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Contents

Keynote Speakers

Predicting variant pathogenicity by combining protein language models and biological features	1
Harnessing real and artificial intelligence in patient-centric medicine	2
Integrating strain-level metagenomics in human and food microbiomes	3
Bioinformatics and proteomic studies of biomacromolecules	4

Invited Speakers

Dissecting the transcriptomic basis of behavioural differences between male morphs of ruffs (<i>Calidris pugnax</i>)	5
Identifying regulatory RNA networks behind therapy-induced senescence (TIS) in breast cancer cells	6
AI-driven bioinformatics: advancing chronic disease management and aging assessment	7
Zero-shot prediction of thermodynamic properties of proteins	8
Nextflow and nf-core: a virtual infrastructure for the next generation of bioinformatics pipelines	9
Rethinking protein complex discovery from network topology to dynamic graph learning	10
Towards causal and interpretable virtual cells	11
Noninvasive biomarker discovery by metabolomics of fingertip sweat to guide individualized anti-PD-1 immunotherapy in melanoma	12
From fatty liver to scar: Exploring the rewiring of gene networks across the human chronic liver disease continuum.	13
Exploiting the linearity of joint embedding spaces to mine cancer precision medicine knowledge	14
AI/ML in the search for novel antivirals	15
Context-dependent remodeling of aging-related and neurodevelopmental pathways in human cerebral organoids across APOE genotypes	16
The role of local energetic frustration across conformational ensembles of disordered proteins	17
Predictive modeling of B- and T-cell epitopes in allergen discovery and immunotherapy design	18
Genetic and phenotypic heterogeneity of type 2 diabetes across Russian ancestry groups	19

A universal toolkit for single-cell and spatial long-read transcriptomic sequencing data	20
Unraveling the molecular effects of nanoplastics on human blood cells using microfluidic chip-based single-cell RNA sequencing	21
Integration and standardization of gene–disease associations in ProDiGenIDB	22
Multi-omics integration approaches for addressing cardio-renal-metabolic multimorbidity	23
Unlocking complex diseases through federated data governance, FAIR multi-modal data, and AI	24
Gut microbiota profiling as a tool for predicting and enhancing immunotherapy response	25
Resolving variants of uncertain significance at scale with calibrated computational and experimental evidence	26
Monitoring heart health at home: Health informatics challenges	27
Cyberbiosecurity threats in the time of AI: Digital vulnerabilities with biological consequences	28

Oral Presentations

Metaplasia as a transitional cell-of-origin state revealed by epigenome-informed cancer prediction	29
EffRank: a bioinformatics scoring pipeline for prioritizing novel type III secreted effectors in the <i>Pseudomonas syringae</i> species complex	30
An integrated single-nucleus landscape of cardiovascular disease	31
Omics-driven agent-based modelling of hepatocellular carcinoma tumours	32
Detection of mosaic copy number alterations in PTA-amplified single cells	33
Structured generative AI for interpretable analysis of complex biomedical data	34
Federated learning in Healthcare across Europe: Challenges and Insights from the BETTER Project	35
A pan-tissue multi-omics framework for aging analysis via non-negative matrix tri-factorization	36
Targeting resistance and survival mechanisms in <i>Pseudomonas aeruginosa</i> using data fusion and machine learning	37
Deeply phenotyped and multi-omics data integration with the Human Phenotype Project	38
Cell-type specific expression and structural features of transcriptional isoform LDLRAD4-217 in rectal cancer	39
Permafrost-preserved Late Pleistocene wolf from Yakutia: palaeogenomic and metagenomic perspectives	40
Pathogenic variations illuminate functional constraints in intrinsically disordered proteins	41
Integrated transcriptomic analysis reveals coordinated network reprogramming and p53 pathway inactivation in temozolomide-resistant glioblastoma A172 cell line	42
Understanding the interplay between positive and negative selection in the human genome	43
A Bayesian framework for quantifying allele count evidence for variant pathogenicity	44

A statistical perspective on the Last Universal Common Ancestor (LUCA)	45
Predicting drug synergy under realistic scenarios	46
Exploring the role of the ZBTB14 transcription factor in U937 macrophages	47
Mechanistic insights into ageing biology from single-cell omics	48
Generalized discovery of chromatin structural patterns across developmental and evolutionary scales	49
CardioSafe: Multi-task prediction of cardiac ion channel activity with reverse-leak audited benchmarking	50
Time- and cost-efficient parameter optimization for 3D bioprinting of composite bioinks via active machine learning with Gaussian Processes	51
CTCF transcription factor as a potential therapeutic target in osteosarcoma	52
Meta-analysis of oral shotgun metagenomes for prediction of caries and periodontitis . .	53
Genetic architecture and AI-assisted phenotyping of alopecia in a large Russian genomics cohort	54
Improving spatial transcriptomics data processing and annotation under suboptimal image-based cell segmentation	55
Study of circulating blood linear RNA nominates CD55 and DLD as novel causal genes and early-stage biomarkers for Parkinson's disease	56
IsoChecker: multisample analysis and interactive browsing of long-read transcript isoforms	57

Flash Talks

Rare variant pharmacogenomic analysis identifies pathways associated with vedolizumab response in Crohn's disease patients	58
Confidence-guided lightweight CNN ensemble for OrganMNIST classification	59
Predicting interaction-specific protein–protein interaction perturbations by missense variants with MutPred-PPI	60
CellAnalyst: delivering single-cell transcriptomic insights from bioinformatics analysts to experimental biologists	61
Integrative multi-layer bioinformatics of transcriptomic data for elucidating molecular mechanisms of action of polyphenol-rich extract on cardiometabolic health	62
Identification and functional characterization of tumor-suppressive microRNAs with potential key roles in breast cancer progression and metastasis	63
Diet reconstruction of a woolly mammoth calf using multiple metagenomic approaches .	64
A multi-omic digital twin network reveals C3-glycosylation as an actionable driver of latent metaflammation	65
A compact genotype sketch for cross-platform sample matching	66
Analysis of total transcriptome from brain tissue of progressive multiple sclerosis patients identifies ferroptosis-related genes relevant for distinction of inactive from chronic active white mater lesions	67
RefusalBench: Why refusal rate misranks frontier LLMs on biological research prompts .	68

Cross-workflow signal reporting in pan-viral metagenomics	69
Temporal dynamics of codon usage in SARS-CoV-2 proteins	70
Cobalt chloride affects neural precursor cell differentiation: A transcriptional study . . .	71
Predicting nephrotoxicity from cell morphological perturbations using high content image-based profiling	72
Cytokine profiles as promising predictive biomarkers for glucocorticoid therapy response in inflammatory bowel disease	73

Poster Presentations

Transcriptomic reanalysis reveals membrane-associated pathways involved in fullereneol C60(OH)24 cytotoxicity	74
Long-range chromatin interaction dynamics and molecular aging signatures in schizophrenia	75
AI-driven concordance engines as a decision-support layer for veterinary import compliance	76
Comparative evaluation of the regenerative efficacy of <i>Lucilia sericata</i> larval secretions across varied storage conditions.	77
Assessing the predictive power of intrinsic disorder and sequence features for plant stress response	78
Hierarchical deep learning for tandem repeat proteins classification	79
Multi-resolution framework for orthology and regulatory region delineation in divergent grass genomes	80
Sex-specific distribution of EGFR rs712829 and rs712830 polymorphisms in East Asian, European and Serbian populations	81
Regulatory landscape of TGF β pathway in gastrointestinal tumors	82
Spatial organization of gene expression in 3-day old zebrafish larvae	83
Towards protein complex identification in dynamic PPI networks using embeddings . . .	84
Bioinformatics identification of extracellular vesicle - derived microRNAs biomarkers in pancreatic cancer	85
Trehalose synthesis is induced in maize roots under low-temperature stress	86
Molecular docking approach in prediction of binding between selected bioactive phenolic compounds and human serum albumin	87
Double-stranded RNA sequencing and bioinformatic analysis reveal viruses in <i>Verticillium dahliae</i>	88
Integrated analysis reveals bias in single-tool miRNA profiling during grapevine viral coinfection	89
Prothrombin reshapes the temporal transcriptomic trajectory of Caco-2 colorectal cancer cells	90
DigestedProteinDB: A compact key-value database for in silico peptide digestion and mass-based search	91
PromoterLab: browser-based application for human promoter design, visualization and sequence analysis	92

Development and validation of a reproducible RNA-seq pipeline for transcriptomic analysis	93
Dynamics in soil microbial community structure under the influence of heavy metal contamination and phytoremediation: a pot trial experiment	94
Precision and recall in rare disease genomics: benchmarking the IMGGE whole genome sequencing pipeline	95
Implementation and clinical utility of the GATK gCNV pipeline for rare disease diagnostics using whole exome sequencing	96
FluxInside: A high-performance Flux Balance Analysis engine for spatial agent-based models.	97
Transcriptomic analysis reveals suppression of the filamentation-associated virulence program in <i>Candida albicans</i>	98
Contribution of ILCs to the development of different forms of multiple sclerosis	99
Assessment of coumarin derivatives potential as cyclooxygenase-2 inhibitors	100
Benchmarking of pre-processing steps in Sarek pipeline on MGI sequenced standard human genome	101
Classification of coronavirus types based on repeats derived from amino acid and nucleotide sequences	102
Integrated chemical and metagenomic profiling of urban wastewater: antibiotic residues, microbial community and resistome at a Belgrade collector point	103
Identification of shared molecular programs and putative role of <i>ankrd1a</i> in zebrafish heart regeneration and skeletal muscle repair using WGCNA	104
In silico approach to proteomic identification of biologically active proteins in an insect non-model species, <i>Ostrinia nubilalis</i>	105
IntelliLung: A human-in-the-loop AI-DSS for ICU ventilation settings	106
<i>Pilosibacter</i> species emerge as leading contributors to glutarate pathway-mediated butyrate synthesis in the human gut	107
Comprehensive genomic profiling of advanced Non-small Cell Lung Cancer in Serbia: Implications for targeted therapy selection	108
Sequence diversity and genomic relatedness of blaVIM-positive clinical <i>Proteus mirabilis</i> isolates	109
Spatial transcriptomic profiling of thrombin-PAR-fibrinogen axis in colorectal cancer	110
Genome-wide analysis of human respiratory adenoviruses in Russia reveals extensive diversity and recombination	111
Coordination geometry-driven selectivity toward tumor-associated carbonic anhydrases in novel Zn(II) dithiocarbamate complexes	112
Development of the amplification strategy and NGS data processing pipeline for HIV-1 genome assembly and resistance genotyping	113
Microbial composition of peloids from Banja Koviljača	114

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Bioinformatics identification of extracellular vesicle - derived miRNAs biomarkers in pancreatic cancer

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Pancreatic cancer is one of the most lethal malignancies worldwide due to the lack of effective early diagnostic tools and its asymptomatic early stages. It is the 6th leading cause of cancer-related mortality, with a 5-year survival rate ranging from 44% in localized disease to 3% in metastatic cases. CA19-9, the most commonly used biomarker, has limited sensitivity and low positive predictive value, restricting its use in screening. Therefore, novel biomarkers are needed, and extracellular vesicle-associated miRNAs (EV-miRNAs) are increasingly recognized for their stability and potential in non-invasive cancer diagnosis. In this study, we performed an *in silico* analysis of plasma EV-miRNA expression profiles using the GEO dataset GSE221185 (BioProject: PRJNA913165), including 16 pancreatic cancer patients and 13 healthy controls. Seventeen differentially expressed miRNAs (5 downregulated and 12 upregulated) were identified, of which nine candidates were prioritized based on network topology metrics, including the number of single-line regulators (NSR) and transcription factor ratio (TFR) ($p < 0.05$). Further refinement using dbDEMOC 2.0 meta-profiling identified five miRNAs (miR-218-5p, miR-221-3p, miR-423-3p, miR-505-3p, and miR-766-3p) exhibiting $|\log_2FC| \geq 2$. Receiver operating characteristic (ROC) analysis demonstrated that only hsa-miR-766-3p achieved statistically significant diagnostic performance (AUC = 0.750, $p = 0.028$). Network analysis using miRNet 2.0 revealed that the nine miRNAs collectively regulate 3,499 target genes. Based on degree and betweenness centrality, three regulatory clusters were identified: high-centrality hub miRNAs (miR-218-5p, miR-221-3p, miR-423-3p), intermediate regulators (miR-590-3p, miR-766-3p, miR-512-3p), and peripheral miRNAs. Functional enrichment analysis indicated involvement of key oncogenic pathways, including *PI3K-AKT*, *EGFR/MAPK*, *JAK-STAT*, cell cycle regulation, apoptosis, angiogenesis, and *TGF- β /EMT* signaling. The four final candidate miRNAs (miR-218-5p, miR-221-3p, miR-423-3p, and miR-766-3p) collectively targeted 1,922 genes, including 14 pancreatic cancer-associated genes. TargetLink2.0 confirmed validated interactions for miR-218-5p (*CDK6*, *E2F2*, *EGFR*, *IKBKB*) and miR-221-3p (*PIK3R1*). External validation using independent serum-based GEO datasets (GSE85589 and GSE59856) demonstrated significant diagnostic performance for miR-221-3p (AUC = 0.734, $p < 0.0001$) and miR-218-5p (AUC = 0.676, $p = 0.016$). Overall, miR-218-5p and miR-221-3p represent promising non-invasive diagnostic biomarkers for pancreatic cancer, warranting further experimental validation.

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Keywords:

Extracellular vesicles, microRNA, Pancreatic cancer