Abstracts

TBIO-26. NON-CANONICAL OPEN READING FRAMES ENCODE FUNCTIONAL PROTEINS ESSENTIAL FOR CANCER CELLS
SURVIVAL
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The brain is the foremost non-gonadal tissue for expression of non-coding RNAs of unclear function. Yet, whether such transcripts are truly non-coding or rather the source of non-canonical protein translation is unknown. Here, we used functional genomic screens to establish the cellular bioactivity of non-canonical proteins located in putative non-coding regions of untranslated regions of protein-coding genes. We experimentally interrogated 553 open reading frames (ORFs) identified by ribosome profiling for three major phenotypes: 257 (46%) demonstrated protein translation when ectopically expressed in HEK293T cells, 401 (73%) induced proliferation, and 375 (68%) induced a viability defect when the endogenous ORF was knocked out using CRISPR/Cas9 in 9 human cancer cell lines. CRISPR silencing and start codon mutagenesis indicated that the biological impact of these non-canonical ORFs requires their translation as opposed to RNA-mediated effects. We functionally characterized one of these ORFs, G029442—renamed GREP1 (Glycinorexin Extracellular Protein-1)—as a cancer-implicated gene with high expression across many cancer cell types, such as gliomas. GREP1 can knockdown in >200 cancer cell lines reduced cell viability in multiple cancer types, including glioblastoma, in a cell-autonomous manner and produced cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic cytokine GDF15, and GDF15 supplementation mitigated the growth inhibitory effect of GREP1 knockout. Taken together, these experiments suggest that the non-canonical ORFeome is surprisingly rich in biologically active proteins and potential cancer therapeutic targets deserving of further study.

TBIO-27. RASOPATHIES AND BRAIN TUMOROGENESIS: ARE SOS1 MUTATIONS CONCERNED?
Nouha Bouayed1, Abdellah Abdelmoula1, Rim Loutati1, Balkiss Abdellah1, and Samir Alouid1. UR17ES36 Genomic of Signalopathies at the service of tumor progression. Two aunts developed blindness and then died subsequently to a familial history revealed other affected children by neurodevelopmental and epileptic disorders. During our genetic counselling for congenital heart disease, mutation c.1655 G>A was confirmed. This mutation affected the PH domain of SOS1. Heterozygous single nucleotide substitution of SOS1 gene: Braf and SOS1, was conducted using HRM analysis and bidirectional sequencing. In >200 cancer cell lines reduced cell viability in multiple cancer types, including glioblastoma. Therefore, we propose the use of Delta-24-AT as a therapeutic approach for glioma tumours. We observed that Delta-24-AT is able to infect and replicate in human glioblastoma cells. As recently performed in the cell-autonomous manner and produced cell cycle arrest via single-cell RNA sequencing. Analysis of the secretome of GREP1-expressing cells showed increased abundance of the oncogenic cytokine GDF15, and GDF15 supplementation mitigated the growth inhibitory effect of GREP1 knockout. Taken together, these experiments suggest that the non-canonical ORFeome is surprisingly rich in biologically active proteins and potential cancer therapeutic targets deserving of further study.

TBIO-28. MUTATIONS ARE CONCERNED?
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THER-01. AWAKENING THE IMMUNE SYSTEM WITH AN IMMUNO-ONCOLYTIC VIRUS AS A THERAPEUTIC STRATEGY FOR DIPGs
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Diffuse intrinsic pontine glioma (DIPG) is an aggressive brain tumour, being the leading cause of paediatric death caused by cancer. Despite all the advances made regarding effective therapies, the survival is dismal. Our lab has engineered the oncolytic virus Delta-24-AT armed with the costimulatory ligand 41BBL in order to increase the anti-tumoral effect of the administration. 41BBL is a costimulatory ligand that promotes the expansion of activated T cells and the generation and maintenance of CD8 T memory cells. Therefore, we propose the use of Delta-24-AT as a therapeutic approach for DIPG tumours. We observed that Delta-24-AT is able to infect and replicate in DIPG cell lines, producing cell death. Mechanistic experiments, showed an increase of T cell infiltration (mainly CD8), decrease of proliferating cells and a reduction of the number of vessels in FFPE brain samples in xenotransplanted mice. We are currently performing a transplantation study in a mouse model of DIPG to assess the changes in the transcriptional immune phenotype of treated versus control mice. In summary, our data suggest that Delta-24-AT is safe and induces a potent antitumor immune response in DIPG models mainly based in the activation of CD8 lymphocytes recruited by the viral particle.

THER-02. EVALUATION OF THE ONCOLYTIC VIRUS DELTA24-RGD AS AN ANTI-TUMOR AGENT IN PRECLINICAL MODELS OF LOCALIZED AND DISSEMINATED AT/RT
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Current therapies for atypical teratoid/rhabdoid tumours (AT/RTs) are suboptimal, resulting in a 2-year OS below 20% and the development of severe side effects. Therefore, we need to explore alternative therapeutic approaches for this disease. Since the virus Delta24-RGD has already demonstrated its efficacy and safety as a therapeutic agent for brain tumours, including pediatric patients, here we propose to evaluate the anti-tumor effect of Delta24-RGD in AT/RT. In vitro, Delta24-RGD infects and replicates in AT/RT cultures followed by oncolysis, obtaining IC50 values below 1 PFU/cell. In vivo, a single local injection of Delta-24-RGD in three intratrabecular AT/RT models (BT-12, CHLA-06 and CHLA-266) extended significantly the median OS (50 to 78 days BT-12; 21 to 31 days CHLA-06; 64 to 110 days CHLA-266). Delta-24-RGD also increased the survival of mice bearing supratentorial CHLA-266 tumors (from 93 to 132 days). Next, we evaluated the efficacy of Delta24-RGD in a model mimicking metastatic disease through intraventricular injection of BT-12-luciferase cells. Administration of Delta24-RGD inhibited tumor growth and development of metastases, leading to an increased OS and nearly 70% of long-term survivors. The interaction between Delta24-RGD and the immune system was evaluated in humanized mice models bearing CHLA-06. In this model, Delta24-RGD treatment extended OS from 23 to 34 days and we characterized the anti-tumor immune landscape in control and treated mice. Our results show that Delta24-RGD as a promising therapeutic option for patients affected by AT/RT.

THER-03. IN VITRO EVALUATION OF THE EFFECT OF CANNABIDIOL ON PEDIATRIC BRAIN TUMOUR CELL LINES USING A PULSED TREATMENT REGIME
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Pediatric brain tumours are the second most common cancer after haemato-oncological malignancies. Intermittent dosing regimens are typical for chemo-