27th - 28th May 2024

Book of Abstracts

Organization:

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Program

Day 1 - May 27 2024

08:00 - 09:30	Arrival and Registration
09:30	Opening Remarks
	SESSION 1: COMPUTATIONAL BIOLOGY | CHAIR: ELISABETTA MEREU
09:40	**Gioele La Manno (École Polytechnique Fédérale de Lausanne, Switzerland)** "No flight of fancy: Manifold-Constrained Statistical Inference on Cellular Process Dynamics"
10:15	**Yanay Rosen (Stanford University, USA)** Selected talk "Uncovering Structure Across the Universal Landscape of Cell Biology Spanning Species, Cell Types and Cell States"
10:35	**Michael Scherer (Centre for Genomic Regulation , Spain)** Selected talk "DNA methylation jointly encodes clonal identity and cell state in single cells"
10:55	**Aitor González (Josep Carreras Leukaemia Research Institute, Spain)** Selected talk "Integrative Analysis of Single-Cell Datasets Reveals Insights into Human Pancreatic Function, Renewal, and Regeneration"
11:15	Coffee Break
	SESSION 2: HEMATOPOIESIS | CHAIR: ALEJO RODRÍGUEZ-FRATICELLI
11:45	**Linde Miles (Cincinnati Children's, USA)** "Single cell analysis of clonal evolution in acute myeloid leukemia (AML)"
12:20	**Indranil Singh (IRB Barcelona, Spain)** Selected talk "Deciphering Cellular Destiny: Pivotal Role of Stem Cell States' in Shaping Responses to Preleukemic Mutations"
12:40	**Lena Nitsch (Berlin Institute of Health at Charité, Germany)** Selected talk "Single-cell resolved longitudinal clonal dynamics in human hematopoiesis and regeneration"
13:00	**Chiara Reggio (Scale Biosciences, Global Distribution Manager)** Sponsor talk "Welcome to single-cell genomics at scale"
13:15	Lunch

SESSION 3: CANCER | CHAIR: ASHLEY SANDERS

14:30	**Sydney Shaffer (University of Pennsylvania, USA)** "Tracking plasticity and cancer evolution in human samples"
15:05	**Viola Hollek (Charité, Germany)** Selected talk "Deciphering Oncogenic Signatures in Colorectal Cancer Through Single-Cell Multi-Omics"
15:25	**Llorenç Solé-Boldo (MDC-BIMSB/BIH/Charité, Germany)** Selected talk "Bone marrow breakout lesions act as key sites for tumor-immune cell diversification and exhaustion in multiple myeloma"
15:45	**Magdalena Pajonk (University Hospital Heidelberg/DKFZ, Germany)** Selected talk "Single-cell metabolic multiomics to identify vulnerabilities in acute myeloid leukemia stem cells"
16:05	Coffee Break
16:30	**John Marioni (Genentech-Roche, EMBL-EBI, CRUK Cambridge Institute, UK)** "Moving Beyond Atlases"
17:30	Poster Session
20:00	Dinner and Party

Day 2 - May 28 2024

SESSION 4: DEVELOPMENT | CHAIR: STEFANIE GROSSWENDT

Time	Program
10:00	**Yonatan Stelzer (Weizmann Institute of Science, Israel)** "Toward a canonical spatiotemporal model of early mammalian development"
10:35	**James Cotterell (EMBL Barcelona, Spain)** Selected talk "Cell 3D Positioning by Optical encoding (C3PO) and its application to spatial transcriptomics"
10:55	**Rory Maizels (The Francis Crick Institute, UK)** Selected talk "Modelling developmental gene regulation from time-resolved single-cell transcriptomics"
11:15	**Jeroen Aerts (Deep Cell, Market Development Manager EMEA)** Sponsor talk "Expanding the single-cell omics toolkit with label-free, AI-driven single-cell morphology analysis using REM-I"
11:30	Coffee Break

SESSION 5: GENOMICS | CHAIR: LEIF S. LUDWIG

Time	Program
12:00	**Mor Nitzan (The Hebrew University of Jerusalem, Israel)** "Learning from the information gaps: inference of multicellular organization"
12:35	**Yu-Hsin Hsieh (Berlin Institute of Medical Systems Biology, Germany)** Selected talk "Single-cell resolved immune and mitochondrial DNA mutational landscapes of human mitochondrial disorders"
12:55	**Martina Macino (MDC/BIMSB, Germany)** Selected talk "DeCRYPTing the somatic mutational landscape in inflammatory bowel disease"
13:15	Lunch

SESSION 6: IMMUNOLOGY | CHAIR: SIMON HAAS

14:30	**Caleb Lareau (Sloan Kettering Institute, USA)** "Viral latency and reactivation at single cell resolution"
15:05	**Laura Jiménez Gracia (Centro Nacional de Análisis Genómico, Spain)** Selected talk "Interpretable Inflammation Landscape of Circulating Immune cells"
15:25	**Maria Sopena-Rios (Barcelona Supercomputing Center, Spain)** Selected talk "Single-cell atlas of the aging circulating immune system"
15:45	**Sara Lobato-Moreno (European Molecular Biology Laboratory, Germany)** Selected talk "Leveraging Single-Cell Ultra-High Throughput Mul7ome sequencing (SUMseq) to inves7gate molecular mechanisms governing the innate immune inflammatory response"
16:05	**Agnieszka Ciesielska (10X Genomics, Science & Technology Advisor)** Sponsor talk "Discover the complexity of immune system with single cell resolution"
16:20	Coffee Break
16:45	**Theodore Alexandrov (EMBL, BioInnovation Institute, Germany)** "Spatial single-cell metabolomics: a powerful technology for revealing metabolic states of cells"
17:45	Closing Remarks

JOHN MARIONI

Genentech Research and Early Development, Genentech-Roche, EMBL-EBI, CRUK Cambridge Institute

"Moving Beyond Atlases"

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THEODORE ALEXANDROV

EMBL, BioInnovation Institute

"Spatial single-cell metabolomics: a powerful technology for revealing metabolic states of cells"

Recent discoveries put metabolism into the spotlight. Metabolism not only fuels cells but also plays key roles in health and disease. In parallel, emerging single-cell technologies opened a new world of cell types and states previously hidden beneath population averages. Yet, methods for discovering links between metabolism, cell states, metabolic plasticity and reprogramming on the single-cell level and in situ are crucially lacking. Our research aims to contribute to bridging this gap. We will present method SpaceM for spatial single-cell metabolomics. SpaceM detects 100+ metabolites or 500+ lipids from thousands of individual cells together with fluorescence and morpho-spatial features. We will show how SpaceM provides insights into metabolism of activated CD4+ T cells from peripheral human blood, on the single-cell level. SpaceM helped characterizing the induced metabolic states of T cells and finding small molecule markers. In the second application, we investigated cells lines and patient-derived xenografts (PDXs) to discriminate epithelial/classical and quasi-mesenchymal (QM) phenotypes in pancreatic ductal adenocarcinoma (PDAC). We found distinct groups of cells associated with each of the phenotypes, with a striking similarity between cell lines and PDXs. Overall, SpaceM can help open novel avenues for understanding metabolism single cells in immuno-oncology.

CALEB LAREAU

Sloan Kettering Institute

"Viral latency and reactivation at single cell resolution"

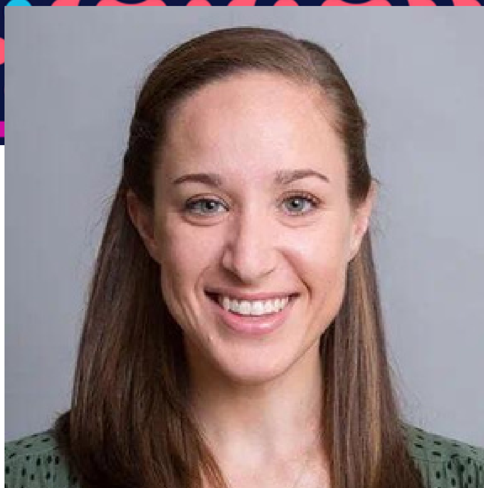
Cell therapies have mediated durable clinical responses for patients with cancer, but the engineering therapeutic human cells ex vivo can lead to unexpected complications. We currently lack a comprehensive mechanistic understanding of underlying toxicities observed in patients receiving T cell therapies, including recent reports of encephalitis caused by reactivation of human herpesvirus 6 (HHV-6). Here, via petabase-scale viral genomics mining, we examine the landscape of human latent viral reactivation and demonstrate that HHV-6B can become reactivated in human CD4+ T cell in vitro cultures. Using single-cell sequencing, we identify a rare population of HHV-6 'super-expressors' (~1 in 300-10,000 cells) that possess high viral transcriptional activity in research-grade allogeneic chimeric antigen receptor (CAR) T cells. By analyzing single-cell sequencing data from patients receiving cell therapy products that are FDA-approved or in clinical studies, we identify the presence of CAR+, HHV-6 super-expressor T cells in patients in vivo. Together, our study demonstrates the utility of comprehensive genomics analyses to implicate cell therapy products as a potential source contributing to lytic HHV-6 reported in clinical trials and may influence the design, production, and monitoring of viral reactivation in autologous and allogeneic cell therapies.

GIOELE LA MANNO

École Polytechnique Fédérale de Lausanne

“No flight of fancy: Manifold-Constrained Statistical Inference on Cellular Process”

In this talk, I will present a novel approach to understanding transcriptome dynamics through the lens of RNA velocity. Traditional RNA velocity algorithms suffer from a lack of statistical control and return predictions that “fly off planet”: they do not align with the gene expression manifold sampled, leading to dynamic inconsistency. I will discuss a generative model of RNA velocity built to tackle these challenges. At the core a Bayesian inference model that ensures manifold-consistent estimations of gene expression change rates. This framework coherently identifies the parameters of a dynamical system, a possibility I demonstrate by focusing on the cell cycle within one-dimensional periodic manifolds. The model's efficacy is validated against live imaging for accurate cell cycle period inference and further tested through sensitivity analyses and multi-sample assessments. I will show examples of VeloCycle application to both in vivo samples and in vitro genome-wide Perturb-seq, highlighting its ability to discern regional proliferation patterns in neural progenitors. The talk will conclude with the implications the method has on expanding the scRNA-seq analysis toolkit, with modular statistically sound approaches for RNA velocity inference.



LINDE MILES

Cincinnati Children's

"Single cell analysis of clonal evolution in acute myeloid leukemia (AML)"

Acute myeloid leukemia (AML) encompasses a heterogeneous class of hematologic malignancies where epigenetic/genetic events lead to aberrant proliferation of immature hematopoietic stem/progenitor cells and concomitant blockade of differentiation. Large scale bulk sequencing efforts over the past two decades have profiled the mutational landscape of AML, uncovering frequent mutations in epigenetic modifier genes, such as DNMT3A, TET2, and IDH1/2, as well as oncogenic signaling mutations like NRAS and FLT3. While these efforts have been instrumental in prognostication and therapy decisions for AML patients, bulk sequencing is unable to completely delineate the clonal framework, mutational trajectories, and aberrant genotype-immunophenotype relationships in AML that may be important for disease development and progression.

Previous work by our lab and others investigated the clonal architecture of AML utilizing single cell molecular profiling of AML patient samples. These studies identified that mutated genes within the same class (i.e. epigenetic modifiers vs. signaling genes) displayed recurrent clonal patterns with regards to mutation order, clonal dominance, and cell state.

To further investigate clonal evolutionary trajectories in AML, we utilized an improved single cell multi-omics platform (scDNA+Protein) to simultaneously analyze genotype and immunophenotype of TET2- and IDH2-mutant AML. Moreover, we performed scDNA+Protein analysis on longitudinal samples from TET2- and IDH2-mutant AML patients who underwent standard induction chemotherapy (7+3) to uncover clonal dynamics at different disease states. We observed multiple patterns of clonal evolution during therapy through multi-omic analysis of this cohort. In certain cases, we found significant alterations to both the clonal architecture and immunophenotype from diagnosis to relapse. However, in other patients, we observed stable genotype and immunophenotype over the course of therapy, suggesting alternative methods of therapeutic relapse. Future studies aim to determine the underlying mechanisms behind these differences and identify evolutionary trajectories important in therapeutic resistance.

MOR NITZAN

The Hebrew University of Jerusalem

"Learning from the information gaps: inference of multicellular organization"

In this talk I will discuss what can be learned about the organization and behavior of multicellular structures when we peel off the layers of information we have already measured or reconstructed from single-cell or spatial omics data. I will present several computational approaches we have recently developed for this challenge, including ones involving spectral, probabilistic kernel-based, and generative deep learning methods for filtering, enhancement, and disentanglement of information about tissue configuration, temporal processes, and interactions from single-cell data. Then, I will discuss what can be revealed by the incongruence between single-cell genomics data and our prior understanding, or annotation, of it, and how this challenge can be approached by analyzing the training dynamics of deep neural networks trained on annotated single-cell and spatial omics data. This approach can be used to address key challenges in the interpretation of genomic data, including auditing and rectifying erroneous cell annotations, identifying intermediate cell states, delineating complex temporal trajectories along development, and characterizing cell diversity and treatment effectiveness for diseased tissues.

SYDNEY SHAFFER

University of Pennsylvania

"Tracking plasticity and cancer evolution in human samples"

Understanding the earliest changes in oncogenesis remains a significant challenge because we lack tools to map these changes in patient tissues. Using single-cell RNA and spatial profiling combined with mitochondrial mutation lineage tracing, we capture the clonality and transcriptional states of early oncogenic lesions from human patients. We find that pre-cancerous metaplasia of the esophagus is polyclonal, similar to normal tissues, and consists of well defined cell types. In contrast, early cancer lesions emerge from a single clone, showing reduced transcriptional and cell-type diversity. Broadly this approach aims to delineate the initial evolutionary steps in cancer progression, offering potential diagnostic and therapeutic targets for cancer interception.

YONATAN STELZER

Weizmann Institute of Science

"Toward a canonical spatiotemporal model of early mammalian development"

My research group aims to address the fundamental challenge of understanding how multicellular organisms achieve variation despite cells having identical genetic information. The development of a single fertilized egg into a complete mammalian embryo is an especially beautiful embodiment of this problem. During this process, cellular differentiation is defined by the capacity of the cell ensemble to acquire increasingly more specialized internal states in a coordinated fashion. Our research program is based on the premise that a comprehensive quantitative model of mammalian development will necessitate the integration of three primary layers of phenomenological information: (i) The natural flux of cell state changes over time. (ii) The sum of extracellular cues affecting cell specification in the three-dimensional embryonic space in a non-cell-autonomous manner and (iii) epigenetic makeup shaping and memorizing cellular states cell-autonomously. We are excited by recent breakthroughs in single-cell transcriptomics and epigenomics since these can capture the emergence of embryonic cellular diversification at incredible resolution. But, we believe there is an urgent need in the field to match descriptive single cell atlases with models and experimental frameworks to derive novel understanding of function and regulation in this process. In my talk, I will present recent progress by my group towards these aims.

SELECTED TALKS

Yanay Rosen

Stanford University

"Uncovering Structure Across the Universal Landscape of Cell Biology Spanning Species, Cell Types and Cell States"

Learning universal representations (embeddings) of single cell RNA-sequencing data is critical for drawing scientific conclusions from diverse omics datasets. While large, self-supervised foundation models hold promise in learning generalizable representations, their direct application from natural language processing overlooks the unique qualities of single-cell data.
To address this, we designed and trained a foundation model that incorporates biological inductive biases to produce a universal cell embedding (UCE) that detects true biological variation despite experimental noise. UCE enables the representation of any cell, regardless of tissue or species, within a fixed biological latent space. Our model can accurately transfer and predict cell types with no model finetuning (in a zero-shot setting), even for novel species that were absent from the training data. We apply UCE to uncover novel cell type hierarchies, sub-types, and trajectories.

Michael Scherer

Centre for Genomic Regulation

“DNA methylation jointly encodes clonal identity and cell state in single cells”

DNA methylation is an epigenetic mark dynamically regulated during cellular differentiation in general and particularly during hematopoietic differentiation. Epimutations, defined as the spontaneous loss (or gain) of DNA methylation at individual CpG dinucleotides, have been explored as potential clonal labels in cancer. However, DNA methylation's utility as a clonal label in healthy differentiation is unclear.

Here we show that targeted, high-confidence single-cell measurements of DNA methylation with scTAM-seq deliver joint information about the cellular differentiation state and clonal identity. The DNA methylation-based map of murine hematopoiesis has a resolution similar to scRNA-seq. CpGs associated with cellular differentiation are enriched for enhancer elements, while clone-specific CpGs are preferentially located in heterochromatic and late replicating regions of the genome. We developed a new method, EPI-Clone, to infer clonal identity from DNA methylation data only, without the need for genetically engineered clonal labels. Clones

defined by EPI-Clone show a high overlap with ground-truth genetic barcodes using the LARRY barcoding system. Using the high-resolution map of hematopoiesis together with clonal identity, we characterized functional behavior of stem cell clones. Analogously to experiments using genetic barcodes, we found heterogeneity within hematopoietic progenitors with respect to blood output and lineage biases.

We found that the clonal identity imprinted in the epigenome was present both in hematopoietic progenitors and in mature myeloid cells. When investigating hematopoietic output during aging, we found the characteristic myeloid shift in an aged in comparison to a young mice that, until today, could only be investigated using genetic barcoding systems such as LARRY.

In summary, we show that single-cell DNA methylation profiles provide information on both the cellular differentiation state and clonal origin of hematopoietic cells. We believe that the joint readout of clonal identity and cell state enables functionally characterizing stem cell clones in systems where genetic barcoding is not applicable.

Aitor González

Josep Carreras Leukaemia Research Institute

“Integrative Analysis of Single-Cell Datasets Reveals Insights into Human Pancreatic Function, Renewal, and Regeneration”

Establishing a comprehensive single-cell reference atlas for the human pancreas stands as a pivotal stride in unraveling the molecular intricacies governing its function and pathology. Such an exhaustive molecular reference atlas is instrumental in providing a detailed roadmap of gene expression patterns and regulatory networks that orchestrate pancreatic development and functionality.

Our current research focuses on delving into multiple adult pancreatic datasets harnessed through diverse single-cell technologies. Specifically, we possess three distinct datasets: a compact multiome dataset featuring two samples from healthy patients (8947 cells), a snRNA-seq set comprising 440332 cells, and an additional 197946 cells sequenced via snATAC-seq.

The inherent distinctions in characteristics and procedural requirements associated with each of these technologies introduce technical variations, presenting a formidable challenge for the unified analysis of the data. Addressing this challenge, we employed the Seurat R package, which facilitates the integration of chromatin accessibility and transcriptomic data through a multiome dataset. Among our initial steps in the analytical pipeline, we prioritized the bridge integration of our snATAC and snRNA-seq datasets. This not only served to test the efficacy of the Seurat method but also allowed us to assess the correlation and integrability between cell types defined through different technologies.

Furthermore, we utilized an internally developed deep learning algorithm known as DeepSCore to facilitate label transfer across diverse modalities. This innovative approach enabled us to establish a secondary source for assessing the correlation between chromatin accessibility and transcriptomic data.

In tandem with our data integration efforts, we conducted an in-depth exploration of the cell renewal process within the human pancreas. Despite the known characteristic of steady turnover of differentiated cell types and its significant regenerative capacity following injury in pancreas, the existence of a dedicated adult pancreatic progenitor population remains a subject of controversy. Various proposed models suggest differing perspectives, with some advocating for certain cell types acting as facultative progenitors, while others propose the presence of more specialized progenitors. Regardless, there is evidence pointing to a degree of transdifferentiation in pancreatic regeneration.

To further investigate this regenerative process, we inferred cell fate trajectories from a specific set of acinar and ductal cells with profiles resembling naive or progenitor cells. Additionally, we employed the recently developed SCENIC+ package to construct enhancer-driven gene regulatory networks based on both paired and unpaired chromatin accessibility and transcriptomic data. These networks aim to unveil transcription factor-region-gene sets governing cell identity, which could play a pivotal role in transdifferentiation processes. This insight is not only valuable for pancreatic regeneration but also holds relevance in the realm of cancer research. Furthermore, SCENIC+ facilitates in-silico gene perturbation studies, providing an additional avenue for investigating key genes involved in transdifferentiation processes.

Indranil Singh

IRB Barcelona

“Deciphering Cellular Destiny: Pivotal Role of Stem Cell States' in Shaping Responses to Preleukemic Mutations”

Tumors with the same driver mutations can display a striking variation in their progression and treatment response, but the origins of this variation are still unclear. In this study, we unveil that heritable stem cell states can have a profound influence on how individual cells respond upon acquisition of the same cancer mutation. Here we develop a new method, scTRAACK, for single-cell Tracking of Recombinase Activation And Clonal Kinetics and apply it to hematopoietic stem cells carrying Cre-conditional leukemia alleles. Tracking the expansion kinetics of a common set of stem clones, with and without the same leukemia mutations, we unveil a striking heterogeneity in the malignant fates of diverse stem cell clones. First, we define that heritable clonal states persist in expansion cultures and affect their clonal composition. Then, using mouse models of the most frequent initiating AML mutation in Dnmt3a, we define that these pre-existent heritable stem cell states influence clonal expansion, differentiation properties, and unique malignant gene expression programs that arise from each cell upon acquisition of mutation. Whereas high-fitness slow-cycling clones expanded regardless of mutational state, cancer mutations significantly increased the survival probability in stem cell clones with higher cycling and lower fitness, which are the most abundant clones within the stem cell compartment. Moreover, mutations reprogrammed the stem cell states of low-fitness clones to maintain a pool of stem cell-like cells.

These findings suggest that whereas both high and low-fitness stem cells can be at the origin of AML, the more abundant low-fitness pool likely represents the cell of origin for the majority of AML.

Lena Nitsch

Berlin Institute of Health at Charité

“Single-cell resolved longitudinal clonal dynamics in human hematopoiesis and regeneration”

The formation of our blood and immune cells is sustained throughout our lifetime by hematopoietic stem cells. A quantitative understanding of the output and activity of individual stem cells in humans, however, is only emerging. Here, we leverage somatic mitochondrial DNA (mtDNA) mutations as natural barcodes to longitudinally trace clonal output and dynamics for up to three years. Via mitochondrial single-cell ATAC sequencing (mtscATAC-seq) of bone marrow and peripheral blood from patients undergoing allogeneic stem cell transplantation, we investigate clonal reconstitution dynamics compared to longitudinally profiled blood from healthy individuals, collectively comprising mutational profiles of over 800.000 cells. As expected, we observe stable cell-type compositions for all healthy individuals over time. Following transplantation, innate immune cells such as monocytes, repopulate the blood earlier than adaptive immune cells. B cells, for example, only appear months a[er transplantation. Leveraging mtDNA variants over time we observe a higher fluctuation of mutational profiles during early time points a[er transplantation potentially indicative of a less stable output immediately following engra[ment. By contrast, later time points resembled a steadier clonal output comparable to what we observe in healthy individuals.
Compared to healthy, we further observe a potential decrease in clonality in the transplantation setting, without prominent lineage biases being detectable outside of immune cell expansions in healthy as well as transplanted individuals. Together, our study provides one of the first longitudinally resolved clonal evaluations at single-cell resolution in hematopoiesis under physiological conditions and regeneration a[er stem cell transplantation.

Viola Hollek

Charité

“Deciphering Oncogenic Signatures in Colorectal Cancer Through Single-Cell Multi-Omics”

Tissue homeostasis is orchestrated by the precise modulation of signalling pathways, guiding cellular fate towards proliferation, differentiation, or apoptosis. This balance can be disrupted by genetic mutations in key signalling molecules, potentially leading to uncontrolled growth. Interestingly, different oncogenic mutations can result in divergent phenotypes, even if the mutated proteins belong to the same signalling pathway. For instance, our research has demonstrated that mutations in KRAS and BRAF, both members of the MAPK pathway, have distinct effects on ERK signalling in colorectal cancer (CRC) patients. This variation suggests a complex relationship between specific oncogenic mutations and the tumorigenesis of CRC, and highlights a critical gap in our understanding of the phenotypic outcomes arising from individual oncogenic mutations.

In this study, we aim to dissect mutation-specific changes in signalling networks, cell fate decisions, and cell behaviour in CRC. We profile a compendium of prevalent mutations in CRC using a bar□coded lentivirus-based oncogene library, analysed across five CRC cell lines with distinct genomic backgrounds. By leveraging specific nucleotide- and peptide-based barcode systems, we are able to perform the oncogene screen in a pooled manner and analyse it by single-cell RNA-sequencing to identify transcriptomic alterations, complemented by mass cytometry and mass spectrometry to map the phospho-proteome and proteome, respectively.

This multi-omics strategy, with single-cell resolution, enables us to construct detailed oncogenic profiles, linking genotypes to a spectrum of phenotypic traits. A comparative analysis with single-cell RNA-sequencing data from CRC patients further refines these profiles, bridging in vitro findings with clinical observations.

Our oncogene screen may pave the way for the use of oncogenic signatures as diagnostic and prognostic tools, offering a more nuanced understanding of genotype-phenotype relationships in CRC. Thus, this project not only contributes to the precision medicine landscape by enhancing tumour classification but also demonstrates the power of single-cell omics in dissecting the complexities of cancer biology.

Llorenç Solé-Boldo

CMDC-BIMSB/BIH/Charité

“Bone marrow breakout lesions act as key sites for tumor-immune cell diversification and exhaustion in multiple myeloma”

Multiple myeloma (MM) is a blood cancer characterized by the expansion of malignant plasma cells in the bone marrow. The bone marrow microenvironment plays a pivotal role in supporting myeloma growth and modulating tumor immunity. As the disease progresses, myeloma cells may become bone marrow independent, leading to extramedullary disease associated with poor prognosis. However, the early processes associated with bone marrow independence and its implications for disease and immune control remain poorly understood. Here, we employed comprehensive single-cell and spatial mapping to identify bone marrow breakout lesions as key events in multiple myeloma pathogenesis, serving as a hotspot environment for tumor-immune cell interactions, diversification and exhaustion. Bone marrow breakout lesions harbored an adapted niche and a unique immune microenvironment conferred by sustained immune□tumor interactions. In particular, tumor-reactive T cells with a highly exhausted phenotype, alongside unique NK cell and macrophage subtypes, specifically expanded in breakout lesions. Spatially-resolved genomic, transcriptomic and cellular analyses uncovered extensive intra-lesion heterogeneity, suggestive of a divergent co-evolution of genomic variation and locally confined T cell responses in distinct subregions. Ultra-high plex imaging revealed that tumor-reactive T cells, together with other immune-regulatory cells and dendritic cells co-expand and exhaust upon sustained tumor interactions in locally confined immune islands. In contrast, immune-regulatory macrophage and NK cell subsets operated, spatially separated, within the myeloma cell desert. Jointly, our analyses uncover bone marrow breakout lesions as major sites for tumor evolution, immune cell diversification and exhaustion, representing a key event in myeloma pathogenesis with significant therapeutic implications.

Magdalena Pajonk

University Hospital Heidelberg/DKFZ

“Single-cell metabolic multiomics to identify vulnerabilities in acute myeloid leukemia stem cells”

Acute myeloid leukemia (AML) is an aggressive myeloid malignancy characterized by substantial inter and intra-patient heterogeneity. While treatment regimens lead to remission in most patients, relapse rates are high and overall survival remains poor. The driving force of relapse are rare leukemic stem cells (LSCs), which based on recent studies differ not only from leukemic blasts but also from hematopoietic stem cells in their metabolism. In this study, we developed a novel flow cytometry panel measuring known surface markers for LSC discrimination together with key metabolic properties at single cell (sc) level. With this tool, over 100 de novo AML patients of all subtypes were characterized to address the heterogeneity within AML and the LSC pool. We were able to identify metabolic features associated with poor overall survival that are shared between almost all subtypes and are enriched in phenotypically defined LSC populations. Further characterization of specifically isolated metabolic populations confirmed their high clonogenic capacity and engraftment potential in xenotransplant experiments. Besides common metabolic features, we also observed subtype-specific signatures.

For a deeper, mechanistic understanding of identified metabolic phenotypes, a small cohort of AML patients was further subjected to metabolic multiomics by index-sort paired with sc-RNAseq using MutaSeq and sc-cultures to simultaneously measure metabolic properties, gene expression, mutational status and clonogenic capacity. In our sc-dataset, we were able to discriminate between heathy, preleukemic and leukemic cells of different clones. By applying a supervised machine learning approach, a growth probability score was calculated for each sequenced cell. Finally, data integration allowed us to link the identified metabolic phenotype to an immature quiescent cell state and high activity of specific metabolic pathways. These by metabolic flux analysis inferred activities are further confirmed on proteome level. Interfering with identified metabolic dependencies of LSCs may pave the way for developing more effective treatments.

James Cotterell

EMBL Barcelona

“Cell 3D Positioning by Optical encoding (C3PO) and its application to spatial transcriptomics”

Current state-of-the-art spatial omics approaches suffer from the drawback that they are tissue section-based and thus inherently 2-dimensional. A full understanding of biological processes will only be possible when such data is available in 3-dimensions (3D). Here, we introduce Cell 3D Positioning by Optical encoding (C3PO) - the first technique capable of reconstructing the 3D positions of cells in a tissue, after they have been fully dissociated for single-cell omics analysis. It imposes a Cartesian coordinate system of positions on the tissue and cells of interest, before dissociation. This is created by multiple orthogonal spatial gradients of active fluorophores, carefully shaped by a 3D bleaching method, such that each position in the tissue is encoded by a unique fluorescent address. Upon dissociation of the tissue the fluorescent addresses of the cells can be read via an appropriate device (such as a FACS machine) to computationally reconstruct the tissue in 3D, before omics are performed downstream. Here, we show two proof-of-principle results for C3PO. First, pure C3PO without omics, to reconstruct the 3D geometry of a developing mouse limb bud. Second, an application of C3PO to spatial transcriptomics, revealing the expression patterns of 42 genes important in limb development. C3PO is a genuinely novel approach to reconstruct the original 3D positions of cells in a tissue after dissociation. Combined with transcriptomics, it can play a significant role in the study of any tissue or organ in which 3D structure and geometry is important, such as developmental biology, cancer biology and neuroscience. It is not an omics technique per se, and in the future could be combined with the growing family of other omics technologies.

Rory Maizels

The Francis Crick Institute

“Modelling developmental gene regulation from time-resolved single-cell transcriptomics”

A major goal of single-cell transcriptomics is to model the gene regulation controlling cell fate decisions in development. For this, it is vital to capture dynamics with sufficient resolution. As such, we established methods for modelling gene expression dynamics with single-cell resolution.
We developed sciFATE2, an optimised combinatorial indexing protocol for metabolic labelling of RNA. This improved protocol provides temporal information at single-cell level and data quality and throughput comparable to commercial platforms. With this, we generated a time-resolved dataset of 45,000 cells from in vitro spinal cord neural differentiations, capturing cell fate decisions from neuromesodermal progenitors through to mature neurons. To explore these cell fate decisions, we developed a deep learning framework (VelvetVAE) that models expression dynamics as a latent dynamical system within a variational autoencoder. This method quantitatively outperforms other velocity inference tools and provides simulations that capture dynamical aspects, such as decision boundaries between bifurcating trajectories and fate-specific expression profiles. We are now extending this approach to an automated pipeline that can target select gene panels and sequence millions of cells per experiment, simultaneously reducing cost and increasing throughput by orders of magnitude. This will allow combinatorial profiling of perturbation and signalling regimes, coupling the output of expression dynamics to changing inputs, providing a highly resolved picture of developmental gene regulation

Yu-Hsin Hsieh

Berlin Institute of Medical Systems Biology

“Single-cell resolved immune and mitochondrial DNA mutational landscapes of human mitochondrial disorders”

Mutations within the mitochondrial genome (mtDNA) may disrupt cellular metabolism and are associated with a broad spectrum of maternally inherited primary mitochondrial disorders (PMD), many presenting with organ-specific phenotypes. We previously reported the purifying selection of pathogenic mtDNA mutations in peripheral blood T lymphocytes in PMDs caused by a mtDNA single nucleotide variant (mtSNV) and single large-scale mtDNA deletions (SLSMDs); however, whether purifying selection is a shared characteristic across a wide spectrum of mitochondrial diseases and how different pathogenic germline mtSNVs affect the transcriptome remain largely unexplored. Here, we profiled >400,000 peripheral blood mononuclear cells (PBMCs) from 16 patients with mitochondrial diseases, encompassing a range of germline mtDNA and nuclear mutations, using a single-cell multi-omics approach. Single-cell mtDNA genotyping revealed diverse and intricate dynamics of purifying selection specific to both mtSNVs and cell types. Furthermore, gene set enrichment analysis (GSEA) unveiled notable changes in pathway activity, including oxidative phosphorylation (OXPHOS), cellular metabolism, and cytokine-mediated signalling, compared to healthy individuals, suggesting cell-type-specific metabolic vulnerabilities and adaptation. Collectively, our study provides novel insights into the relationship between mtDNA genotypes and cellular phenotype, contributing to a holistic perspective on this crucial aspect of mitochondrial biology.

Martina Macino

MDC/Berlin Institute of Medical Systems Biology

“DeCRYPTing the somatic mutational landscape in inflammatory bowel disease”

Somatic mutations arise constantly in the cells of our bodies which can result in the development of disease. While this phenomenon is very well characterized in carcinogenesis, it is yet to be deeply studied in other complex or inflammatory diseases. Recently it was discovered that the intestinal epithelium of inflammatory bowel disease (IBD) patients has increased rates of somatic single nucleotide variants and indels, which correlate with age and disease duration. This is coupled with an increased risk of developing gastrointestinal cancers, which are known to bear complex genomic alterations, including somatic structural variants (SVs). The role somatic SVs play in IBD pathogenesis, however, is still unknown. To determine their presence and significance in IBD, we are applying Strand-seq, a single-cell, single-strand DNA sequencing technology, to intestinal crypt organoids derived from primary patient biopsies. Strand-seq can distinguish between the sequences of homologous chromosomes, allowing the detection and functional characterization of copy-neutral variants, like inversions and complex DNA rearrangements – all at the single-cell level. We are using this data to construct single-cell mutational landscapes of patient samples to study the full spectrum of SVs arising in intestinal epithelial cells.

Our preliminary results reveal a global increase in genomic instability in the affected intestinal area compared to unaffected areas of the tissue. Moreover, we identified an enrichment of recurrent genomic rearrangements that converge on genes never associated with IBD before. These results suggest that common mutational processes may be acting in the intestinal epithelial cells of different patients and that shared somatic mutational profiles might emergevin the context of inflamation. We believe that our approach will shed new light on the players involved in IBD pathogenesis, which ultimately will help define new therapeutic targets.

Laura Jiménez-Gracia

Centro Nacional de Análisis Genómico

“Interpretable Inflammation Landscape of Circulating Immune cells”

Inflammation is a biological phenomenon involved in a wide variety of physiological and pathological processes. Although a controlled inflammatory response is beneficial for restoring homeostasis, it can become unfavorable if dysregulated. In recent years, major progress has been made in characterizing acute and chronic inflammation in specific diseases. However, a global, holistic understanding of inflammation is still elusive. This is particularly intriguing, considering the crucial function of inflammation for human health and its potential for modern medicine if fully deciphered. Here, we leverage advances in the field of single-cell genomics to delineate the full

spectrum of circulating immune cell activation underlying inflammatory processes during infection, immune-mediated inflammatory diseases and cancer. Our single-cell atlas of >2 million peripheral blood mononuclear cells from 356 patients and 18 diseases allowed us to learn a foundation model of inflammation in circulating immune cells. The atlas expanded our current knowledge of the biology of inflammation of acute (e.g. inflammatory bowel disease, sepsis) and chronic (e.g. cirrhosis, asthma, and chronic obstructive pulmonary disease) disease processes and laid the foundation to develop a precision medicine framework using unsupervised as well as explainable machine learning. Beyond a disease-centered classification, we charted altered activity of inflammatory molecules in peripheral blood cells, depicting functional biomarkers to further understand mechanisms of inflammation. Finally, we have laid the groundwork for developing precision medicine diagnostic tools for patients experiencing severe acute or chronic inflammation by learning a classifier for inflammatory diseases, presenting cells in circulation as a powerful resource for patient stratification.

Maria Sopena-Rios

Barcelona Supercomputing Center

“Single-cell atlas of the aging circulating immune system”

Age-associated decline in immune function, known as immunosenescence, predisposes individuals to infection, autoimmune disorders, and cancer. Immune function decline manifests as chronic low-grade inflammation (inflammaging) and impaired responsiveness to stimuli.

Single-cell RNA sequencing (scRNA-seq) is a powerful tool to uncover the cellular and molecular dynamics of immunosenescence among immune cell populations. However, studying immune cell type dynamics and cell state changes during human aging requires extremely large sample sizes.

Here, we leverage a scRNA-seq dataset of 982 individuals encompassing over 1 million human peripheral blood mononuclear cells (PBMCs) to systematically investigate the effect of aging on the human circulating immune system. Differential expression (DE) analysis with age reveals opposite expression patterns across distinct immune cell populations. Specifically, CD8+ T naive and B memory cells exhibited the largest number of down-regulated genes, while Natural Killer (NK) cells and CD8+T effector memory cells (CD8+ TEM) showed the largest number of up-regulated genes with age. This opposing pattern led us to identify a group of cell types, including CD8+ T Naive, CD4+ T Naive, MAIT and B cells, showing a coordinated down-regulation of both inflammatory response and translation-related processes with age.

Conversely, the remaining cell types, which include all CD4+ T cell types, CD8+ T Memory and CD8+Treg cells, monocytes and NK cells, have the same pathways up-regulated including inflammation and translation processes. When looking at genes DE in multiple cell types, we confirm that the discordant directionality pattern in the pathways is driven by the same genes that are up-regulated in one group of cell types and down in the other. These cell-type opposite responses go beyond lineage or functional classifications, highlighting a heterogeneity in aging trajectories within the immune cell repertoire.

We then performed differential cell type composition analysis with age and observed a strong consistency between changes in cellular proportions and gene expression patterns. Specifically, cell types with significant down-regulated genes, such as CD8+ T Naive and B memory, decreased in proportion. Conversely, cell types with substantial age-related up-regulation, including NK and CD8+ TEM, showed a corresponding increase in their proportions. This underscores the intrinsic link between cell population dynamics and gene expression during aging.

Finally, we carried out a sex-stratified differential expression analysis with age. This revealed sex-specific patterns within certain immune cell populations. Specifically, CD8+ TEM in females displayed up-regulated gene expression. Conversely, B memory cells in males showed down-regulation of numerous genes. These findings suggest that sex might be a primary driver of the previously observed age-associated gene expression changes. Finally, we find that up-regulated gene signatures in cell types with pro-inflammatory phenotypes (e.g. NK, CD8+ TEM, CD4+ CTL) are enriched for autoimmune disease exclusively in elderly females. These results elucidate the crucial role of sex in immune system aging, highlighting the need to include a sex perspective in immunity studies.

Overall, our study unveils a dual aging trajectory across immune cell types coupled with joint responses in gene expression and cell type composition and provides unprecedented insights into the cellular and molecular dynamics underlying immunosenescence.

Sara Lobato-Moreno

European Molecular Biology Laboratory

"Leveraging Single-Cell Ultra-High Throughput Multiome sequencing (SUMseq) to investigate molecular mechanisms governing the innate immune inflammatory response"

Simultaneous profiling of chroma4n accessibility and gene expression at single-cell resolution enables the exploration of the molecular mechanisms underlying cell differentiation and function in health and disease states. Currently available single-cell multiomic methods are, however, limited in scalability and multiplexing capacity, constraining their widespread applicability. Here, we present SUMseq, a Single-cell Ultra-high throughput Multiome sequencing method that allows profiling hundreds of samples at the million-cell scale without increased library prepara4on costs. Notably, SUMseq outperforms existing high-throughput single-cell multiome methods in data complexity. We used SUM-seq to dissect the gene regulatory mechanisms governing the innate immune inflammatory response in two contexts: 1) macrophage polarization to pro- and anti-inflammatory phenotypes as a model of infection and 2) microglia-neuron co-cultures treated with inflammatory stimuli as a model of neuro-inflammation.

For the infection model, we captured dis4nct signatures of both polarization trajectories and identified gene regulatory programs driving macrophage polarization. Furthermore, integration with genome-wide association studies (GWAS) data revealed an association of the ISGF3 regulon, a key driver of pro-inflammatory macrophage differentiation, to immune diseases.

For the neuro-inflammation model, we focused on understanding the impact of different isoforms of ApolipoproteinE (APOE), a key factor in the pathogenesis of sporadic Alzheimer's Disease (sAD), on the response of neurons and microglia to neuroinflammation. The E4 variant of APOE is the highest genetic risk factor associated with sAD. To investigate the effect of the E4 allele on each cell-type, we co-cultured iPSC-derived neurons and microglia expressing APOE3 or APOE4 in homozygosity in all four combinations. We stimulated the co-cultures with inflammatory molecules and found a set of genes that are specifically upregulated in neurons when both cell-types express the APOE4 isoform, indicative of a synergistic effect of APOE4 expression in both cell-types. Remarkably, some of these genes are linked to sAD development, providing molecular insights into the mechanisms underlying APOE4-associated pathogenicity.

In conclusion, SUMseq emerges as a highly flexible pla\orm to study key gene regulatory mechanisms and genetic programs governing diverse cellular processes in health and disease in a high-throughput manner.

SPONSOR TALKS

Chiara Reggio

Scale Biosciences

“Welcome to single-cell genomics at scale”

At ScaleBio, our single cell library preparation methods enable increased levels of sample multiplexing, increased cell throughput, and support for a broad range of single cell genomics applications. Cells and samples can be scaled exponentially to enable single cell experiments with 100s of thousands to millions of cells, at a fraction of the cost of on-market singl cell isolation instruments. In this presentation, we will discuss our latest kits to support scRNAseq, single-cell methylation, and CRISPR guide enrichment.

Jeroen Aerts

Deepcell

“Expanding the single-cell omics toolkit with label-free, AI-driven single-cell morphology analysis using REM-I”

Cell morphology reflects changes in cellular genomics and proteomics, offering valuable insights into phenotype and function. Analyzing high-dimensional morphological features in a quantitative manner is crucial for unlocking this biological information. The achieve this, the REM-I platform integrates high-resolution brightfield imaging of individual cells in flow with deep learning Foundation Models to analyze cell morphology, thus revealing a comprehensive “-ome”, the "morpholome". By projecting high-dimensional descriptions of cell features onto morphology UMAPs, this platform enables visualization and exploration of cell groups without constraints. This technology facilitates the assessment of morphological changes at the single cell level, providing insights into tumor heterogeneity, drug resistance, cell state, differentiation status, target discovery, and more.

Agnieszka Ciesielska

10x Genomics

“Discover the complexity of immune system with single cell resolution”

The vast complexities of biology require approaches to build a complete picture, starting from single cells to tissues and beyond. At 10x Genomics, we provide single cell, spatial, and in situ technologies that fuel scientific discoveries and drive exponential progress. Unravel highly complex biological systems, while bringing into focus the details that matter most.

Join us to learn how single cell and spatial transcriptomics applications can help you push the boundaries of your research. Uncover molecular insights, dissect cell-type differences, investigate the adaptive immune system and detect novel subtypes and biomarkers at scale with high definition.

Enabling deeper insight into immunology, 10x Genomics gives researchers the ability to see biology in new ways.

POSTER SESSION

Poster 1

Benedict Monteiro

Max Delbrück Center for Molecular Medicine

“Unravelling the clonal dynamics of somatic mutations to learn mechanisms of selection in human disease”

To date a wide range of somatic mutations have been detected in the genomes of human disease samples, including single nucleotide variants (SNVs), small insertions and deletions (indels) and large-scale structural variations (SVs). The clonal prevalence of somatic mutations is dynamic throughout disease progression, which can be used to make biological inferences of the selective pressures acting on the affected tissue. However, to our knowledge there is no method to characterise the complete spectrum of somatic mutations and its clonal evolution in the same sample.
To address this, we are developing computational frameworks to identify all classes of somatic mutation in data from Strand-seq, a haplotype-resolved single strand DNA sequencing technique that has greater power to resolve SVs than conventional methods. We are then leveraging mutational profiles to unravel the phylogenetic relatedness of both subclones and individual cells, which will allow us to model the contribution of different mutation types to the clonal architecture of inflammatory diseases. Our preliminary results indicate that disease heterogeneity can be better resolved by integrating additional mutation classes into our SV-based phylogenies. For example, we have identified somatic mtDNA mutations that emerged exclusively in the inflamed region of an inflammatory bowel disease patient's colon, which can therefore be used to trace clonal lineages. By integrating new variant classes into our analyses, we expect to gain new insights into how different selective pressures influence human disease.

Poster 2

Julia Rühle

Centre for Genomic Regulation

"Genomic encoding of lineage-specific gene regulation in the hematopoietic system at single-cell resolution"

Gene regulatory programs acting in cis tightly control cellular differentiation and determine cellular identity. These regulatory programs result from the complex interplay between gene regulatory elements (GREs), such as enhancers and promoters, and associated transcription factors (TFs). Massively parallel reporter assays (MPRAs) have enabled the simultaneous investigation of GRE activity of endogenous, as well as synthetic elements at the bulk level in isolation from their larger chromatin context. However, the regulatory logic underlying cellular progression along continuous developmental trajectories cannot be sufficiently explained by bulk data.

To this end we develop a lentiviral single-cell MPRA (scMPRA), that allows us to trace the regulatory activity of GREs along their course of differentiation. Briefly, we use a lentiviral vector to deliver specific GREs coupled to a barcoded reporter to the single cells. At the same time, we are leveraging a second independently expressed barcode to identify the incorporated GREs, allowing us to measure GRE activity and presence at single cell resolution. Using this assay, we will compare the activity of endogenous and synthetic regulatory elements during the continuous differentiation of hematopoietic stem cells into six blood lineages. Ultimately, these data, together with targeted scRNAseq information from the same cells, will be ideally suited to train predictive models that quantitatively infer GRE sequence activity during hematopoietic differentiation.

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Poster 3

Carla Mölbert

Max Delbrück Center for Molecular Medicine in the Helmholtz Association

"Constructing the gene regulatory network underlying the B2 to B1 cell state transition"

It has been shown that mature B2 cells have the potential to transition into bona fide B1 cells, when acquiring a B1 cell-typical self-reactive B-cell receptor. But it is yet unknown which transcription factors (TFs) play a crucial role in the cell state transition and how they interact with each other. One possibility of getting to know the role a TF in the transition, is the knock□out of this TF in the lab. However, running knock-out experiments for every possible TF is both time consuming and expensive. Here, we are introducing a method to construct a gene regulatory network (GRN) that focusses specifically on the TF-target interactions driving the cell state transition between B2 and B1 cells, by making use of coupled scATAC-seq and scRNAseq data together with the cell annotation into three distinct states (B1, B2 and intermediate).

The construction of the GRN consist of two major steps; (1) prediction of an unweighted, directed base network using the scATAC-seq data based on motif enrichment analysis, and (2) the calculation of edge weights based on scRNA-seq data. The resulting GRN can then be used in downstream analysis to identify the driving TFs and their interactions using both the visualization of the GRN, as well as statistical measures, such as the betweenness centrality and degree centrality. We aim to demonstrate that clustering TFs based on their interactions in the GRN and examining target genes for known B1 and B2 markers can predict a (pseudo)temporal order of TFs. This enhances understanding of the cell state transition. The TFs that are identified as relevant for the transition process can then be tested in downstream analysis in the lab.

Poster 4

Núria Gumà

Hospital del Mar Research Institute

"Applying single-cell omics technologies to unravel the impact of E. coli on Crohn's disease immunopathology"

Crohn's disease (CD) is a chronic and relapsing inflammatory condition of the gastrointestinal tract characterized by exacerbated immune responses to the gut microbiota in genetically predisposed individuals. Although a causative role has not been identified, Escherichia coli has been suggested to contribute to CD pathogenesis. Yet, it remains unclear whether the presence of E. coli plays a role in disease heterogeneity by triggering distinct adaptive and innate immune responses in the gut mucosa. It is also unknown how the presence of specific microbiome profiles in CD associate with a unique mucosal antibody repertoire. To address these important questions, we initially conducted an analysis of the composition of the mucus embedded microbiota through 16S rRNA sequencing in both non-IBD controls (n=29) and CD patients (n=20). Our preliminary data revealed a general contraction of certain beneficial bacterial species and a massive expansion of E. coli in a subset of CD patients. Building up on these results, we selected an exploratory cohort consisting of non-IBD controls, and CD patients with or without a substantial expansion of mucus-embedded E. coli for single-cell RNA and V(D)J sequencing (n=10) to map the mucosal immune responses. Our analysis revealed a differential proportion of mucosal immune cell subsets and cellular modules driving inflammation in patients with expanded E. coli. Moreover, these patients display an increased frequency of IgG-expressing plasma cells and extensive B cell clonal expansion. Altogether, our preliminary findings suggest that the presence of E. coli might be promoting a unique inflammatory signature in CD and may trigger mucosal humoral immunity.

Poster 5

Guoping Gu

University of Edinburgh

“Spatiotemporal transcriptomics deciphers tumor cell heterogeneity and tumor-macrophage interactions in bone metastatic prostate cancer”

Prostate cancer is the most prevalent form of cancer among men in western societies. When it advances to metastatic castration-resistant prostate cancer (mCRPC), it no longer responds to androgen deprivation therapies (ADT) and becomes the most severe stage of the disease. This advanced stage is responsible for over 90% of deaths related to prostate cancer. In this study, we employed single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics technology (Stereo-seq) to investigate the complex landscape of bone metastatic CRPC. Our primary objective was to identify resistant tumor cells, characterize their unique molecular signatures, and elucidate the spatial dynamics within the tumor microenvironment. By integrating scRNA-seq data, we successfully pinpointed resistant tumor cell sub-populations, revealing novel clusters distinguished by their gene expression profiles that contribute to drug resistance. Subsequent spatial analysis allowed us to precisely locate these resistant clusters within the tumor architecture, providing insights into their distribution and spatial organization. Furthermore, we explored the interactions between these resistant tumor cells and the tumor microenvironment, with a specific focus on metastasis associated macrophages. Our findings demonstrate a significant presence of infiltrated macrophages surrounding the identified resistant tumor cells, suggesting a critical role of tumor-macrophage interactions in supporting and possibly enhancing tumor resistance. Through detailed analysis of the interaction patterns, we aim to illustrate mechanisms by which macrophages contribute to the survival of resistant tumor cells, and potentially offering new targets for therapeutic intervention.

Poster 6

Irepan Salvador-Martínez

Centro Nacional de Análisis Genómico

“Quantifying adaptive evolution of the human immune cell landscape”

The human immune system is under constant evolutionary pressure, primarily through its role as first line of defence against pathogens. Accordingly, population genomics studies have shown that immune-related genes have a high rate of adaptive evolution. These studies, however, are mainly based on protein-coding genes without cellular context, eaving the adaptive role of cell-types/states uncharted. Inferring the rate of protein-coding genes adaptation in human immune cells at cellular resolution, we found cell-types from lymphoid and myeloid compartments to harbour significantly increased adaptation rates (eg foetal Pre-ProB and adult Trm CD8+). In the Lung, we found that cell-types with high adaptation localise to barrier tissues, suggesting an adaptive response to external challenges such as respiratory pathogens. We further analysed iPSC-derived macrophages responding to various challenges, including pro- and anti-inflammatory cytokines or bacterial and viral infections, the latter simulating the evolutionary arms race between humans and pathogens. Here, we found adaptation in early immune responses, suggesting host benefits to adapt to early infection stages to control pathogen spread.

Together, our study reveals spatio-temporal and functional biases in human immune populations with evidence of rapid adaptive evolution, providing a retrospect of forces that shaped the complexity, architecture, and function of the human body.

Poster 7

Julia Bandres-Meriz

Center for Biomarker Research in Medicine

“Metabolic reprograming in diffuse gliomas and response to drug therapy ”

Background: Mutation on the isocitrate dehydrogenase (IDH) gene is the main criteria for classifying adult-type diffuse gliomas into glioblastoma (IDH wild type, wt) or astrocytoma and oligodendroglioma (IDH mutant). Mutation of the IDH gene results in production of the onco-metabolite α-ketoglutarate, alterations in Krebs cycle and cell energetics. Current efforts to treat diffuse gliomas focus on understanding the intricate metabolic reprograming taking place in the tumor. We hypothesize that IDH mutation leads to metabolic differences in IDH wt and mutant diffuse gliomas, ultimately affecting tumor growth and response to therapy.

Methods: Tumor tissue and matched plasma samples (n=39) were collected during surgical resection. Untargeted metabolomics was performed using HILIC-HRMS. In addition, drug response in patient derived cells (PDCs) was assessed in a drug screening platform. Downstream data analysis included supervised and unsupervised methods as well as pathway and network analyses.

Results and conclusions: IDH wt and IDH mutant showed different metabolic fingerprints. Approximately 25% of metabolites were present at higher levels in IDH wt tumors. Interestingly, the concentration of microbiome-derived uremic toxins, i.e., indoxyl sulphate and p-cresyl sulphate, were significantly higher in tumor tissue of IDH wt patients ($p<0.001$). In plasma, p-cresyl sulphate ($p=0.039$) concentration, but not indoxyl sulphate concentration, was also higher in IDH wt. Moreover, there was a positive correlation in p-cresyl sulphate and indoxyl sulphate concentration between plasma and tumor tissue ($r=0.678$, $p<0.001$; $r=0.461$, $p<0.005$). Higher levels of uremic toxins in tumor tissue were associated with better response to Panobinostat, Vorinostat, Temsirolimus, Bortezomib, Ixazomib, Lazertinib and Dacomitinib treatments. Further single-cell omics studies investigating the distribution and subcellular localization of uremic toxins in tissue are necessary to elucidate their potential role in diffuse gliomas.

Poster 8

Inés Sentís

Centro Nacional de Análisis Genómico

"Exploring Tumourigenic Transformation in Rhabdoid Tumours Through Spatial Multi-Omic Analysis"

Rhabdoid tumour (RT) is a paediatric cancer characterised by the biallelic inactivation of SMARCB1 (subunit of a chromatin remodelling complex). Alterations in this remodeller have effects in the epigenome and ultimately the transcriptome (Wang et al., 2017). Despite their low mutation burden (Gröbner et al., 2018), previous studies have shown high infiltration of immune cells in RTs (Chun et al 2019, Leruste et al., 2019). While molecular subgrouping based on genome-wide DNA methylation and bulk gene expression profiles aids in disease stratification, a comprehensive exploration of intra- and inter-tumoural heterogeneity could elucidate the regulatory mechanisms and tumour microenvironment interactions within these tumours. To this purpose we have retrospectively sequenced a cohort of 12 RTs of different locations (intracranial and extracranial). For each donor resection, we have used Single Cell Multiome ATAC + Gene Expression kit (10x Genomics) which simultaneously profiles gene expression and open chromatin from the same cell, and Visium Spatial Gene Expression (10x Genomics) technology to obtain mRNA molecular profiling in its tissue context. We observed that RTs display a high level of heterogeneity and reveal themselves as different phenotypic entities. Spatial transcriptomic analysis display immune-infiltrated regions within the tumours of most patients, underscoring the immunogenicity of RTs. Inference of spatial co-expression patterns, outline metabolic and stromal-related gene programs active across all tumour tissues of the examined cohort. Deconvolution of the visium spots, coupled with paired single-cell data, shows a general pro-tumorigenic profile of macrophages and T-cells in the infiltrated areas. Notably, intracranial versus extracranial tumour location shows most differences transcriptomically, mirroring findings from previous studies on methylation patterns (Chun et al 2019). Despite the inter-tumoural differences, some similarities emerge among them revealing potential key deregulators of these tumours. Among these, hepatocyte growth factor (HGF) and its receptor, Mesenchymal epithelial transition factor (c-MET), are expressed in a subset of our cohort, presenting as plausible therapeutic targets for RTs.

Poster 9

Juan Luis Melero
Omniscope

“Spatio-temporal tracking of therapy-induced T cell immunity against pediatric rhabdoid tumors - A success case report”

Malignant rhabdoid tumors (RT) are rare childhood tumors that initiate during embryonic development and manifest in the kidney, soft tissues, or the brain. Current treatment strategies include surgery, chemotherapy, and radiation, without biomarkers to personalize therapy selection or duration and extremely poor patient prognosis. Immune checkpoint inhibitors (ICI) may serve as a first-line therapy, with PD-L1 expression as a biomarker for patient selection.
Anti-PD-L1 therapy has been shown to be highly effective in high mutational burden adult solid tumors, but evidence for its value for pediatric RT therapy is poorly investigated. Here, we report a comprehensive spatiotemporal profiling of the T cell repertoire for complete remission (CR) of a pediatric RT patient following adjuvant combinatorial treatment with chemotherapy (doxorubicin, cyclophosphamide, and etoposide) and anti-PDL1 (atezolizumab). Single-cell RNA and TCR sequencing identified an inflamed tumor microenvironment (TME) with extensive clonal expansion of the effector-memory CD8 and T helper/regulatory CD4 compartment. We found notable overlap between tumor-infiltrating and lymphocytes in circulation using ultra-deep single-cell T cell receptor sequencing (OS-T, Omniscope), which allowed us? to quantify the T cell dynamics throughout ICI treatment (1-year follow-up) and informing clinical decisions in real-time. Therapy-induced clonotypes, identified through noise-modeling of the overall deep repertoire distribution, were mostly newly activated CD8 T cells with conserved clonal sizes observed across all time points. We also found a late expansion of tumor-resident CD4 T helper cells that may form long-lasting anti-tumor immunity. To confirm tumorigenicity, we now validate the anti-tumor activity of tumor-resident and therapy-induced clonotypes against the primary tumor in vitro, shortlisting candidate T cells for potential second-line TCR-based therapy. In summary, ultra-deep T cell sequencing of immune cells in circulation enabled the quantification and tracking of therapy-induced T cell clonotypes. Clonal persistence pointed to the induction of long-lasting anti-tumor activity for patient monitoring and identified candidates for personalized TCR-based T-cell therapies.

Poster 10

Anna Dostalova

Immunai

“Leveraging Proprietary CITE Panels for Enhanced Single Cell Omics Analysis: A Focus on Immune Cell Typing”

Recent advancements in single-cell omics technologies have revolutionized our understanding of cellular heterogeneity and function. Here we present Immunai's innovative approach to single-cell RNA sequencing (scRNAseq) data curation, with a specific emphasis on immune cell type identification. Central to our methodology is the development of a proprietary scRNAseq processing pipeline, uniquely tailored to make use of proprietary CITE (Cellular Indexing of Transcriptomes and Epitopes) panels. By integrating CITE information into our analysis, we significantly enhance the accuracy and granularity of cell type identification, surpassing the limitations of RNA-only datasets. Furthermore, we extend the utility of CITE data beyond direct integration by constructing comprehensive immune cell type references using internally processed CITE datasets. Leveraging these references, we can effectively annotate RNA-only datasets with unprecedented cell type granularity, akin to datasets containing protein information. Using this approach we have been able to construct a comprehensive single-cell database mostly consisting of datasets from immuno-oncology and autoimmune therapeutic areas. By consistently annotating cell types across diverse datasets, our database serves as a robust knowledge base, empowering us to help our partners to address a myriad of biomedical questions with confidence and accuracy.

Poster 11

Jessica Kanglin Li

Josep Carreras Leukaemia Research Institute

“DeepSCore: a comprehensive, flexible, and user-friendly single-cell multimodal data annotation tool”

The development of single-cell multimodal sequencing technologies allows for the simultaneous profiling of transcriptomes and epigenomes within the same cells. This presents a distinctive opportunity to collectively analyse multimodal data at the single-cell level, facilitating the orthogonal characterization of distinct cell types and states. However, the mapping of different data sources remains a statistical and computational challenge.
In this project, we introduce deepSCore, a novel R package designed as a reference-based classifier for annotating single-cell multiomics data. Leveraging a flexible deep learning model, deepSCore classifies cell types based on the reference data, empowering users to adapt the model training process by adjusting parameters according to their specific input data.
The tool encompasses three main applications: an intra-modality classification, where the model learns from scRNA-seq (or scATAC-seq) data to predict another scRNA-seq (scATAC-seq) dataset; a cross-modality classification, utilizing scRNA-seq data as the reference to predict scATAC-seq data, thereby connecting gene activity matrices between these two modali4es; and a bridge classification, relying on scMultiome data to transfer labels from the reference peak multi-assays to any query peaks assay.
Extensive testing using different human healthy datasets, including PBMC, pancreas, and kidney, demonstrated high-quality cell type annotations for each case. Comparative analysis against established cell type annota4on tools such as Seurat, SCINA, scID, scMAP, and CHETAH revealed deepSCore’s significantly high performance.
Ongoing efforts involve further tes4ng in diverse biological scenarios, comparing results across general cell types and specific cell subtypes, as well as distinguishing between healthy and pathological cells. In conclusion, deepSCore emerges as a highly versatile tool, allowing users to tailor model parameters to their needs and effectively handling distinct single-cell modalities, thereby exhibi4ng exceptional performance across challenging substates.

Poster 12

Davide Maspero

Centro Nacional de Análisis Genómico

“Unveiling the Landscape of Inflammation: A Machine Learning Approach exploiting Single-Cell Genomics”

Inflammation, a fundamental biological process, plays a pivotal role in various physiological and pathological conditions. While its controlled manifestation is vital for maintaining homeostasis, dysregulation can lead to adverse outcomes. Despite significant strides in understanding inflammation within specific diseases, achieving a comprehensive global perspective remains elusive. This underscores the importance of deciphering inflammation for advancing personalized medicine.

In this study, we leverage cutting-edge machine learning techniques alongside recent advancements in single-cell genomics to unveil the intricate landscape of circulating immune cell activation during inflammatory processes across immune-mediated disorders, infections, cancer, or other inflammatory diseases.

After constructing a single-cell atlas comprising over 2 million peripheral blood mononuclear cells (PBMC) from 356 patients across 18 diseases, we accomplished two primary goals.

Firstly, we proposed a machine-learning approach based on Gradient Boosted Decision Trees and post-hoc interpretation with SHAP (SHapley Additive exPlanation) values to delineate altered inflammatory molecule activity in PBMCs, offering functional biomarkers to elucidate inflammation mechanisms.

Secondly, we devised a patient classifier that exploits the integrated cell embedding to assign the correct disease to each patient. Performance evaluation using a 5-fold cross-validation strategy, with balanced accuracy scores higher than 0.9, suggests that our approach lays the groundwork for utilizing single-cell atlases as precision medicine diagnostic tools, leveraging cells as a robust resource for patient stratification.

Overall, our study enhances comprehension of both acute (e.g., inflammatory bowel disease, sepsis) and chronic (e.g., cirrhosis, asthma, chronic obstructive pulmonary disease) inflammatory processes and paves the way for a precision medicine framework utilizing advanced unsupervised and interpretable machine learning techniques

Mohmed Abdalfttah

Centro Nacional de Análisis Genómico

“Deciphering Astrocyte Heterogeneity and Function in Neurodegenerative Diseases through Single-Cell and Spatial Transcriptomics”

Astrocytes are pivotal in brain function and response to injury, with their complexity and roles in CNS health and disease now appreciated through advances in single-cell transcriptomics. This approach has uncovered their heterogeneity, highlighting the nuanced roles of astrocytes in protective and detrimental responses across various disease contexts. Seminal studies by Batiuk et al. (2020) and Habib et al. (2020) have been instrumental in identifying distinct astrocyte subtypes, each characterized by unique molecular signatures and functional roles, particularly in neurodegenerative diseases like Alzheimer's and Parkinson's.

Building upon these findings, our project has developed an extensive atlas of astrocyte diversity, analyzing data from approximately 500,000 astrocytes across 18 public datasets. These datasets cover a range of conditions, including Alzheimer’s disease, Parkinson’s disease, Multiple Sclerosis, Temporal Lobe Epilepsy, COVID-19, Autism Spectrum Disorder, Lewy Body Diseases, and Frontotemporal Dementia. Our analysis identified four populations of reactive astrocytes, three types of fibrous astrocytes, and eight types of protoplasmic astrocytes. Transcription factor activity analysis revealed heightened activity in NFkB, AP1, RELA, KLF4, and STAT1 in reactive astrocytes populations. Differential expression (DE) analysis highlighted disease-specific signatures, with IRF3 upregulated in Parkinson’s disease, MEGF10 in COVID-19, CD44 in Multiple Sclerosis, and CX3CL1, GFAP, CPEB1 in Alzheimer’s disease.

Furthermore, our atlas integrates spatial transcriptomics to map astrocyte populations within healthy and Alzheimer’s disease-affected CNS regions. This spatial mapping distinguishes between astrocyte populations in the gray and white matter, offering unprecedented insights into their distribution and functional states across different brain regions. This comprehensive atlas not only elucidates the diversity of astrocytes but also enhances our understanding of their specific contributions to neurodegeneration and various neurological disorders.

Poster 14

Fumio Nakaki

EMBL Barcelona

“Probability of stealth multiplets in sample-multiplexing for droplet-based single-cell analysis”

One of the critical technical limits in droplet-based single-cell RNA sequencing (scRNA-seq) is the stochastic presence of a multiplet, a droplet containing multiple cells by chance. Recent advances in sample-multiplexing scRNA-seq (mx-scRNA-seq) enable us to detect part of these multiplets and reduce the occurrence of undetectable ones, allowing us to evaluate more cells and samples simultaneously. However, the probability of potential undetectable multiplets in mx-scRNA-seq remains to be quantitatively examined. Here, we developed a simple theoretical model to predict four classes of possible multiplets in mx-scRNA-seq: Homogeneous stealth, partial stealth, multilabelled, and unlabelled. We estimated each class’s probability and found that the partial stealth multiplet, which has yet to be described in detail, may substantially affect the whole data when the sample labelling process was suboptimal. In addition, we illustrated their presence in actual mx-scRNA-seq datasets when the sample labelling was suboptimal and when a sample-demultiplexing algorithm was unsuitable for the dataset to be analysed. Our results emphasise the necessity of optimising the labelling procedure and offer a theoretical basis to estimate the probability of each type of multiplets to ensure the integrity of mx-scRNA-seq.

Poster 15

Tarran Rupall

Wellcome Sanger Institute

"Single-cell gene expression and surface protein expression in the stimulated peripheral immune systems of healthy Mexican individual"

Genetic variation is associated with immune disease susceptibility and influences the ability to respond to pathogens. Latin Americans are genetically diverse populations but are only represented in less than 2% of genomic studies. Therefore, we aim to understand how genetic variation, within a Mexican cohort, contributes to the heterogeneous response to stimulus within different immune cells, by stimulating peripheral blood mononuclear cells (PBMCs) and applying single-cell multiomic assays.

We stimulated PBMCs from 5 Mexican healthy donors in vitro, with anti-CD3, IFNα and R848 and generated single-cell RNA-seq and CITE-seq (130 cell surface proteins) data at 0h, 16h, 40h and 64h post stimulation. To increase throughput, we overloaded 10x Chromium and implemented a multiplexing strategy that combines cell hashing and genotype-based multiplexing, pooling up to 20 samples, within one reaction.

Overloading Chromium enhances cell recovery by 125% per lane and in total we recover 136,868 cells (average 2,851 per sample). We detect changes in cellular composition upon stimulation over time. The proportion of B cells increases significantly at 64h compared to earlier time points after IFNα and R848 stimulations. Stimulating with IFNα also resulted in a significant increase in the proportion of monocytes at 64h compared to earlier time points. Principal component analysis reveals distinct transcriptional changes that occur within PBMCs upon stimulations, with the biggest changes occurring after anti-CD3 stimulations. Samples stimulated with IFNα had the biggest differences at 64h, with samples at 16h and 40h clustering closer to 0h. We conducted single-cell differential gene expression analysis for each stimulus across time points and detected 100s of differentially expressed genes within the main cell subtypes.

This dataset demonstrates our approach enables rapid and robust data generation for large patient cohorts. Initial analysis has already revealed distinct compositional and transcriptional changes upon stimulation over time at the single-cell level. We are scaling this approach up by generating single-cell gene expression and cell surface protein expression data for 200 Mexican donors, in addition to generating whole genome sequencing data for each individual.

María del Carmen Martos

Immuneo therapeutics

"BITAP: an Integrative Pipeline for Immunogenic Peptide Prediction and Immunotherapy Efficacy Evaluation in Cancer"

Cancer is the second leading cause of mortality worldwide, necessitating the development of innovative approaches for effective treatment and improved patient outcomes. Immunotherapy has emerged as the fifth pillar of cancer treatment alongside surgery, radiotherapy, chemotherapy, and targeted therapy thanks to Next Generation Sequencing (NGS). In particular, epitope-based immunotherapy has garnered significant attention due to its potential to induce targeted and specific immune responses against tumor antigens. Neoepitopes play a critical role in initiating and modulating immune responses.

Bioinformatics pipelines for epitope design have become an essential tool to create personalized vaccines in the field of cancer immunotherapy. These pipelines utilize computational algorithms (NetMHCpan, MHCflurry, NetCTLpan...) and databases (CEDAR, IEDB) to predict and identify potential epitopes that can elicit T-Cell immune responses against cancer cells. However, the effectiveness of these pipelines heavily relies on the accuracy and efficiency of the underlying algorithms and methodologies employed and only a few predicted neoantigens are highly immunogenic.

In our work, we present BITAP (Bioinformatics Targeted Adressed Peptides), an novel pipeline that combines the whole epitope process for immunogenicity prediction, (TAP proteasome, processing, epitope and MHC binding and TCR complex), and the immunoreaction in cancer at the single-cell level. We add scRNAseq analysis to characterize interplays between cancer prognosis and CD8+ T-cell populations. In conclusion, our integrated BITAP pipeline predicts personalized immunogenic epitopes according to the immune subtypes that enhance a immune response.

Poster 17

Nair Varela

CINBIO - Universidade de Vigo

“Challenges and applications of single-cell genomics for the study of circulating tumor cells”

Preclinical and clinical evidence indicates that circulating tumor cells (CTCs) play a key role in the metastatic process. CTC clusters correspond to cell aggregates that detach from primary tumor sites and are generally associated with unfavorable clinical outcomes and up to 100-fold greater metastatic potential when compared to individual CTCs. While the prevailing hypothesis posits that cells within CTC clusters cooperate during dispersal, dissemination, and colonization processes, the extent of cellular heterogeneity and clonality within these clusters remains unclear.

On this basis, we use whole-exome sequencing data stemming from primary tumor, metastasis, single CTCs, and CTC clusters from multiple mouse xenografts of a triple-negative breast cancer cell line. We have developed a pipeline to identify genetic variants in single CTCs and CTC clusters that take advantage of calls made for primary and secondary bulk samples. In particular, analyzing CTC clusters is very challenging because we have to handle amplification biases for groups of 2 to ~5 cells. This specific pipeline allows us to explore the genomic intratumor heterogeneity and evaluate the origin and clonal composition of these CTC clusters.

Daniel Steiert

Charite/MDC/BIH

"Multi-Omics characterization of the first Waldenström Macroglobulinemia -like mouse model"

Waldenström Macroglobulinemia (WM) is a cancer, genomically characterized by its founder mutation in MYD88 (>90% of patients) and a unique gain-of-function mutation in CXCR4 (30-40% of patients) in B-cells. Clinically, this disease is characterized by a monoclonal IgM surge and infiltration of lymphoid organs by lymphoma cells.

To investigate this cancer, we generated the first genetically engineered mouse model to mimic the characterization of B-cell proliferation in the human WM. This was achieved by a CXCR4C1013G and a conditionally expressed constitutively active Myd88L252P mutation in all B-cells (CD19Cre) or in activated B-cells (AIDCre). B-cells from mice were first characterized by their transcriptomic profile and their immunophenotype to verify the expected similarity to the human condition. Subsequently, an in-depth analysis of the transcriptomic profile has been performed with state-of-the-art single cell technologies by leveraging single-cell proteogenomics (Abseq) and spectral flow cytometry.

The RNAseq data revealed a high level of similarity with a gene-set derived from a WM cohort, sequenced with as well with RNAseq. With the transcriptomic depth of single-cell proteogenomics and the high number of cells from the spectral flow cytometry, we anticipate the detection of not just the cancerous B-cells but also their microenvironment. This high-plex data and their integration will provide a deep characterization on the developmental trajectories of the cancerous B-cells driven by MYD88 and CXCR4 related signaling signatures.

With this first WM-like mouse model coupled with these unique insights into the disease, we will establish a comprehensive basis for the formulation of WM treatment hypotheses and their in-vivo validation.

Poster 19

Rosario Astaburuaga-García

Charité – Universitätsmedizin Berlin

“RUCova: An R package to Remove Unwanted Covariance in mass cytometry data of intracellular signal”

Mass cytometry enables the quantification of protein abundance in single-cells using antibodies conjugated with metal isotopes, facilitating the investigation of intracellular signals that determine the state and response to treatments. However, challenges such as heterogeneous cell volume and labeling efficiency confound the data, leading to spurious correlations between markers and hindering comparisons between cell lines, perturbations, and cell states. To address this, we developed RUCova, an R package designed to remove unwanted covariances in mass cytometry data.

Since cell volume and labeling efficiency cannot be directly measured with mass cytometry, we propose the use of four Surrogates of Unwanted Covariance (SUCs): mean iridium (DNA), mean used barcodes, total ERK, and pan AKT. Principal Component Analysis (PCA) applied to these SUCs yields the main axis of unwanted covariance (PC1), which strongly correlates ($\rho = 0.79$) with the median cell area quantified via microscopy images across 8 different head-and-neck cell lines and two conditions (0 and 8 Gy of ionizing radiation). Subsequent PCs do not correlate with the cell area, suggesting they represent other sources of unwanted covariance.

The RUCova method involves fitting a multivariate model for the expression of each measured protein y, based on either (A) the user-defined number of PCs, or (B) directly on the SUCs. If multiple samples are included in the data set and the relationship between protein abundance and SUCs varies across samples, an interaction model is appropriate. After applying RUCova, the new value of the protein is then independent of the SUCs.

Following the application of RUCova, several improvements are observed: (I) marker distributions become narrower, indicating the removal of unwanted heterogeneity, (II) spurious correlations are eliminated while authentic correlations are retained, enabling differentiation between activated and non-activated signalling pathways, (III) relationships between different samples change, facilitating reliable detection of differences between perturbations, and (IV) protein abundance within small and large cells becomes comparable.

Poster 20

Emanuele Pitino

Centro Nacional de Análisis Genómico

“Deciphering Clonal Expansion and the Immune Landscape in Non-Small-Cell Lung Cancer Patients to Identify Biomarkers for Immune Checkpoint Blockade Response”

Over the past decade, the landscape of Non-Small Cell Lung Cancer (NSCLC) treatment has been transformed by the advent of immunotherapies. Notably, Pembrolizumab and Atezolizumab, targeting the programmed death 1 (PD-1) and programmed death-ligand 1 (PD-L1) pathways, respectively, have been adopted as first-line treatments. However, despite these advances, the effectiveness of such treatments is limited by the development of resistance and the absence of reliable biomarkers for predicting therapeutic responses. The current standard, which relies on PD-L1 tumor expression, falls short in addressing these challenges. This highlights a critical need for research into novel biomarkers that can more accurately predict responses to immunotherapy in NSCLC patients.

In our study, we applied single-cell RNA and deep TCR sequencing to explore clonal expansion in 10 NSCLC patients undergoing anti-PD-L1 therapy. Our findings indicate that responders exhibit localized clonal expansion within specific CD4 and CD8 populations, particularly effector memory CD8+ T-cells, compared to non-responders where the clonal expansion exhibit a broader distribution across various populations. Additionally, responders demonstrated a predominant type 1 immune response, whereas non-responders showed an increase in Th2 clones and Tregs expansion.

Responders also exhibited an expansion in cytotoxic CD4 populations, indicating that CD4's role might extend beyond traditional helper functions, actively contributing to the immune response.

By elucidating the distinct patterns of immune response and clonal expansion in NSCLC patients, our study aims to enhance the current understanding of biomarker-driven immunotherapy, opening new pathways for personalizing treatment strategies and improving patient outcomes.

Mario Acera

Josep Carreras Leukaemia Research Institute

“Evaluation of single-cell multi-modal data integration for comprehensive human organ atlas”

The field of single-cell analysis is advancing rapidly, offering unprecedented tools to dissect the complexity of organs at a granular level. Techniques ranging from RNA sequencing to ATAC sequencing (for studying DNA packaging within cells) provide complementary insights, enabling researchers to profile individual cells with remarkable precision. By correlating open chromatin regions—markers of active regulatory elements—with gene expression profiles, these methodologies facilitate a comprehensive understanding of cellular functions and identities.

While previous benchmarking efforts have primarily focused on comparing experimental techniques within specific single-cell assay categories, these studies offer valuable yet incomplete insights into the methodologies' capabilities to characterize complex tissues.

Consequently, our study presents a thorough comparison across multiple single-cell data modalities, offering a wider perspective on how the integration of multi-omics data can capture tissue complexity more effectively.

Taking the kidney as a representative model for complex organs, we conducted a multicenter comparative study across a collection of 19 kidney samples. Our investigation included 3' single-cell RNA sequencing (scRNA-seq), 5' scRNA-seq, and combined snRNA/ATAC-seq experiments. By integrating these diverse single-cell transcriptomic and epigenomic datasets, we aimed to evaluate their effectiveness and determine their complementary or redundant roles in defining cell types and states within both matched and unmatched kidney samples.

Additionally, we assessed the reproducibility of data collection methods across different research sites.

Our benchmarking of single-cell multi-modal data focused on assessing "specificity," "concordance," and "biological predictability." Drawing inspiration from the established concept of anchors in single-cell multiomics integration, we devised a novel three-part analytical framework. This framework facilitates the comparison of data across different dimensions: horizontal (matching similar data types from diverse sources), vertical (combining different data types from the same cells), and diagonal/mosaic (integrating various data types from different cells).

Through a systematic evaluation employing multiple metrics, we determined the contribution of each technology/modality to the resolution of specific cell types in complex tissues and examined how multi-modal data integration enhances our comprehension of tissue cell biology beyond what is possible with any single modality.

Ultimately, our findings aim to provide guidelines for generating reproducible, high-quality tissue atlases. By aiding researchers in making informed choices about combining single-cell omics techniques, our work lays the groundwork for the development of comprehensive human reference atlases and the advancement of groundbreaking discoveries.

Poster 22

Marta Casado-Pelaez

Josep Carreras Leukaemia Research Institute

“Mapping the Spatial Transcriptomes of Cancer of Unknown Primary to Unveil Origin and Treatment Vulnerabilities”

Metastasis is the primary cause of mortality in over 90% of cancer patients. In about 2% of these cancers, standardized diagnostic work-up fails to identify the site of primary origin of the metastasis, a fact that seriously hinders clinical management and treatment choice, since nowadays cancer treatment is predominantly based on the primary tumour. This heterogeneous group of orphan metastases, diagnosed as Cancers of Unknown Primary (CUP), is characterized by early dissemination, aggressive clinical course, unpredictable metastatic patterns, intrinsic treatment resistance and dismal prognosis.

Our research leverages the unprecedent power of Spatial Transcriptomics to address two main objectives: the identification of the tumour's origin and the assessment of CUP biology. This cutting-edge method enables a detailed examination of the complex cellular ecosystems within the CUP, including malignant, stromal, and immune cells, and the exploration of the spatial context of the tumour microenvironment (TME), which plays a pivotal role in cancer progression and response to treatment.

We have generated detailed transcriptomic maps from 34 CUP samples across 15 anatomical sites, providing crucial insights into the intricate biological core of these tumours. Building on the tissue-specificity of transcriptomic profiles, many gene expression programs found in primary tumours are preserved in metastases. By analysing extensive public single-cell datasets of a wide variety of cancer types, we have identified unique cancer-type-specific signatures. Integrating this approach with advanced computational tools that do not rely on single-cell references (e.g., SpaCET and UniCell Deconvolve), we provide an innovative way to identify the potential tissue of origin in CUP samples. Our strategy overcomes the challenges posed by the lack of primary tumour information and the diversity of anatomical sites. Identifying the primary origin could aid treatment decision-making and clinical management of patients afflicted by this dismal disease.

Concurrently, our research into the TME has shed light on the tumour architecture and the spatial interplay among different cell types within CUP tumours, by comparing with both in-house and public spatial transcriptomics datasets of metastasis of known primary. These efforts advance our understanding of CUP biology and could open new avenues to guide targeted therapeutic interventions in patients with CUP.

Poster 23

Patrick Weidner

Belin Institute for Medical System Biology

“Dissecting the impact of somatic mutations in the adaptive immune cell compartment on inflammatory bowel disease patients”

Inflammatory bowel disease (IBD) is complex disorder characterized by the dysregulation of the adaptive immune system leading to chronic inflammation of the gastrointestinaltract. Somatic mutations were recently implicated in the pathology of IBD, in which patient-derived epithelial crypt cells show an elevated rate of somatic point mutations that converge on pro-inflammatory pathways. However, outside the scope of single nucleotide variation and short Indels, larger genomic structural variation like copy-neutral inversions remain notoriously challenging to resolve using conventional single-cell sequencing approaches and typically escape detection. As a consequence, whether many classes of naturally occurring somatic structural variants affect susceptibility to IBD or its disease progression in the adaptive immune cell compartment remains a long-standing question in the field. To systematically address this knowledge gap, we recently adapted the haplotype aware single-cell, single strand sequencing technology Strand-seq for full discovery and genotyping of the somatic mutations arising in primary T cells of both IBD patients and healthy controls. By establishing the types and rates of somatic mutations that emerge in T cells during IBD progression, we are generating complete somatic mutational “landscapes” that cluster cells based on recurrent mutational profiles across health and disease. We then aim to directly measure the functional consequences of somatic mutational profiles by integrating our genomic readouts with epigenetic (single-cell nucleosome occupancy profiling) and transcriptomic (scRNA-seq and immune repertoire profiling) molecular phenotyping. We expect that somatic mutations occur frequently in the T cell compartment of IBD patients, where they could impact key pro-inflammatory pathways that lead to positive selection of disease specific subclones contributing to IBD pathology.

Oleksandra Aust

Institute of Pathology, Charité-Universitätsmedizin

“Reporter-assisted classification of resistance mechanisms to broad-spectrum RAS inhibition in KRAS-mutant colorectal cancer”

KRAS activating mutations are observed in 40% of colorectal cancer (CRC). Recently, RAS inhibitors targeting a limited spectrum of mutations have demonstrated preclinical eaicacy in RAS-mutant cancer models and promising results in the clinic. However, emerging resistance to RAS inhibition limited long-term eaicacy. Here, we aim to identify and categorize mechanisms of resistance to a broad-spectrum active-state RAS inhibitor (hereafter termed RASi) in CRC cell lines. To this end, we employed a compartment-specific dual-color ERK activity reporter system to classify emerging resistant cell populations by KRAS eaector pathway reactivation, and use single cell multi-omics for characterization.

We found that KRAS-mutant CRC cell lines are universally sensitive to RASi. RASi treatment halted proliferation and resulted in inhibition of the RAS-RAF-MEK-ERK axis, as we demonstrated by western blot analysis and the ERK reporter. Long-term dose escalation of RASi in stably ERK reporter-expressing CRC cell lines revealed multiple patterns of ERK reactivation in emerging resistant cell populations. Cells sorted according to their ERK activity patterns were characterized on the genome, phosphoprotein and transcriptome levels by exome sequencing, mass cytometry, and single cell RNA-sequencing, respectively. The resistant subpopulations showed distinct genomic mutations as well as heterogeneous signal network states and transcriptomic signatures, casting light on the resistance mechanisms.

In summary, our observations validate reporter-assisted screening together with single cell analyses as a powerful approach for dissecting the complex landscape of therapy resistance.

Poster 25

Lorna Wessels

European Center for Angioscience

“Multi-view Latent Variable Models for Unsupervised Single-cell Data Integration Across Cell-types and Samples”

As the availability of large-scale single-cell atlases increases, the complexity of data integration tasks escalates. Thereby, multiple axes of comparisons have to beaddressed such as, combining independent datasets under similar conditions, measuring sample variability across distinct cell types, and integrating data across different model species. Here we propose to leverage different existing multi-view latent variable models to integrate, at the sample-level, single-cell datasets of distinct experimental design complexity. We show how by operating in latent spaces, these models preserve the inherent structure and relationships present in the original data across multiple cell-types without the need for a one-to-one mapping procedure at the feature or single-cell resolution. Furthermore, we show that the unsupervised nature of these latent variable models are adaptable to large, heterogeneous datasets, making them well-suited for addressing the multifaceted challenges of data integration in modern research settings. Moreover, these models allow for the use of prior-knowledge facilitating its contextualization and the interpretability of sample-level integration. By applying these techniques in different case studies, we highlight the efficacy of leveraging latent spaces for different types of complex, unsupervised integration tasks in the era of large-scale single-cell atlases.

Shubhra Ashish Bhattacharya
Josep Carreras Leukaemia Research Institute

"Characterizing gene expression changes in myelodysplastic syndrome patients with mutations in cohesin subunit STAG2 at bulk and single cell resolution"

Background: Loss of function mutations in the cohesin complex subunit stromal antigen 2 (STAG2) frequently occur in myelodysplastic syndrome (MDS). STAG2 mutations are associated with an increased risk of progression towards acute myeloid leukemia (AML) and an overall worse prognosis. The ring-like cohesin complex regulates the three-dimensional organization of chromatin and affects gene regulation. However, exact genetic changes caused by loss of STAG2 In MDS and how they may contribute to disease progression are still to be understood.

Aims: By studying the transcriptomic changes in bone marrow of patients with myelodysplastic syndrome (MDS) at bulk and single cell level, we aim to identify specific genes and pathways deregulated in STAG2 mutant (STAG2mut) samples. Finally, these findings will be functionally characterized in cell models.

Methods: We analyzed whole transcriptome sequencing (WTS) data from bone marrow samples of a cohort of 753 MDS patients including 48 samples with STAG2 mutations. Differential gene expression analysis was performed on STAG2mut v/s STAG2wt with DESeq2 and affected pathways were identified using gene set enrichment analysis. To infer changes in cell populations we used XCell and correlated these results with available cytomorphology data. To gain a higher resolution on cell types affected in abundance and their gene expression, we used 3' single cell sequencing on bone marrow samples from 4 MDS patients with and 3 without STAG2 mutation. The scRNA data was processed by filtering mitochondrial gene expression, removing doublets, correcting for ambient mRNAs and regressing out cell cycle effects. Cell clusters were identified using the Seurat pipeline and annotated using two different sources of dif fering resolutions.

Results: Consensus clustering on WTS data revealed that patients with mutations in the STAG2 consistently clustered together indicating a distinct transcriptomic class associated with this mutation. 398 were differentially expressed genes between STAG2mut and STAG2wt samples. We observed an increased enrichment of hematopoietic stem cells (HSC) and granulocyte progenitor (GMP) in STAG2 mutants. There was also an apparent myeloid bias as indicated by the downregulation of important B cell commitment genes such as PAX5 and VPREB3. These patients further had increased counts of immature cell populations such as myeloblasts and promyelocytes in cytomorphology analysis thus indicative of differentiation block. At the pathway level, STAG2mut were associated with a downregulation of several inflammatory response pathways. This association was independent of the context of frequent co-mutations in ASXL1, RUNX1 and SRSF2. Deregulated genes included IER3 and FFAR2 involved in neutrophil polarization and CCL7 involved in monocyte polarization indicative of aberrant immune activation. A thorough analysis of single cell transcriptomic data is ongoing, initial analysis have indicated that STAG2mut samples have a higher number of hematopoietic stem-like cells compared to wildtype samples. Currently we are performing pseudo bulk analysis and pathway analysis on the single-cell data set.

Summary/Conclusion: Our analysis shows that bone marrow samples from STAG2mut MDS patients display distinct transcriptional alterations. Specifically, a downregulation of inflammatory pathways accompanied by increases in immature cell populations. We are currently testing the hypothesis of how these two observations are functionally linked.

Poster 27

Andrea Martí

University of Barcelona

"Cellular imbalance in Dravet Syndrome: A single-cell RNA sequencing approach in Dravet Syndrome-derived brain organoids"

Dravet Syndrome (DS) is a severe developmental epilepsy linked to a dysfunction in the Sodium Voltage-Gated Channel Alpha Subunit 1 (NaV1.1), encoded in humans by the SCN1A gene mostly in inhibitory populations. In this study, we have generated brain organoids from induced pluripotent stem cells (iPSCs) carrying a loss-of-function mutation in SCN1A (p.A371V:GCT>GTT) and compared them with control healthy organoids by single-cell RNA sequencing (scRNAseq). Our findings shed light on the molecular underpinnings of DS, suggesting intrinsic transcriptional changes happening in the cortical excitatory populations. Genes coding for neurotransmitters controlling the excitatory-inhibitory balance are shifted towards a generalized epileptic phenotype.

Sonal Rashmi

Centro Nacional de Análisis Genómico

“Enti-typer: A Regression-Based Approach for Quantifying Multiclonality in Hematological Malignancies Using Single-Cell Genomics”

Single-cell genomics has emerged as a rapidly evolving domain within computational genomics, enabling in-depth understanding of the heterogeneous and complex nature of hematological malignancies. Despite the availability of numerous effective treatments, the multiclonality of these diseases poses significant challenges for therapy. Comprehensive monitoring of Minimal Residual Disease (MRD) can help assess the early relapse risk and supervise the precise tailoring of treatment strategies accordingly. While machine learning techniques offer a promising avenue for achieving this, the challenge lies in effective feature selection, compounded by limitations in the number of available samples at the bulk level.

Here, we developed a binary regression-based technique called Enti-typer to classify samples and cells into hematological malignancy entities using single-cell RNA-seq data. Going one step further into the classification within tumors, we tested the models for hematological malignancies subtypes using the case of chronic lymphocytic leukemia and their categorization based on IGHV mutational status into mutated (M-) and unmutated (U-) CLL cases. We compiled single-cell RNA sequencing (scRNA-seq) data for a total of 200,000 cells from eight different hematological malignancies and healthy tissues, sourced from publicly available databases. The dataset includes cells from Chronic Lymphocytic Leukemia (CLL, 40,000 cells across 10 patients), Diffuse Large B-Cell Lymphoma (DLBCL, 13,000 cells across 11 patients), Follicular Lymphoma (FL, 16,000 cells across 8 patients), Multiple Myeloma (MM, 1,000 cells), Reactive Lymphadenitis (4,000 cells across 3 patients), transformed Follicular Lymphoma (tFL, 5,000 cells across 4 patients), and Acute Lymphoblastic Leukemia (ALL, 60,000 cells across 13 patients).

Poster 28 (cont)

An in-house Seurat wrapper was used for quality control and regression of all mitochondrial, ribosomal, and repertoire-related genes. We split the normalized expression data into 70% as the training dataset and 30% as the validation dataset. There are three parts to model building using a binary regression-based technique for classification:

- Calculate AUC based on logistic regression for each gene per meta-variable present (disease entity or disease subtype) in the training.
- A threshold of 0.7 and 0.6 was used on the calculated AUC to rule out randomly expressed genes in the case of disease and disease-subtype, respectively. Since there is a fine line between mutated and unmutated CLL, we had to lower the threshold for AUC to 0.6. A range-iteration-based technique was used to obtain a combination of features that could best characterize and predict the meta-variable.
- Lastly, the model was used on the 30% test data to find the AUC for the combination of features.

Additional 5 CLL cases with 57,000 cells were included to the existing CLL cohort to be used for the disease subtypes, i.e., M- and U- CLL cells classification.
Overall, we obtained a very high AUC with selected features of 0.9-0.96 in the predictions of haematological malignancy entities with respect to the test data. Further, with the subtype model classification of CLL cases we could distinguish U-CLL cells from M-CLLs with an accuracy of 0.998. Furthermore, since the model is trained using both tumoral and TME fractions across tissues, we were able to classify tumor microenvironment (TME) cells along with the associated disease.
This logistic regression classifier could potentially be used for more external datasets as well as for the identification of multiclonal disease subtypes within the same patient. Since the training is conducted using different cells from the patient, bulk averaged expression data could be utilized for testing. Furthermore, it could be explored for cell annotation using either single-cell or bulk datasets.

Poster 29

Dmitrii Olisov

European Molecular Biology Laboratory

“Profiling of DNA Rearrangements in Colorectal Cancer”

Colorectal cancer (CRC) tumorigenesis is a complex process initiated by the progressive accumulation of mutations, transitioning from benign adenoma polyps to aggressive invasive carcinoma. Structural Variants (SVs) play a crucial role in driving this cancer progression. Although previous studies have identified patterns and mechanisms of SVs in CRC, the question of what DNA rearrangement processes occur during CRC tumor evolution and what role they play in tumor microenvironment spatial structure remains relevant. Using advanced single-cell and spatial sequencing technologies, we aim to explore how DNA rearrangements interact with the tumor microenvironment and affect treatment responses.

As part of a pilot study, colorectal cancer liver metastasis organoids samples were provided by the Rene Jackstadt group from the Heidelberg Institute for Stem Cell Technology and Experimental Medicine. One sample was found to resistant to the standard chemotherapy treatment, while the other was sensitive. Both samples were sequenced with Oxford Nanopore technology and single-cell DNA template strand sequencing (Strand-seq). In brief, both specimens have an aneuploid karyotype with varying copy number of chromosomes, and each sample had over 40 thousand structural variants detected, prevalently insertions and deletions, while a few dozens individual SVs were found only in the resistant sample. Preliminary analysis showed that some of the duplicated genes are involved in the drug metabolism and cellular transport, for example gene ABCC3, reported to be strongly associated with chemotherapy resistance. The next step is the generation of single-cell transcriptomic maps of the samples using Xenium technology to profile the cells within the tumor microenvironment. By developing a multi-omics integration approach for two data layers, we can pinpoint the functional consequences of specific DNA rearrangements on the cell composition of the tumor ecosystem transcriptional states of its components.

Poster 30

Aida Ripoll

Barcelona Supercomputing Center

"An integrative meta-analysis framework to uncover the role of sex and age in circulating immune cells at single-cell solution"

Single-cell RNA sequencing (scRNA-seq) has enabled deciphering the human transcriptome at an unprecedented resolution. Its popularity for studying how donor characteristics affect gene expression has grown tremendously now that scale, cost, and sensitivity have significantly improved. As population-based datasets continue to increase, there is a need for a framework that avoids sharing privacy-sensitive data and rerunning analyses of datasets every time new ones are available.

Here, we have developed such an integrative statistical meta-analysis framework that can work with both discrete and continuous variables. To show proof-of-concept, we used this framework to identify sex- and age-associated changes in gene expression, cell type composition, and gene expression variability. For this, we applied the framework to three peripheral blood mononuclear cell datasets within the sc-eQTLGen consortium, together spanning 140,306 cells from 154 donors.

We show that our meta-analysis methodology substantially increased the statistical power to detect differentially expressed (DE) genes over taking the union of the DE genes from the individual datasets separately. Sex-DE genes detected in the individual datasets are all located in the sex chromosomes, as these have generally large effect sizes. Our meta-analysis identifies additional sex-DE genes, including many with smaller effect sizes located in autosomal chromosomes. In addition, the meta-analysis considerably increased the number of detected age-DE genes up to hundreds, especially in CD8+ T cells. The age-DE genes significantly overlap with previously identified age-related genes, but we also discovered many novel ones.

Importantly, unlike expression changes with sex, most of the gene expression changes with age in CD8+ T cells are driven by naïve to memory cell type proportion shifts. This observation required us to identify the true gene expression changes at higher cellular resolution. These findings underscore the benefit of single-cell data to distinguish cell type and state composition from gene expression level changes.

Leveraging the single-cell resolution of scRNA-seq, we investigated cell-to-cell variability disparities associated with individual traits. Sex-differentially variable (DV) genes exhibited significant biases towards either depending on the cell type, whereas age-DV genes displayed significant bias only in CD4+ T cells towards young individuals. Notably, our analysis revealed a low overlap between DE and DV genes for both sex and age, suggesting distinct underlying mechanisms governing gene expression and its variability.

Lastly, to validate that our meta-analysis framework works robustly when a large number of datasets are available, we applied the framework to nine peripheral blood scRNA-seq datasets from 565 donors. We identify many additional sex- and age-associated gene expression changes, especially in the less abundant cell types. Our findings using the extensive meta-analysis setup were highly replicated in a single large dataset of 983 donors (Yazar et al., 2022).

Altogether, we show that our meta-analysis framework can robustly and computationally efficiently identify gene expression, cell type composition, and gene expression variability changes that would otherwise remain hidden. This framework can be easily expanded to associate other single-cell molecular phenotypes, such as single-cell chromatin accessibility or single-cell DNA methylation, with other demographic traits or environmental conditions.

Poster 31

Fabian Imdahl

Helmholtz Institute for RNA-based Infection Research

“Growth-condition specific global transcriptomes of individual bacteria captured by single-cell RNA-seq”

RNA molecules are very powerful proxies to report cellular identities and physiologies. The development of RNA-seq and dual-RNA-seq has opened a new window to decipher host-pathogen interactions. However, most of these methods have been averaging the signal of thousands of cells disregarding the heterogeneity of every individual infected cell. Recently the breakthrough of single-cell RNA-seq paved the way to understand cell-by-cell variability notably the opportunity to discover cellular subpopulations that convey chronic infections. Also, single cell analysis promises to reveal the variety of strategies used by both the pathogen and the host to promote or control disease spread. Although, single-cell RNA-seq is a well-established technique for eukaryotic cells, it failed so far to capture the transcriptome of single bacteria. Bacterial RNAs are extremely rare compared to their eukaryotic counterparts (one hundred less in abundance) and their transient nature in the order of few minutes has prevented easy adaptation of existing protocols.

Here, using a poly(A)-independent single-cell RNA-seq, we report for the first time the development of a protocol that faithfully captures growth-dependent gene expression patterns in individual Salmonella bacteria, across all RNA classes and genomic regions.

We analyzed Salmonella grown in three different conditions: (i) ‘stationary phase’ after overnight growth reflecting mainly resting cells; (ii) ‘oxygen tension’ mimicking the intestinal environment; and (iii) ‘salt stress’ caused by increased sodium chloride concentration in the medium. Furthermore, principal components were identified bioinformatically, enabling us to differentiate between the different populations under the specific conditions. To validate our findings, we compared our results to previously published comprehensive bulk RNA-seq data. On this basis, we were able to conclusively relate the upregulated genes detected in our studies to the corresponding conditions in the bulk assay data. These transcriptomes provide important reference points for single-cell RNA-seq of bacterial species. Future applications could have the potential to elucidate insights of host-pathogen interactions and mixed microvial communities.

Liliya Sokalchuk

IDAEA-CSIC

"Integration of single-cell omics to unravel modes of action of endocrine-disrupting chemicals"

Endocrine Disrupting Chemicals (EDCs) are widely spread global pollutants and are considered a threat to human health, wildlife, and the environment. These compounds are structurally diverse, with some examples found as polybrominated flame retardants (Tetrabromobisphenol A, TBBPA) and organotins (tributyltin, TBT) or synthetic hormones (levonorgestrel and mifepristone). EDCs are referred to as interfering with sex-related signaling mechanisms, however, other studies evidenced the existence of environmental disruptors acting through other hormonal systems, like the glucocorticoid, neuroendocrine, thyroid, or retinoic acid systems.

Our recent research reveals important effects of EDCs exposure at bulk level in zebrafish (neurotoxicity, hepatotoxicity, retinoic acid, and fatty acid metabolism, among others), but little is known about their effects at the cellular level. Single-cell analysis allows to study of discrete cell populations enabling the discovery of new key molecular mechanisms of action of contaminants that are not revealed when performing bulk-omic analysis.

Herein, we investigated the effects of these compounds in zebrafish embryos. Exposures were performed from 0 to 4 or 4 to 5 days post-fertilization and effects on developmental parameters and survival rates were evaluated. After a preliminary dose-response assay in which transcriptomic responses were evaluated by real-time PCR, remarkable concentrations of each compound were selected to perform deeper omic studies and bulk transcriptomic analysis was assessed by RNA sequencing. Then, we optimized a dissociation protocol to obtain viable single-cell preparations embryos. Single-cell RNA-seq analysis was performed in disaggregated zebrafish eleuthero embryo pools and data analysis is pending. Soon, using a holistic approach, we aim to characterize lipidomic responses at the single-cell level to better understand the mode of action of these compounds.

Poster 33

Pauline Kautz

BIH/MDC

“Single-cell mitochondrial genomics reveals the age-related accumulation of thousands of somatic mitochondrial variants and their single-cell distribution shaped by genetic drift and selection”

Mitochondria are essential for cellular energy metabolism and carry their own multi-copy genome. As we age, somatic mutations accumulate in the mitochondrial genome (mtDNA) which has been associated with mitochondrial dysfunction contributing to aging and associated disorders. A quantitative understanding of i) the rate of acquisition of somatic mtDNA mutations during aging, ii) their distribution across cell types as well as iii) their phenotypic impact, in human organ systems is however only emerging. To systematically characterize this, we profiled >300,000 human immune cells from cord blood, peripheral blood, and bone marrow from donors of different ages via mitochondrial single-cell ATAC-sequencing (mtscATAC-seq). While we detect only a limited number of somatic mtDNA variants in human cord blood, this number rises rapidly to a thousand in pediatric samples and several thousand somatic mtDNA variants in adults revealing increasing mtDNA variation with age. At the single-cell level, this corresponds to an overall linear increase in the somatic mtDNA mutational load during aging and at comparable rates across the spectrum of immune cell types. While mostsomatic mtDNA variants are present at low heteroplasmy levels, a small subset of individual variants expands and reaches high allele frequency levels over time, likely due to random genetic drift. Via analysis of the single-cell distribution of somatic mtDNA variants, we observe an enrichment of lowly constrained variants among high heteroplasmy events across age groups and cell types. Furthermore, we observe a decreased frequency of truncating somatic mutations as compared to missense and synonymous variants and an enrichment of variants in different regions of the mtDNA, suggesting potential mechanisms of selection against specific mtDNA mutations. Finally, we observe an age-related increase in the transversion/transition ratio indicative of increasing oxidative damage in the mitochondrial genome. Together, we provide a detailed characterization of somatic mtDNA mutational landscapes and their phenotypic effects across immune cell types as a function of age, thereby advancing our quantitative understanding of how somatic mtDNA mosaicism arises andcontributes to cellular heterogeneity.

Poster 34

Franz Ake

Bellvitge Institute for Biomedical Research

“SCALPEL, a new tool for isoform quantification at single cell resolution”

Alternative polyadenylation (APA) is a widespread mechanism of gene regulation that that affects more than 70% of human genes. mRNA isoforms generated though APA have distinct 3' ends, which can affect mRNA regulation as well as the resulting proteins. APA is well known to be regulated during cell differentiation and it is a major source of gene regulation in the brain. Yet, it is not known till which extend APA contributes to the transcriptomic variability across individual cell populations.
The rapid development of 3’tag-based single-cell RNA sequencing (scRNAseq) has enabled the study of gene expression and the implementation of methods for describing isoform usage at single cell resolution. Yet, most of these methods have low sensitivity when quantifying isoforms at the single-cell level. Here we present SCALPEL, a Nextflow based tool to quantify and characterize isoform differential usage at the single-cell level. Benchmark analysis shows that SCALPEL outperforms current published tools in the identification of genes expressing multiple isoforms and detecting changes in isoform usage. We used SCALPEL to study the changes in isoform usage during the differentiation of human induced pluripotent stem cells (iPSCs) to neural progenitor cells (NPCs). The results from our analysis show clear changes in 3’end usage between iPSCs and NPCs. We aim to use SCALPEL to investigate the role of APA during neural differentiation and how these changes are altered in neurodegenerative diseases.

Robert Castelo

Universitat Pompeu Fabra

“GSVA 2.0: Pathway-centric analysis at single-cell and spatial resolution”

GSVA (https://bioconductor.org/packages/GSVA) is an R/Bioconductor package that enables pathway-centric analyses of data produced by high-throughput molecular profiling technologies.
The interpretation of biological findings from such data is one of the cornerstones of biomedical research, and GSVA facilitates that goal by performing a conceptually simple but powerful change in the functional unit of analysis, from genes to gene sets. Here we describe our efforts to adapt GSVA to data produced at single-cell and spatial resolution, increasing its robustness and scalability, and improving the user interface and documentation. By using pathways instead of gene-centric features, this new version of GSVA contributes to improve exploratory data analysis, and to develop lower-dimensional statistical and machine learning models, on data from single-cell and spatial transcriptomics experiments.

Poster 36

Orane Guillaume-Gentil

Swiss Federal Institute of Technology in Lausanne

"Live-Seq: Live Single-Cell Transcriptomics"

Live-seq is a transformative method for single-cell gene expression profiling that eliminates the need for cell isolation and lysis. Live-seq utilizes Fluidic Force Microscopy (FluidFM) to extract cytosolic picolitre biopsies from living cells, which are subsequently analysed with a highly-sensitive RNA-seq protocol. While the resulting gene expression profiles faithfully represent lysed cell transcriptomes, the sampled cells remain alive and functional in their original microenvironment. Live-seq thus enables spatially-resolved, longitudinal profiling and phenotyping of individual cells, offering exciting opportunities for investigating cellular dynamics and cell-cell communication.

Poster 37

Francesco Cardamone

Max-Planck-Institute of Immunobiology and Epigenetics

"Chromatin dynamics at cis-regulatory regions dictate cell fate identities in early embryos"

Establishing distinct germ layers during early development is imperative for defining cellular identities and facilitating proper body formation to adulthood. Maternally inherited transcription factors, proteins, and histone modifications serve as primary drivers in this process. Yet, the intricate events connecting these dynamics and governing the selective activation or repression of specific genes in a cell-type-specific manner remain poorly understood. In this study, we conducted through 10x Multiome a comprehensive and simultaneous dissection of the epigenetic and transcriptomic states of individual nuclei, from wild-type, E(z), and CBP-depleted Drosophila embryos at the onset of zygotic genome activation (ZGA). Our investigation aimed to unveil the complex interplay between chromatin states, regulatory elements and cell fate specification. We propose that the early epigenetic landscape at cis-regulatory regions plays an instructive role in cell fate specification through two mechanisms: a novel pre-zygotic H3K27me3-repressive action safeguarding aberrant expression of germ layer-specific genes in other tissues and an H3K27ac-active zygotic role guiding cells from a pluripotent state towards proper germ layer specification. Intriguingly, our findings demonstrate that chromatin accessibility is established in the absence of zygotic transcription, nevertheless the co-activator CBP is required for proper cell fate specification, suggesting an inherent role for chromatin maturation independent of transcription and acetylation.

Poster 38

Amos Muench

Charité - Universitätsmedizin Berlin

"Morphology-aware dimensionality reduction and correlation of transcription and histology in spatial transcriptomics"

While novel spatial transcriptomics (ST) technologies have proven effective in identifying tumor domains, routine diagnostics still rely heavily on tissue morphology. Gene expression (GE) and cell morphology describe the state of a cell in different modalities; their integrated observations consist of a shared subspace, a complementary and modality-specific subspace as well as noise. We aim to exploit both the shared and the complementary subspace to integrate histology and ST. To quantify morphology at individual tissue locations and derive informative, human-readable features, several convolutional neural networks both for image classification and segmentation were tested.

Morphology-aware dimensionality reduction for ST data

Among the best performing dimensionality reduction methods for ST data is spatialPCA, which is based on probabilistic PCA. It factorizes a gene-by-location matrix by assuming that its spatial factors follow a multivariate normal distribution. The covariance of the spatial factors is estimated with a Gaussian Kernel on spatial coordinates. We hypothesized that both spatial proximity and morphological similarity shape the transcriptional covariance between locations, and developed an approach that estimates their covariance by summing a Gaussian on the spatial coordinates as well as a Gaussian on MF. Thereby, we account for morphological structures corresponding to functional tissue domains. Our results in healthy and diseased tissues indicate a greater congruence of spatial domains with ground truth annotations, as domains align to fine-grained morphological structures.

Correlation between morphology and gene expression

MF that are associated with transcriptional hallmarks of the tumor's pathology may be inferred from the correlation of MF with GE. To investigate this correlation directly, we applied canonical correlation analysis (CCA) on paired MF and GE data across ST spots. We aim to extend this approach across samples using multiCCA with a structured penalty derived from the spatial neighborhood structure between spots. In a second approach, we intend to uncover MF-GE correlation via predicting marker gene expression from histological images using a neural network and deriving explanation with layer-wise relevance propagation. The uncovered morphological features associated with tumor marker genes will be evaluated by pathologists for their applicability in routine diagnostics..

Poster 39

Aina Rill

Josep Carreras Leukaemia Research Institute

“Unraveling Transcriptomic Signatures of Inflammation in Alcohol-Related Liver Disease Through Single-Cell RNA Sequencing”

Alcohol-related liver disease (ALD) encompasses a spectrum of liver pathologies, with alcohol-associated hepatitis (AH) representing its severe manifestation. Despite its clinical significance, the precise transcriptomic alterations in distinct cell populations during ALD progression remain unknown. This study aimed to elucidate the transcriptomic landscape of liver cellular subsets across ALD stages using single-cell RNA sequencing (scRNA-seq). We collected liver biopsies from patients with AH (n=6), compensated ALD (n=3), and healthy donors (n=3), and unraveled their transcriptomic heterogeneity by single-cell transcriptomics. Our analysis identified more than 60 distinct cell subpopulations based on gene expression paNerns. Notably, 12 subpopulations exhibited significant enrichment in AH, delineating specific AH-associated cellular identities.
More specifically, the biological characterization of AH subpopulations revealed diverse inflammatory and matrix remodeling signatures. These findings correspond to the distinct molecular profiles exhibited by various liver cell types during ALD, including hepatocytes showing upregulation of cytokine signaling, cholangiocytes displaying characteristics of hepatic progenitor cells, endothelial cells expressing cytokine production genes, and hepatic stellate cells exhibiting an activated phenotype associated with inflammation and fibrogenesis. Additionally, lymphocytes and macrophages demonstrate stress-related and inflammatory profiles, respectively.
This comprehensive analysis provides novel insights into the dynamic transcriptomic alterations occurring at the single-cell level during ALD progression. Moreover, the identification of specific AH-associated subpopulations elucidates common inflammatory and matrix remodeling processes across diverse cellular compartments.
Understanding these molecular signatures may offer valuable targets for therapeutic intervention in AH and advance personalized treatment strategies for patients with ALD.

Poster 40

Helena Crowelll
Centro Nacional de Análisis Genómico

“Spatio-temporal dissection of colorectal cancer initiation using whole transcriptome imaging”

Revolutionizing clinical pathology requires new technologies that excel currently applied approaches which multiplex selected biomarkers to guide clinical decision making. While higher-plex techniques recently became available, none is scalable to appreciate the full spectrum of molecular biomarkers holistically. We argue that biomarker discovery requires substantially more features to cope with the complexity of diseases. Here, thousands of genes or proteins, imaged at single-cell resolution, will be required to comprehensively identify diagnostic and therapy-prognostic biomarkers.

Single-cell transcriptomics technologies are contributing to such a leap in feature numbers and have been successfully applied to understand the complexity of healthy and diseased tissues. However, their implementation in clinical practice has been slow, due to the lack of examples of direct clinical utility. On the other side, spatial transcriptomics currently provides too low resolution (spatial capture methods) or too low definition (imaging methods) to be considered as viable next-generation pathology approaches and new high-resolution and high-definition technologies are needed to increase the feature space, while conserving spatial single-cell resolution.

Spatial molecular imaging (SMI) that provides a whole-transcriptome solution at molecule resolution now enables spatial single-cell profiling at a definition, previously only achieved through scRNA-seq. Thus, next-generation pathology is in sight, digitalizing full transcriptomes in tissue sections and improving the current state-of-the-art methods in pathology by orders of magnitude. We applied SMI’s full transcriptome solution to spatially resolve tumor evolution within colon tissues, progressing from premalignant lesions to malignant colorectal cancer (CRC). We generated spatially resolved single-cell landscapes of Tubullovillous adenoma, a premalignant stage with the potential to progress to distinct CRC subtypes. Using the CosMxTM SMI assay, we detected vast spatial heterogeneity, with intertwined cell state niches resembling stem cells or differentiated colonocytes, phenotypes previously linked to the progression into different CRC subtypes. Appreciating the whole transcriptome spatial resolution, we associated gene programs of stemness, fetal development and immunogenicity, the latter spatially correlated with immune-cell infiltration patterns. Importantly, the enrichment of clinically relevant pre-malignant cell states at discrete niches could only be resolved using whole transcriptome spatial data and not through scRNA-seq, providing evidence of the method’s superiority in enabling next-generation pathology strategies

Léonard Hérault

Ludwig Institute for Cancer Research Lausanne

“Metacells facilitate the analysis of single-cell multiomics data”

Single-cell multiomics enables the measurement of different modalities (e.g., chromatin accessibility, RNA, proteins) in the same cell. Combined with the increased throughput of single-cell technologies, these advances hold the promise for a better understanding of cell-type specific transcriptional regulation and its potential alteration. Computational tools play a central role in the analysis of such complex data to cope with their high sparsity, which blurs the correlation between the modalities, and the constant increase in cell and sample numbers in single-cell atlases. To address these needs, metacells, defined as groups of disjoint and very similar cells corresponding to highly granular cell states, can be identified in single-cell omics data and used for downstream analyses.
To date, few studies have focused on metacell approaches for multiomics data and the tools currently available only consider one modality for metacell identification despite evidence that cellular heterogeneity is better resolved with multimodal analysis. Thus, building upon the SuperCell framework developed by our lab for single-cell RNA-seq data [2], we have developed a new metacelll identification approach relying on multimodal graph clustering and investigated how multiomics metacells preserve or enhance the biological signal observed at the single-cell level.
Our results on single-cell multiomics datasets of peripheral blood mononuclear cells (PBMCs) show that metacells preserve the overall structure of the single-cell data and reconcile the different modalities. In particular we observe a significant increase between gene body chromatin accessibility and its expression for 10X multiome data and between gene expression and the corresponding surface protein abundance for CITE-seq data. We then integrated at the metacell level a CITE-seq atlas composed of 18 samples gathering more than 160’000 PBMCs on a standard laptop in 30 minutes, highlighting the interest in metacell approaches to save computational resources. Finally, we were able to show that our multimodal approach outperformsother tools in terms of accuracy and speed.
Overall, we have demonstrated that metacells can be used to analyze single-cell multiomics and improve the consistency between different modalities while significantly accelerating and facilitating data analysis.

Sandra Garcia-Mulero

Bellvitge Institute for Biomedical Research

"Integrative single-cell and spatial analysis to study SLIT-ROBO guidance cues in the immune modulation of pancreatic cancer"

Pancreatic ductal adenocarcinoma (PDAC) is a highly deadly disease, with up to 80% of patients diagnosed at late stages and with a high recurrence rate. These facts highlight the need to find new therapeutic options for PDAC tumors. Remarkably, PDAC tumors are characterized by an extensive presence of stroma, which can constitute up to 90% of the tumor mass. PDAC microenvironment is characterized by high proportion of cancer associated fibroblasts, low vascularization, and high presence of immunosuppressive cells. Genomic profiling of large patient cohorts highlighted the SLIT ligand-ROBO receptor signaling pathway to be disrupted in up to 30% of patients.

Underpinned by spatial transcriptomic analyses from RNAscope data, we have defined the landscape of SLIT and ROBO expression in human PDAC samples. Our preliminary data indicates that SLIT-ROBO determines the spatial positioning of stromal and immune cells. Additionally, analyses on single cell RNA-seq data suggest that SLIT-ROBO axis plays a prominent role in controlling immune suppression in PDAC. Given the prevalent alterations in SLIT-ROBO, unveiling the role of the pathway in the human setting may translate into innovative tailored therapies for PDAC patients.

Poster 43

Lukas Balsevicius

University of Copenhagen

Introduction: Differentiating colorectal cancer patients based on mismatch repair (MMR) mechanism has been established as a major genetic biomarker for the response to immunotherapy. Regardless of MMR status, colorectal cancers exhibit a dynamic range of immune cell infiltration, with a specific set of recurring and spatially organized cellular interactions coordinating the immunological response in both genetically distinct subtypes. Moreover, the immune cell profile was shown to be a superior factor to MMR status in predicting multiple patient outcomes. Hence, we hypothesize that cellular and molecular profile of patient's tumour immune microenvironment, regardless of its genetic status, might define the response to immunotherapy more accurately.

Method: Patient tumours (n = 45) from two phase II clinical trials of treatment with PD-1 inhibitor will be investigated by employing multi-technological single-cell profiling approach. Post-treatment biopsies will be collected and processed for cellular indexing of transcriptomes and epitomes by sequencing (CITE-seq). Cell type specific molecular differences between responders and non-responders will be used as an input to generate antibody panels for spatial tumours profiling (Miltenyi MACSima™ Imaging Cyclic Staining technology) of both pre- and post-treatment biopsies – identifying immunotherapy altered functional cell-to-cell coordination networks in the tissue.

Results: In a preliminary spatial proteomics experiment, we profiled 93,428 cells from two phenotypically distinct treatment-naïve colorectal tumours. Analysis of 30 protein targets from a single formalin fixated tissue slide revealed extensive differences in cellular composition between tumours. Whilst both tumours showed a rich immune infiltration in stroma, intraepithelial immune cell profile was vastly different. One tumour had comparably rich cellular immune profile in the cancer epithelium that included multiple types of innate immune cells (e.g. macrophages and dendritic cells) and T cells (both CD4+ helper and CD8+ cytotoxic cells); whilst the other resembled nonimmunogenic tumour with an epithelium completely deserted of T cells.

Conclusion: We demonstrated the applicability of cyclical staining and imaging technology to accurately define spatial immune cell communities in colorectal tumours. By doing so, we are now enabled to develop further strategies to identify functional immune cell profiles predictive of immunotherapy response in the main clinical cohort.

Poster 44

Agostina Bianchi

Centre for Genomic Regulation

“Single-cell multiomics analysis unravels DNA methylation heterogeneity in lymphoma-specific enhancers across healthy B cells”

Tumors evolve from a single ancestor cell through multiple rounds of clonal selection, in which oncogenic hits accumulate promoting malignant transformation. However, the early steps of this evolutionary process remain largely unstudied due to technical limitations. Recent studies have evidenced positive selection of clones carrying oncogenic hits even within healthy tissues. Nevertheless, the analysis of oncogenic events in healthy cells has mostly focused on somatic mutations. Here we hypothesize that, concurrent with the accrual of somatic mutations in our tissues, there is a parallel acquisition of epigenetic alterations, specifically in the form of DNA methylation changes.

To assess the presence of epigenetic hits in healthy cells, and its potential role in early tumorigenesis, we analyzed lymphoma-specific epigenetic alterations in B cells from healthy donors, focusing on mantle cells lymphoma (MCL). To that end, we leveraged scTAM-seq, which enables the targeted profiling of the DNA methylome, together with the analysis of somatic mutations, B cell receptor identity, and cell-surface markers in thousands of single cells.

We have so far analyzed ~25.000 healthy B cells, sorted from blood (n=7). Focusing on naive B cells (~8.000 cells), we observed 19 co-demethylation events involving 12 unique CpGs located in MCL-specific enhancers. Naive B cells showing co-demethylation of 5 or more of these CpGs represent a small non-clonal population (2% average) present in 6 out of 7 samples. Interestingly, these cells overexpress cell-surface markers associated with lymphoma and other diseases.

To further characterize this population, we linked the 12 CpGs of interest to their closest upregulated gene in MCL and defined a 10-gene signature. We next performed a scRNA-seq target capture approach of this signature to assess its expression in a sensitive manner, combined with whole transcriptome and B-cell receptor identity readouts (Singleron®). Preliminary analyses of ~50.000 B cells (sorted from bone marrow and blood), allowed us to identify the expression of the signature in a minor proportion of precursors and early B cells.

Overall, these findings point out the existence of a small and, so far, unexplored B-cell subpopulation that can be associated with MCL and may shed further light on the early events of lymphomagenesis.

Poster 45

Mercedes Guerrero-Murillo

Josep Carreras Leukemia Research Institute

“Single cell multiomic analysis of clonal and transcriptional programs of CD19 CAR T cells in the immunotherapy response”

CD19-directed CAR T-cell therapy has shown high rates of complete response against relapsed/refractory B-ALL, but it is only maintained in 50% of patients after a year. The impact of CAR T-cells' phenotypic, clonal, and functional heterogeneity on clinical outcomes remains unclear. Thus, a deeper examination of how clonal kinetics and diversity of CAR T-cells translate into short-term effectiveness and long-term persistence is crucial to pinpoint.
scTCR-seq and scRNA-seq were used to analyze samples from manufactured Infusion Product (IP) and peripheral blood during the CAR T-cell expansion peak (Peak) of five B-ALL patients. Our study revealed that patients with higher CD4 T-cell proportions at IP had a larger response, while patients with higher exhaustion scores had worse prognosis regardless of the presence of the CAR. At Peak, a significant increase in clonally expanding CD8+ T-cells was observed but impressive expansion of cytotoxic γδ T-cells correlated with patient outcome pointing out its importance. These findings provide insight into the interplay between the immune response and CAR-T-cell therapy and could contribute to the development of more effective treatments.

Poster 46

Mirko Francesconi

Ecole normale superieure de Lyon, CNRS

"Inferring cells physiological age from the transcriptome with raptor"

Large-scale -omics atlases are being produced at fast pace. The challenge is now to develop computational strategies that exploit these atlases for integrative analyses that help the interpretation of small-scale more focused datasets. We have recently developed RAPToR, a simple method that precisely estimates the real physiological of age (not pseudotime) of a biological sample from its transcriptome, exploiting existing time-series data as reference(Bulteau and Francesconi Nature Methods, 2022). RAPToR is flexible and works in multiple model organisms and humans, on bulk, dissected tissue, single-animal and single-cell data for both development and aging. Moreover, when provided with tissue-specific information it can output tissue-specific age estimates from whole animal data. Importantly it is extremely robust to differences in experimental conditions and genetic background.
Therefore, its estimates are comparable even across species. Comparing inferred physiological with chronological age precisely quantifies the time-specific effects of genetic or environmental perturbations on developmental or aging speed. Using RAPToR estimates helps remove age as a confounder and allow recovery of the signal of interest in differential expression analyses. RAPToR was however limited to infer age on a single trajectory. We are now developing "multi-trajectory RAPToR" which is capable of both inferring the physiological age and cell type/state of single cells of a query dataset, exploiting reference atlases. We provide a proof of concept using C. elegans embryogenesis both for early lineages differentiation and for later differentiation of specialized neurons.

Gerard Deuner Cos

Centro Nacional de Análisis Genómico

“Single cell characterization of the tumor microenvironment upon immunotherapy in colorectal and endometrial tumors: the SERPENTINE trial”

Immune checkpoint inhibitor (ICI) therapy has proved efficacy in patients with microsatellite instability (MSI) metastatic colorectal cancer (CRC) and endometrial cancer (EC) while its applica9on in patients bearing microsatellite stable (MSS) tumors still remains a challenge. Previous studies have demonstrated that the tumor immune microenvironment (TIME) is a key player in mediating tumor progression, dissemina9on and response upon ICI treatment. However, the TIME dynamics following ICI therapy in MSS CRC and EC tumors have not been fully understood, and the underlying mechanisms driving resistance are unclear. Here we conducted single-cell RNA sequencing and T cell receptor (TCR) repertoire analysis of liver metastases from 5 MSS CRC/EC patients across two timepoints, pre-treatment and post-treatment. Thus, we provide a single-cell characterization of the TIME composition, complexity and expansion dynamics upon an9-PD-L1 and anti-CTLA4 treatment. Our ongoing study reveals a shiT in immune cell composition post-treatment towards increased frequency of effector-memory, proliferation and exhaustion of CD8+ and CD4+ T cells, with enriched T follicular helper phenotypes. The integration of matched T cell receptor (TCR) data pointed to an infiltration of new clonotypes, rather than the expansion of pre-existing tumor-resident T cells, with distinct degrees of dysfunctional phenotypes between the clones. Our research uncovers biological insights into the mechanisms of resistance to ICI therapy in this type of patients.

Despite these dynamics, the treatment did not improve the response rate in patients with metastatic MSS CRC and EC, emphasizing the potential relevance of CD4+ cells in immune evasion as a potential approach to treat these tumours.

Poster 48

Bernat Gel Moreno

Germans Trias i Pujol Research Institute

"The PNF-ANF-MPNST progression at single cell level: delving deep into NF1-related tumors"

Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft-tissue sarcomas and the leading cause of NF1-related mortality. Over the years several efforts have been made to genomically characterize these tumors using different bulk technologies. However, some questions cannot be addressed without information at the single-cell level.

For this, we characterized 16 samples from the progression from benign PNFs to pre-malignant ANF to the malignant MPNST (4 control nerves, 4 PNF, 4 ANF and 4 MPNST) using five complementary technologies (scRNA-seq, scATAC-seq, SMARTseq2, scDNA-seq with cell surface markers and spatial transcriptomics) to obtain a complete picture of the genomics, epigenomics and transcriptomics of the whole progression at single-cell resolution. This is a unique dataset since it can be used to integrate multiple technologies in each single tumor type, multiple tumor types with a single technology or perform a full integration of multiple technologies in multiple tumor types. We performed different controls on sample preparation. Basic scRNA-seq analysis revealed that the main cellular composition of the tumors matched the expected proportions determined by histology. A custom bioinformatic quality control analysis identified and corrected cellular stress signals in some samples.
By performing an integrated analysis of all technologies across all tumor types, we were able to completely determine the cellular composition of each tumor type, including minor cell populations and different differentiation states along the neural crest - Schwann cell axis. We also characterized the systematic changes in cellular composition along the PNF-ANF-MPNST progression. The combination of this information with SMART-seq data allowed us to determine the exact cell populations affected by the inactivation of NF1, CDKN2A and PRC2 and thus identify the cells most probably originating the different tumor types.

We also detected large cellular identity changes on the transformation from ANF to MPNST and explored the epigenetic changes determining them by combining transcriptomics and scATAC-seq data. Estimation of copy-number profiles from scRNA-seq data revealed different subclones within MPNSTs and their different transcriptomic patterns. Finally, we were able to map the different cell populations on tumor tissue using spatial transcriptomics, revealing the spatial organization of these tumors.

In summary, we performed an analysis of the whole PNF-ANF-MPNST progression at single-cell resolution using multiple techniques. The integration over different tumors and different data types revealed new biological insights into the mechanisms of MPNST progression with potential translation to the clinics. This whole dataset will be open and a valuable asset for the whole research community.

Alessia Buratin

Josep Carreras Leukaemia Research Institute

“New trick for old drugs: targeting multiple myeloma plasma cell therapeutic heterogeneity in single cell RNA-seq data”

Despite the advances in treatment in the last decade, Multiple Myeloma (MM) remains an incurable disease, and almost all patients relapse. The development timeline for treatment against the progressive nature of the disease can be significantly reduced by re-using drugs already available on the market, a concept known as drug repurposing (DR). To date, very little single cell (sc) research exists regarding drug repurposing in MM.
Using scRNA sequencing data of 40 individuals along the MM progression spectrum we pursued to develop a system biology pipeline for the identification of drug repurposing candidates. Exploring the immune microenvironment together with plasma cells from the same individual may highlight potential new targets for immunotherapy and could predict response to specific treatments. To achieve that, we propose a twostep approach including at first the identification of tumor cell (TC) subpopulations and then the prediction of drug responses.
To predict drug perturbation to TC subpopulations we will use public datasets for comprehensive molecular and pharmacological characterization of cancer cell lines, providing gene expression signatures with drug response and treatment sensitivity.
In this situation, it is reasonable to hypothesis that drugs (or drug combinations) capable of targeting TC at sc resolution can be identified by integrating drug response profiles and scRNA-seq data in order to discover therapeutic clusters of cells, which we define as groups of cells with a similar drug response. Through this drug datasets screening we will suggest new knowledge-driven treatments. In this framework we will also address the tumor therapeutic complexity, revealing the impact of tumor heterogeneity in response to drugs and the scope of tumor cells whose therapeutic approach could be managed with approved drugs, clinical trials or drug repositioning strategies.
Using newly generated sc ex vivo sensitivity of 61 selected immunotherapies in MM (Myelomics), we will validate our computational approach comparing the perturbation score of predicted and validated drugs. In this way we can further investigate and evaluate the reuse of drugs in a completely new rational way and accelerate therapeutic applications using single cell technologies.

Poster 50

Gema Fuerte

Mission Bio Inc

"Single Cell Multi-Omic Correlation of Single Nucleotide Variants, Copy Number Variation and Surface Epitopes for Clonal Profiling of Myeloma"

Introduction

Multiple myeloma (MM) is a cancer of plasma cells with approximately 200,000 new diagnoses each year and a 54% 5-year overall survival rate. As myeloma cells expand, clonal genetic differences lead to relapse due to acquired resistance in 100% of patients, mandating regular, long-term surveillance. Improvements in precision medicine for MM have come from monoclonal antibodies and antibody-drug conjugates that specifically target the offending cells. However, while existing diagnostic modalities offer exquisite sensitivity, they are unable to correlate subclonal genetic changes and putative antibody targets on refractory myeloma cells, leaving the clinician to use their best judgment on salvage therapy which can lead to unnecessary expense and toxicity to patients due to ineffective treatment. Specifically knowing actual targets on refractory MM cells would better facilitate precision medicine and potentially improve outcomes.

Methods

Cryopreserved, human MM patient samples were processed on the Mission Bio Tapestri platform, enabling simultaneous single cell quantification of subclones by single nucleotide variants (SNV), copy number variants (CNV), and surface protein analysis. These single time point samples were thawed from frozen bone marrow mononuclear cells previously enriched for CD38, stained with a 45-plex antibody-oligo cocktail to label common heme-specific surface markers for sequencing analysis, and processed with a 733-plex DNA primer panel that combined whole-genome CNV coverage with MM gene hotspots. Sequencing was performed on an Illumina system with the raw data being analyzed using Mission Bio proprietary algorithms.

Results

We show complex clonal evolution of MM in individual samples as copy gains and losses that were sequentially acquired and correlated with expression changes of MM markers. Across the cases analyzed, complex branching phylogenetic trees were reconstructed with as many as five subclones and clear associated shifts in MM-marker expression. When averaged across subclones, each sample had CNV profiles that matched patient bulk genome-wide array records, ranging from a single gene-level copy gain to arm-level gains and losses across the majority. The progression of protein expression within different samples could be mapped together on a single plot and correlated with generally higher MM-markers as subclonal genetic variants were acquired, though occasional branches saw reversal. The fraction of other cell phenotypes, such as T-cells and those with low viability, decreased within subclones as mutational burden grew.

Conclusion

This high-resolution, single cell assay offers a potential new modality for the diagnosis and surveillance of patients with suspected MGUS, SGUS or high-risk MM. We have demonstrated: 1) exceptional results from cryopreserved human specimens, 2) the ability to use genetic lesion profiling to positively identify subclonal MM and, most importantly, 3) correlate cell surface protein expression of potential therapeutic targets with each clonal and subclonal population.

Ines Hofer

Queen Mary University of London

"Haematopoietic landscape dynamics during human cytomegalovirus latency establishment and maintenance"

Haematopoiesis involves stringent genetically and epigenetically dictated patterns of gene expression that is only partially understood. Human cytomegalovirus (HCMV) establishes latency in CD34+ haematopoietic progenitors (HPCs) but preferentially persists in the myeloid lineage where it ultimately reactivates. Hypothetically, understanding how HCMV re-programmes CD34+ cells could reveal new insight into myelopoiesis.

Human CD34+ HPCs were infected with HCMV and cultured in homeostatic conditions for a total of 17 days post infection – with infection confirmed by qPCR for viral transcription which persisted throughout the culture period. Cells were harvested at day 0 (baseline), day 10 and day 17 along with uninfected controls. Samples were subjected to single cell multiome (RNAseq and ATACseq) analyses to concomitantly define transcription and epigenetic profiles with the aim of defining virus specific changes in the host transcriptome. Our results show a temporal differentiation trajectory between the sampling points, with 21 cell types annotated, and distinct clusters between myeloid and lymphoid/erythroid populations. We detect viral transcripts in approximately 7% of the cells in infected samples. Most importantly, we identify changes in the different subpopulation of cells in the infected samples compared to uninfected ones. These changes in cell populations are characterised by cell specific gene expression programmes which are linked with distinct epigenetic states. In this work, we are defining virally induced changes in CD34+ HPCs with a view to identifying the specific changes required for myelopoiesis. Overall, our results add to the growing understanding of how HCMV manipulates HPC identity and illuminate the mechanisms governing haematopoietic differentiation.

Poster 52

Valérie Marot-Lassauzaie

Berlin Institute for Medical Systems Biology

“Identifying cancer cells from calling single-nucleotide variants in scRNA-seq data”

Single cell RNA sequencing (scRNA-seq) data is widely used to study cancer cell states and their heterogeneity. However, the tumour microenvironment is usually a mixture of healthy and cancerous cells and it can be difficult to fully separate these two populations based on gene expression alone. If available, somatic single nucleotide variants (SNVs) observed in the scRNA-seq data could be used to identify the cancer population. However, calling somatic SNVs in scRNA-seq data is a challenging task, as most variants seen in the short read data are not somatic, but can instead be germline variants, RNA edits or transcription, sequencing or processing errors. Additionally, only variants present in actively transcribed regions for each individual cell will be seen in the data. Because of these difficulties, there have been efforts to identify cancer cells by quantifying mutational events that are easier to capture than point mutations, such as copy number variations or mutations in mitochondrial DNA. However, in the absence of CNVs and usable MVs in the sample, recovery of cell lineages from scRNA-seq data has not been addressed up to date.

To address these challenges, we develop CCLONE (Cancer Cell Labelling On Noisy Expression), an interpretable tool adapted to handle the uncertainty and sparsity of SNVs called from scRNA-seq data. CCLONE jointly identifies cancer clonal populations, and their associated variants. We apply CCLONE on two acute myeloid leukaemia datasets and one lung adenocarcinoma dataset and show that CCLONE captures both genetic clones and somatic events for multiple patients. CCLONE is able to use the signal present in these SNVs to gather new insight into the course of the disease and the origin of cancer cells in scRNA-seq data.

Poster 53

Chiara Schiller

Institute for Computational Biomedicine

“Comparison of computational methods analyzing cellular colocalization in tissues”

Studying the spatial characteristics of tissues is essential for understanding their function in health and disease. Spatial omics technologies have enabled the study of single cells in their native spatial context, providing valuable information about cellular architecture and interactions. A variety of methods for spatial cellular neighborhood analysis have been developed to retrieve these spatial patterns from biological data. However, these methods differ in their biological and algorithmic approaches, and have not been systematically compared. In this project, we identified a common algorithmic framework of neighborhood analysis tools that allows for a modular understanding of the methods. Within this framework, we conducted a systematic comparison of cellular colocalization methods by assessing the ability to differentiate tissue cohorts based on their analysis results. To generate cohorts with different tissue architecture, we utilized the recently released framework for in silico tissue simulation. This allowed us to also evaluate the tool's ability to recover ground-truth cellular colocalizations, which is not possible with biological tissue data.

After identifying advantageous algorithmic features across cell type co-localization tools, we combined them to create an optimal co-localization method. The method can recover directionality of co-localizing cell types and is sensitive to variations in co-localization strength among different cohorts. Our study serves as a first comprehensive guide for users and method developers in the field of neighborhood analysis, while offering a novel approach to evaluate spatial omics tools.

Poster 54

Chiara Reggio

Scale Bio

“Scaling Single-Cell Sequencing: Multiplexing Innovations for Cost-Effective High-Throughput Profiling”

Single-cell sequencing technology has vastly enriched our understanding of biology across various domains. However, its widespread application in high-throughput endeavors such as screens, atlases, and large cohorts has been hindered by the high costs associated with instrumentation and reagents, along with challenges related to throughput and sample diversity. Addressing these constraints, combinatorial indexing exploits the inherent characteristics of individual cells as reaction compartments, allowing sequential barcoding of samples within a plate-based workflow. This innovative approach obviates the necessity for complex and expensive instrumentation, offering a robust, cost-effective, and high-throughput protocol.

Scale Bio offers flexible avenues for sample multiplexing, accommodating a broad range of cell inputs into both the fixation workflow and the 3-level plate based single-cell RNA workflow. Scale fixation can be optimized for lower cell numbers, minimizing volumes and post-fixation losses for precious samples. The Scale low cell input fixation protocol accommodates up to 1 million cells in a 1.5mL tube, streamlining the fixation process. Complementing this capability, ScalePlex enables further multiplexing with hash oligos during the fixation protocol upstream of the Scale scRNA workflow. This versatility is particularly advantageous for scenarios involving samples collected and fixed at different times, low cell numbers, or the pooling of samples from diverse sources, streamlining sample handling without compromising data integrity and saving valuable time.

The modular scRNA plate-based workflow incorporates 96 unique barcodes in its initial step, theoretically allowing for 96 distinct fixed samples. Additionally, the Scale scRNA kit can efficiently handle cell inputs ranging from 2,500 to 10,000 cells per well, ensuring robust downstream cell recovery and sequencing metrics. Augmenting throughput capacity, Scale introduces extended throughput kits, enabling recovery of up to 500,000 cells throughout the assay, effectively encompassing the input and output ranges.

Scale Bio's comprehensive range of bespoke single-cell RNA sequencing offerings is adept at handling a wide spectrum of cell inputs, ensuring increased recovery rates, providing a variety of fixation solutions, and ultimately streamlining sample processing with the incorporation of ScalePlex. These innovative solutions empower researchers across a multitude of disciplines, enabling cost-effective and high-throughput investigations that drive the frontiers of biological knowledge.

Alina Batzilla

EMBL Barcelona

"Using an in vitro 3D blood-brain barrier model and single-cell RNA sequencing to study pathogenic mechanisms in Cerebral Malaria"

Cerebral Malaria (CM) is a severe neurovascular pediatric complication of Plasmodium falciparum infections characterized by the disruption of the blood-brain barrier (BBB) causing vasogenic edema and brain swelling. The adherence of P. falciparum-infected red blood cells (iRBC) and the accumulation of immune cells in the microvasculature of CM patients are thought to be important factors contributing to BBB breakdown and CM pathogenesis. To improve our incomplete understanding of how these cells interact with the brain microvasculature and how they might induce BBB damage we are using an engineered, perfusable in vitro 3D-BBB model seeded with endothelial cells, pericytes, and astrocytes. To investigate the effects of iRBC binding and factors released upon rupture of the iRBC on the BBB, we perfused the 3D-BBB model with different stages of iRBC as well as parasitic products released after parasite egress of the iRBC. The malaria perfused 3D-BBB model was dissociated it into a viable single-cell solution and we performed single-cell RNA sequencing. Our results show an activation of the JAK/STAT pathway, ferroptosis and antigen presentation pathways in all three cell BBB cell types upon exposure to P. falciparum products. Endothelial cells show a downregulation of junctional and actin cytoskeleton pathways. In contrast, the binding of iRBC alone only induced minor transcriptomic changes in the BBB cells. To study the effect of immune cell binding in the microvasculature, we are currently performing single-cell RNA sequencing of the 3D-BBB model perfused with P. falciparum-stimulated leukocytes (Pf-leukocytes) in order to characterize the activation of Pf-leukocytes, the leukocyte populations predominantly adhering within the 3D-BBB model, and the transcriptomic mechanisms of BBB disruption. Using this combination of a perfusable in vitro 3D BBB model and single-cell RNA sequencing we aim increase our understanding of how iRBC and immune cell accumulation in the brain affect the BBB during CM pathogenesis.

Poster 56

Thomas Weber

European Molecular Biology Laboratory

"Automated Strand-seq data processing and analysis using MosaiCatcher v2 and its ecosystem"

Introduction: Single-cell DNA template strand sequencing (Strand-seq) is a pre-amplification-free single-cell short-read sequencing technique that generates strand-specific libraries by targeting and sequencing specifically template strand during DNA replication. Strand-seq enables a wide range of genomic analysis including hromosome length haplotype phasing and structural variation (SV) calling in individual cells. Here, we present MosaiCatcher v2, a computational workflow that allows scientists to easily process and analyse Strand-seq data.

Methods: MosaiCatcher v2 addresses both the computational challenges and the evolving needs of researchers working with Strand-seq data, by providing a framework, written in Snakemake, that integrates a broad range of functionalities. This includes an automated upstream Quality Control (QC) and assembly sub-workflow that relies on multiple genome assemblies and incorporates a new multistep normalisation module. Moreover, the integration of the single-cell nucleosome occupancy and genetic variation analysis module (scNOVA) allowing SV functional characterization (scNOVA), and of the arbitrary genotyping tool (ArbiGent), extend downstream possible analysis. Leveraging Snakemake functionalities, the workflow is highly portable, scalable, reproducible, and provides a user-friendly and shareable web report.

While this major update of MosaiCatcher provides already a milestone through a user-friendly toolbox to analyse Strand-seq data, additional tools were developed in order to address technical challenges related to computational workflow execution on a cluster environment.

This ecosystem includes MosaiWatcher, a monitoring system that detects the availability of new Strand-seq runs generated by a core facility and automates the trigger of MosaiCatcher v2 on these. Finally, Strand-Scape the latest addition to the ecosystem, provides a graphical web interface for easier data management, data visualisation, and results interpretation through a modern cloud-based microservices platform.

Results and Discussion: MosaiCatcher v2 application to more than 41 000 single-cell Strand-seq libraries, demonstrated its ability to efficiently and reliably process diverse biological samples. We provide today a homogeneous and modular framework that allows researchers, in an automated way, to process Strand-seq data and generates SV calls and their functional impact, at the single-cell level. The development of additional tools and platforms such as MosaiWatcher and Strand-Scape provides an integrative ecosystem that allows users to reduce the technical burden of triggering manually workflows, but also a streamlined and interactive access to results, paving the way towards production environments.

Poster 57

Colin Cess

Max Delbrück Center

“Compound-SNE: soft alignment of single-cell embeddings”

Data visualization is a key step in examining single-cell omics data, most often done by embedding high-dimensional data into low-dimensional space. However, visualizing multiple datasets at once poses a challenge due to batch effects, when comparing datasets across patients, or biological differences when comparing data modalities (such as scRNA and scATAC). While data integration methods are necessary for downstream analyses, these methods obscure visual comparisons as they force cells of the same type into the same regions of feature space before embedding in visualization space. This leads to visualizations of datasets that are indeed well-aligned with each other, at the cost of the dissolution of unique local embedding structures that can be seen when datasets are embedded independently of each other, which could help identify inter-dataset differences. Therefore, we developed an embedding method, Compound-SNE, that performs a soft alignment of multiple datasets based on cell annotations, yielding embeddings that are visually aligned with each other, while preserving dataset-specific local embedding structures.

Aikaterini Symeonidi

Helmholtz Center Munich

“epiAneufinder: identifying copy number variations from single-cell ATAC-seq data”

Single-cell measurements of chromatin architecture using the single-cell Assay for Transposase-Accessible Chroma (scATAC-seq) are a powerful technique to study functional heterogeneity between single cells. Here we introduce epiAneufinder, a new algorithm that uses read count information from scATAC-seq data in order to identify genome-wide copy number variations (CNVs) for individual cells.
EpiAneufinder allows the incorporation of CNV information to any scATAC-seq experiment, offering the opportunity to study CNV heterogeneity within a sample at the single-cell resolution.

We applied epiAneufinder to several cell lines and primary tumor scATAC-seq datasets, and identified intratumor clonal heterogeneity within populations of single cells, based on their CNV profiles. These CNV profiles aligned with those deduced from (sc)WGS data of the same samples, demonstrating the performance of epiAneufinder. Moreover, epiAneufinder outperformed methods that call CNVs based on scRNA-seq data, allowing to better study aneuploidy in disease.

With the advent of the new single-cell multiomic sequencing, it is now possible to measure scRNA and ATAC simultaneously for the same cells. We are expanding the capabilities of epiAneufinder to accommodate multiome data, with the aim of enhancing its utility in understanding genomic heterogeneity at the single-cell level.

Pascal Wetzel

Max-Delbrück-Center

"Detecting footprints of transcription factors in single-cell data"

The identification of active transcription factors (TFs) is an important part of understanding the complex mechanisms of gene regulation in cells. Using the information from chromatin accessibility assays, genomic footprints indicate TFs that are bound to DNA and can therefore identify putative active TFs. The rise of single-cell sequencing technologies unlocks the potential to apply this concept on a more fine-grained level, which could potentially help to identify cell type or state specific TF activities.

To examine the feasibility of single-cell footprinting, we analyzed the TF activity in single-cell ATAC-seq data from human blood cells. First, known cell type annotations were used to compare the activity of TFs across different cell types. Next, the signal of similar single cells was combined to build pseudo bulk clusters. Subsequently, the genomic footprints were compared across the clusters to identify cluster specific TF activity.

Comparing the genomic footprints of the different cell type clusters revealed cell type specific patterns that are linked to TFs, that act as known cell type markers, such as GATA1.

Furthermore, the analysis of the pseudo bulk clusters shows that single-cell footprinting allows the detection of individual TF activity profiles across heterogeneous data. However, while the global activity of a TF seems to be covered by this approach, it remains challenging to detect the activity of a specific TF at individual sites, due to the lack of data from current single-cell technologies. Nevertheless, with future advances that hopefully lead to higher coverage from single-cell sequencing, this method has the potential to detect TF activity in single cells at individual regions.

Poster 60

Mara Santarelli

Institut Pasteur

"A Supervised Non-Negative Matrix Factorisation Approach for the Identification of Resistance-Related Gene Expression Programs in Clinical Single-Cell RNA sequencing Data"

In single-cell RNA-sequencing analysis, gene expression programs, which represent groups of coherently expressed genes corresponding to specific biological processes, are often identified by condensing the vast number of genes using dimensionality reduction methods such as non-negative matrix factorisation (NMF).
While NMF is known to be able to identify principal gene expression programs, it remains challenging to identify subtle gene expression patterns, particularly in complex clinical datasets from multiple cancer patients, which are characterised by extensive inter- and intra-patient heterogeneity.
In this context, patient or sample level annotations are often available but ignored at this stage of data analysis. In cases where one searches for expression programs that are ultimately associated with specific phenotypes, this association can be used to constrain NMF, and therefore facilitate the identification of weak expression programs.
We propose a supervised NMF approach that implements this idea by extending the standard NMF algorithm by a classifier module that enables the identification of general expression programs as well as programs that are associated with a phenotype. We tested our approach on simulated data to systematically evaluate the accuracy improvement of our method in uncovering weak signatures compared to conventional NMF.
In preliminary data, we show the application of our approach to a clinical scRNA-seq data set from 42 high-grade serous ovarian cancer patients to identify gene programs associated with chemotherapy resistance.

Poster 61

Akshay J. Ganesh

Institut d'Investigació Biomèdica de Bellvitge

“Understanding the role of the RNA-binding protein Staufen 2 during neurogenesis using single cell transcriptomic”

Neurogenesis is a crucial process involving the formation of new neurons in the developing cortex of embryos, which is regulated by multiple factors, including RNA-binding proteins (RBPs). Staufen 2 (STAU2) is an RBP implicated in the asymmetric distribution of mRNAs in radial glial cells (RGCs), thereby dysregulating the balance between neural stem cell maintenance and differentiation. However, the molecular mechanisms of STAU2-mediated regulation in human neurogenesis remain largely unknown. To characterize these regulatory mechanisms, we performed single-cell RNA-seq (scRNA-seq) on different neurogenic populations derived from STAU2 KO and control human induced pluripotent stem cells (hiPSCs). The samples were sequenced at multiple timepoints, reflecting the transition from hiPSCs to mature neuronal and glial cell types. Clustering analysis revealed that the expected cell types such as iPSCs, committed progenitor cells, and mature neurons were recapitulated in the integrated scRNA-seq dataset. Using differential expression analysis we discovered that most differentially expressed genes were in the neuroepithelial cell cluster.
Immunohistochemical and qPCR analyses of neuroepithelial cells show increased expression of neuronal markers such as MAP2 in STAU2 KO compared to control cells. Based on these results, we propose a model where STAU2 regulates human neurogenesis at the neuroepithelial stage. To understand how such regulation affects differentiation trajectories, we are performing pseudotime and RNA Velocity analyses. Gene Regulatory Network analysis could provide further insights into the active regulatory modules that may be contributing to the accelerated differentiation observed in STAU2 KO.

Poster 62

Giovanna Mantica

University of Cambridge

"Integrated single cell analyses reveal altered myeloid differentiation dynamics in DNMT3A R882 mutant human haematopoietic stem cell"

The number of human haematopoietic stem cells (HSCs) contributing to blood cell production at any time is estimated between 10,000 and 200,000. In young, healthy individuals, the HSC pool composition is highly diverse, with individual HSCs displaying heterogeneous functional behaviour. With age, the acquisition of somatic mutations leads a few HSC clones to expand and dominate blood production. HSC oligoclonality and the resulting clonal haematopoiesis(CH) are an inevitable hallmark of ageing and are linked to pro-inflammatory clinical manifestations and increased mortality. Yet, so far, research on inflammation in CH has mainly focused on the effect of CH driver mutations on mature immune cell behaviour. Focusing on DNMT3A R882 mutant CH, we reveal that the immune dysregulation in CH already starts at the top of the hematopoietic hierarchy.

Leveraging single-cell HSC cultures and whole-genome sequencing, we characterised in depth the HSC compartment of one individual with DNMNT3A R882 CH. We reconstructed phylogenetic relationships of HSCs and studied their in-vitro differentiation output by flow cytometry and methylation sequencing analysis. This revealed an altered myeloid differentiation of DNMT3A mutant HSCs compared to their wild-type (WT) counterparts. Expanding our study cohort to 6 DNMT3A R882 CH carriers and assessing the differentiation output of 1627 individual HSCs, we found that DNMT3A R882 MUT HSCs display a decreased CD14+ monocyte output and an increased output and tempo of neutrophil differentiation. Coupling single-HSC differentiation assays with scRNA-seq analysis, we defined cell states and transcriptional programmes in mutant and WT HSC myeloid differentiation within the same individual.

Overall, our study demonstrates that DNMT3A R882-driven CH is characterised by functional differentiation alterations at the HSC level, yielding an unbalanced production of innate immune cells. Further studies will need to assess how this phenomenon impacts CH-linked inflammation and its potential contribution to the leukaemia predisposition of DNMT3A R882 mutation carriers.

Poster 63

Marcel Schilling

Institut d'Investigació Biomèdica de Bellvitge

“Post-transcriptional regulation in iPSC derived neural and glial cells from Alzheimer disease patients”

Alzheimer’s disease (AD) is the most common cause of dementia, but its pathogenesis remains poorly understood. Post-transcriptional regulation, e.g. via RNA-binding proteins (RBPs) or alternative cleavage and poly-adenylation (APA) has been shown to be implicated in AD. However, whether the corresponding alterations play a causative role or rather represent consequential symptoms has not yet been shown. To address this question, and ideally identify potential new therapeutic targets, we investigate the transcriptional landscape of single cells progressing from iPSCs derived from AD patients and healthy controls throughout their differentiation to neural and glial cells. Analysing thousands of genes in tens of thousands of cells, we recover all major cell types (iPSCs, NECs, NPCs, neurons and astrocytes) including various precursor and mature cell populations in both AD and control samples. Differential gene expression analyses identified time point and cell type specific gene abundance changes between cells derived from AD patients compared to controls. Several of those differentially expressed genes have previously been linked to AD. Amongst the differentially expressed genes were several RNA binding proteins (RBP)s, implicating the corresponding regulatory networks as potential targets for further investigation. Differential 3’ UTR usage analyses using SCALPEL revealed that several genes previously implicated in AD pathogenesis display changes in APA between AD and control cells. Here we present those results and our ongoing efforts to validate individual findings, and integrate these lines of evidence towards a better understanding of post-transcriptional regulation in pre-neurodegeneration AD.

Laura Kida

Sloan Kettering Institute

"The landscape of NK cell dynamics and memory in viral infection"

Natural killer (NK) cells as part of the innate immune system shield the host against external health threats as well as internal malignant cells without requiring prior priming. Different from B or T cells, NK cells do not experience gene rearrangement to acquire clonally arranged antigen-specific receptors. Diverse stimuli, like viral antigens or inflammatory cytokines can imprint NK cell function lastingly, establishing NK memory-like phenotypes in a variety of conditions. In patients infected with human cytomegalovirus (HCMV) or hantavirus, rapid expansion and long-term persistence of memory NK cells has been demonstrated, but our overall understanding of innate immune cell memory remains limited. Importantly, NK cells are utilized in innovative cell therapies, on the rise for the treatment of cancers that have shown poor prognosis in the past. However, the response to treatment and therefore patient outcome remains widely inhomogeneous, demanding further investigations of the mechanisms of NK cell memory.
Furthermore, viral infections pose frequent challenges in the clinical setting for patients in oncological treatment, featuring an immunocompromised status and motivate the study of the role of NK cells specifically in the context of viral infections. In contrast to NK cells, the establishment of an immunological memory of cells descending from B or T cell lineage has been studied more robustly. Here, we aim to provide insights into the role of innate memory in viral infections by mapping NK cell dynamics in humans diagnosed with SARS-CoV-2, Influenza, or HHV-6 infections and contrast the findings to known T cell biology. Despite our increasing understanding of memory-like NK cells, a comprehensive evaluation across infections and disease.

To establish a consensus understanding of NK cell heterogeneity, we comprehensively surveyed publicly available scRNA-seq data sets from human patients. Sequencing data from 25 individuals featuring confirmed viral infection, e.g. SARS-CoV2, Influenza virus, HCMV, or HHV-6, as well as healthy controls were compiled to generate a comprehensive landscape of NK cells in viral infection. We characterized the transcriptional landscape of NK cells throughout viral infections on single-cell level. Our comprehensive integration and interrogation of NK cell states across a range of infections identifies recurrent and distinct modes of NK cell subtypes that may be critical across a range of infections. These cell states motivate future mechanistic work about how the clonality, epigenetic memory, and evolution of NK cells occurs in diverse physiologic contexts.

Rubén Chazarra-Gil

Barcelona Supercomputing Center

"Alternative splicing variability between human populations at single-cell resolution"

Transcriptional response to immune challenges varies between individuals of different genetic ancestries which impacts susceptibility to infectious disease. While humans of different populations differ in their expression of inflammatory genes upon immune stimulation, population variation in the alternative splicing landscape due to infection has been less explored, being restricted to isolated cell types or heterogeneous tissues. With a growing consensus in the contribution of alternative splicing to immunity and common disease risk, characterizing immune related alternative splicing differences between individuals becomes a priority. Single-cell RNA technologies can emerge as a valuable tool in order to understand population differences in the response to infection across a broad range of cell types. Until recently, the limited number of samples available and the sparsity of single-cell data made the study of alternative splicing at single-cell resolution extremely challenging. With the advent of large cohort scRNA-seq datasets available including hundreds of donors and thousands of cells, we can now for the first time uncover alternative splicing interindividual differences at single-cell resolution.

Here, we developed a computational framework to identify alternative splicing differences in 3' scRNA-seq datasets. In this framework, we first perform pseudo-alignment of 3' biased scRNA-seq reads to obtain transcript counts per cell. Next, based on this quantification, we perform differential transcript usage by modeling isoform ratios of each gene between conditions per cell type leveraging a nonparametric multivariate approach. Permutation analysis shows that our analysis is robust and shows extremely low false positives.

We apply our framework to study alternative splicing differences between 45 European and 45 African descent individuals in a scRNA-seq dataset of immune blood cells before and after influenza infection. First, we evaluate differences in transcript usage between populations at baseline, finding changes in transcript ratios in all immune cell types. We highlight RPL10 gene, component of the 60S ribosome subunit, and ACD gene, involved in telomere function, for displaying differential transcript usage between populations across all immune cell types. We find significant overlaps of our population differentially spliced genes with bulk RNA-seq data from blood immune cells.

When focusing on influenza infected samples, we again find population differences in transcript usage across all cell types. Notably, only one third of these differences are shared with baseline. Finally, we assess differences in transcript usage between non and infected samples in each population separately. As expected, we find a marked remodeling of the isoform repertoire in monocytes given they are the primary cell type infected by influenza.

In summary, we present a computational strategy to study alternative splicing variation in the circulating immune system between individuals of different genetic background and upon viral infection. Our approach can be readily scalable to associate differences in transcript ratios with other individual traits or environmental factors in future studies.

Zana Kapustina

Atrandi Biosciences

“Ultra-High Throughput Single-Microbe Sequencing Enabled by Semi-Permeable Capsules”

Whole-genome and targeted sequencing open a window to understanding the diversity and function of unculturable microorganisms. On one hand, metagenomic sequencing is attractive for its straightforward sequencing library preparation from bulk environmental samples but only offers limited resolution into individual species. On the other hand, single-microbe sequencing offers true single-clone resolution but can only meaningfully address the high biological diversity expected in environmental samples if performed on thousands of individual cells in parallel. To satisfy the need to study such large numbers of single-microbes per sample, well- and droplet-based approaches keep evolving in parallel to provide single-cell compartmentalization required during sequencing library preparation. However, these approaches suffer from a fundamental trade-off between throughput and versatility. Our Semi-Permeable Capsule (SPC) technology combines the throughput of droplets with the versatility of wells by enabling a virtually unlimited number of processing steps on genetic material from thousands of individual microbes in parallel. This study aimed to demonstrate the use of SPCs for barcoding >10,000 individual microbial genomes to obtain single-microbe whole genome sequencing data of unprecedented quality. For proof-of-concept evaluation, we encapsulated well-characterized E. coli and B. subtilis bacteria into SPCs, lysed cells at alkaline conditions (pH 13), amplified their genomes, and employed a split-pool approach to add unique cellular barcodes. Upon sequencing of an aliquot of ~3,000 cells, important technical metrics, such as cross-contamination and genome recovery, were measured to assess the performance of the workflow. The results showed excellent genome retention within SPCs, with <1% of cross-contaminated genomes. Genome recovery analysis yielded a median coverage of 90% at a median sequencing depth of 8X for B. subtilis cells (1690 cells sequenced), and a median coverage of 63% at a median sequencing depth of 3X for E. coli cells (2023 cells sequenced) with clear indication for higher recovery at saturating sequencing. We conclude that the compartmentalization of microbial cells into SPCs allows the generation of high-quality whole-genome data at scale, and further apply the method on environmental samples.

Poster 67

Maria Tsagiopoulou

Centro Nacional de Análisis Genómico

“Immune Cell Meta-Atlas: Exploring Organ-Specific Gene Expression Patterns”

Despite significant progress in understanding the immune system, there remains a gap in our understanding of the behavior, diversity, and specificity of immune cells across various organs. This study is designed to address this gap by analyzing the composition of immune cells in healthy human organs, identifying changes within the host organs, and exploring the mechanisms by which they enter host organs, using single-cell RNA sequencing data.

We combined 162 samples from 12 Human Cell Atlas studies and used Seurat to analyze 14 organs. CD45 expression was used to identify immune cells, while UMI count, gene detection, and mitochondrial expression were used to filter out low-quality cells. Batch effect correction was performed using Harmony. Cell annotation was accomplished manually using the most significant markers in each cluster, alongside comparing the results with SingleR and EnrichR tools in R. Transcription factors activity analysis was performed by pySCENIC (topEnrichment) and pathways using GSEA and Hallmark database (FDR<0.05). Moreover, doublet scores were calculated using scDblFinder package in R.

We report a meta-atlas of 114,275 CD45+ immune cells from 14 organs. The transcriptome landscape of immune cells was constant across organs; however, organ-specific cell types (i.e., macrophages and Tregs) and organ-specific expression of genes were discovered.

Specifically, our analysis has revealed unique expression of GTPX3 in the kidney, and DNTT and ACVR2B in the thymus. Interestingly, these genes are expressed across all immune cell types within these organs, suggesting a dynamic interaction and adaptation to their microenvironment since they are expressed in the dominant cells of these respective organs.

To validate our findings, we extended our analysis to independent cohorts, including kidney single-cell RNA sequencing datasets (Lake et al., 2023, Nature; Liao J et al., 2020, Sci Data; Stewart et al., 2019, Science) and thymus bulk RNA-seq data from sorted CD19+ thymic cells (Cepeda et al., 2018, Cell Rep). These independent datasets support the observed gene expressions within the respective tissues.
In terms of functional changes, the “TNF-signaling via NFkB” pathway was the only one that was detected across organs (>3 organs) and in all immune compartments. Using the target genes of NFkB from pySCENIC analysis, we scored the activation of NFkB in each cell.
Regarding the immune cell types, macrophages that are highly organ specific exhibited the highest NFkB activation. Moreover, we observed an activation of the NFkB target genes in specific organs where such immune cells are not commonly found (e.g., B cells in thymus) supporting a potentially critical role of NFkB in immune cell homing. We validated the activation of NFkB target genes in thymic B cells using the previously used independent cohort (bulk RNA-seq from sorted CD19+ thymic cells) affirming the activation of the NFKB pathway (FDR=4.52e-15 compared to negative control- T cell markers). Finally, different repertoires of NFkB family genes and regulated cytokines were expressed in different organs and immune cell types, demonstrating its significance in cell localization.
This study prioritizes biological relevance over detailed cell characterization, providing a meta-atlas that reveals organ-specific gene expression patterns in immune cells influenced by their host microenvironments. A key finding highlights the involvement of the NFkB pathway in cellular positioning. Understanding how immune cells adapt to and interact with diverse organ environments is crucial for insights into health and disease.

Poster 68

Anna Siewert

Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn

“Insights from human embryonic single-cell RNA sequencing data suggest involvement of epithelial cells and pharyngeal arches in non-syndromic cleft lip ± cleft palate”

Background/Objectives:
Non-syndromic cleft lip with/without cleft palate (nsCL/P) is one of the most common birth defects. Although over 45 risk loci have been identified, the function of the associated risk alleles at these loci and the affected cell types are mostly unknown. We here aimed to identify nsCL/P-related cell types by analyzing gene expression from genome-wide association study (GWAS) candidate genes and generated co-expression networks to suggest novel candidate genes based on joint expression patterns.

Methods:
We re-analyzed published single-cell RNA sequencing (scRNA-seq) data from the heads of unaffected human embryos aged 4-6 weeks (GSE157329) using Seurat. We investigated the association of cell types with the joint expression of nsCL/P GWAS candidate genes using single-cell disease relevance scores (scDRS). Then, we generated co-expression networks from these cell types using high dimensional weighted correlation network analysis (hdWGCNA) and performed pathway enrichment analysis in these networks using clusterProfiler.

Results:
We found that nsCL/P candidate genes are significantly associated with the epithelium ($p < 0.01$) and HAND2+ pharyngeal arches ($p < 0.05$). The marker genes of the associated epithelial cells included established nsCL/P candidate genes (e.g. IRF6, TFAP2A, ESRP1).

Furthermore, significant within-cell type scDRS heterogeneity ($p < 0.05$) was observed in epithelial cells. In each of the two cell types we identified 18 co-expression modules and prioritized three modules each based on their percentage of nsCL/P candidate genes (e.g. known candidate genes such as IRF6, TFAP2A, ARHGAP29, etc. in one of the epithelial gene modules). These gene modules contain interesting new candidate genes (e.g. CALD1, PDGFC) and were further analyzed with regard to their enriched pathways.

Discussion:

Our results indicate that specific subsets of epithelial cells and the pharyngeal arches may be involved in nsCL/P development. For some nsCL/P candidate genes, genetic interaction has already been shown (e.g. IRF6 and TFAP2A). However, our co-expression networks suggest additional genetic associations and interactions between known and novel candidate genes that require further investigation.

Poster 69

Francesc Muyas

AstraZeneca

"De novo detection of somatic mutations in high-throughput single-cell profiling data sets"

Detecting somatic mutations at single-cell resolution is essential to study genetic heterogeneity, clonal mosaicism in non-neoplastic tissues, and to identify the mutational processes operative in malignant and phenotypically normal cells. However, the identification of mutations in individual cells is still challenging from a technical and algorithmic standpoint. Here, we present SComatic, an algorithm designed to detect somatic mutations de novo in single-cell transcriptomic and ATAC-seq data sets. Using more than 2.6 million single cells from 688 single-cell RNA-seq and ATAC-seq data sets, we show that SComatic can detect somatic mutations not only in tumour samples, but also in differentiated cells from polyclonal tissues not amenable to mutation detection using existing methods. In addition, SComatic allows the accurate estimation of mutational burdens and de novo mutational signature analysis at cell-type resolution. Using matched DNA sequencing and single□cell RNA-seq data, we show that SComatic has higher precision (> 4-fold) than existing algorithms for detecting somatic mutations without compromising sensitivity. Overall, SComatic permits the study of somatic mutagenesis at unprecedented scale and resolution using high-throughput single-cell profiling data sets.

Poster 70

Barbara Zita Peters Couto

Institute for Computational Biomedicine

Chemotherapy resistance poses a significant challenge in the treatment of high-grade serous ovarian cancer (HGSOC), with the majority of patients experiencing relapse and subsequent mortality. To identify novel strategies for overcoming treatment resistance, we aim to better characterise the mechanisms of heterogeneous drug responses in HGSOC. To this end, we perform combinatorial drug perturbation experiments in ovarian cancer cell lines, with varying drug concentrations and schedules of clinically used drugs. We use a cell hashing method to do a single cell RNA sequencing run per 96 well plate and map each cell to its original well. Then, we computationally examine signalling pathway activity changes under these different drug perturbations and create a drug condition ranking for each pathway, denoting which of these drug conditions leads to the strongest short term effect on pathway activity. By leveraging single cell data, our analysis has the potential to take into account the variability in cell populations treated with the same perturbation, hence potentially enabling a comprehensive investigation of heterogeneous drug responses in ovarian cancer cell lines in vitro.

Poster 71

Christophe Fleury / Alfonso Troyano

Oxford Nanopore Technologies

“Single-cell transcriptomics unlocks cellular diversity using full-length cDNA sequencing, providing high-resolution analysis”

Poster 72

Mari Carmen Romero-Mulero

Max Planck Institute of Immunobiology and Epigenetics

“Preservation of cardiac function via forced hematopoietic stem cell quiescence upon myocardial infarction”

Located at the top of the hematopoietic system, bone marrow (BM) quiescent hematopoietic stem cells (HSCs) have the unique capability to sustain long-term hematopoietic replenishment, including myeloid cells. Myeloid cells play a critical role in tissue repair after myocardial infarction (MI). However, excessive MI-triggered myelopoiesis can worsen scarring and impair cardiac function. Here, employing comprehensive single-cell methodologies and functional assays, we show that human BM HSCs suffer detrimental activation and loss of functionality in post-MI patients. Our investigations reveal a myeloid-primed signature in MI□HSCs, accompanied by a persistent inflammatory state and a distinctive temporal distribution of BM monocytes upon MI. Through lineage tracing experiments, we identify HSCs as key contributors of pro-inflammatory myeloid cells infiltrating the cardiac tissue after MI. These discoveries open potential therapeutic avenues through the modulation of the root of the hematopoietic system following MI by forcing HSC quiescence, thereby dampening the downstream myeloid production and preserving long-term cardiac function. The implications extend beyond MI, encompassing immune-mediated disorders driven by HSC overactivation.

Poster 73

Tamás Gerecsei

Cytosurge

“Cytoplasmic live-cell Biopsies for Temporal Single-Cell Profiling”

Advances in single-cell transcriptomics have greatly contributed to the understanding of cellular heterogeneity and dynamic gene expression patterns. However, conventional RNA-seq methods require cell lysis - which is terminal in nature, making the understanding of cell lineage decisions and fate determination, challenging, and relying on complex bioinformatic models.

With FluidFM Nanosyringes, cytoplasmic biopsies from cultured single cells can be retrieved (Guillaume-Gentil, Cell, 2014), while preserving their viability. In combination with a highly sensitive, low-input RNA-seq protocol, it was demonstrated that transcriptome snapshots can be captured (Live-seq) and are faithful representations of lysed cell transcriptomes (Chen, Nature, 2022).

Preserving cell viability maintains temporal information, complements high-throughput datasets, and facilitates the unraveling of subtle cell trajectories. Here we show how the FluidFM OMNIUM platform enables the serial collection of cytoplasmic biopsies, streamlining the workflow from the insertion of the FluidFM Nanosyringe into the cytoplasm, to the ejection of the biopsy into lysis buffer droplets for downstream analysis.

Poster 74

Yanay Rosen

Stanford University

"Uncovering Structure Across the Universal Landscape of Cell Biology Spanning Species, Cell Types and Cell States"

Learning universal representations (embeddings) of single cell RNA-sequencing data is critical for drawing scientific conclusions from diverse omics datasets. While large, self-supervised foundation models hold promise in learning generalizable representations, their direct application from natural language processing overlooks the unique qualities of single-cell data.
To address this, we designed and trained a foundation model that incorporates biological inductive biases to produce a universal cell embedding (UCE) that detects true biological variation despite experimental noise. UCE enables the representation of any cell, regardless of tissue or species, within a fixed biological latent space. Our model can accurately transfer and predict cell types with no model finetuning (in a zero-shot setting), even for novel species that were absent from the training data. We apply UCE to uncover novel cell type hierarchies, sub-types, and trajectories.

Poster 75

Ritika Kulshreshtha

Singleron

"Solutions for Simultaneous Single Cell Transcriptome Analysis and Immune Receptor Profiling"

The adaptive immune system is composed of a diverse array of antigen binding T-cells and B-cells. The diversity in T-cell and B-cell receptor sequences are governed by the recombination and somatic hypermutation in the V (variable), D (diversity) and J (joining) gene segments. Advancements in multi-omics approaches at a single cell resolution has led to adapting the cutting-edge GEXSCOPE® microwell-based technology, to provide gene expression data combined with the T-cell or B-cell receptor sequences, including the sequence information on the complementarity determining region 3 (CDR3). The improved performance of our GEXSCOPE® Single Cell V(D)J Kit enables high pairing rate of immunoreceptors. For full length immunoreceptor single cell sequencing, Singleron offers the sCircle® Single Cell Full Length Immunoreceptor Library Kit, which enables full length V(D)J region sequencing at a single cell level. Characterization of therapeutic antibodies or T-cell therapies at a single cell level can now be conveniently performed on both, human and mouse immunoreceptors. Singleron's immune profiling kits, offer unique high-throughput single cell solutions for clonal analysis, antibody discovery, or developing CAR-T therapies, which can be used without the necessity of specialized equipment.

Michael Scherer

Centre for Genomic Regulation

“DNA methylation jointly encodes clonal identity and cell state in single cells”

DNA methylation is an epigenetic mark dynamically regulated during cellular differentiation in general and particularly during hematopoietic differentiation. Epimutations, defined as the spontaneous loss (or gain) of DNA methylation at individual CpG dinucleotides, have been explored as potential clonal labels in cancer. However, DNA methylation's utility as a clonal label in healthy differentiation is unclear.

Here we show that targeted, high-confidence single-cell measurements of DNA methylation with scTAM-seq deliver joint information about the cellular differentiation state and clonal identity. The DNA methylation-based map of murine hematopoiesis has a resolution similar to scRNA-seq. CpGs associated with cellular differentiation are enriched for enhancer elements, while clone-specific CpGs are preferentially located in heterochromatic and late replicating regions of the genome. We developed a new method, EPI-Clone, to infer clonal identity from DNA methylation data only, without the need for genetically engineered clonal labels. Clones

defined by EPI-Clone show a high overlap with ground-truth genetic barcodes using the LARRY barcoding system. Using the high-resolution map of hematopoiesis together with clonal identity, we characterized functional behavior of stem cell clones. Analogously to experiments using genetic barcodes, we found heterogeneity within hematopoietic progenitors with respect to blood output and lineage biases.

We found that the clonal identity imprinted in the epigenome was present both in hematopoietic progenitors and in mature myeloid cells. When investigating hematopoietic output during aging, we found the characteristic myeloid shift in an aged in comparison to a young mice that, until today, could only be investigated using genetic barcoding systems such as LARRY.

In summary, we show that single-cell DNA methylation profiles provide information on both the cellular differentiation state and clonal origin of hematopoietic cells. We believe that the joint readout of clonal identity and cell state enables functionally characterizing stem cell clones in systems where genetic barcoding is not applicable.

Jeroen Aerts

Deepcell Inc

"High-dimensional morphological profiling powered by AI enables label-free cellular insight"

Information encoded by cell morphology has traditionally been used as a readout of cell identity, state, and function. However, scalability, interpretation, and sorting based on morphology has remained challenging. The Deepcell® platform, REM-I, performs high-dimensional morphology analysis of unlabeled, single cells using deep learning on high-resolution brightfield images. As unlabeled cells flow through the microfluidic channel on a chip in the instrument, high resolution (0.16 µm/pixel) brightfield images are captured and sent to deep learning models for characterization and identification in real-time.

Morphology analysis of single cells is achieved by a self-supervised deep learning foundation model. The current model iteration, termed "Human Foundation Model" (HFM) was trained and validated on millions of cell images across a wide range of human cell types for broad human applicability. The HFM is a hybrid architecture that combines self-supervised learning (SSL) to extract deep learning embeddings and computer vision to extract morphometric features. Of the 115 morphology dimensions of each cell in a sample that the HFM extracts, 64 are deep learning embeddings and 51 are morphometric features.The HFM extracts these 115 quantitative dimensions of morphology and our software projects this data onto a morphology UMAP where each dot represents an individual cell. Using Leiden clustering, users are able to see distinct morphotypes that cluster together in UMAP space giving a view of the heterogeneity within any given sample. And for a view into differences between samples, users can also examine morphology density UMAPs that show what morphotypes are present or predominant in any given sample.

Results show combining deep learning and morphometrics improve interpretability of data and enable rapid characterization and classification of cells with high accuracy (tumor, drug resistant, gene edited, etc.). Using the high-dimensional morphology data output - morpholomics - investigators can define and save cell populations of interest and reproducibly use them for subsequent runs and to sort them into six collection wells for further multi-omic and functional analysis. Proof-of-concept applications, including profiling heterogeneity in dissociated tumor tissue, enriching carcinoma cells in malignant body fluids, and analyzing immune cell activation/differentiation states, have revealed associations between morphological fingerprints and cell state, function, and identity. Thus, application of the Deepcell platform can help usher in quantitative morphology as a new image cytometry modality by revealing high-dimensional morphology-based information critical for biological discoveries.

Viola Hollek

Charité

“Deciphering Oncogenic Signatures in Colorectal Cancer Through Single-Cell Multi-Omics”

Tissue homeostasis is orchestrated by the precise modulation of signalling pathways, guiding cellular fate towards proliferation, differentiation, or apoptosis. This balance can be disrupted by genetic mutations in key signalling molecules, potentially leading to uncontrolled growth. Interestingly, different oncogenic mutations can result in divergent phenotypes, even if the mutated proteins belong to the same signalling pathway. For instance, our research has demonstrated that mutations in KRAS and BRAF, both members of the MAPK pathway, have distinct effects on ERK signalling in colorectal cancer (CRC) patients. This variation suggests a complex relationship between specific oncogenic mutations and the tumorigenesis of CRC, and highlights a critical gap in our understanding of the phenotypic outcomes arising from individual oncogenic mutations.

In this study, we aim to dissect mutation-specific changes in signalling networks, cell fate decisions, and cell behaviour in CRC. We profile a compendium of prevalent mutations in CRC using a bar□coded lentivirus-based oncogene library, analysed across five CRC cell lines with distinct genomic backgrounds. By leveraging specific nucleotide- and peptide-based barcode systems, we are able to perform the oncogene screen in a pooled manner and analyse it by single-cell RNA-sequencing to identify transcriptomic alterations, complemented by mass cytometry and mass spectrometry to map the phospho-proteome and proteome, respectively.

Poster 79

Indranil Singh

IRB Barcelona

“Deciphering Cellular Destiny: Pivotal Role of Stem Cell States' in Shaping Responses to Preleukemic Mutations”

Tumors with the same driver mutations can display a striking variation in their progression and treatment response, but the origins of this variation are still unclear. In this study, we unveil that heritable stem cell states can have a profound influence on how individual cells respond upon acquisition of the same cancer mutation. Here we develop a new method, scTRAACK, for single-cell Tracking of Recombinase Activation And Clonal Kinetics and apply it to hematopoietic stem cells carrying Cre-conditional leukemia alleles. Tracking the expansion kinetics of a common set of stem clones, with and without the same leukemia mutations, we unveil a striking heterogeneity in the malignant fates of diverse stem cell clones. First, we define that heritable clonal states persist in expansion cultures and affect their clonal composition. Then, using mouse models of the most frequent initiating AML mutation in Dnmt3a, we define that these pre-existent heritable stem cell states influence clonal expansion, differentiation properties, and unique malignant gene expression programs that arise from each cell upon acquisition of mutation. Whereas high-fitness slow-cycling clones expanded regardless of mutational state, cancer mutations significantly increased the survival probability in stem cell clones with higher cycling and lower fitness, which are the most abundant clones within the stem cell compartment. Moreover, mutations reprogrammed the stem cell states of low-fitness clones to maintain a pool of stem cell-like cells.

These findings suggest that whereas both high and low-fitness stem cells can be at the origin of AML, the more abundant low-fitness pool likely represents the cell of origin for the majority of AML.

Poster 80

Maria Sopena-Rios
Barcelona Supercomputing Center

"Single-cell atlas of the aging circulating immune system"

Age-associated decline in immune function, known as immunosenescence, predisposes individuals to infection, autoimmune disorders, and cancer. Immune function decline manifests as chronic low-grade inflammation (inflammaging) and impaired responsiveness to stimuli.

Single-cell RNA sequencing (scRNA-seq) is a powerful tool to uncover the cellular and molecular dynamics of immunosenescence among immune cell populations. However, studying immune cell type dynamics and cell state changes during human aging requires extremely large sample sizes.

Here, we leverage a scRNA-seq dataset of 982 individuals encompassing over 1 million human peripheral blood mononuclear cells (PBMCs) to systematically investigate the effect of aging on the human circulating immune system. Differential expression (DE) analysis with age reveals opposite expression patterns across distinct immune cell populations. Specifically, CD8+ T naive and B memory cells exhibited the largest number of down-regulated genes, while Natural Killer (NK) cells and CD8+T effector memory cells (CD8+ TEM) showed the largest number of up-regulated genes with age. This opposing pattern led us to identify a group of cell types, including CD8+ T Naive, CD4+ T Naive, MAIT and B cells, showing a coordinated down-regulation of both inflammatory response and translation-related processes with age.

Conversely, the remaining cell types, which include all CD4+ T cell types, CD8+ T Memory and CD8+Treg cells, monocytes and NK cells, have the same pathways up-regulated including inflammation and translation processes. When looking at genes DE in multiple cell types, we confirm that the discordant directionality pattern in the pathways is driven by the same genes that are up-regulated in one group of cell types and down in the other.

These cell-type opposite responses go beyond lineage or functional classifications, highlighting a heterogeneity in aging trajectories within the immune cell repertoire.

We then performed differential cell type composition analysis with age and observed a strong consistency between changes in cellular proportions and gene expression patterns. Specifically, cell types with significant down-regulated genes, such as CD8+ T Naive and B memory, decreased in proportion. Conversely, cell types with substantial age-related up-regulation, including NK and CD8+ TEM, showed a corresponding increase in their proportions. This underscores the intrinsic link between cell population dynamics and gene expression during aging.

Finally, we carried out a sex-stratified differential expression analysis with age. This revealed sex-specific patterns within certain immune cell populations. Specifically, CD8+ TEM in females displayed up-regulated gene expression. Conversely, B memory cells in males showed down-regulation of numerous genes. These findings suggest that sex might be a primary driver of the previously observed age-associated gene expression changes. Finally, we find that up-regulated gene signatures in cell types with pro-inflammatory phenotypes (e.g. NK, CD8+ TEM, CD4+ CTL) are enriched for autoimmune disease exclusively in elderly females. These results elucidate the crucial role of sex in immune system aging, highlighting the need to include a sex perspective in immunity studies.

Overall, our study unveils a dual aging trajectory across immune cell types coupled with joint responses in gene expression and cell type composition and provides unprecedented insights into the cellular and molecular dynamics underlying immunosenescence.

Alejo Rodriguez Fraticelli

IRB Barcelona

"Heritable states influence stem cell fates upon acquisition of cancer mutations"

Tumors with the same driver mutations can display a striking variation in their progression and treatment response, but the origins of this variation are still unclear. In this study, we unveil that heritable stem cell states can have a profound influence on how individual cells respond upon acquisition of the exact same cancer mutation. Here we develop a new method, scTRAACK, for single-cell Tracking of Recombinase Activation And Clonal Kinetics and apply it to hematopoietic stem cells carrying Cre-conditional leukemia alleles. Tracking the expansion kinetics of a common set of stem clones, with and without the same leukemia mutations, we unveil a striking heterogeneity in the malignant fates of diverse stem cell clones. First, we define that heritable clonal states persist in expansion cultures and affect their clonal composition. Then, using mouse models of the most frequent initiating AML mutation in Dnmt3a, we define that these pre-existent heritable stem cell states influence clonal expansion, differentiation properties, and unique malignant gene expression programs that arise from each individual cell upon acquisition of mutation. Whereas high-fitness slow-cycling clones expanded regardless of mutational state, cancer mutations significantly increased the survival probability in stem cell clones with higher cycling and lower fitness, which are the most abundant clones within the stem cell compartment. Moreover, mutations reprogrammed the stem cell states of low-fitness clones to maintain a pool of stem cell-like cells. These findings suggest that whereas both high and low-fitness stem cells can be at the origin of AML, the more abundant low-fitness pool likely represents the cell of origin for the majority of AML.

Poster 82

Leone Albinati

Centre for Genomic Regulation

“Tracing the accumulative pattern of epigenetic and transcriptomic events in early lymphoma formation”

Tumor formation is the consequence of the accumulation of genetic and epigenetic aberrations in time. While for some tumors the tumorigenesis path has been deeply characterized at the genetic and epigenetic level, for other types of tumors the chain of events that leads to the tumoral transformation of a normal cell is yet to be completely identified. This is the case for Mantle Cell Lymphoma (MCL), an aggressive Non-Hodgkin lymphoma, whose development and progression cannot be fully explained by genetic driver mutations only.

To better understand the early steps of lymphomagenesis, we are analyzing bone marrow samples from patients with MCL using a single-cell multi-omics approach. We collected gene expression information and B-cell receptor sequencing from ~20,000 healthy B cells at various differentiation stages from 4 patients with MCL. From one of the samples, we also have information about methylation and mutational status at the single-cell level. Analyzing these samples, we aim to (i) uncover the presence of epigenetic events, typical for the fully developed lymphoma cells, that occur in the early pre-malignant stages of lymphomagenesis and (ii) characterize alterations of the early B cell lineage caused by the presence of the tumor in the bone marrow.

Altogether, with this data we aim to create a better understanding of early MCL pathogenesis.

Poster 83

Karina Cancino

Centre de Recherches en Cancérologie de Toulouse

“Diving deeper into the development of Non-Cirrhotic liver cancer”

Hepatocellular carcinoma (HCC) is one of the major causes of cancer-related death worldwide. Typically, HCC afflicts individuals in their maturity years (~60 y.o.) in the background of cirrhosis; however, about 20% of cases can develop in a non-cirrhotic liver (NC-HCC). In Peru, NC-HCC’s incidence is abnormally elevated (~90%) and develops at a younger age (~40 y.o.). In addition, most of these patients present huge tumors (>10cm in diameter), high levels of alpha-fetoprotein (>10,000ng/mL), and hepatitis B virus infection. The molecular analysis of this atypical HCCrevealed a peculiar mutational spectrum (primarily insertions and deletions) and an unusual hypermethylation pattern. Recently, our group described liver clear cell foci in the non-tumoral liver parenchyma of NC-HCC patients (20%), but the malignant potential of these lesions has not been assessed yet.
Therefore, we aimed to characterize at the single-cell level the cellular transition from a pre-malignant to a malignant state in NC-HCC. Preliminary results of paired frozen liver and tumor samples (~100,000 cells) from NC-HCC patients (n = 8) were obtained. Considering that samples come from a developing country, we adapted the recommended sample preparation guidelines.
Next, accurate and precise cell phenotype identification was automated using an artificial intelligence tool. We undertook the description of the adjacent non-cirrhotic liver parenchyma to uncover pre-malignant features. Surprisingly, most of the cells presented liver progenitor-like features. The normal liver spatial organization (zonation) was explored using previously reported transcriptomic signatures. As a result, a singular group of cells with a loss of hepatocyte identity and high carcinogenic potential were identified in the adjacent non-tumoral liver parenchyma. For further validation, spatial transcriptomics and multiplexing tissue imaging will be used. Moreover,pseudo-temporal inference unveiled the intricate biologic background of carcinogenesis, where cancer cells display blended features of the two major HCC molecular classes. This approach provided further insights into cancer cell transformation, which is paramount for biomarker identification tailored for NC-HCC.