9th COLLOQUIUM OF GENETICS

Book of Abstracts

September 29th 2023
Piran

GENETIC SOCIETY OF SLOVENIA
IN COLLABORATION WITH THE
SLOVENIAN SOCIETY OF HUMAN GENETICS

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Špela Mikec

9:25 - 9:40 **Archaeogenetic analysis of Late Pleistocene and Holocene Bison fossils from the Slovenia and Hungary**
Lars Zver

9:40 - 9:55 **Identifying introgression between domestic (*Capra hircus*) and Alpine ibex *(C. ibex)*: a case study from Slovenian part of Alps**
Neža Pogorevc

9:55 - 10:10 **Hybridization and population genetic structure of European wildcats from Dinaric Alps and Pannonian Basin**
Felicita Urzi

10:10 - 10:25 **The conjugation frequency of the pOX38:Cm conjugative plasmid in *in vitro* models of human intestine and in an anaerobic chamber**
Aleksander Lesar

10:25 - 10:40 **A small bacteriophage protein determines the hierarchy over co-residential jumbo phage in *Bacillus thuringiensis* serovar *israelensis***
Anja Pavlin

PROGRAM

10:40 - 11:15 OPENING LECTURE

Chair: Maja Čemažar

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Andrej Sečnik

12:00 - 12:15 **Genome-wide association study and population structure of different hemp (*Cannabis sativa* L.) genotypes**
Marjeta Eržen

12:15 - 12:30 **Irradiation and gene electrotransfer of plasmid DNA encoding chemokines CCL5 and CCL17 induce immunomodulatory effects in murine tumors**
Tim Božič

12:30 - 12:45 **HPV-positive mouse model of oral squamous cell carcinoma: development and transcriptome analysis**
Živa Modic

12:45 - 13:00 **Molecular and clinical characteristics of Slovenian patients with small cell carcinoma of the ovary, hypercalcemic type**
Ana Blatnik

PROGRAM

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	Announcement of GENETIKA 2024
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OPENING AND CLOSING LECTURES

HOW A BIASED GENETIC CODE CREATES ORDER FROM PROTEINS' DISORDER

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Protein dosage is regulated to maintain cellular homeostasis and health. The dosage of proteins containing disordered low complexity domains (LCDs) must be particularly well-controlled, yet no mechanism to maintain their mutual homeostasis has been identified. Here we report a mutual homeostatic mechanism that controls the concentration of such proteins, termed 'interstasis', in which proteins with similar LCDs co-regulate their combined dosage through mRNA-mediated negative feedback. We focused on the mechanism that exploits the fundamental multivalency of GA-rich RNA regions that encode charged LCDs, including those with arginine-enriched mixed charge domains (R-MCDs). Modest variations in the abundance of an R-MCD protein change the properties of nuclear speckles, a protein-RNA condensate, selectively trapping multivalent GA-rich mRNAs to promote their nuclear retention. This interstasis depends on the inherent characteristics of the genetic code, and on codon biases that are most pronounced in amniotes, which enhance the multivalency of GA-rich regions encoding the charged LCDs. The threshold of interstasis is modulated by CLK kinases, which affect the nuclear speckle localisation of proteins such as TRA2B, key binder of GA-rich RNAs. Notably, many classes of LCDs are encoded by RNA regions containing multivalency-enhancing codon biases, each preferentially bound by specific proteins, suggesting that interstasis might co-regulate many classes of functionally related LCD-containing proteins through dose-sensitivity of various types of protein-RNA condensates.

CODING AND NON-CODING RNA IN DORMANT CELLS

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cl

Specific signals can interrupt relentless cell proliferation by inducing quiescence, a dormant cell state characterized by a reversible exit from cell cycle that can be sustained over long periods of time. Whereas quiescence is likely to have emerged early in evolution to facilitate survival of micro-organisms during periods of limitation, it has since then spread widely in phylogeny to suit specialized purposes in diverse biological contexts. Indeed, quiescence is known to play essential roles in tissue differentiation, resistance to stress, aging, and longevity of organisms, while its disruptions underlie cancer. However, understanding quiescence is limited by lack of efficient, broad-spectrum inducing conditions. In this lecture, I will discuss our recent finding that rapid depletion of two highly conserved ribozymes, RNases P and MRP, induces a dormant, quasi-quiescent state in commonly studied mammalian cells in culture. I will focus on altered processing of the most abundant coding and non-coding RNA species and how this may contribute to the induction and reversibility of cell dormancy.

ABSTRACTS OF SHORT LECTURES

GENETIC VARIATION IN POLYADENYLATION SIGNALS OF DIFFERENTIALLY EXPRESSED mRNA ISOFORMS IN MOUSE SELECTION LINES FOR FATNESS AND LEANNESS

I1

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Alternative polyadenylation (APA) regulates genes by creating distinct 3'-ends of transcripts by selecting different polyadenylation (PA) sites along genes, thereby influencing transcription termination and poly(A) tail addition [1]. Using the Whole-Transcriptome Termini Site Sequencing (WTTS-seq) method [2], we determined PA sites in the hypothalamus of the Fat and Lean selection mouse lines, models for polygenic obesity and healthy leanness [3]. A total of 29,091 PA sites were expressed in both lines in the hypothalamus, a major brain region regulating energy balance. They were located within 13,837 genes (86.9 %), predominantly protein-coding (93.6 %). There were 472 differentially expressed PA (DE-PA) sites in the Fat compared to the Lean line, located within 357 genes (log2FC ≥ |1.5|, $p_{adj} < 0.05$). Due to APA, 5,733 genes (41.4%) had multiple PA sites that could lead to mRNA isoforms. After additional filtering, we obtained 4,753 genes with more reliable PA sites producing mRNA isoforms. One hundred forty-eight of these genes had at least one DE-PA site, with 13 having multiple. Gene Ontology enrichment of 148 genes highlighted processes important for neuronal communication, including behavior, cognition, and synaptic signaling, suggesting altered brain activity in both lines. Additionally, these genes influence intracellular protein transport and organization, indicating potential changes in cellular structure and protein distribution in the Fat and Lean mice. Moreover, six genes with mRNA isoforms and DE-PA sites had SNPs within polyadenylation signals (PAS); and PAS-SNPs. Each line had three PAS-SNPs within three distinct genes: *Nrsn2* (rs27369860), *Ric3* (rs36754429), and *Rpl14* (rs263963399) genes in the Fat line; *Hlf* (rs229072835), *Taf1a* (rs32656801), and *Ints11* (rs227466545) genes in the Lean line, the latter aligning with our previous study [4]. PAS-SNPs altered the canonical AATAAA PAS motif, possibly resulting in decreased expression of these DE-PA sites, suggesting that PAS-SNPs might affect their expression. Future functional studies are needed to explore the role of PAS-SNPs and mRNA isoforms due to APA in polygenic obesity, extend the research to other metabolic tissues, and evaluate APA isoforms as novel potential therapeutic targets for obesity management.

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ARCHAEOGENETIC ANALYSIS OF LATE PLEISTOCENE AND HOLOCENE BISON FOSSILS FROM SLOVENIA AND HUNGARY

I2

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During the last 130,000 years, two species of Bison were traditionally acknowledged to be present in Europe: the steppe bison (*Bison priscus*) and the European bison or wisent (*Bison bonasus*) [1]. The steppe bison appeared already during the Middle Pleistocene and occupied an area ranging from Western Europe to North America [1,2]. It became extinct in Europe at the end of the Late Pleistocene [3], while in some areas, it survived into the Early [4] or Middle Holocene [5]. The morphologically distinct osteological remains of the extant European bison first appear in the fossil record around the start of the Holocene [6]. Phylogenetic inferences based on mitochondrial DNA prove the existence of three clades in the last 50,000 years: Bp, which includes the steppe bison, and Bb1 and Bb2, which are related to the European bison [1,7-9]. Although several dozen Late Pleistocene and Holocene bison remains have been discovered in Slovenia and Hungary, genetic analysis has been mostly performed on (sub)fossils from Western Europe, Poland, the Caucasus, and Siberia. Therefore, our study aimed to analyse the ancient DNA (aDNA) from teeth/bones found in these two regions in order to assign the specimens to the existing clades. For this purpose, we extracted the DNA from 8 samples from the second half of the Late Pleistocene from Slovenia, and 2 Holocene samples from Hungary, morphologically attributed to *Bison sp.*, in a specialised aDNA laboratory. After using an amplicon-based approach with a maximum amplicon length of 198 base pairs, targeting the 12S, 16S, ND2, COI, ND4, and cytochrome b regions of the mitochondrial DNA, we sequenced the amplicons on the Ion Torrent S5 next-generation sequencing system. We processed the sequencing results using an in-house developed bioinformatics pipeline and constructed phylogenetic trees using the BEAST software. We successfully amplified and sequenced aDNA from the samples, followed by the assembly of 2,195 bp long concatenated consensus sequences of the mitochondrial DNA. Our results show that most of the Late Pleistocene *Bison* from the studied areas belong to the Bp clade, while the one close to the Pleistocene/Holocene transition and the Holocene specimens belong to the Bb2 clade. We did not detect the presence of the extinct Bb1 clade. Our research can, therefore contribute new insights into the paleogeography of

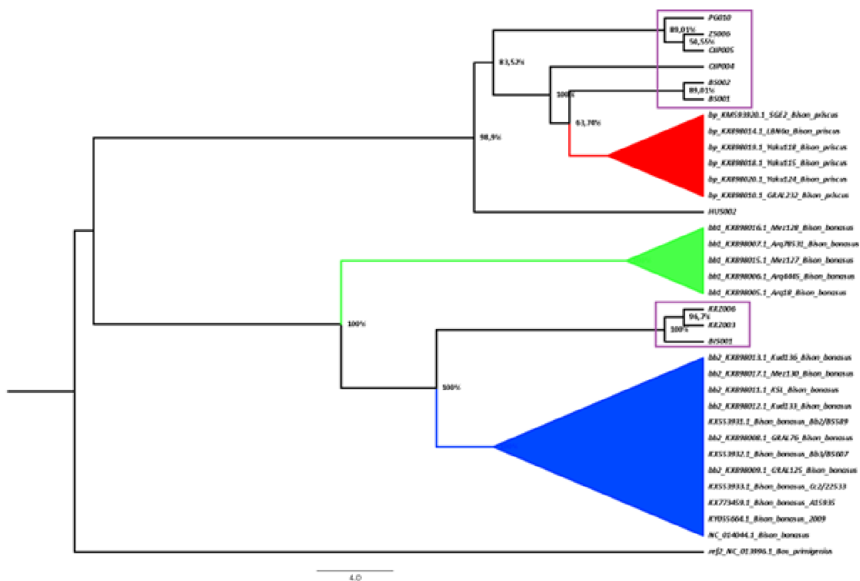

Fig. 1: Phylogenetic tree, constructed with BEAST. The tree shows the relationship between the clades: Bb1 (green) and Bb2 (blue) are closely related to each other, and the Bp clade (red) represents the steppe bison. Our samples are marked with a purple square.

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IDENTIFYING INTROGRESSION BETWEEN DOMESTIC GOAT (*CAPRA HIRCUS*) AND ALPINE IBEX (*C. IBEX*): A CASE STUDY FROM SLOVENIAN PART OF ALPS

I3

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Domestic goats and Alpine ibexes share a habitat on mountain pastures. The spatial overlap occasionally results in hybrid offspring, as reported in Italy, France, Switzerland, and Austria [1]. In addition, we encountered three hybrids in the Slovenian part of the Alps. Confirmation of their identity and survival potential raises the question about the actual extent of such interspecific hybridisations and the possibility of ancient or more recent introgression events since the hybrids seem to be fertile. To address this, we analysed whole genome sequences from more than 160 animals belonging to domestic goat breeds and wild goat species from Africa, Asia, and Europe. We performed clustering based on the identity by state distances [2] and examined the genomic structure of the populations [3]. Our analyses confirmed that the hybrids have ancestries from both populations, the Slovenian Drežnica goat breed and the Alpine ibex. Furthermore, D-statistics [4] were used to detect and quantify gene flow between the sampled populations. The D-statistic test considers a scheme with four taxonomic units: two sister taxa (P1 and P2), of which P2 is a candidate for gene flow with an external group (P3), and an outgroup (O) that is used to determine ancestral alleles. Significant values were found in comparisons between European ibex species (Alpine and Iberian) and domestic goats. The similarity of values among domestic goats with both ibex species suggested that introgression was ancient. This inference was further supported by identifying introgressed genome sequences using the program IBDmix [5]. Despite the discovered hybrids, we did not detect any signs of recent introgression in the existing Drežnica goat and Alpine ibex populations. As the latter could represent a threat to both endangered populations, our analysis is the first step towards developing genomic tools for monitoring and coexistence of both populations in Slovenia and other countries.

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HYBRIDIZATION AND POPULATION GENETIC STRUCTURE OF EUROPEAN WILDCATS FROM THE DINARIC ALPS AND PANNONIAN BASIN

I4

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Fragmentation and habitat loss have contributed significantly to the demographic decline of European wildcat *(Felis silvestris silvestris)* populations, and hybridization with domestic cats *(F. catus)* poses a threat to the loss of genetic purity of the species. Here, we explore the genetic structure, demographic history, and population differentiation based on whole genome data of the endangered European wildcat in the intersection of the Dinaric Alps and the Pannonian Basin in Slovenia, Croatia, and Serbia, with the particular focus on historical and recent introgression between wild (sub)species and domestic cats.

We whole-genome sequenced 32 wildcats. Twenty-two of the individuals included in the study were identified as pure wildcats (69%) and ten as hybrids (31%) between wildcats and domestic cats by microsatellite markers (Urzi et al. 2021*). We have included 17 samples downloaded from GenBank in the analysis. These samples belong to the following taxa: *F. catus*, *F. s. silvestris* and *F. s. lybica*.

Using microsatellite markers, we found that wildcat populations were divided into two genetic clusters, largely consistent with a geographic division into a genetically diverse northern group and a genetically eroded south-eastern group. The WGS data also revealed a statistically significant? divergence of populations in two clusters, but the introgression pattern differs from the microsatellite analysis. In Serbia, we found that half of the Serbian "hybrids" (as indicated by microsatellite markers) resulted from introgression from *Felis silvestris lybica*. This may reflect a historical introgression that persists in the area and may result from a historical contact zone between *F. s. lybica* and *F. s. silvestris* in the region.

We found population differentiation of European wildcats, including recent persecution-driven population divergence. The low level of domestic introgression

found in this study indicates a substantial level of “resistance” of this elusive species towards major anthropogenic impacts, such as the omnipresence of domestic cats as well as substantial habitat fragmentation. In Serbia, we likely found introgression that is a consequence of historic contact zone, but a more detailed study of selective pressures is needed.

THE CONJUGATION FREQUENCY OF THE pOX38:Cm CONJUGATIVE PLASMID IN *IN VITRO* MODELS OF HUMAN INTESTINE AND IN AN ANAEROBIC CHAMBER

I5

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Bacterial antimicrobial resistance (AMR) has emerged as one of the leading public health threats of the 21st century [1]. AMR can occur by mutations or by acquisition of antibiotic resistance genes (ARGs) via horizontal gene transfer (HGT), the latter is considered to be the most important factor in the AMR spread. Of the three canonical mechanisms of HGT, conjugation is thought to have the greatest influence on the dissemination of ARGs [2]. The human gut microbiome is a known 'melting pot' for conjugation, with ARG transfer in this environment widely documented [3].

The aim of this study was to determine the conjugation frequency of the plasmid pOX38:Cm from the *Escherichia coli* N4i strain, the probiotic Nissle 1917 strain with gentamicin resistance, (=MSE259) [4] to the DL82, a uropathogenic *E. coli*, under different conditions in TNO intestinal models (TIM-1 and TIM-2) and in anaerobic atmosphere. The TIM-1 is a multi-compartment model of the upper gastro-intestinal tract with four compartments mimicking conditions in the stomach and three parts of the small intestine, while the TIM-2 mimics the colon containing the human microbiota. The effect of MSE259 and DL82 on human microbiota present in TIM-2 was also studied by sequencing. In addition, similar conjugation experiments were performed in an anaerobic chamber. The obtained conjugation frequencies were compared with the those obtained in mating assays performed aerobically in LB liquid medium. In TIM-1 pOX38:Cm conjugation was successful, albeit the conjugation frequency was lower compared to the one obtained aerobically in LB liquid medium. From assays in TIM-2 conjugation frequency could not be calculated, as no transconjugants were detected, which could be due to lower conjugation frequency under anaerobic conditions, lower viability of the MSE259 or DL82 in TIM-2 and presence of other microbiota. Addition of MSE259 in TIM-2 provoked a change in the structure of microbiota: a significant increase of 5 taxa and a decrease of 10 taxa was observed. In order to exclude an effect of the peristaltic movement, which is simulated in the TNO intestinal models, mating assays with mixing of the mating mixture were performed. The mixing did not have any significant effect on conjugation frequency. Further, to exclude the effect of lower pH, mating assays in liquid LB with lower pH were performed. A decrease in conjugation frequency in such medium

was observed, but not as much as in the TIM-1 model. Mating assays performed in TIM-1 medium (a special solution with salts and no nutrients) aerobically in test tube, did not affect conjugation frequency. From mating assays performed in liquid LB in anaerobic chamber conjugation frequencies were calculated, a significant decrease in conjugation frequency was observed, when compared to the conjugation frequency obtained aerobically in LB liquid medium.

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A SMALL BACTERIOPHAGE PROTEIN DETERMINES THE HIERARCHY OVER CO-RESIDENTIAL JUMBO PHAGE IN *BACILLUS THURINGIENSIS* SEROVAR *ISRAELENSIS*

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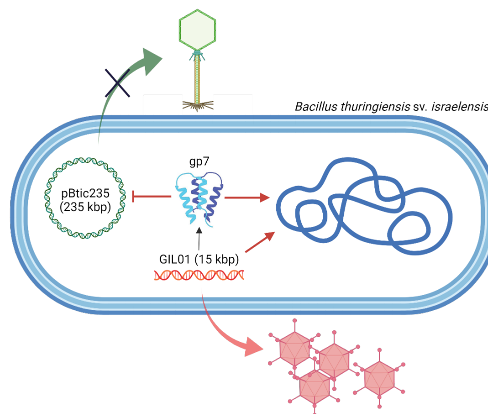
Bacillus thuringiensis serovar *israelensis* is the most widely used biopesticide against insects, including vectors of animal and human diseases. Among several extrachromosomal elements, this endospore-forming entomopathogen harbors two bacteriophages: a linear DNA replicon named GIL01 that does not integrate into the chromosome during lysogeny and a circular-jumbo prophage known as pBtic235. Here, we show that GIL01 hinders the induction of cohabiting prophage pBtic235. The GIL01-encoded small protein, gp7, which interacts with the host LexA repressor, is a global transcription regulator and delays the induction of pBtic235 after DNA damage to allow GIL01 to selectively produce its own progeny. In a complex with host LexA in stressed cells, gp7 down-regulates the expression of more than 250 host and pBtic235 genes, many of which are involved in the cellular functions of genome maintenance, cell-wall transport, and membrane and protein stability. We show that gp7 homologs that are found exclusively in bacteriophages act in a similar fashion to enhance LexA's binding to DNA, while likely also affecting host gene expression. Our results provide evidence that GIL01 influences both its host and its co-resident bacteriophages.



EPIGENETIC INSIGHTS INTO VIROID PATHOGENESIS FROM GENOME-WIDE DNA METHYLATION ANALYSIS OF CBCVd-INFECTED HOP PLANTS (*HUMULUS LUPULUS* VAR. 'CELEIA')

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Viroids are small, single-stranded RNA molecules, that exploit host factors for propagation, causing severe diseases in agricultural crops. Notably, recent outbreaks such as the Citrus bark cracking viroid (CBCVd) in hop plants in Slovenia, Germany, and Brazil, emphasize the urgency to study the underlying mechanisms underscoring viroid pathogenesis and host response to infection.

Prevailing research delineates the intricate interplay between viroid RNA and host RNAi factors, orchestrating nuanced changes in gene expression, metabolic pathways, and the observable phenotype. Further investigation has discerned viroid-induced alterations in DNA methylation patterns via the RNA-directed DNA methylation pathway. Previous study has indicated consistent whole-genome DNA methylation levels between CBCVd-infected and healthy hop plants. Our current work, however, employs a comprehensive bisulfite sequencing approach, illuminating subtle yet significant shifts in specific parts of the DNA methylation landscape of the hop genome consequent to CBCVd infection. In addition, our study analysed important epigenetic marks of CBCVd-infected hop plants, providing novel evidence for host DNA methylation's role in defence against viroids. We found that genes linked to pathogen interaction pathways, such as MAPK signalling and LRR, exhibited hypomethylation, potentially enhancing host defence. Conversely, key RNA transcription genes like POL II, POL IV, and POL V displayed hypermethylation, highlighting DNA methylation's defensive significance. Additionally, our efforts were centred on establishing a vital connection between DNA methylation and pre-existing transcriptomic data, allowing us to gain a more comprehensive insight into plants' defence against viroids. Interestingly, the mediator subunit MED7a was upregulated and hypomethylated, suggesting impaired RNA transcription and emphasizing mediator complexes' role in viroid-infected plants.

GENOME-WIDE ASSOCIATION STUDY AND POPULATION STRUCTURE OF DIFFERENT HEMP (*CANNABIS SATIVA* L.) GENOTYPES

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Hemp (*Cannabis sativa* L.) is one of the oldest cultivated plants in the world, with a multipurpose range of uses. It is a dioecious annual plant [1]. Due to wind pollination, heterozygous plants frequently occur within varieties, leading to appearance of different phenotypes, which can manifest at both the chemical and genetic levels.

Based on visual traits, different phenotypes were identified within three different hemp varieties (Carmagnola selected, Tiborszallasi, and Finola selection). DNA of selected phenotypes was isolated and shotgun NGS libraries were designed using an in-house protocol. Libraries were hybridized with 4537 capture probes for target loci enrichment. After enrichment, samples were loaded on the Ion v3 chip and sequenced using the Ion Proton Sequencer. Two chips were used for 171 samples. Raw UBAM reads were imported into CLC Genomic Workbench 22. Reads were trimmed for low quality regions and mapped to the reference genome cs10. SNP calling was performed using the Genome Analysis Toolkit (GATK). Population structure analysis was conducted using ADMIXTURE 1.3.0 [2]. Cross-validation (CV) error was calculated for each group. Genome-wide association studies (GWAS) were conducted using Tassel 5 software [3]. Genotyping data were filtered, resulting in 3670 SNP positions out of 4537. These data were combined with three recorded phenotype traits (CBD, THC, and anthocyanin coloration of leaf petiole). Kinship analysis was performed to minimize false positives. GWAS analysis was done using MLM model, incorporating filtered and combined genotype and phenotype data, along with kinship matrix and PCA results. GWAS results were visualized using Manhattan plots with p-values in RStudio and the 'qqman' package [4]. False discovery rate (FDR) was determined using the 'qvalue' package at thresholds of 0.01, 0.001, or 0.0001. According to ADMIXTURE, genotype data were divided into 5 groups with a minimum CV value of 0.44940. The Carmagnola selected variety was homogeneous with no distinct groups, while Tiborszallasi and Finola selections were divided into two groups. GWAS revealed 14 significant SNPs above the FDR threshold associated with THC. Among them, two were on chromosome 1, one on chromosome 2, two on chromosome 4, one on chromosome 6, two on chromosome 7, four on chromosome 8, and one on chromosome 10, which was also the most significant SNP with a p-value of 0.000135 and an R^2 of 0.3474. For CBD, one significant SNP was associated with

chromosome 10. The p-value was 0.0000795, the marker's effect on the trait was 0.1479, and R^2 was 0.04245. Anthocyanin coloration of leaf petiole was associated with three statistically significant SNPs, all on chromosome 8. The most significant SNP had a p-value of 0.000504, a marker effect of 0.05, and an R^2 of 0.35167. Based on significant SNPs, genes located at defined positions were determined for each phenotypic trait. The GWAS analysis results serves as a valuable tool for understanding the mechanisms and functions of genes correlated with specific hemp traits.

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IRRADIATION AND GENE ELECTROTRANSFER OF PLASMID DNA ENCODING CHEMOKINES CCL5 AND CCL17 INDUCE IMMUNOMODULATORY EFFECTS IN MURINE TUMORS

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Chemokines regulate immune cell migration. The degree and the type of immune cells in the tumor affects disease progression and correlates with the efficacy and outcome of immunotherapies. Similarly, beneficial immunomodulatory effects were also observed after irradiation. Therefore, we sought to investigate gene electrotransfer (GET) of proinflammatory chemokines CCL5 or CCL17 in combination with irradiation, as a potential therapeutic strategy for cancer therapy.

Tumor models were chosen to correspond to an inflamed (CT26 murine colon cancer) or immunosuppressive (4T1 murine breast cancer) immunophenotype. First, chemotactic properties of investigated chemokines were examined *in vitro*. Next, the potential of chemokines to induce the extravasation of fluorescently labelled splenocytes was determined using intravital microscopy of tumors in dorsal window chamber model (DWC). The antitumor effectiveness of combined therapy utilizing GET of chemokines and two irradiation regimes (single dose of 10 Gy and fractionated dose of 3× 5 Gy) was then determined *in vivo*. Lastly, qRT-PCR was used to evaluate gene expression of several cytokines in tumors after the therapies, while changes in the abundance of CD4+, CD8+ cells and vasculature (CD31+ cells) were determined with immunofluorescent staining.

Both chemokines CCL5 and CCL17 induced the migration of murine macrophages RAW264.7 *in vitro*. Similarly, in both CT26 and 4T1 tumors growing in DWC, GET of chemokines showed increased retention of splenocytes compared to control. CT26 tumor growth delay after combined therapy of GET of chemokines and both irradiation regimes was significantly longer compared to control and led to tumor cures. In the case of 4T1 tumors, only GET of chemokines combined with irradiation led to a pronounced tumor growth delay but without tumor cures. Gene expression analysis showed increased expression of both chemokines after corresponding therapies. Moreover, increased expression of CXCL9 and CXCL10, two potent chemoattractants of cytotoxic CD8+ T lymphocytes, was determined in tumors after

most of the combined therapies. Immunofluorescence showed increased numbers of CD4+ and CD8+ T lymphocytes in tumors after GET of chemokines, however their numbers decreased whenever irradiation was used. Our results show that combined therapy elicits an antitumor immune response in inflamed tumors CT26 and to some extent in immunosuppressive tumors 4T1, indicating the potential of chemokines in cancer immunotherapy.

HPV-POSITIVE MOUSE MODEL OF ORAL SQUAMOUS CELL CARCINOMA: DEVELOPMENT AND TRANSCRIPTOME ANALYSIS

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Head and neck cancers are a heterogeneous group of tumors that includes oral squamous cell carcinoma (OSCC). One of the risk factors for the development of some anatomical subtypes of OSCC is persistent infection with high-risk human papillomavirus (HPV) strains, such as HPV-16. Clinical data indicate, that some patients with HPV-positive OSCC have a better prognosis and respond better to various treatment modalities, including radiotherapy and immunotherapy. To evaluate the differences between HPV positive and HPV negative OSCC and therapies based on adaptive immune response, immunocompetent mouse models are essential. Nevertheless, HPV-positive mouse model of OSCC are still scarce due to the species specificity of HPV. Therefore, the aim of our study was to establish and characterize a mouse model of HPV-positive OSCC. We established two monoclonal MOC1-HPV cell lines (MOC1-HPV K1 and MOC1-HPV K3) by transduction of murine OSCC cell line MOC1 with LXSN16E6E7 retrovirus, which encodes the HPV-16 E6 and E7 open reading frames. We confirmed a stable expression of HPV-16 E6 and E7 in both monoclonal MOC1-HPV cell lines on mRNA and protein level with quantitative PCR and immunofluorescent staining, respectively. *In vitro* characterization demonstrated differences in cell morphology and cell migration capacity, where MOC1-HPV K3 cells migrated significantly slower in the wound healing assay compared to the parental or MOC1-HPV K1 cell line. *In vivo*, we characterized the tumor microenvironment (TME) of the newly established tumor models by immunofluorescent staining of blood vessels (CD31), hypoxic areas (EF5), and proliferation (EdU). MOC1-HPV K1 tumors were less hypoxic, with a higher proportion of proliferating cells compared to MOC1 and MOC1-HPV K3 tumors. Both in vitro and in vivo results correlate with transcriptome analysis results of the three cell lines, where the 20 most enriched gene ontology “biological process” terms in MOC1-HPV K1 cell line include terms related to angiogenesis and blood vessel formation, as well as cell migration and motility. In conclusion, we established a mouse model of HPV-positive OSCC that can be used to study basic characteristics as well as tumor microenvironment and immune responses to different therapies of HPV-positive OSCC.

MOLECULAR AND CLINICAL CHARACTERISTICS OF SLOVENIAN PATIENTS WITH SMALL CELL CARCINOMA OF THE OVARY, HYPERCALCEMIC TYPE

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Description Small cell carcinoma of the ovary, hypercalcaemic type (SCCOHT) is rare and usually affects young women with median age at diagnosis of 241. It has a poor prognosis with long-term survival of 30% in early-stage disease [1]. Germline and somatic variants in SMARCA4 gene, which encodes a catalytic subunit of the SWI/SNF chromatin-remodelling complex, are associated with SCCOHT. Penetrance of *SMARCA4* germline pathogenic variants (PV) and incidence of SCCOHT is currently unknown. The aim of our study was to identify all cases of SCCOHT in Slovenia from 1991 to 2021 and present their genetic testing results, histopathological and clinical characteristics. We also aimed to estimate the incidence of SCCOHT and assess the penetrance of germline *SMARCA4* PVs in Slovenian families.

First, we analysed medical records and data from the Slovenian Cancer Registry. In patients suspected of having SCCOHT, histopathological review with immunohistochemical staining for SMARCA4/BRG1 was undertaken to confirm the diagnosis. In cases of confirmed SCCOHT, both germline and somatic genetic analyses using next-generation sequencing were performed. We identified 7 patients who developed SCCOHT aged 21-41 in a population of two million during a 30-year period and estimate the minimal incidence of SCCOHT to be 0.12/million/year [2]. Two cases were sporadic, with presumably biallelic *SMARCA4* variants in their tumours not seen on germline testing. In one patient, a *SMARCA4* PV and loss-of heterozygosity was identified in tumours tissue, but germline testing was unfeasible. In four cases from two different families, two novel germline loss-of-function variants in SMARCA4, c.1423_1429delTACCTCA p.(Tyr475Ilefs*24) and c.3216-1G>T were identified. Based on pedigree analysis,

they appear to be associated with relatively high penetrance. In all tested tumours, tumour mutational burden was low. Five tumours consisted of small cells with scant cytoplasm. In two cases, there was a predominance of large cells with eosinophilic cytoplasm, i.e. large cell variant of SCCOHT. Patients included in our study were diagnosed with stage FIGO IA-III disease and died due to SCCOHT within 36 months. In one case, temporary disease stagnation was achieved using immunotherapy.

In conclusion, our study presents all known cases of SCCOHT diagnosed in Slovenia between 1991 and 2021. We offer a first estimate of SCCOHT incidence in the non-paediatric population, but expect studies in larger populations will lead to more accurate assessments.

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ISOLATE-SPECIFIC DIFFERENCES IN MACROPHAGE RESPONSE TO GROUP B *STREPTOCOCCUS*

I12

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Although Streptococcus agalactiae (Group B Streptococcus, GBS) is primarily an opportunistic pathogen that colonizes the gastrointestinal and genitourinary tracts of 15-30 % of healthy individuals, it remains a major cause of invasive disease in neonates [1]. The latter can occur in the first week of life and usually manifest as sepsis or pneumonia (i.e., early-onset-disease), or at 1 week to 3 months of age, which usually manifests as meningitis (i.e., late onset disease) [2]. To date, 10 different serotypes (Ia, Ib, II – IX) have been described, distinguished by their virulence and the immune response they elicit [3]. Since intrapartum antibiotic prophylaxis can only prevent early-onset, but not late-onset disease and infections in the elderly and immunocompromised individuals, further studies are needed to investigate GBS-associated pathogenesis. Because adaptive immunity is underdeveloped in neonates, cells of innate immunity play a critical role in protection against pathogens [4]. Of the latter, macrophages are particularly important as they are involved in pathogen recognition, phagocytosis, and elimination [5]. Because disease severity depends on both the GBS isolate and the immune status of the individual, we investigated possible isolate-specific differences in the immune response of THP-1 macrophages to 12 different previously genotyped GBS isolates [6]. Significant isolate-specific differences were observed in phagocytic uptake and expression of macrophage polarization markers [7]. Furthermore, by measuring inflammatory and anti-inflammatory cytokines and chemokines at the protein level, as well as expression of genes involved in antimicrobial activity and inflammation at different time points, we observed that different isolates have different potential to become invasive or remain colonizing. In addition, by measuring IL-1B, IL-18, and caspases 1 and 3 at the mRNA and protein levels, LDH secretion, and caspase-1 activity, we demonstrated that some isolates were significantly more cytotoxic to macrophages and induced pyroptosis. By measuring glycolysis and oxidative phosphorylation using Seahorse extracellular flux analyzer and analyzing the expression of glycolytic genes, we have shown that different GBS isolates activate macrophage metabolism differently and that differences in metabolism lead to differences in macrophage effector functions [7].

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ABSTRACTS OF POSTERS

ENGINEERED M13 FILAMENTOUS BACTERIOPHAGE AS A NOVEL ANTIGEN DELIVERY SYSTEM FOR VACCINATION IN THE TREATMENT OF MALIGNANT MELANOMA

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Bacteriophages are prokaryotic viruses that are the most abundant organisms on Earth and can be found in virtually every environment, including the human body [1]. With many intriguing characteristics, bacteriophages have become a favorable tool in bioengineering. In the field of cancer immunotherapy, bacteriophages have been exploited to create powerful vaccine platforms designed to overcome the tolerogenic tumour microenvironment while inducing specific immune responses [2,3].

Our study aimed to create a novel vaccine for malignant melanoma treatment by constructing non-lytic M13 filamentous bacteriophages expressing MAGE-$A1_{161\text{-}169}$ tumour peptides as fusion proteins with pIIII minor and pVIII major coat proteins. Using the phage display technology, recombinant bacteriophages were released from Escherichia coli, after transformation with recombinant phagemids pComb3XSS-MAGE-A1 or pComb8-MAGE-A1 followed by super-infection with VCSM13 helper bacteriophages. Phage-displayed tumour peptides were confirmed by proteomic analysis. C57BL/6 mice were vaccinated intraperitoneal, receiving 10^{12} pfu of genetically engineered bacteriophages per mouse three times at two-week intervals, with PBS and wild-type M13 bacteriophage used as controls. One week after each vaccination, sera were collected and analysed by ELISA. One week following the second vaccine boost, splenocytes were harvested to assess T-cell cytotoxicity by co-culture assay. Phage-displayed tumour peptides were confirmed by nano-LC-MS/MS (protein pVIII-MAGE-A1) and Western blot (protein pIII-MAGE-A1). We have shown that administration of the M13 filamentous phage vaccine to mice is safe, with no evidence of side effects. Despite variations in display valencies, both pIII and pVIII display techniques effectively stimulated humoral responses, leading to increasing anti-phage and anti-MAGE-A1 antibodies which exhibited an increase from the first to the third phage dose. Moreover, the elicited anti-MAGE-A1 antibodies demonstrated the capacity to bind to naturally expressed epitopes on B16F10 tumor cells *in vitro*. Splenocytes from recombinant bacteriophage-vaccinated mice also demonstrated enhanced induction of CD8+ T cell cytotoxicity against B16F10 cells compared to control groups.

To summarize, vaccination with engineered M13 filamentous bacteriophages displaying the MAGE-A1 tumor peptide is safe and triggers adaptive immune responses. We believe that an M13 filamentous bacteriophage-based vaccine holds significant promise for the treatment of malignant melanoma.

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p1

IDENTIFICATION OF VIRUSES IN CV. MALVASIA (*VITIS VINIFERA* L.) AND EFFECT OF THERMOTHERAPY ON THEIR ELIMINATION AND DIFFERENTIAL EXPRESSION OF GENES INVOLVED IN THE RNA SILENCING PATHWAY

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Grapevine (*Vitis vinifera* L.) is one of the most widely used fruit crops and it is important for its economic properties. However, it can harbour more than 86 viruses and viroids [1], which cause yield reduction, alterations in chemical and sensorial quality of grape and wine, developmental and morphological malformations in vine organs, and affect the length of vine life [2]. In Slovenia, according to the Official Gazette of the RS N°93/05 and 101/20 all propagated vine material must undergo mandatory testing on viruses ArMV, GFLV, RpRSV, TBRV, GVA, GVB, GRSPaV, GLRaV-1/-3 and GFkV (only for rootstocks). Testing is recommended on GLRaV-2 and GLRaV-4–9, as well as for emerging virus GPGV.

In our study, we tested 17 vines of cv. Malvasia on 14 viruses with obligatory and recommended testing with RT-PCR and RT-qPCR. Six viruses were identified (GRSPaV, GFLV, GFkV, GLRaV-2, GLRaV-3, GPGV). The most prevalent viruses were GRSPaV and GFLV, followed by GPGV. In addition, due to its symptomatology and the physiological characteristics of the tested Malvasia variety, we hypothesised that grapevine asteroid mosaic-associated virus (GAMaV), which causes peripheral vesiculation of the chloroplasts, could also be associated with a star-like structure at the distal end berry rupture point, but it was only detected in two vines using RT-PCR and in five vines using RT-qPCR. In the second part of our study, we conducted thermotherapy in combination with shoot tip culture in order to study the elimination efficiency of detected viruses. Thermotherapy is often used as a successful sanitation method for obtaining virus-free planting material due to inhibition of virus replication or virus RNA degradation [3]. It is also associated with an antiviral immune defense mechanism, RNA silencing [4,5]. A 100 % elimination rate was observed for viruses GFLV, GFkV, and GLRaV-2, the hardest to eliminate was GRSPaV, for which successful elimination was obtained only for one vine. In the third part of our study, we monitored the effect of high temperature on the differential expression of Dicer-like, Argonaute and RNA-dependent RNA-polymerase genes in grapevine, which could contribute to an improved response to viral infection. Differences in expression were observed with RT-qPCR for all genes considered (DCL1/2/3, AGO1/2a/4, and

RDR2), with significant differences for all genes except DCL3. The biggest expression differences between treated and untreated samples with high temperature were observed for the genes DCL1 and DCL2. We suggest further studies in the field of RNA silencing in grapevine, as our results indicate significant changes in the expression profiles of core RNAi proteins.

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VIRULENCE AND RESISTANCE GENOTYPES OF ESBL-PRODUCING *E. COLI* RECOVERED FROM LOWER RESPIRATORY TRACT BETWEEN 2018–2022) IN RELATION TO BIOFILM FORMATION

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) can cause a variety of different infections for which treatment options are limited, primarily due to the increasing prevalence and spectrum of antimicrobial resistance. Of particular concern are extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC), especially the pandemic *E. coli* sequence type 131 clone (ST131).

This study aims to investigate the biofilm formation ability and its correlation with genotypes of clinical ESBL-EC isolates from lower respiratory tract (LRT) samples.

A total of 116 ESBL-EC, isolated from LRT, were assigned to phylogroups, sequence type, and clonal groups. Isolates were also screened for virulence-associated (VAGs) and antimicrobial resistance genes, as well as for class 1, 2, and 3 integrons. Furthermore, we examined the biofilm formation ability by culturing the bacterial isolates for 24 and 48 hours in a 96-well Calgary Biofilm Device (CBD) in two different growth media. The biofilms formed were stained with 1% w/v crystal violet and spectrophotometrically quantified.

The resistance profile of the 116 ESBL-EC isolates showed a high prevalence of group 1 $bla_{CTX\text{-}M}$ (75.9%) and a dominance of phylogenetic group B2 (59.5%), which was also significantly associated with group ST131. After 24 hours of incubation, strong biofilm formation was observed in 18.1% and 51.7% of ESBL-EC isolates in LB and MG with 0.02% glucose, respectively. Spearman's rho correlation matrix showed a statistically significant positive correlation between strong biofilm formation in the MG medium after 24 hours of incubation and sequence type 131, phylogenetic group B2, $bla_{CTX\text{-}M}$ group 9 genes, the biocide resistance gene emrE and the VAGs *afa/dra, fluA, fyuA, iha, iroN, irp2, kpsMTII, sat, usp*. When isolates were grown in the nutrient-rich Luria–Bertani (LB) media, we found statistically significant positive correlations with phylogenetic group C and with virulence genes *papC* and *papGII*.

Our results suggest that ST131 ESBL-producing *Escherichia coli*, a multidrug-resistant human extraintestinal clonal group, has the ability to form biofilms, especially under

nutrient deprived conditions, and that biofilm production is associated with certain virulence factors. Our results suggest a possible reason for the successful global spread of ST131, particularly in clinical settings.

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DEVELOPMENT OF qPCR AND dPCR APPROACH FOR DETECTION OF CRISPR/Cas9-INDUCED MUTATIONS IN CABBAGE PROTOPLASTS

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Targeted genome editing with CRISPR/Cas9 is nowadays the most common method for creating new plant varieties because it is efficient, simple, and faster than conventional breeding. The invention of new methods for rapid and sensitive detection of mutations in genome-edited plants is crucial to shorten the time to obtain modified plants. At present, there are several methods for detecting mutations after CRISPR/Cas9 genome editing, such as qPCR, Sanger sequencing, HRMA, T7 endonuclease, etc., but they are less suitable for detecting mutations in polyploid plants or when we have low editing efficiency. On the other hand, next-generation sequencing (NGS) has high sensitivity but is cost-efficient when larger number of samples are analyzed. Therefore, we decided to develop a new, fast, and efficient method using qPCR and dPCR to detect mutations after genome editing using CRISPR/Cas9.

For our samples, we used transformed protoplasts of three distinct cabbage cultivars (Huzaro F1, Reball F1, Rebecca F1), all of which had different mutation ratios in the *CENH3* gene revealed by NGS. In our experiments, we used a TaqMan duplexed assay with a non-mutated reference gene (*PDS*) which allowed us to quantify mutations in our samples. However, before the dPCR method could be used to determine the percentage of mutations in edited samples, it had to be optimized. In the first part of the experiment, designed probes for C*ENH3* and *PDS* genes were tested using qPCR. The same method was also used to determine primer concentrations to achieve optimal amplification of both genes in the duplex reaction. Since we did not obtain optimal amplification of both genes, new primers for *PDS* gene were designed which gave us shorter amplicons and required amplification efficiency. After the optimization, the assay was used in dPCR to check whether this method is suitable for detection of mutations. We were able to detect the mutated samples and distinguish them from the wild-type samples. The percentage of mutations was calculated by dividing positive partitions of CENH3 with positive partitions of *PDS* gene. The range of mutations in our samples were between 2.8% - 26.4%. The same samples were also analyzed by qPCR, where in general the percentage of mutations was higher (0.65% - 46.9%). Detection of mutations with qPCR is faster, requires fewer reagents and a lower amount of initial DNA. We also compared the results of dPCR and qPCR with results of NGS method and concluded

that they are comparable.

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IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF PATHOGENIC GENETIC VARIANTS CAUSING HUMAN MONOGENIC CONDITIONS WITH RNA-TRANSCRIPT ANALYSIS

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Next-generation sequencing represents an important step in diagnostics of monogenic conditions, as it allows the analysis of the majority of the human genome, including non-coding regions. However, characterization of the pathogenicity of variants identified by these approaches and their functional assessment remain a challenge. The aim of this research was analysis of cases with undiagnosed genetic conditions that harbor genetic variants of uncertain clinical significance, and cases in which conventional genetic approaches identified no causal variants.

We sought to establish that by analyzing RNA transcripts, we can improve the diagnosis of pathological variants situated in non-coding regions, thereby augmenting the insights obtained from exome and genomic sequencing approaches. Through targeted sequencing of gene transcripts, we clarified both the pathological variants present, as well as the underlying mechanisms that led to their appearance and resulted in the clinical picture. Based on the functional data, we reassessed the pathogenicity of variants according to ACMG and ACGS standards and we found that the clinical classification of pathogenicity has improved in every case. In four cases we confirmed exon skipping, in three cases a frameshift mutation, and one case of each intron retention and novel splice site introduction. The findings demonstrate a noteworthy enhancement in sensitivity through RNA transcript analysis methods. Focusing on specific regions and increasing sequencing depth offers advantages in terms of data volume and investigation accessibility. In accordance with the 2022 study by Wai et al. [1], which focused on the advantages of targeted sequencing of (shorter) RNA-transcripts over classical RNA-seq, we also confirmed the higher accuracy of targeted sequencing compared to whole-genome sequencing, considering that we clarified pathogenicity in most cases. In terms of economics and efficiency, this approach proves more cost-effective than whole genome sequencing. It's a crucial aspect in clinical settings, as it's user-friendly and doesn't necessitate high-performance sequencers. These methods hold significant applicability. They allow for detecting residual isoform expression that might otherwise degrade in the nonsense-mediated decay process. Furthermore, they enable identification of a broad spectrum of exon splicing mechanisms, a feat unattainable with previous-generation methods. In contrast to

concerns raised by de Klerk and 't Hoen in 2015 [2] about the limitations of Illumina technology based on short read length mapping, our study yielded precise and consistent data with high coverage. In the case of an unexplained clinical picture, we are only left with the sequencing of wider regions for which we assume the presence of pathological variants, which prolongs the diagnostic process, but at the same time does not allow us to clarify the genopathy in the subject.

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EFFECTIVE GENE THERAPY WITH COMBINATION OF IL-2 AND IL-12 IN TWO DIFFERENT MURINE TUMOUR MODELS

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Immunotherapy has become an important approach in the treatment of cancer. One example is the use of cytokines such as interleukin 2 (IL-2) and 12 (IL-12), which stimulate immune cells directly in the tumour. In this way, the tumour microenvironment could be altered by recruiting and activating tumour-specific immune cells, leading to tumour eradication [1,2]. The application of the above cytokines was primarily in the form of recombinant proteins, but is nowadays being replaced by the gene therapy approach [3]. Electroporation represents a safe and effective method of introducing genetic material (gene electrotransfer) into the cell [4]. The aim of our study was to compare the antitumor effects of gene electrotransfer of plasmids encoding IL-2 (pIL-2) and IL-12 (pIL-12) to B16F10 and CT26 tumour models *in vivo*.

Tumour growth delay was measured and the concentrations of different immunostimulatory cytokines in tumour and serum samples were determined. Moreover, histology analysis was also performed in tumour samples to assess different immune cell populations inside the tumour and also to determine the extent of tumour vascularization. Tumour growth delay was observed in all therapeutic groups, the highest delay was observed in group with gene electrotransfer of both plasmids (combination group). Furthermore, complete tumour regression was observed only in the groups with gene electrotransfer of pIL-12 alone and in combination group. In the latter, long-term anti-tumour immunity after tumour rechallenge was also observed. After gene electrotransfer, increased expression of different cytokines with potent anti-tumour activity was detected, mainly in combination group. In addition, immune infiltration in tumour was the highest in combination group and more pronounced in B16F10 in comparison to CT26 tumour model. The antiangiogenic effect of the therapy was observed in the group with geneelectrotransfer of pIL-12 alone and in

combination group in both tumour models.

To conclude, we demonstrated the anti-tumour effectiveness of gene electrotransfer of IL-2 and IL-12 in murine B16F10 melanoma and CT26 colon carcinoma.

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WHOLE TRANSCRIPTOME EXPRESSION ARRAY ANALYSIS OF HUMAN COLON FIBROBLASTS CULTURE TREATED WITH *HELICHRYSUM ITALICUM* SUPPORTS ITS USE IN TRADITIONAL MEDICINE

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Helichrysum italicum (HI) is a Mediterranean plant with well-reported use in traditional medicine for a wide range of applications, including digestive and liver disorders, intestinal parasitic infections, wound healing, stomach ache and asthma [1]. However, little is known about the global mechanism behind its pleiotropic activity. The aim of our study was to explain the mechanism behind the previously demonstrated effects of HI and to justify its use in traditional medicine.

A microarray-based transcriptome analysis was used to discover the global transcriptional alterations in primary colon fibroblasts (CCD112CoN) after exposure to HI infusion for 6 h and 24 h. Microarray results were verified by quantitative real-time PCR. In addition, functional *in vitro* assays were performed to confirm the functional annotation of the differentially expressed genes.

Transcriptome analysis identified a total of 217 differentially expressed genes compared to non-treated cells, of which only 8 were common to both treatments. Gene ontology term enrichment analysis showed that 24 hour treatment with HI infusion altered the expression of genes involved in cytoskeletal rearrangement and cell growth, whereas pathway enrichment analysis further revealed the importance of interleukin signalling and transcriptional regulation by TP53. For the 6 hour treatment only the process of hemostasis appeared in the results of both enrichment analyses. In functional assays, HI infusion increased the migration potential of Caco-2 cells and conditioned media of CCD112CoN cells decreased blood clotting and prothrombin time, whereas HI infusion alone did not affect blood coagulation.

With the careful evaluation of the role of individual genes, especially SERPING1, ARHGAP1, IL33, and CDKN1A, represented in the enriched pathways and processes, we propose the main mode of HI action, which is wound healing. In addition to its

indirect prevention of diseases resulting from the impaired barrier integrity, HI also effects inflammatory and metabolic processes directly, by regulating genes such as LRPPRC, LIPA, ABCA12, PRKAR1A, and ANXA6 [2].

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DEVELOPMENT OF 3D SPHEROIDS FROM MURINE CELL LINE K7M2 AS MODELS FOR OSTEOSARCOMA TUMORS

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Osteosarcoma (OS) is a common primary bone cancer that mostly affects adolescent children between ages 10 to 14 and has a poor prognosis in case of metastatic disease. Current established therapy includes surgical amputation with adjuvant standard chemotherapy [1,2]. Due to no important advances in treatment for the last 50 years there is a need for advancement. One novel therapy, that could be used for the treatment of OS, is electrochemotherapy (ECT). ECT is a local tumor ablation therapy that combines a physical delivery method; electroporation, with cytotoxic drugs, such as bleomycin and cisplatin. It has already proved effective in treating tumors of different histological types, including bone metastasis of various primary tumors [3,4]. However, there is still a lack of information on its suitability to be employed as a treatment for osteosarcoma tumors.

Effects of ECT on osteosarcoma tumors can be studied on different preclinical models, from 2D cell cultures to homologous animal models. For that purpose, we developed 3D spheroids from murine cell line K7M2. Spheroids of different sizes (5000 and 10 000 cells per spheroid) were incubated for 1 or 2 weeks in a rotary incubator Clinostar® (CelVivo, Inc, Chevy Chase, MA, USA), specifically suited for 3D cell models. Spheroids were then electroporated at 2 different voltage to distance ratios. We measured the proportions of apoptotic and necrotic cells in spheroids and determined cytotoxicity at multiple time points after electroporation. Smaller spheroids, formed from 5000 cells and growing for 1 week, turned out to be a promising research tool, as they have a lower proportion of apoptotic and necrotic cells than other groups. However, additional molecular and genetic testing will be applied to further confirm, how suitable spheroids are as a model for evaluation of ECT effectiveness in treatment of OS tumors.

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DEVELOPMENT OF AN ARTIFICIAL VIROID INOCULATION METHOD FOR HOP TISSUE CULTURES

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Common hop (*Humulus lupulus L.*) is a herbaceous perennial plant in the botanical family Cannabacae [1]. In Slovenia hop plantations are threatened by various pathogens, including three different viroids: hop latent viroid (HLVd), hop stunt viroid (HSVd) and citrus bark cracking viroid (CBCVd). Viroids are of great economic importance, as they negatively affect the quality of hop cones. In general, the mass of the crop decreases, and the content of plant metabolites, which affect the aroma and taste of beer, also decreases. The presence of viroids negatively affects the rooting of cuttings, chlorosis, slower plant growth and even plant death can be observed [2].

Our goal was to examine a large number of hop cultivars for resistance, tolerance and sensitivity to infection with viroids (HLVd, HSVd and CBCVd), which threaten Slovenian hop farms.

During the experiment, 13 different hop cultivars were grown in in-vitro conditions: we prepared the culture medium, subcultivated the plants onto fresh culture media, and monitored the growth of the plants. Viroid transcripts were prepared, the quality of the transcripts validated with the Agilent Bioanalyser 2100 device, and then used for hop plants infection in tissue cultures. The growth and development of infected and uninfected plants was monitored for eight weeks. The success of viroid hop infection was confirmed using the RT-PCR method and agarose gel electrophoresis.

Our data showed that using our newly established method of viroid inoculation we have successfully infected all hop cultivars using viroid transcripts. Interestingly, the rate of infection was the highest for HSVd transcripts (100% for all 13 cultivars), followed by CBCVd (100% for 9 cultivars and 75% for 4 cultivars) and the lowest for HLVd (75% for 3 cultivars, 50% for 5 cultivars and 25% for 3 cultivars). Up to date, we did not observe any difference in growth and development of infected and uninfected plants and we did not discover any possible resistance or tolerance in tested varieties.

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GENE UPREGULATION IN SPECIFIC STAGES OF CAR T CELL-MEDIATED CYTOTOXICITY: A QUANTITATIVE REAL-TIME PCR APPROACH TO ENHANCE MONITORING OF CYTOTOXIC EFFICACY AND THERAPY PROGRESSION

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Chimeric antigen receptor (CAR) T cell therapy holds enormous potential for treating hematologic malignancies. Despite its advantages, it is still used as a second line of therapy mainly because of its severe side effects and/or patient unresponsiveness. To improve the therapeutic efficacy, a better understanding of the complex mechanisms behind effective tumor cell killing is necessary. The aim of this study is to identify upregulated genes at specific stages of the CAR T cell cytotoxic process with which the assessment and monitoring of the cytotoxic activity of CAR T cells and therapy progress with quantitative real-time PCR (qPCR) could be obtained.

After infusion into the patient, CAR T cells move through the bloodstream to the tumor sites. This process is enhanced by chemokines and adhesion molecules, with genes such as CXCR4, ICAM1, VCAM1, and SELL [1] possibly playing pivotal roles in ensuring the cells reach their intended destinations. Once in the tumor vicinity, CAR T cells identify specific tumor-associated antigens, a process governed by genes like CBP, Unc119, and IFT20 [2,3]. Upon antigen recognition, the CAR T cell, guided by genes involved in cell adhesion and signaling such as BACH2, EZH2, and TGF-β1 [4-6], attaches to the tumor cell and forms the immunological synapse, a unique junction between the CAR T cell and its target. To exert their cytotoxic effects on tumor cells, CAR T cells undergo a dynamic reorganization. Key cellular organelles, responsible for cytotoxic actions, migrate toward the immunological synapse (Golgi apparatus, mitochondria, etc.). The upregulated genes in these migrations are believed to be e.g., DISC1, GLUT1, MAPRE1, GMAP210, and others [7-9]. Actin filaments, influenced by the upregulation of e.g., BBS2 and ARPC1B [3,10] genes, bolster the structural integrity of the lamellipodium at the synaptic area. Concurrently, the centrosome, guided by genes associated with cytoskeleton organization such as MAP4 and CIP4 [11], aligns with the reconfiguring cytoskeleton. The culmination of these processes is the formation of lytic granules which are transported through the immunological synapse into target cell, where target cell apoptosis is induced. The upregulated genes in these final stages of target cell killing are e.g. HkRP3, SERPINB9, GZMB, GZMA, and PRF1 [6,11,12].

We have identified genes that are potentially upregulated during specific stages of the cytotoxic process, suggesting their potential use as markers for monitoring the cytotoxic efficacy of CAR T cells. The subsequent phase of our research will focus on testing this designated set of genes to evaluate their marker potential. With this knowledge, we would be able to monitor the cytotoxic processes and efficacy of the CAR T cells and consequently improve the course of this complex immunotherapy.

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UPREGULATION OF CYTOSOLIC PATTERN RECOGNITION RECEPTORS AFTER GENE ELECTROTRANSFER OF PLASMID ENCODING IL-12

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During gene therapy, therapeutic genes are inserted into cells and recognized by cell defense mechanisms as exogenous nucleic acids and consequently as pathogen-associated molecular patterns (PAMPs) [1]. Exogenous nucleic acids are then recognized by endosomal and cytosolic nucleic acid-specific pattern recognition receptors (PRRs) [2]. Activation of PRRs induces a specific immune response that may lead to antitumor effects [3]. One of the gene therapies that has already been proven to be safe and effective, having good local control and an abscopal effect in the treatment of melanoma skin metastases, is gene electrotransfer (GET) of plasmid DNA coding for cytokine interleukin-12 (IL-12) [4]. Plasmid DNA introduced into cells by GET could activate different cytosolic PRRs, therefore the aim of our study was to evaluate the effect of GET on plasmid DNA coding for IL-12 on PRRs.

Parallel electrodes with a 2-mm gap and a clinically used pulse protocol were used for GET; 8 times 1300 V/cm pulses of 100-microsecond duration at a frequency of 5 kHz were applied with CLINIPORATOR®. Plasmid DNA encoding murine IL-12 (1 mg/ml, pmIL12), noncoding backbone plasmid DNA (1 mg/ml, pScramble), and a plasmid coding for a green fluorescent protein (1 mg /ml pEGFP,) were used for GET into B16-F10 melanoma and CT26 colon carcinoma cells. Transfection efficiency was determined 2 days after the electrotransfer of pEGFP using fluorescence microscopy. Cell viability was determined 3 days after treatment using the PrestoBlue™ Cell Viability Reagent. The expression of 15 different PRRs (DNA sensors:DAI, IFI16, IFI204, DDX60, DHX9, DHX36, AIM2, cGAS, STING, DDX41, LRRFIP1, Ku70, and RNA sensors: MDA5, LGP2, RIG-I) was determined using qRT–PCR 4, 24 and 48 hours after treatment. Clinically used electroporation protocol led to 24 % of transfected B16-F10 cells and only about 6% of transfected CT26 cells. Cell viability was significantly reduced after IL-12 and non-coding DNA plasmid GET. Contrary to our expectation, GET of pmIL12 and noncoding pScramble mainly led to upregulation of RNA binding PRRs. Further studies are needed to elucidate underlying processes for such cellular response.

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DIGITALISATION OF BIODIVERSITY

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We are in a time of accelerated biodiversity loss and environmental degradation, and ex situ conservation and archiving of molecular samples are of immense importance as they represent important resources for future research, especially for the study of species under different scenarios (endangered species, non-native species, unpredictable occurrence, voucher specimens, etc.). Specimens must be collected in an appropriate manner, and storage and preservation of biological material must follow the best available practices. Biorepositories must ensure integrity, authenticity, and availability of the associated data to describe the stored samples in detail.

Quality checks were performed on archived DNA samples of *Mawia benovici*, a rare scyphozoan species described from the northern Adriatic Sea [1]. Tissue samples, DNA extraction, and amplification of several genetic markers were performed on 60 individuals in 2013. DNA was extracted using the commercial kit E.Z.N.A. Mollusc DNA Kit (Omega Bio-Tek) or KAPA Express Extract. In 2023, several parameters describing the quality of the archived samples were investigated and evaluated (DNA concentration, fragmentation, purity, DNA integrity) by spectrophotometric measurements (Nanodrop) and automated microfluidic-based electrophoresis (TapeStation System, Agilent). A re-examination of the quality criteria is required to meet the basic criteria for continued storage and to provide guidelines for maintaining and preserving the cnidarian specimens. Currently, the biorepository at the Marine Biology Station in Piran (Slovenia) includes 10 cnidarian species with 4272 records.

This contribution is part of the initiative LIFEWATCH-SI (co-financed by the Republic of Slovenia, the Ministry of Education, Science and Sport, and the European Union from the European Regional Development Fund) to promote the digitalisation of biodiversity and the principles of open science.

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AN AMPLITUDE-BASED MULTIPLEX DDPCR ASSAY FOR DIAGNOSIS OF HEREDITARY α-TRYPTASEMIA

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Hereditary α tryptasemia (HαT) is an autosomal dominant trait characterized by germline multiplication on one single allele in the *TPSAB1* gene encoding α-tryptase [1]. The determination of HαT is being discussed as an important biomarker to be included in risk assessment models and future diagnostic algorithms for patients with mastocytosis [2-4]. Patients with HαT are also prone to more severe anaphylaxis [1,5-7]. Therefore, tryptase genotyping should be considered in the clinical evaluation of individuals with a history of, or at risk for, severe anaphylaxis [8]. Due to the complex genetic structure at the human tryptase locus, genetic testing for HαT is presently notably limited and infrequently pursued [9,10]. The aim of this study was to develop, optimize and validate a multiplex droplet digital PCR (ddPCR) assay that can reliably quantify α- and β-tryptase encoding sequences in a single reaction well. Custom primers and probes targeting sequences encoding α- and β-tryptases were reported previously [1]. For the detection of reference copy number invariant locus, additional primers, and probes targeting *AP3B1* and *AGO1* genes were designed. To optimize the ddPCR conditions and establish an amplitude-based multiplex ddPCR assay, a thermal gradient with varying annealing temperatures, different primers/probe concentrations, and various initial DNA quantities were tested. The multiplex ddPCR performance was compared with separate duplex ddPCRs in 113 DNA samples. An annealing temperature of 60°C, DNA quantity of 55 ng and optimized primer and probe concentrations enabled a clear separation of positive droplets and clear, distinct differentiation of each target set within unique clusters. Results obtained from the multiplex ddPCR were concordant with those achieved with the duplex ddPCRs. Utilizing this multiplex ddPCR assay, in contrast to conducting distinct duplex ddPCRs, presents noteworthy benefits for tryptase genotyping. These advantages encompass a substantial threefold decrease in material costs and considerable time savings. Consequently, this approach exhibits a high degree of suitability and particularly captures interest for routine clinical implementation.

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RRADIATION RESULTS IN MOLECULAR AND TRANSCRIPTIONAL SHIFTS OF TUMOR ENDOTHELIAL CELLS

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Aberrant tumor vasculature is characterized by insufficient blood flow and oxygen supply, which leads to the formation of hypoxic regions, resistant to radiotherapy (RT) [1]. Irradiation (IR) kills cancer cells, but also affects the tumor microenvironment, including tumor vasculature. The effect of IR on tumor endothelium is time- and dose-dependent. Importantly, IR can cause tumor endothelial cell (TEC) apoptosis, but, can also lead to vascular remodeling/normalization or activation of endothelial cells (EC), thus possibly reducing hypoxia and enhancing the immune cell infiltration into tumors [2]. However, the impact of changes in tumor vasculature and TECs due to IR on tumor response to RT is yet to be elucidated. Hence, in this research, we aimed to clarify the vascular response to IR focusing on vascular remodeling and activation of ECs. We first irradiated human EA.hy926 and Hulec5a, and murine bEnd.3, 2H11, and SVEC4-10 EC lines with single doses of 0 – 10 Gy, which resulted in the reduction of proliferation and increased EC death, depending on the dose received. Next, we irradiated CT26 and MC38 colon carcinomas grown in Balb/c or C57Bl/6 mice, respectively with a single dose of 15 Gy or the fractionated dose of 5 x 5 Gy, which led to the reduction of tumor growth rate. RNA sequencing analysis of TECs isolated from irradiated tumors showed higher expression of genes (*Cd47, Vcam-1, Vwf, Il6, etc.*) and pathways related to ECs activation and immune response. Finally, frozen tumor sections of irradiated tumors were immunofluorescently stained for endothelial (CD31, MECA-79, and ERG), immune infiltration (CD4 and CD8), and transcription (IRF9) markers. To sum up, IR reduces EC proliferation and survival. Furthermore, a single dose of 15 Gy changes the TEC transcriptional profile and usage of pathways that support TEC activation and augment anti-tumor immune response.

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BIOINFORMATICS ANALYSIS OF RNA-seq DATA OF COLORECTAL CANCER AND LIVER METASTASES ELUCIDATES GENES ASSOCIATED WITH METASTASIZING OF COLORECTAL CANCER

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Colorectal cancer (CRC) is a significant cause of cancer-related morbidity worldwide, and patients with advanced CRC have a low five-year survival rate despite new treatment options. Most genetic events associated with tumor development occur early, leading to a need to define biomarkers responsible for malignant transformation and metastasis.

To identify differentially expressed genes (DEGs) associated with CRC metastasis, we analyzed RNA-seq data from primary tumors and liver metastases and performed pathway enrichment analysis (PEI) and protein-protein interaction analysis (PPI). By comparing DEGs to those from TCGA data, we identified 668 DEGs, performed PEI and PPI analysis. The identified DEGs contribute to the understanding of gene expression patterns involved in metastasizing of CRC. Genes *MMP1* and *MMP3*, were among the most highly differentially expressed, associated with degradation of the extracellular matrix are interesting candidates for further validation. Additionally, 20 other strongly differentially expressed, and highly significant genes that were only differentially expressed between CRC and liver metastases are candidates for further validation for involvement in development/maintenance of liver metastases. Our results could upon further validation provide new prognostic markers, or targets for treating advanced CRC.

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