a highly conserved developmental regulator that maintains cellular identity by dynamically silencing key genes involved in differentiation. Alterations in PRC2 have been shown to play a driving role in many cancers. EZH2 is the major catalytic methyltransferase of PRC2 and found to be commonly overexpressed in solid tumors. Nevertheless, the role of EZH2 in solid tumors, such as colorectal cancers (CRC) has not been sufficiently explored. Specifically, EZH2 is overexpressed in 66% of CRC, and its expression appears to inversely correlate with patient survival and advanced disease. Thus, EZH2 is an attractive therapeutic target, although its role and targets in CRC is unknown.**

CRC is one of the leading causes of cancer deaths worldwide, and advanced metastatic disease is still incurable. Thus, there is significant unmet clinical need for treatments for CRC, especially those with activating mutations in KRAS. Many drugs that target classic oncogenic kinases are ineffective therapies as single agents, such as MEK inhibitors for KRAS mutant solid tumors. Therefore, one approach is to develop more effective combination therapies that might enhance the sensitivity of cells to MEK inhibitors and/or prevent resistance. Interestingly, we have found that EZH2 inhibitors are frequently effective when combined with MEK inhibitors in KRAS mutant CRC. We hypothesize that co-targeting EZH2 along with key oncogenic pathways may lead to cooperative killing of CRC by clamping down on crucial oncogenic signals at both the kinase level and the transcriptional level.
EZH2 and MEK inhibitors kill KRAS mutant CRC in a variety of in vitro, in vivo xenograft, and organoid models, which reveals a novel approach for treating this advanced disease. Using a combinatorial RNA-seq and CUT&RUN approach, we find that EZH2/MEK inhibitors induce a shift in the differentiation state of the cell by modulating key transcription factors and regulators involved in colonic development. EZH2/MEK inhibitors broadly suppress oncogenic intestinal Wnt signaling pathways and promote an upregulation of markers associated with differentiated intestinal cells. Once differentiated, EZH2/MEK inhibitors kill CRCs by cooperatively triggering apoptotic signals by upregulating the pro-apoptotic protein BMF. We show that BMF is regulated by EZH2 via H3K27me3 repression, and EZH2 inhibition primes cells for apoptosis by the induction of BMF expression. Together, these findings identify a promising therapeutic strategy for advanced CRC and illustrate a new paradigm for epigenetic-based combination therapies to treat aggressive tumor types.

Raquel Blanco, PhD student, PROMINENT, Institute for Research in Biomedicine Barcelona, ES
Poster 31
Deciphering the molecular signatures of cancer promotion through the analysis of normal tissues

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Cancer in adults is thought to mainly originate through the accumulation of mutations over decades. However, some of the specific alterations driving tumorigenesis have also recurrently been found in normal tissues and associated with clonal expansion. This finding and others suggest other non-mutagenic mechanisms are involved in cancer initiation. In relation with this, recent studies have also proven many known carcinogens do not exert their effect through a mutagenic mechanism. Altogether, this raises the possibility that there is a fundamental step in cancer development in which pre-existing mutant cells acquire a growth advantage when exposed to specific promoting stimuli.

The importance of the promotion stage of carcinogenesis was first proposed at the beginning of the 20th century. Berenblum and Shubik used mouse models to demonstrate that initiation needs to precede promotion for cancer to develop and that initiated cells are essentially permanent but completely dormant, unless exposed to additional stimuli. Little is known about the mechanisms allowing mutant cells to persist in normal tissue for long periods of time, or about the factors that stimulate single mutant clones to undergo transition into a preneoplastic state.

This research project, framed within the Normal Phenotypes Cancer Grand Challenge addressed by the PROMINENT team, aims to test the hypothesis that the promotion stage of carcinogenesis constitutes a rate-limiting step in cancer development. For that we will use a collection from IARC of 2000 human tumor and normal tissues of 8 different cancer types coming from high and low cancer risk regions, together with detailed clinical information including the level of exposure to potential promoters. Applying computational methods to data obtained from deep sequencing and single cell profiling, we intend to capture the differences in clonal structure and evolution across the normal tissues of individuals exposed to different promoters. Together with microenvironment and immune infiltrate information, we expect to find specific promotion signatures per cancer type and/or promoter. The results of this study could lead to new strategies to inform cancer prevention more effectively and revert the early stages of tumorigenesis.

Richard Stephens, patient advocate, STORMing Cancer
Poster 32
Team: STORMing Cancer
Challenge: Determine the mechanisms that cause cancer without known mutagenesis, such as obesity, in order to devise novel interventions

Saumya Bollam, PhD candidate, PROMINENT, University of California San Francisco, US
Poster 33
Mechanisms of promotion in Ras mutant mouse skin
The PROMINENT team is addressing fundamental questions of tumorigenesis by exploring mutant cells in healthy and malignant tissue. Recent studies highlighting the presence of mutated cells in healthy tissue have reinforced the idea that oncogenic mutations are not sufficient for the initial stages of tumorigenesis. The additional promotion then required for a mutated cell to begin neoplastic growth may take many forms, such as environmental exposures, tissue damage, chronic inflammation, and others currently unknown. In this study, we aim to investigate the mechanisms by which promotion, or non-mutagenic processes, can influence the selection of mutant cells which eventually populate a tumor. Through the use of a well-established mouse model of chemical carcinogenesis, we can induce tumors with either oncogenic Hras or oncogenic Kras mutations. With this model, we have demonstrated distinct signaling modules that are activated by oncogenic Hras or oncogenic Kras. The proposed study aims to characterize promotion mechanisms as they coordinate with these distinct oncogenic Ras signals. We plan to characterize the transcriptional landscape activated by the promoting agent TPA, and define the coordination between TPA and oncogenic Ras signaling. As team PROMINENT continues to uncover fundamental components of the earliest stages of tumorigenesis, the findings from this study will provide mechanistic understanding of how promotion may influence selection of mutant cells.

Sara Wakeling, patient advocate, NexTGen
Poster 34
Team: NexTGen
Challenge: Develop novel therapies to target unique features in solid tumours in children

Sarah Moody, senior staff scientist, Mutographs, Wellcome Sanger Institute, UK
Poster 35
A mutagenic exogenous exposure present in kidney and liver cancers from Japan

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Evidence of mutagenic exogenous exposures in human cancers can be detected via the distinct patterns of somatic mutations (mutational signatures) they produce. As part of the Mutographs Grand Challenge project, whole genome sequencing was performed on 962 renal cell carcinoma (RCC) cases from 11 countries of varying incidence, to look for evidence of unknown causes of cancer and to investigate the unexplained global variation in RCC incidence rates. Initial analysis of average mutational spectra revealed an enrichment of T>C mutations in Japanese cases (n=36) compared to other countries. Principle component analysis of relative mutation counts was able to separate two clusters from the main population. The first cluster was expected and is due to enrichment of T>A mutations caused by Aristolochic Acid, a mutagenic exogenous exposure previously found in RCC from Romania, with the second cluster consisting of Japanese cases with enrichment of T>C mutations. K-means clustering on the same data was also able to identify a cluster unique to Japan. Both methods suggest a unique component in the RCC mutational spectra in Japan which could be explained by the presence of an exogenous exposure. Using SigProfilerExtractor we extracted a de novo mutational signature which was enriched in Japan but not in any of the other countries studied. Decomposition of this signature to the COSMIC reference signature panel identified a mixture of SBS5 (a clock like signature associated with age found in most normal and cancer tissues) and SBS12 (unknown aetiology). The SBS12 component was subsequently found to be responsible for the observed enrichment of the de novo signature in Japan and was present in 72% of Japanese RCC, whereas SBS5 was found in RCC from all countries. No association between SBS12 activity and RCC risk factors was found. SBS12 was first identified in hepatocellular cancers (HCC) and using ICGC (International Cancer Genome Consortium) datasets which include HCC cases from Japan, we were able to show the same enrichment of T>C mutations in Japan compared to HCC from other countries. In summary, we have identified evidence of a mutagenic exogenous exposure present in both RCC and HCC from Japan. Future studies should focus on identifying the biological mechanism driving this mutational signature and fully establishing the geographic extent of the exposure.
**Poster 36**

**Mutational signatures indicate multiple mutagens in kidney cancer genomes**

Sergey Senkin1*, Sarah Moody2*, Ludmil Alexandrov3,4,5, Michael Stratton2, Paul Brennan1 on behalf of the Mutographs Grand Challenge.

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Global variation in cancer incidence is indicative of unknown mutagenic exposures that are difficult to identify with conventional epidemiology. For clear cell renal cell cancer (ccRCC), established risk factors include obesity, hypertension and tobacco smoking, but these cannot explain the high incidence of this cancer in parts of central and northern Europe. In order to uncover additional risk factors, we have analysed mutational signatures from 962 ccRCC genomes from patients recruited across 11 countries of high and low incidence. Somatic mutational profiles varied across countries, most noticeably in Romania, Serbia and Thailand where two mutational signatures suspected to be caused by aristolochic acid were present in most cases, and largely absent elsewhere. Another signature characterized by T>C mutations was present in >70% of cases from Japan, and <2% of cases elsewhere (p<5 × 10e−78) indicating exposure to an unknown mutagen specific to the Japanese population. Globally, we find evidence of a mutational signature (called SBS40b) that was present in all countries, although at increased levels in countries with higher incidence of ccRCC (p<6 × 10e−18). An untargeted metabolomics scan identified a strong correlation with blood-based markers of general renal function and this mutation signature (p< 7 × 10e−6), implying the presence of a nephrotoxin as an underlying cause of SBS40b. These results highlight the presence of multiple exposures, both known and unknown, which may contribute to renal cancer incidence. No mutational signatures were associated with obesity and hypertension, implying that they act via non-mutagenic mechanisms in causing ccRCC.

**Poster 37**

**A living biobank of patient-derived ductal carcinoma in situ Mouse-INtraDuctal xenografts identifies factors associated with risk of invasive progression**

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Ductal carcinoma in Situ (DCIS) is a non-obligate precursor of invasive breast cancer (IBC) treated similar to early IBC even though the minority of untreated DCIS cases eventually become invasive, due to a lack of biomarkers able to distinguish high- from low-risk DCIS. We provide 130 well characterized patient samples, Mouse-INtraDuctal (MIND) DCIS models reflecting the full heterogeneity observed in DCIS patients. Utilizing the unique possibility to follow the natural progression of DCIS combined with extensive omics and imaging data we reveal multiple prognostic factors for high-risk DCIS including high grade, HER2 amplification, expansive 3D growth and a high copy number aberration burden.
Additionally, sequential transplantation of xenografts showed minimal changes in phenotype and genotype over time indicating invasive behavior is an intrinsic phenotype of DCIS with minimal evolution, supporting a multiclonal evolution model. Moreover, this provided a unique collection of 19 transplantable DCIS MIND models including all molecular subtypes. Ultimately providing a resource that can bring the treatment of DCIS one step closer to a more tailored approach.

**Tim Martin, trainee researcher, SPECIFICANCER, Brigham and Women’s Hospital and Harvard Medical School, US**

**Poster 38**

**Tumor suppressor gene inactivation drives cancer immune evasion**

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Cancers are driven by mutations and amplifications of oncogenes (OG) and loss of function mutations and deletions of tumor suppressor genes (TSGs). These genetic events ultimately lead to tumorigenesis, metastasis, and eventually, mortality. During tumorigenesis, cancer cells must evolve to evade the immune system and are known to do this by disrupting the genes involved in antigen processing and presentation or upregulating inhibitory immune checkpoint genes. We performed in vivo CRISPR screens in syngeneic mouse tumor models to examine requirements for tumorigenesis both with and without adaptive immune selective pressure. In each tumor type tested, we found a striking enrichment for the loss of tumor suppressor genes (TSGs) in the presence of an adaptive immune system relative to immunocompromised mice. Nearly one third of TSGs showed preferential enrichment, often in a cancer- and tissue-specific manner. These results indicate that clonal selection of recurrent mutations found in cancer is driven largely by the tumor’s requirement to avoid the adaptive immune system.

**Viktor Ljungstroem, post doctorate, SPECIFICANCER, Harvard Medical School, US**

**Poster 39**

**Detection of KRAS permissiveness mechanisms through single cell RNA sequencing analyses of normal tissues**

Viktor Ljungström1*, Hu Jin1, Peter Park1

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A still unanswered question in cancer biology is why most recurrent gene mutations are observed only in a few cancer-types. Mutations in KRAS are detected in up to 25% of all cancer but are only recurrently mutated in some tumor types such as pancreatic ductal carcinoma (PDAC), lung adenocarcinoma (LUAD), and colorectal adenocarcinoma (CRC). PDAC is the prototypical KRAS mutated malignancy where more than 90% of all cases carries a mutation but rarely mutations in other MAPK pathway genes.

Using bulk RNA sequencing data from TCGA PDAC tumors and GTEx normal pancreatic tissue, we established a PDAC gene signature including 36 genes with high log fold change in the tumors. Analysis of normal single cell RNA sequencing (scRNA seq) data from the Tabula Sapiens consortium revealed that a small subpopulation of pancreatic ductal cells had high expression of the PDAC signature genes and is highly prevalent in pancreatitis and PDAC.

Analysis of tissues outside of pancreas showed that several mucin producing cell types also had high scores and that their related tumor types (e.g., invasive mucinous adenocarcinoma of the lung and mucinous adenocarcinoma of the colon) had higher scores compared to other tumor types in the same tissue. Finally, by comparing cell types with high vs low PDAC gene signature scores, we propose novel genes and processes that could be involved in dictating the cellular responses to KRAS mutations.

Taken together, this study has characterized a pancreatic ductal subpopulation that could be a cell of origin for PDAC, identified cell types and tumor subtypes in other organs with expression profiles resembling PDAC, and proposed a set of candidate genes that could influence cell type specific responses to KRAS mutations.
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<td><strong>Targeting Clonogenic Cells of the Barrett’s-Dysplasia-Cancer Axis</strong></td>
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Wa Xian*, Jaffer Ajani, Frank McKeon

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The concepts surrounding the cancer stem cell and its role in tumor progression and chemotherapy resistance remain in flux. Here we employ novel single-cell technology to clone patient-matched stem cells of Barrett’s, dysplasia, and esophageal adenocarcinoma (EAC). These clones meet all stem cell criteria and respectively differentiate to intestinal metaplasia, low- and high-dysplasia, and cancer. Genomic analysis of these clones readily details the phylogenetics underlying the evolution of precursors to the index EAC, as well as clades that did not. Unexpectedly, dysplasia and EAC stem cell clones display a near absolute genomic stability throughout cloning, expansion, and months of tumor formation in xenografts, arguing for a prior “Big Bang” source of intratumor diversity and against genomic instability in the evasion of chemotherapeutics. Finally, we leverage Barrett’s and normal mucosal stem cells in parallel drug screens for prospective preemptive therapeutics, and show these same drugs simultaneously and effectively eliminate stem cells of dysplasia and EAC.