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#1

Integrative Single-Cell Analysis of 2D and 3D Breast Cancer Models Reveals a Transcriptional Signature of Tumour Architecture and Drug Sensitivity

Fabiana Arco
TIGEM

Breast cancer is a heterogeneous disease characterized by distinct molecular and clinical subtypes that influence therapeutic response. Conventional classification based on receptor expression (ER, PR, HER2) provides important clinical guidance but fails to capture the complexity of intra-tumoral heterogeneity, which contributes to drug resistance and treatment failure. To address this limitation, our group previously developed the first Breast Cancer Single-Cell Atlas, revealing significant transcriptional variability within and across subtypes.Building on this foundation, the current project expands the atlas by integrating single-cell transcriptomic data from 2D cell lines and 3D Single-Cell Tumor Spheroids (SCTS) for a total of 36 cell lines and more than 80k single cell profiles. These 3D models more accurately mimic the architecture and microenvironment of breast tumors, offering a better platform for studying heterogeneity and drug sensitivity. Specifically, comparative analyses revealed that several cell lines exhibit increased transcriptional heterogeneity under 3D growth conditions. Differential gene expression and pathway enrichment analyses (3D vs 2D) were used to generate a consensus gene expression signature caused by the 2D to 3D transition. This signature was then used to computationally screen for compounds, eliciting transcriptional effects either "similar" or "opposite" to those observed in 3D cultures building on our previous work. We then excluded those compounds that were known to be toxic, and we experimentally validated 20 different compounds to identify those with differential activity in cells grown in 2D vs cells grown in 3D. Experimental validation in three selected breast cancer cell lines confirmed the predictive power of the model. Among the predicted compounds, we identified one that demonstrated a significant, selective effect only on 3D spheroid growth and viability, with minimal activity in 2D cultures. These findings suggest that this compound can prevent spheroid formation and may offer a potential therapeutic avenue to prevent metastases in breast cancer.Future work will extend the validation to additional breast cancer cell lines and add an in vivo demonstration of the potential of the compound to inhibit metastasis.

#2

HR-Defective Phenotype Induced By Small Molecules To Trigger Synthetic Lethality In Pancreatic Cancer Cells

Bagnolini Greta (1) , Ferrandi Giovanni (1,2), Falchi Federico (1), Poppi Laura (2), Masi Mirco (2), De Franco Francesca (3), Girotto Stefania (4), Roberti Marinella (1), Cavalli Andrea (1,2,5)

(1) Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy (2) Computational and Chemical Biology, Istituto Italiano di Tecnologia, 16163 Genoa, Italy (3) TES Pharma S.r.l., I-06073 Corciano, Perugia, Italy (4) Structural Biophysics Facility, Istituto Italiano di Tecnologia, 16163 Genoa, Italy (5) Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland

Synthetic lethality (SL) has transformed oncology as a cornerstone of precision medicine. Beyond identifying genetic vulnerabilities, SL is emerging as a framework for designing synergistic drug combinations that exploit specific tumor genotypes. This strategy enables pairing existing drugs with novel candidates, reducing costs and time, broadening patient eligibility, and mitigating resistance often seen with targeted monotherapies.In this study, we exploited the synthetic lethal pair BRCA2 and PARP to induce SL in homologous recombination (HR)-proficient cancers, typically unresponsive to PARP inhibitors (PARPi). Given the poor druggability of BRCA2, we sought to inhibit its interaction with RAD51, the recombinase essential for HR-mediated DNA repair and cancer cell survival. We established a workflow to develop small-molecule inhibitors of the RAD51–BRCA2 protein–protein interaction (PPI), aiming to impair HR and induce SL in combination with clinically approved PARPi in pancreatic cancer, selected as a representative model of high unmet need. Virtual screening of the RAD51 surface at PPI hotspots (PDB 1N0W), followed by ELISA validation and chemical optimization, yielded ARN26912. This compound inhibited HR in a dose-dependent manner, as shown by reduced RAD51 nuclear foci, impaired DNA repair upon cisplatin treatment, and increased micronuclei formation. Importantly, ARN26912 combined with PARPi induced apoptotic cell death in pancreatic cancer models, notably in 2D and 3D BxPC-3 and HPAC cells with olaparib, and in HPAC cells with talazoparib. ARN26912 was ineffective in CAPAN-1, consistent with BRCA2 deficiency, and non-toxic to normal pancreatic cells, indicating selective activity.Overall, ARN26912 demonstrates that disrupting the RAD51–BRCA2 PPI can mimic a BRCAness-like phenotype, enabling SL with PARPi in HR-proficient cancers. This work underscores SL as a powerful accelerator of anticancer drug discovery and therapy innovation.

#3

Deep generative multi-omics integration from pre-clinical to clinical cancer models

Ana Rita Baião* (1,2), Susana Vinga (1,2), Emanuel Gonçalves (1,2)
"(1) INESC-ID, 1000-029 Lisboa, Portugal, (2) Instituto Superior Técnico (IST), Universidade de Lisboa, 1049-001 Lisboa, Portugal"

*Advances in high-throughput sequencing and other assay technologies have enabled the extensive molecular and phenotypic characterization of cancer cells, generating large and complex multi-omics datasets. While these datasets offer unprecedented opportunities to improvetreatment strategies and advance precision medicine, their integration remains a significant challenge due to the high dimensionality, heterogeneity, and frequency of missing values across data types. Recent progress in machine learning, particularly in deep learning, has demonstrated strong potential to address these challenges and develop models that represent and integrate diverse types of features.This project aims to develop a deep generative learning framework to integrate, augment, and expand multi-omics datasets from both preclinical and clinically relevant cancer models. Specifically, we focus on integrating Cancer Dependency Map (DepMap) datasets from cancer cell lines and organoids with tumor data from TCGA. To achieve this, we propose a mosaic integration strategy designed to unify these diverse sources and bridge the gap between preclinical models and patient tumors. As an initial step, we present preliminary results using a conditional adversarial variational autoencoder (VAE) applied to gene expression data, performing horizontal integration of cell lines, organoids, and tumors. These results demonstrate the feasibility of aligning datasets into a unified representation space.Building on this foundation, the adversarial VAE framework will be extended to integrate additional omic modalities, handle incomplete data, and enable synthetic data augmentation. Ultimately, this approach will support patient-specific prediction of treatment outcomes and the prioritization of effective drug therapies for clinical validation.**Work supported by national funds through Fundação para a Ciência e a Tecnologia, I.P. (FCT) under projects UID/50021/2025, UID/PRR/50021/2025, and SYNTHESIS (https://doi.org/10.54499/LISBOA2030-FEDER-00868200)*

#4

Computational identification and prioritisation of antibody-drug conjugate targets for new anti-cancer drug development

Lorenzo Mathieu Brochier* (1,3, 4), Laura Riva (2), Francesco Iorio (1)
"(1) Human Technopole(2) Nerviano Medical Sciences(3) University of Milan ""La Statale""(4) European School of Molecular Medicine (SEMM)"

Antibody drug conjugates (ADCs) represent a novel class of anti-cancer therapeutic agents, exhibiting high target specificity towards cell surface antigens. They are composed of three components: an antibody (Ab) which targets specificity surface antigens/proteins, a payload which is a cytotoxic drug, and a linker which connects the payload to the antibody.With several ADCs approved for clinical use and a robust pipeline in development, there is a growing need for computational models to identify and prioritise novel ADC targets. Making use of publicly available cancer functional genomic data, the objectives of this project are the following: a) identifying cancer type-specific surface protein expression; b) identify new surface antigens / payload combinations.We curated a list of Surface Protein Genes (SPGs) (~400 genes) from Gene Ontology and Amigo2 and a list of approved and clinical trials ADC targets.For the first analysis (a) We used data from the Cancer Genome Atlas to identify differential expression (DE) of SPGs across 16 cancer types contrasting tumour versus normal samples. Once identified the most differentially expressed SPGs, we further prioritised them considering a metric of toxicity that we designed on purpose. This Toxicity Index (TI) is a weighted sum of the basal expression levels of a given SPG in healthy tissues from the Genotype-Tissue Expression (GTEx) database. We focused on only target with a TI lower than the maximum TI for approved ADC targets. For the second (b) analysis, we retrieved CRISPR-derived genetic dependency data from the Cancer Dependency Map (DepMap). We first scaled this data with the CoRe package, then filtered out common essential, never essential genes and genes essential in less than 5 cell lines for each tissue.The first analysis (a) identified a total of 164 DE genes in 1 or more cancers. Of these 133 were below the toxicity Index. Among these genes 4 are already approved ADC targets, 37 are in ADC clinical trials and 92 are potential new ADC targets. The most differentially expressed genes (with relative cancers where found over-expressed) are: CA9 (KIRC), CD70 (KIRC), ENPP3 (KIRC). Results of the second analysis (b) yielded 606 significant gene expression-dependency tests in 8 tissues (Lung, Lymphoid, Skin, Brest, Esophagus, Brain, Bowel, Ovary). The best surface protein gene – dependency associations (with respective tissues were found associated) are: CD79A-EBF1 (Lymphoid), EDNRB-SOX10 (Skin), ERBB3-FOXA1 (Breast).The presence of differentially expressed SPGs in specific cancers can be leveraged todesign new ADCs. The dependency analysis can instead be used to guide towards thedevelopment of new ADCs carrying targeted therapy towards the found dependencies,instead of the classical cytotoxic molecules. Furthermore, those SPGs identified in bothanalyses could be used to develop an even more selective ADC therapy, allowing tofind, in a cancer, a subpopulation that could be targeted in such a selective fashion.

#5

Gene mutant dosage determines prognosis and metastatic tropism in 60,000 clinical cancer samples

Nicola Calonaci* (1), Eriseld Krasniqi* (2), Daniel Colic (3), Stefano Scalera (2), Giorgia Gandolfi (1), Salvatore Milite (4), Konstantin Bräutigam (5), Andrea Sottoriva (4), Trevor A. Graham (5), Leonardo Egidi (6), Biagio Ricciuti (7), Marcello Maugeri-Saccà (2), Giulio Caravagna (1,8)
(1) Department of Mathematics, Informatics and Geosciences, University of Trieste, Italy, (2) IRCCS Regina Elena National Cancer Institute, Rome, Italy, (3) Institute for Research in Biomedicine, Barcelona, Spain, (4) Centre for Computational Biology, Human Technopole, Milan, Italy, (5) Centre for Evolution and Cancer, Institute of Cancer Research, London, United Kingdom, (6) Department of Economics, Business, Mathematics and Statistics, University of Trieste, Italy, (7) Lowe Center for Thoracic Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, (8), Area Science Park, Trieste, Italy

The intricate interplay between somatic mutations and copy number alterations critically influences tumour evolution and patient prognosis. However, traditional genomic analyses often treat these alterations independently, overlooking gene mutant dosage – a key emergent property of their interaction. Here, we develop an innovative computational framework that infers allele-specific copy number alterations directly from clinical targeted sequencing panels without requiring matched normal samples. Using this approach, we derived gene mutant dosage statistics for over 500,000 mutations across 60,000 clinical samples spanning 39 cancer types. By stratifying more than 20,000 patients according to mutant dosage across multiple oncogenes and tumour suppressor genes, we identified 46 tumour type-specific biomarkers predictive of overall survival. Notably, 13 of these biomarkers across 12 tumour types were undetectable using standard binary mutant/wild-type models. Additionally, 26 biomarkers were recurrently associated with metastatic spread in 10 tumour types, and 24 predicted organ-specific metastatic tropism in 6 tumour types. Alongside confirming known roles for established oncogenes and tumour suppressors, our method reveals, for the first time, gene mutant dosage patterns as independent predictors of prognosis, metastatic potential, and site-specific dissemination across diverse solid tumours. This augmented insight into genomic drivers enhances our understanding of cancer progression and metastasis, holding the potential to foster biomarker discovery significantly.

#6

Dissecting the contribution of ZBTB18 and TIP60 complex to the mesenchymal/quiescent state in GBM

Roberto Ferrarese* (1), Eyleen Corrales (2), Lucrezia Zanzi (1), Sofia Munari (1), Stefan Tholen (3), Greta Forlani (1), Geoffroy, Andrieux (2) and Maria Stella Carro (1)

"(1) Department of Medicine and Technological Innovation, School of Medicine, University of Insubria, Varese, Italy(2) Institute of Medical Bioinformatics and Systems Medicine, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany(3) Institute of Clinical Pathology, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany"

Glioblastoma (GBM) is the most malignant type of brain tumor with a median survival of 15 months, due to a high recurrence rate and therapy resistance. The mesenchymal-like (MES-like) state of GBM has been associated with its most aggressive features. MES-like tumors contain a high proportion of slow-cycling cells, which are responsible for therapy resistance, tumor recurrence and the establishment of the GBM immunosuppressive environment. We have previously pinpointed ZBTB18 as a transcriptional repressor of mesenchymal genes and a tumor suppressor in GBM. In order to get more insight into ZBTB18-mediated transcriptional repression mechanism in GBM, we performed co-immunoprecipitation experiments, followed by mass spectrometry (MS), in brain tumor stem-like cells (BTSC), which express ZBTB18. The analysis revealed that many components of the NuA4/TIP60 acetyltransferase complex are putative ZBTB18 interactors. The NuA4/TIP60 complex is a large protein complex, which contains at least 16 subunits and at least three interrelated enzymatic activities, including histone H4/H2A acetyltransferase. The catalytic subunit KAT5 has been recently shown to oppose the quiescent state in GBM. Based on previous reports and on our preliminary data, we hypothesize a role for ZBTB18 and TIP60 complex/KAT5 in regulating the expression of genes important for a mesenchymal/quiescent GBM state. Indeed, by using a mesenchymal genetic tracing vector (MGT#1-mVenus) synthetic reporter, we could observe that depleting ZBTB18 or KAT5 in BTSCs by CRISPR/Cas9, results in a small increase in mVenus fluorescence. We then examined the acetylation status of histone H4 by ChIPseq in BTSCs, upon ZBTB18 or KAT5 ko. The results showed a global enrichment of acetylated peaks at genes previously linked to qGBM cells and associated with mesenchymal traits upon both ZBTB18 and KAT5 ko. In particular, our data suggest that ZBTB18 and TIP60 complex may inhibit the MES/qGBM state by interfering with TGFB3-mediated gene activation.

#7

Predict, perturb, and process: a systems biology pipeline for identifying synthetic lethality

Rosalind Juihsuan Chou* (1, 2), Chenchu Lin (2), Veronica Gheorghe (2), Sabriyeh Alibai (2), Subin Kim (1, 2), Lori Wilson (2), Xingdi Ma (2), Traver Hart (2)
(1) UTHealth Graduate School of Biomedical Science, The University of Texas MD Anderson Cancer Center, Houston, TX, USA, (2) Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

The synthetic lethality principle holds great promise for cancer therapy, but identifying synthetic lethals presents enormous challenges. Synthetic lethality, where loss of two genes causes cell death but loss of either gene alone does not, is difficult to assay because combinatorial perturbation technologies are inefficient and because the search space is well beyond the capacity of current experimental systems: 19,000 protein coding genes offer more than 180 million candidate gene pairs. To address this, we combine network-driven predictive models with the In4mer CRISPR/Cas12a combinatorial knockout platform to conduct tractable pairwise knockout screens with high probability of identifying synthetic lethals. Even with these advances, effective methods for quantifying GIs in these screens remain limited. To address this gap, we developed GRAPE (Genetic interaction Regression Analysis of Pairwise Effects), a novel regression-based method for analyzing GIs in all-by-all gene knockout (KO) library designs. GRAPE infers single-gene KO fitness from multiplex CRISPR arrays, predicts combinatorial gene KO fitness, and identifies synthetic lethals that deviate from this expectation. Since no gold-standard exists for benchmarking, we built a simulation framework to systematically evaluate GRAPE, demonstrating improved computational efficiency, adaptability, and precision-recall performance over existing methods. Using the network-driven experimental design, In4mer screening, and GRAPE analysis, we screened all pairwise combinations of 205 genes involved in receptor tyrosine kinase (RTK) signaling, all pairs of 167 genes in DNA damage response (DDR) pathways, and over 4,000 paralog pairs across 12 diverse cancer cell lines. Our DDR results closely align with findings reported by other studies, while our RTK network provides novel insight into ER-mediated protein modification and oncogenic signaling dependencies. Overall, our efforts confirm that we can predict and detect both global and background-specific genetic interactions, advancing the state of the art in functional genomics and cancer target finding.

#8

CRISPR-powered discovery of long non-coding RNA therapeutics in cancer

Michela Coan* (1, 2, 3), Yifan Yu (2, 3), Nivedha Balaji (2, 3), Sunandini Ramnarayanan (2, 3), Clodagh Murphy (3), Xingyu Guo (2, 3), Jack Roban (2, 3), Bhavya Dhaka (2, 3), Ai Ming Tham (2, 3), Rory Johnson (2, 3)
(1) School of Medicine, University College Dublin, (2) School of Biology and Environmental Science, University College Dublin, (3) Conway Institute for Biomolecular and Biomedical Research, University College Dublin

Long non-coding RNAs (lncRNAs) represent a promising yet largely unexplored class of targets for RNA-based therapeutics in cancer. In particular, lncRNAs exhibit disease, tissue, and cell type-specific expression patterns, along with high variability among individuals, which highlights their potential for developing targeted and personalized therapies with reduced toxicity to healthy cells and organs. Despite their known involvement in cancer progression, identifying effective therapeutic targets has been hindered by a lack of effective screening technologies. CRISPR genome editing has revolutionized our ability to study lncRNAs by enabling high-throughput manipulation of their genes with unprecedented precision.We leveraged a functional CRISPR-based screen to comprehensively map lncRNAs that promote lung cancer progression. By systematically perturbing over 160 lncRNA gene loci in four KRAS-mutant non-small cell lung cancer (NSCLC) cell lines and two non-cancer cell lines, we identified a subset of 32 high-confidence oncogenic lncRNAs with significant potential as therapeutic targets and with minimum toxic effect. These were further analysed through multi-omics approaches, revealing desirable therapeutic characteristics such as cancer specificity and an association between lower expression levels and improved overall survival, confirming their therapeutic potential. Moreover, employing antisense oligonucleotides (ASOs) knockdown, we validated the tumorigenic activity of 5 of the best candidates from the CRISPR screens, demonstrating the applicability of our discovery.In summary, we developed a CRISPR-based drug discovery pipeline to create new RNA therapies for cancer, and we are using it to identify the most promising novel lncRNA targets for NSCLC therapy.

#9

CRISPR-Cas9 Knock-out Screening of lncRNAs highlights New Therapeutic Targets for Cancer Treatment

Katia Grillone (1)*, Serena Ascrizzi (1), Paolo Cremaschi (2), Jussara Amato (3), Nicoletta Polerà (1) , Ottavio Croci (2), Roberta Rocca (1,4), Caterina Riillo (1), Francesco Conforti (5), Raffaele Graziano (3), Diego Brancaccio (3), Daniele Caracciolo (1), Stefano Alcaro (4,6), Bruno Pagano (3), Antonio Randazzo (3), Pierosandro Tagliaferri (1) , Francesco Iorio (2,7) , Pierfrancesco Tassone (1)
"(1) Department of Experimental and Clinical Medicine, Magna Græcia University, Catanzaro, Italy(2) Computational Biology Research Centre, Human Technopole, Milano, Italy(3) Department of Pharmacy, University of Naples Federico II, Napoli, Italy(4) Net4Science srl, Magna Græcia University, Catanzaro, Italy(5) Pathology Unit, Annunziata Hospital, Cosenza, Italy(6) Department of Health Sciences, Magna Græcia University, Catanzaro, Italy(7) Cancer Dependency Map Analytics, Wellcome Sanger Institute, Genome Campus, Hinxton, Cambridge, UK"

Introduction. *The genomic landscape of cancer hides numerous alterations in coding and non-coding genes. The molecular dissection of tumors revealed the key role of long-non coding RNAs (lncRNAs) in cancer progression and drug response. LncRNAs are non-coding transcripts longer more than 200 nucleotides, which control gene expression at multi-omic levels. They are considered promising biomarkers and therapeutic targets but most of them remain unexplored.*

Material and Methods. *We i) performed a CRISPR-Cas9 screens to knock-out (KO) 671 lncRNAs in Multiple Myeloma (MM) cell lines sensitive and resistant to the conventional drug Bortezomib (BZB); ii) developed a bioinformatic prioritization pipeline able to integrate functional data from in vitro screens with patients-derived prognostic and genomics data from public datasets; iii) functionally validated the role of the most promising target RP11-350G8.5 via cellular and molecular studies in vitro and in vivo; iv) structurally characterized RP11-350G8.5 via biophysical assays including Thioflavin T, 1H-NMR spectroscopy and circular dichroism v) applied computational tools for predicting the putative binding regions of selective therapeutics vi) performed pan cancer bioinformatic analyses to identify biomarkers associated with the expression of our candidate lncRNA, and to evaluate its differential expression and prognostic value in other cancer types.*

Result and discussion. *Our CRISPR-screen revealed 8 lncRNAs whose KO reduced cell viability in MM cells, and that are associated with high expression and poor prognosis in MM patients'. Among them, RP11-350G8.5 emerged as the most promising target for MM also in BZB-resistant models. We highlighted that the loss of RP11-350G8.5 i) reduces tumour cell viability, proliferation and 3D colony-formation in vitro and impairs tumour growth in vivo ii) induces apoptosis and sensitizes cells to BZB iii) modulates the unfolded protein response system and triggers an Immunogenic Cell Death. We identified 2 G-quadruplex and 3 hairpin- forming regions and focused on specific 3D conformations for the development of selective drugs. In addition, we revealed RP11-350G8.5 overexpression and correlation with poor prognosis across different cancer types in addition to MM, including kidney renal papillary cell carcinoma, and predicted a genetic signature related to the expression of our candidate lncRNA that may help the choose of anti-tumoral therapeutic regimens.*

Conclusion. *Our study (Grillone K. et al., Blood 2024) highlighted novel genetic vulnerabilities never associated before to MM, among which the previously uncharacterized RP11-350G8.5 emerged as the most promising therapeutic target. We then moved forward the design of innovative anti-cancer compounds and to the investigation of the oncogenic role of our candidate lncRNA in a pan cancer setting.*

#10

ReGenT-seq identifies RNA transport directing cancer cell invasion

Carlotta Duval1, Andrea Lauria1, Alessandro Croce1, Chiara Levra Levron1, Livia Caizzi1, Francesca Anselmi1, Gabriele Piacenti1, Elisa Balma1, Mika Watanabe2, Ken Natsuga2, Valentina Proserpio1, Salvatore Oliviero1, Giacomo Donati1.
"1 Molecular Biotechnology Center, Department of Life sciences and Systems Biology, University of Turin, Torino, Italy.2 Department of Dermatology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan."

"In epithelia, wound-healing stem cells and invasive cancer cells share a stress-induced transcriptional program that promotes motility through the formation of cell protrusions. A very detailed mechanism of protrusion initiation has been shown involving actin polymerization. However, less is known about later stages of protrusion maturation and how their heterogeneous composition affects their function. Using a newly developed pooled genetic approach (ReGenT-seq) we screened 615 chromatin factors controlling stress-induced epidermal cell activation. The screen unexpectedly revealed an RNA-binding protein critical for cell protrusions. Beyond its nuclear activities, we revealed a cytoplasmic moonlighting function, essential for governing the subcellular localization of specific mRNAs within invadopodia of squamous carcinoma cells. Strikingly this mRNA transport is required for protrusion maturation. Therefore, we revealed a multilayered control of gene expression to regulate carcinoma cell motility. This regulatory network includes a unidirectional chromatin-to-cell-protrusion mRNA transport that represents a potential therapeutic target to contrast pioneering cancer cells that penetrate the basement membrane and invade surrounding connective tissue.-*ReGenT-seq (REciprocal GENetic Test) is an integrated pooled genetic screening implemented with two transcriptional read out systems engineered with either a drugs-resistance or -susceptibility reporter genes. Since even the top-ranking hits of successful pooled screens may contain false positives, which can arise from biases in the assay or random fluctuations, our approach aims to integrate two reciprocal screens to avoid this issue. In general, ReGenT-seq allows to genetically screen for a desired transcriptional readout with high stringent detection of gene hit."*

#11

A generalised epigenetic clock allows the identification of age-related drug responses and genetic dependencies in cancer cells

Irene Fernández-Rebollo* (1,2,3), Alessandro Digilio* (1), Athanasios Oikonomou (1), Lucia Trastulla (1), Manel Esteller (4), Francesco Iorio (1)
"(1) Computational Biology Research Centre, Human Technopole, Milan, Italy(2) University of Milan ""La Statale"", Milan, Italy(3) European School of Molecular Medicine (SEMM)(4) Cancer Epigenetics Group, Josep Carreras Leukaemia Research Institute, Barcelona, Spain"

Epigenetic clocks are mathematical models that estimate biological age based on DNA methylation patterns. These clocks have become powerful tools in the field of aging research, providing insights into biological processes related to aging that are not captured by chronological age alone. While current epigenetic clocks have been effective in predicting the age of healthy samples, they perform poorly on tumour data due to the altered epigenetic patterns present in cancer. The aim of this study is to develop a generalised epigenetic clock which provides an accurate estimation of epigenetic aging in both healthy and cancerous states and apply it to investigate therapeutic potential and analyse genetic vulnerabilities in a large panel of multi-omics-characterized cancer cell line models from the Cell Model Passport platform.We developed a novel epigenetic clock using elastic net regression trained on a pan-cancer cohort of DNA methylation primary tumour samples from The Cancer Genome Atlas (TCGA). The epigenetic signature provided by the model, consisting of 4862 CpGs, was used to predict the epigenetic age of cancer in vitro models, thus to identify and dissect age-related genetic and pharmacological vulnerabilities, by exploiting the wealth of data from the Cancer Dependency Map (DepMap) and Genomics of Drug Sensitivity in Cancer (GDSC) projects repositories.This new epigenetic model can accurately predict age in both malignant and healthy samples, with Pearson correlation coefficients of 0.786 and 0.85, respectively. These results suggest that DNA methylation patterns may potentially affect common age-related biological pathways that are preserved even under altered conditions of cancer. Given the intrinsic limitations of using chronological age data in cell lines, where “age at sampling” information cannot reflect the true aging of the cells due to numerous confounding factors of in vitro models, the age predicted by our epigenetic clock serves as a new measure for assessing aging-related epigenetic effects in the quest for biomarkers of cancer drug response and genetic dependencies on a very large scale in both pan-cancer and cancer-specific settings. Using this metric, we uncovered distinct patterns of drug sensitivity and gene essentiality between epigenetically “young” and “old” cancer models, with certain subtypes showing specific age-dependent vulnerabilities.This study highlights the potential of epigenetic aging as a molecular signature in the context of cancer, opening new directions for identifying age-related drug response patterns and genetic dependencies.

#12

A computational approach for the discovery of transcriptomic correlates of synthetic lethal interactions in common cancers

Syed Haider* (1), Rachel Brough (1,2), Santiago Madera (1), Jacopo Iacovacci (1), Aditi Gulati (1), Andrew Wicks (1,2), John Alexander (1), Stephen J. Pettitt (1,2), Andrew N. J. Tutt (1), Christopher J. Lord (1,2)

(1) The Breast Cancer Now Toby Robins Breast Cancer Research Centre, (2) CRUK Gene Function Laboratory, The Institute of Cancer Research, London, SW3 6JB, UK

To maintain cell fitness, the deleterious effects of gene defects are often buffered by compensatory changes in additional genes. In cancer, these buffering processes could, in principle, be targeted for therapy exploiting the principle of synthetic lethality. However, despite the large-scale identification of synthetic lethal (SL) effects in pre-clinical models, evidence that these operate in the clinical disease is limited to a small number of examples, a problem that impedes the wider application of the concept. Using an integrated analysis of isogenic and non-isogenic in vitro SL screens in cell models with the molecular profiles of >9,000 cancers, we show that transcriptomic buffering of tumour suppressor gene (TSG) loss by hyperexpression of SL partners is a common phenomenon in human cancers and extends to multiple TSGs and cancer histologies. This effect is also seen in cancers that phenocopy those with specific TSG loss, such as cancers with BRCAness, where a metagene of hyperexpressed BRCA1/2 SL genes correlates with clinical outcome and therapy response. In addition, SL genes that exhibit transcriptomic buffering of BRCA1/2 dysfunction also represent more robust effects, being identified in both isogenic and non-isogenic CRISPR screens. These observations have implications for understanding how tumour cells tolerate TSG loss, in part explain the transcriptomic architecture of cancers, and highlight a series of pre-clinically identified SL effects that are represented by buffering relationships in human cancers.

#13

Systems biology analysis for a large dual-gene knock out screen for combination therapy

H. Horn*1,2, L. Koob*1,3, P. Weidemueller1,2, D. O'Hanion1,2, E. Lockyer3, I. Barrio-Hernandez1,3, E. Duncan3, R. Silva1,3, E. Souster3, L. Dwane1,3, R. Hosseini1,2, E. Karakoc1,3, A. Beck1,3, F.M. Behan3,4, E. Curry3,4, D. Voukantsis3,4, I. Valtingojer3,5, A. Bassett1,3, S. Patel1,3, P. Zalmas3, E. Petsalaki1,2* ,M.J. Garnett1,3*

1Wellcome Sanger Institute, Wellcome Genome Campus, UK; 2European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, UK; 3Open Targets, Wellcome Genome Campus, UK; 4GlaxoSmithKline, Stevenage, UK; 5Sanofi, Reading, UK.

Monotherapy remains the standard of care in oncology and has significantly improved patient survival. However, many tumors eventually develop resistance during treatment, often due to factors such as intrinsic tumour heterogeneity or escape mutations. One strategy to overcome this resistance is combinatorial therapy, i.e. the simultaneous use of multiple agents with different targets. This approach not only has the potential to enhance treatment efficacy but can also allow for lower drug dosages, making previously intolerable drugs viable as well as minimising side effects. However, identifying effective combinations is challenging due to the combinatorics of experiments required to test multiple target pairings across diverse conditions or cell types.We conducted a high-throughput combinatorial screen using a CRISPR-based dual-guide anchor-library strategy (Burgold et al., 2024). This involved constructing a library comprising 40 anchor genes and 1,600 library genes (~ 64,000 pairs), which we screened in cell lines from two cancer types with significant unmet clinical needs: KRAS-mutant colorectal cancer (CRC) and triple-negative breast cancer (TNBC). To assess synergistic interactions, we applied established methods such as HSA and Delta, alongside Valinor, our newly developed Bayesian-based analysis tool.Our initial analysis shows that we capture context dependent synergies for multiple anchor genes, as well as the effects of known and expected paralog pairs observed in other studies. We integrate cell type specific data such as gene expression to understand context and use XGBoost to discover biomarkers for response to dual gene knock-out. Despite the dataset having a strong bias towards cancer genes, we still find significantly enriched pathways (e.g. EGFR or ErbB associated) in the observed synergies.In summary, we expect that our high throughput double knockout experiments will generate novel insights into the molecular signalling pathways as well as their interplay in cancer cells and thereby accelerate the development of combinatorial therapy in oncology.

#14

Anticipating therapy resistance via targeted base editor screening

Daniele Migliorati (1)*, Nicole Cona (1)*, Vincenza Vigorito (1), Alice Rossi (2), Sara Buscarini (2), Dorothea Maneta (3), Chiara Becht (3), Nicola Perotti (1), Giovanni Novi Inverardi (4), Gianluca Lattanzi (4), Alessandro Provenzani (1), Alberto Ciccia (5), Manuel Kaulich (3), Ana Casañal (2), Luca L. Fava (1)
(1) Department of Cellular, Computational and Integrative Biology-CIBIO, University of Trento, Trento, Italy, (2) Fondazione Human Technopole, Milan, Italy, (3) Goethe University Frankfurt, University Hospital, Institute of Biochemistry II, Frankfurt am Main, Germany, (4) Department of Physics, University of Trento, Trento, Italy, (5) Department of Genetics and Development, Columbia University Irving Medical Center, New York, USA

Targeting RNA modifications has recently emerged as a promising therapeutic strategy in cancer. However, as with other targeted approaches, the clinical efficacy of these compounds may be undermined by the evolution of drug resistance. Anticipating how cancer cells escape inhibition is therefore essential for the successful development of this new class of therapies.Here, we sought to identify genetic alterations that confer resistance to small-molecule inhibitors of RNA-modifying enzymes, with the goal of informing strategies for more durable treatments. Using a base editor screen under selective pressure from multiple inhibitors, we introduced single-nucleotide variants across the coding sequence of the target gene. Candidate resistance mutations were then validated by individually testing top-scoring guide RNAs for their impact on drug sensitivity.The screen revealed several enriched edits clustering within the catalytic region of the enzyme. Functional assays confirmed that these alterations reduced the activity of some inhibitors, while others retained substantial potency, highlighting distinct modes of target engagement.Together, these findings demonstrate that mutations in the catalytic domain of RNA modification enzymes can limit inhibitor efficacy but also underscore opportunities to develop next-generation compounds resilient to resistance. This work provides a framework to anticipate and counteract resistance mechanisms, advancing the clinical potential of RNA modification–based therapies.

#15

Exploring the functional impact of lncRNAs through CRISPRi screens in CRC models with KRAS and BRAF mutational background

Mihaljevic A. (1,2), Laleci E. (1,2), Davalan T. N. (1,2) Guillen-Ramirez H. A.(3), Chouvardas P. (1,4), Esposito R. (1,2)
"(1) Department for BioMedical Research, University of Bern, 3008 Bern, Switzerland.(2) Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland. (3) Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland. (4) Department for BioMedical Research, Urology Research Laboratory, University of Bern, 3008 Bern, Switzerland."

Long non-coding RNAs (lncRNAs), with their oncogenic roles and cell type-specific expression, are promising targets for precision therapies. Efficiently targeting BRAF- and KRAS-mutated colorectal cancer (CRC) remains a major challenge. We hypothesize that unexplored lncRNAs downstream of these driver genes could offer novel therapeutic avenues. To uncover such targets, we developed a screening strategy integrating scRNA-seq and CRISPRi in 2D and 3D CRC models.To build our lncRNA library, we developed a custom bioinformatic pipeline to analyze scRNA-seq data from 29 CRC patients, identifying lncRNAs enriched in epithelial cells, the primary origin of CRC. We curated a list of 996 epithelial lncRNAs (1963 transcripts), from which 523 genes (1026 transcripts) were selected for CRISPRi experiments, each targeted by 7 distinct sgRNAs. The library also includes 186 positive controls and 500 neutral controls, totaling 6545 sgRNAs.We established multiple CRC cell lines stably expressing the ZIM3-dCas9-KRAB fusion protein, inducing robust knockdown without causing non-specific effects on cell viability and gene expression4. Screening was conducted in a pooled format across both 2D and 3D settings in eight cell lines, including four KRAS-mutant and four BRAF-mutant models. This represents the most extensive screen performed to date in this context. Preliminary analysis, based on the depletion or enrichment of sgRNAs between the baseline (T0) and the screening endpoint, reveals several consistent oncogenic lncRNA hits, with a comparable number of hits observed in both 2D and 3D settings. Furthermore, our preliminary results indicate that lncRNAs with tumor-suppressor functions predominantly emerged as hits in the 3D screens, underscoring their suitability for identifying such regulators. Promising lncRNA candidates are being validated in CRISPR-engineered patient-derived organoids (PDOs), with the potential to uncover novel therapeutic targets for next-generation CRC treatments.

#16

Deep interrogation of multiomics data for discovery of cancer 'Achilles Heels' and context-specific gene function, towards new clinical tools

Adeline McKie* (1), Mark Wappett (2), Madhulika Verma (1), Hans vanDierendonck (3), Lisa Crawford (1), Ian Overton (1)
(1) Patrick G Johnston Centre for Cancer Research, Queens University Belfast, UK(2) Almac Discovery, Belfast, UK (3) School of Electronics, Electrical Engineering and Computer Science, Queens University Belfast, UK

We developed SynLeGG (www.overton-lab.uk/synlegg, NAR 2021;49:W613) and the MultiSEp R package (manuscript in preparation) to interrogate mutually exclusive loss signatures in multiomics data, towards context-specific synthetic lethal drug targets and companion diagnostic biomarkers. MultiSEp takes a reasoned approach, grounded in biostatistical first principles to reveal gene dependency relationships; unsupervised assignment of samples into clusters is defined by regularized Gaussian Mixture Modelling of 'omics data and depletion patterns characteristic of Synthetic Lethality (SL) are assessed over all pairwise gene combinations. Accordingly, MultiSEp does not require training, avoiding bias; thus is suitable for prediction of SL with non-coding genes and poorly characterised or orphan proteins, illuminating the 'dark genome' and predicting novel targets for drug discovery. MultiSEp performs well in benchmarking on multiple established SL datasets including tissue-specific and 'context-independent' SL gene pairs, taking input data from several different functional genomics technologies. Application of MultiSEp to data from Multiple Myeloma (MM) patients and cytogenetic subtypes reveals context-specific SL networks that inform fundamental biology, for example around the Ubiquitin-Proteasome System and for poorly characterised genes. Almost all MM patients relapse and succumb to therapy-resistant disease; accordingly, more effective treatments are urgently needed. Analysis of our predicted MM patient SL networks reveals individual 'nexus' genes where the SL network neighbourhood genes are collectively mutated in a relatively high proportion of MM cohorts, representing attractive drug targets. Laboratory follow-up of a novel candidate drug target further supports the value of our approach.

#17

Detecting potential novel targets in retroperitoneal liposarcomas

Stefano Percio, Noemi Arrighetti, Alessia Beretta, Valentina Zuco, Claudia Aurelio, Soffientini Cesare, Elena Di Blasi, Marta Barisella, Paola Collini, Giampaolo Dagrada, Silvia Brich, Alessia Bertolotti, Elena Catalani, Loris De Cecco, Chiara Colombo, Silvia Stacchiotti, Roberta Sanfilippo, Alessandro Gronchi, Nadia Zaffaroni, Sandro Pasquali
Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italia

Well-differentiated liposarcoma (WDLPS) is a low-grade, locally recurrent tumor, whereas dedifferentiated liposarcoma (DDLPS) emerges as an aggressive progression of WDLPS, often associated with metastases. Current liposarcoma-specific therapeutic options remain limited. We applied an integrative transcriptomic approach to investigate the molecular underpinnings of WDLPS and DDLPS and select functional drivers of progression, identifying novel biomarkers. By analyzing matched samples of normal adipose and tumor tissue components, we characterized key alterations in gene expression, signaling pathways, and immune profiles. Beyond the shared MDM2 and CDK4 amplifications, DDLPS exhibited secondary dependencies that include dysregulation of cell cycle control, lipid metabolism, and immune-related processes; specifically, gene set enrichment analyses highlighted upregulation of E2F targets, epithelial to mesenchymal transition, and G2M checkpoint pathways. In addition, several candidate targets were linked to mitotic spindle assembly and DNA damage response. Finally, deconvolution method and immunohistochemistry validation revealed that immune infiltration, particularly of T cells, although present are associated to gene signatures of immune evasion. To explore therapeutic implications, we performed drug-target interaction analysis, identifying candidate compounds acting on oncogenic DDLPS drivers (MDM2, CDK4, HMGA2) and the selected secondary dependencies (CHK1, AURKA, EZH2). To evaluate the efficacy, selected single-agent treatments were tested in in-house generated patient-derived cell lines (N=3) and patient-derived xenograft (N=2) models that recapitulate the clinical tumor of origin and, at least in part, the heterogeneity of DDLPS. Transcriptomic profiling post-treatment was conducted to assess drug-induced molecular changes and potential resistance mechanisms. For instance, MDM2 inhibition resulted in different effects in the two analyzed models (median tumor volume inhibition, mTVI% 83% vs. 35%) while CHK1 let to opposite effect (mTVI% 50% vs. 93%). In conclusion, this work provides a comprehensive molecular framework that identifies secondary dependencies, beyond the oncogenic driver MDM2 and CDK4, that represent new therapeutic vulnerabilities in DDLPS. Validation in patient-derived models suggest different level of resistance to these targets and allow investigations on mechanisms of resistance, that may shed lights on potential combinations to improve therapeutic effectiveness.

#18

Dependency-Shock transcriptomic footprints as predictive tools in early anti- cancer drug discovery

Ludovica Proietti (1)*, Gianluca Vozza(1)*, Ottavio Croci(1), Vanessa Spagnolo(1) , Athanasios Oikonomou(1), Nevenka Radic(1), Gianluca Frera(1), Mathew Garnett(2) , Francesco Iorio(1)
(1)Human Technopole, Computational Biology Research Centre, Milan, Italy(2)Wellcome Sanger Institute, Cambridge, United Kingdom

The oncogenic shock model describes the interplay between pro-survival and pro-apoptotic signals that follow an oncogene's inactivation and lead to cancer cell death. Building on this model, we call cancer dependency shock (DepSHOCK) the signal dynamics' interplay that follows the ablation of any gene essential for cancer survival, i.e., cancer dependency gene. Any DepSHOCK triggers the sudden inactivation of pro-survival pathways and activates death-inducing signals, with both events impacting down-streaming transcription factors, leaving a measurable gene expression footprint. Each DepSHOCK footprint can be deconvoluted to infer transcriptional markers of signals that emanate from a dependency gene and drive cancer cell survival through the constitutive activity of specific biological pathways. Our goal is to investigate this hypothesis across tissues, oncogenic addictions, and novel candidate cancer therapeutic targets. We established an experimental/computational platform for the generation and the analysis of transcriptional data generated upon genetic perturbation of cancer cell lines and therapeutically relevant dependency genes. We generated a post-perturbational dataset profiling 10 cancer cell lines with 9 drugs, such that each drug targets at least a cell line-specific cancer driver gene. We apply a variety of unsupervised machine learning methods to this data to identify genetic signatures that can be used to sense the presence of specific pro-survival signals, thus drug sensitivity and genetic dependencies in independent cancer samples. Our preliminary results show that the basal expression of specific dependency-shock signatures is predictive of drug response and genetic dependency in independent cancer samples comparably to pathway/gene-addiction signatures derived with much more complex supervised machine learning methods applied to much larger datasets of clinical and multiomic datasets. Our main goal is to study the information content of the DepSHOCK footprints and their potential use as computational tools in early anti-cancer drug discovery through new statistical and machine-learning methods delivering mechanistically grounded therapeutic markers.

#19

Mapping Genetic Dependencies Of Drug-Resistant Cancer Cells For The Rational Design Of Combinatorial Treatment

Nevenka Radic* (1), Ottavio Croci (1), Katia Grillone (2), Pierfrancesco Tassone (2), Francesco Iorio (1)
(1) Human Technopole, (2) University Magna Graecia of Catanzaro

Chemotherapy is a primary treatment for various oncogenic malignancies; however, its efficacy is frequently hampered by acquired drug resistance of the tumour. Uncovering dependencies of drug resistance mechanisms that can serve as drug targets to overcome them is crucial for improving therapy efficiency. We are developing a Cancer Drug Sensitivity Recovery Map – a comprehensive resource aimed at identifying cancer cell dependencies that can circumvent, or reverse acquired resistance to conventional chemotherapy drugs. Our experimental pipeline leverages genome-wide CRISPR/Cas9 knockout screens in parental and drug-adapted cell lines to uncover context specific cancer cell dependencies. As a proof of concept, we performed a CRISPR/Cas9 knockout screen in the multiple myeloma AMO-1 cell line and its ABZB clone resistant to bortezomib, a proteasome inhibitor crucial in the treatment of multiple myeloma. We also characterised the transcriptional landscape of these cell lines to further understand the underlying biology. We uncovered genetic targets unique to drug resistant cell lines. Specifically, we identified molecular players in the NF-kB pathway as having an important role in the bortezomib resistance of the AMO-1 cell line. These findings were supported by transcriptional differences observed between the bortezomib-resistant and parental cell lines, further validating our approach. Our work highlights the capability of the Drug Sensitivity Recovery Map to elucidate context-specific dependencies, thus offering promising avenues for better therapy targets. Future integration with publicly available datasets will foster a more holistic understanding of therapy resistance and strategies for overcoming it.

#20

High resolution evaluation of the N6-methyladenosine RNA modification pathway as a driver of tumor proliferation and drug resistance

Martina Scichilone, Paride Pelucchi PhD, Cinzia Cocola PhD, Arianna Consiglio PhD, Alessandro Orro PhD, Maria Ilaria Curci, Tommaso Selmi PhD
(1) Università degli Studi di Milano, (2) Consiglio Nazionale delle Ricerche, Istituto di Tecnologie Biomediche, IT

The methylation of RNA adenosines into N6-methyladenosine (m6A) is the most abundant internal mRNA modification in mammalian cells. It is regulated by writers (e.g., methyltransferase-like 3 (METTL3)) and erasers, while readers mediate its molecular effects at the level of different biological processes (e.g., RNA translation and decay). Consequently, m6A affects multiple cellular functions like cell proliferation. For instance, downregulation of METTL3 affects Acute Myeloid Leukaemia (AML) and Colorectal Cancer (CRC) cell proliferation, and a small molecule inhibitor of METTL3 is now on phase I clinical trial. This opens new questions regarding the mechanisms by which all the m6A effectors contribute to the function of m6A in cancer and in METTL3i resistance.The aim of this project is to study m6A in cancer cell proliferation and drug resistance by means of a pooled CRISPR-cytosine base editing (CBE) screening.We developed a CRISPR-base editing platform for the high-density mutagenesis (C>T) of the genes that are active in the m6A-pathway to determine the association between specific single-nucleotide mutations and the proliferation phenotype, either in presence or absence of METTL3i (STM2457).In details, we designed a sgRNA library targeting m6A and control genes (essential and non-essential genes, non-targeting and intergenic sequences) in CRC and AML cell lines.We generated cell lines stably expressing CBE or Cas9, which are currently being used for the proliferation screens. Following proliferative challenge (METTL2i/DMSO), NGS of sgRNAs at T1 vs T0 will identify significantly depleted/enriched sgRNAs. The data analysis will highlight 1) domains associated with proliferation phenotype, that might represent druggable sites and 2) m6A gene mutations associated with METTL3i resistance. The study will investigate the role of m6A writers, erasers and readers in CRC and AML proliferation and drug resistance, and expect to provide molecular insights for the development of novel treatments.

#21

Histone deacetylase activity represents a cancer dependency limiting response to EZH2 inhibition-based therapy in epithelioid sarcoma

Cesare Soffientini* (1), Noemi Arrighetti (1), Valentina Zuco (1), Loredana Cleris (1), Luca Sigalotti (2), Roberta Maestro (2), Stefano Percio (1), Marta Barisella (3), Paola Collini (1), Gianpaolo Dagrada (1), Anna Maria Frezza (1), Alessandro Gronchi (1), Nadia Zaffaroni (1), Silvia Stacchiotti (1), Sandro Pasquali (1)
(1) Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, (2) Centro di Riferimento Oncologico (CRO) IRCCS di Aviano, (3) ASST Fatebenefratelli Sacco

Epithelioid sarcoma (ES) is an aggressive and ultra-rare soft tissue sarcoma (STS) defined by SMARCB1 loss, which drives aberrant EZH2 activity. The EZH2 inhibitor tazemetostat is FDA-approved for advanced SMARCB1-deficient ES, but clinical benefit remains modest. Defining ES dependencies that sustain resistance to EZH2 inhibition is therefore critical to improve therapeutic outcomes.We established two ES patient-derived xenograft (PDX) models, ES-1 and ES-2, with matched cell lines, to investigate responses to tazemetostat and doxorubicin, the first-line treatment for advanced sarcomas. In ES-1, the combination stabilized tumor growth in vivo, although relapse occurred after treatment cessation. ES-2 displayed primary resistance to all regimens, providing complementary models to dissect mechanisms of limited efficacy.Transcriptomic profiling revealed divergent adaptive programs. In resistant ES-2, combined treatment activated chromatin organization pathways, including histone deacetylases (HDACs). Protein analyses showed that H3K27 acetylation, a transcription-associated histone mark, was restored only in ES-1, suggesting that HDAC activity counteracts the chromatin remodeling induced by the combination.To directly address this, cells were exposed to tazemetostat, doxorubicin, and their combination, in the presence of the pan-HDAC inhibitor vorinostat. In ES-2, this triple blockade reinstated H3K27 acetylation, triggered apoptosis, and showed synergy. These results indicate that HDAC activity functions as a resistance mechanism limiting the efficacy of tazemetostat-doxorubicin therapy, and represents a targetable liability in SMARCB1-deficient ES.While EZH2 remains a primary vulnerability linked to SMARCB1 loss, HDAC activity emerges as a resistance-associated dependency that constrains the response to tazemetostat. Targeting HDACs may offer a rational strategy to exploit ES dependencies, enhance EZH2-based therapies and overcome drug resistance in this ultra-rare sarcoma.

#22

Dissecting Cancer Dependencies and Therapy Resistance through CRISPR Functional Genomics

Vanessa Spagnolo(1)*, Athanasios Oikonomou(1)*, Ludovica Proietti(1)*, Nevenka Radic(1), Aurora Savino(1), Lidia Avalle(2), Andrea Lobascio(2), Jahnavi Srivasta(2), Gianluca Vozza(1), Ottavio Croci(1), Valeria Poli(2), Francesco Iorio(1)
1 Computational Biology Research Centre, Human Technopole, Milan, Italy
2 Department of Molecular Biology, University of Turin, Italy

Background: Cancer cells can rely on oncogenic drivers or non-oncogenic adaptations for survival. Perturbation of oncogenes typically triggers an "oncogenic shock" in the respective cancer cell line, disrupting survival pathways and leading to cell death in most cells. However, compensatory pro-survival signals can arise, leading to therapy resistance. In cancers lacking strict oncogene addiction, chemotherapy remains the standard treatment, yet resistance frequently arises, especially through microenvironmental influences such as cancer-associated fibroblast (CAF)-derived signalling.Aim: Projects I am involved in investigate the molecular consequences of genetic perturbations in oncogene-addicted models and genetic plus pharmacological perturbations in non-oncogene addicted cancer models in which CAF-conditioned medium alters chemotherapy responses. By mapping the interplay between pro-survival and pro-apoptotic signals, we aim to identify vulnerabilities and resistance mechanisms exploitable for therapy design.Methods: We are generating post-perturbation datasets from panels of cancer cell lines. In oncogene-addicted models, perturbations are induced via CRISPR/Cas9 targeting key driver genes, without drug treatment, followed by bulk RNA-seq at multiple time points. In non-oncogene addicted breast cancer models, cells are exposed to chemotherapies under standard or CAF-conditioned culture Medium. CRISPR screening under these contexts will allow us to pinpoint genetic drivers of CAF-mediated resistance. Results: We established and validated some stable Cas9-expressing cancer cell lines for our dependency studies. In our breast cancer models, we confirmed that chemotherapeutic efficacy observed under standard culture is reversed when cells are grown in CAF-conditioned medium, establishing a robust platform for resistance-focused CRISPR screening.Conclusion: Our experimental framework investigates oncogene dependency mapping and microenvironment-modulated resistance models, extending CRISPR functional genomics beyond viability readouts. These efforts will uncover conserved and context-specific vulnerabilities and contribute to the development of biomarker-guided therapeutic strategies.

#23

Nuclear mRNA processing masks CRISPRko phenotypes

Sebastian Süsser* (1), Chiara Becht (1), Thomas Rohde (2), Martin Wegner (1, 12), Florian Heigwer (3, 4), Gabriel M. C. Longo (5), Alessandro Vinceti (6), Sweta Talyan (7), Siu Wang Ng (3, 8), Ronay Cetin (1), Antonella Vera Guapi (1), Koraljka Husnjak (1), Francesco Iorio (6), Vassilis Roukos (5, 9), Michael Boutros (3, 8), Mario Looso (7), Maximilian Billmann (2), Manuel Kaulich (1,10,11,#)

"(1) Institute of Biochemistry II, Goethe University Frankfurt am Main, Faculty of Medicine, Frankfurt am Main, Germany,(2) Institute of Human Genetics, University of Bonn, School of Medicine and University Hospital Bonn, Bonn, Germany,(3) Division of Signaling and Functional Genomics, German Cancer Research Center (DKFZ) and Heidelberg University, Heidelberg, Germany,(4) University of Applied Sciences Bingen, Bingen, Germany,(5) Institute of Molecular Biology (IMB), Mainz, Germany,(6) Human Technopole, Milano, Italy,(7) Max-Planck Institute for Heart and Lung Research, Bad Nauheim, Germany, (8) Institute for Human Genetics, Medical Faculty Heidelberg, Heidelberg University, Heidelberg, Germany,(9) Department of Biology, Medical School, University of Patras, Patras, Greece,(10) Frankfurt Cancer Institute, Frankfurt am Main, Germany,(11) Cardio-Pulmonary Institute, Frankfurt am Main, Germany,(12) Present address: Vivlion GmbH, Frankfurt am Main, Germany,# correspondence: kaulich@em.uni-frankfurt.de"

Genome-wide CRISPR screens have revolutionized the study of genetic interactions, but their scale limits the feasibility of combinatorial approaches. Here, we show that leveraging phenotype-consistent guide RNAs (PCGs) enhances signal-to-noise, boosts confidence in single and combinatorial phenotypes, and enables efficient query-by-genome combinatorial CRISPR knockout (CRISPRko) screens in human cells. Using this approach in non-tumorigenic cells, we uncover a dependency on mRNA processing pathways that emerges specifically under combinatorial perturbation and correlates with the strength of the query gene's main effect. Extending this strategy, we identify over 3,000 genes whose cellular fitness depends on RNA processing capacity, dramatically expanding the known landscape of context-dependent gene essentiality. Mechanistically, our results support the existence of a transcriptome surveillance checkpoint (TSC), comprising the nuclear RNA exosome, minor spliceosome, and nuclear pore complex, that senses and responds to perturbation-induced RNA stress to preserve cellular homeostasis. Together, our work suggests the presence of a genetic buffering network that safeguards cellular viability.

#24

Cracking NFYA dependency: genome-scale screens reveal resistance and vulnerability mechanisms

Camilla Vitali*(1), Giacomo Graziano (1), Alessia Canevotti (1), Giulia Pagani (1), Paolo Gandellini (1)
(1) Department of Biosciences, University of Milan, 20133 Milan, Italy

Oncogene addiction, the dependency of tumors on specific genes, extends to transcriptional vulnerabilities, highlighting transcription factors as promising cancer targets. NFYA, the regulatory subunit of the NFY heterotrimeric complex, is frequently overexpressed in cancers and associated with poor prognosis. Despite its oncogenic role, NFYA therapeutic potential remains unexplored.To assess NFYA addiction, we analyzed genome-scale CRISPR and RNAi loss-of-function screens (DepMap) across over 1,000 cancer cell lines using a combined scoring strategy (60% CRISPR, 40% RNAi). Approximately 30% of cell lines were selectively dependent on NFYA. Compared to other NFY subunits, NFYA displayed lower overall essentiality but greater selectivity, suggesting a favorable therapeutic window. NFYA dependency did not correlate with NFYA mRNA expression, protein levels, or copy number, indicating specific molecular programs driving sensitivity and resistance.To identify determinants of NFYA dependency, we examined genes whose expression correlates with the combined score. Resistance genes, whose higher expression is linked to lower dependency, are enriched in interferon response pathways. Vulnerability genes, whose higher expression associates with higher dependency, are involved in estrogen response pathways. We derived a single score based on determinants' expression to predict NFYA dependency in non-screened cell lines and patient tissues. Fibroblasts ranked among the least dependent, suggesting lower sensitivity of non-neoplastic cells. In TCGA, tumors had lower scores than matched normal tissues, supporting NFYA inhibition as a low-toxicity therapeutic strategy. The single score also correlated with immune infiltration, indicating immune-cold tumors may benefit from NFYA targeting. Thus, NFYA represents a selective vulnerability in tumors with high estrogen response and low immune infiltration. The search for compounds that downregulate resistance signatures may expand the use of NFYA-targeted therapy also to resistant cancers.Our findings define key determinants of NFYA sensitivity and resistance, providing a framework for predictive biomarkers and combination strategies for NFYA-targeted therapy.

#25

The Power of Perturbation: Accelerating discovery with CRISPR screening

Elena Vialetto (1), Sebastian Süsser (2), Ronay Cetin(1,2), Angela Hinchie(1), Jessica Martyn(1), Martin Wegner* (1) & Manuel Kaulich (1,2)
1 Vivlion GmbH 2 Institute of Biochemistry II, Faculty of Medicine, Goethe University Frankfurt

At Vivlion, we continually strive to enhance CRISPR library and screening solutions. We introduce Alexandria, our flagship genome-wide PRCISR™ CRISPR knockout library, available in single-targeting and fixed-pair formats. The fixed-pair design delivers two sgRNAs per cell for the same gene (or predefined gene pairs), boosting editing efficiency and enabling combinatorial strategies. Alexandria broadens discovery by including ~1,800 newly annotated genes overlooked by previous reagents and covers 20,381 genes (~99.8% of ENSEMBL) with 4 sgRNAs per gene (2 pairs) to support confident hit calling across cell types and states. All PRCISR™ libraries are produced with Vivlion's 3Cs technology, which utilizes the oligo pool directly and avoids PCR bias and cloning artefacts—yielding highly uniform gRNA distributions that preserve representation even in down-scaled screens (e.g., limited primary or iPSC material). Building on this, our library formats decouple sequence diversity from distribution, so users can deploy single-targeting, fixed-pair, or multiplex designs as their biology demands. In practice, Alexandria enables researchers to identify context-dependent dependencies, explore pathway mechanisms, paralog buffering, and prioritise actionable targets that standard screens may miss. To streamline execution, Vivlion offers pooled and optical pooled screening, bioinformatics support, leveraging ReCo, our automated NGS read-counting pipeline for single and combinatorial CRISPR data that simplifies downstream analysis. By combining advanced CRISPR screening with uniform, low-coverage reagents like Alexandria, researchers can accelerate discovery across disease and non-disease contexts alike.