with resistance to therapy and poor prognosis. KRAS is still not directly druggable, therefore current therapeutic strategies for KRAS mutant cancers aim at identifying susceptibilities in downstream signalling pathways. One unresolved aspect of KRAS biology with potential to translate into patient stratification criteria is the difference between distinct KRAS activating mutations in terms of downstream signalling and drug sensitivity. Understanding the biochemical and biological differences among specific KRAS mutants is essential to discover new actionable vulnerabilities for mutant KRAS.

**Material and methods** To study the role of different KRAS mutants in a controlled and reliable genetic setting, we developed an isogenic KRAS MUT inducible system that lacks endogenous HRas/NRas and harbours conditional CREERT2, controlled KRaslox alleles (KRaslox KRAS MUT system). This system allows direct and robust comparison between different KRAS oncogenic isoforms and rigorous evaluation of the in vitro and in vivo impact on tumour progression and response to MEK/ERK inhibition.

**Results and discussions** Our data confirm differential GTP-hydrolysis properties among different KRAS mutants (G12C, G12D, G12V, G12A, G13D and Q61H). This intrinsic feature is translated into distinct proliferation rates in vitro and in vivo, as well as differential responsiveness to MEK/ERK inhibitors in specific KRaslox KRAS MUT cell lines, with up to 12-fold IC50 variability. Interestingly, the Q61H mutant, known to exhibit the lowest intrinsic GTP-hydrolysis rates, is the most sensitive to MEK/ERK inhibition, suggesting that intrinsic biochemical properties of specific KRAS mutants affect drug response. Moreover, in our KRaslox KRAS MUT cell lines expressing specific KRAS mutants, we observed differential reactivation of upstream RTKs in response to MAPK inhibition, suggesting the existence of a functional crosstalk between specific KRAS mutations and upstream receptors that may be modulated by oncogenic signalling, with potential implications on drug resistance mechanisms.

**Conclusion**

- KRAS specific mutants retain unique GTP-hydrolysis features
- KRAS specific mutants have distinct growth properties in vitro and in vivo
- KRAS specific mutants show differential responsiveness to MEK/ERK inhibitors in vitro and in vivo.

**Senescence**

**PO-210 ANTICANCER DRUG-INDUCED SENESCENCE IN Glioblastoma Cells Is Associated With Changes in DNA Repair Capacity**

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10.1136/esmoopen-2018-EACR25.245

**Introduction** Despite extensive research, malignant glioma remains the most aggressive and fatal type of brain tumour. Following resection, therapy is based on radiation concomitant with the methylating agent temozolomide (TMZ), followed by adjuvant high dose TMZ treatment. The success of glioma therapy depends largely on the DNA repair capacity of the tumour cells, notable repair by MGMT, which confers resistance to TMZ. Beside DNA repair, other mechanisms involved in tumour protection may also play a role. These include the induction of a transient cell cycle arrest that provides time for repair and the induction of an irreversible cell cycle arrest in the form of senescence.

**Material and methods** MZ-induced senescence and repression of DNA repair was analysed in LN229, U87, LN308 and U138 glioblastoma cells using different techniques, including immunodetection, RT-qPCR, ChIP, Co-immunoprecipitation, ELISA, β-Gal staining, C12FDG staining, AnnexinV/PI staining, flow cytometry, pharmacological inhibition and si-RNA mediated knockdown.

**Results and discussions** We show that following single and chronic TMZ exposure most glioma cells evade apoptosis and enter a senescent state. TMZ-induced senescence occurs in the G2/M phase of the cell cycle and is initiated by the ATR/CHK1-mediated degradation of CDC25c. It is further sustained by activation of p21 and NF-kB. Analysing the transcriptional regulation of DNA repair factors upon TMZ exposure, we found a strong repression of the mismatch repair (MMR) proteins MSH2, MSH6 and EXO1 as well as of Rad51, the central component of the homologous recombination pathway. The repression of these genes was regulated by the disruption of the E2F1/DP1 complex and is a specific trait of TMZ-induced senescent cells.

**Conclusion** Repression of DNA repair in senescent cells may result in acquired drug resistance to TMZ and could render these cells susceptible to the accumulation of additional genomic alterations induced by anticancer therapy. In case of an escape from senescence, these genetically altered cells could contribute to increased aggressiveness and therapy resistance in a recurring tumour.

**PO-211 PROFILING THE ALTERNATIVE SPLICING LANDSCAPE OF SENESCENT CELLS**

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10.1136/esmoopen-2018-EACR25.246

**Introduction** Cellular senescence, defined by an irreversible cell cycle arrest in response to potentially oncogenic stimuli, has been described as a protective mechanism in tumourigenesis and a therapeutic target in cancer. The senescence-associated secretory phenotype (SASP) is a pro-inflammatory response by senescent cells involving the release of cytokines, chemokines, growth factors and proteases that, in a cancer progression context, may be beneficial by the elimination of senescent cells or deleterious when triggering angiogenesis, cell proliferation and epithelial-tomesenchymal transition. Despite senescence’s importance in cancer and the suggested role of alternative splicing in its regulation, the transcriptional heterogeneity of senescent cells has, to our knowledge, been extensively characterised only at the gene expression level.

**Material and methods** Next-generation sequencing of RNA (RNA-seq) allows alternative splicing quantification with unprecedented precision. The inclusion level of an exon is commonly quantified by its percent-spliced-in (PSI) value, i.e. the proportion of RNA-seq reads providing evidence supporting its inclusion. However, a PSI ratio does not incorporate information about the number of reads used in the quantification of the cognate alternative splicing event, directly proportional to the precision of its estimate. Beta distributions can be exploited in modelling inclusion levels, using reads supporting exon inclusion and exclusion as surrogates of the distribution’s shape parameters. We employed a computational
pipeline, based on fitted beta distributions, to accurately quantify and compare alternative splicing across different types of senescent cells, relying both on public and in-house RNA-seq datasets.

**Results and discussions** Our analyses reproducibly identified, at a transcriptome-wide level, the alternative splicing changes specifically related with replicative and different types of induced senescence in multiple types of cells. For instance, Ras-induced senescence appears to associate with alterations in the splicing of genes involved in the secretory pathway and intracellular trafficking.

**Conclusion** Differential splicing analyses based on beta distribution modelling contribute to elucidate the specific alternative splicing signatures of different types of senescent cells, providing insights for targeting senescence in cancer therapies.

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### Cancer Cell Metabolism

**PO-212 MORPHOLOGICAL HETEROGENEITY OF HEPATOSPLENIC GAMMA/DELTA T-CELL LYMPHOMA**

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**Material and methods** All patients were male and presented at a mean age of 23 years. One was treated with azathioprine for inflammatory bowel disease, the two others had no history of immunosuppression. All patients were admitted to the hospital with high fever and hepatosplenomegaly. Two presented severe abdominal pain and lymphadenopathy.

Laboratory studies of the three patients were different:

- **Case 1:** thrombocytopenia and bone marrow infiltration with 69% of large lymphomatous blast cells and minimal peripheral blood infiltration.
- **Case 2:** pancytopenia without excess of blasts in the peripheral blood and moderate bone marrow infiltration in cytometry. Morphological and immune-phenotypical studies of the spleen (after splenectomy) showed a massive infiltration by lymphocytic small cells.
- **Case 3:** pancytopenia with 25% and 45% of blast cells in the peripheral blood and bone marrow aspirate respectively.

Immunophenotyping showed a T lymphocytic population double negative (CD3pos CD4neg CD8neg to dim) with gamma-delta expression, CD1a- CD2 +CD5 CD7+CD56 +CD57 in all 3 patients.

Two patients underwent an initial treatment with Cyclophosphamide, Doxorubicin, Vincristine and Prednisone: the first died after 4 months, and there is no response to treatment in the second patient. Treatment was recently initiated in the third patient.

**Results and discussions** In our series, HSTL confirmed a predilection to develop most often in young men with hepatosplenomegaly. Variable degrees of hematologic abnormalities were observed. Thrombocytopenia was the most striking finding in all. Bone marrow involvement is described in approximately two thirds of patients but was observed by immunophenotyping in our 3 cases. We show that immunophenotyping seems to be the best method for the rapid characterisation of the lymphoma cells morphologically heterogeneous and difficult to identify.

**Conclusion** HSTL is an infrequent, rare aggressive tumour. The diagnosis is difficult. There is no treatment consensus and the prognosis remains poor.

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**PO-213 HIGH GLUCOSE AFFECTS ER+ BREAST CANCER CELL METABOLISM**

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**Material and methods** MCF-7 breast cancer cell line were cultured in DMEM with high glucose (HG 25 mM) and low glucose (LG 5 mM). Metabolic fluxes analyses were performed with Seahorse Bioanalyzer. Live cell imaging for reactive oxygen species (ROS) content was performed by confocal microscopy by using DCF-DA as selective probe. Mitochondrial DNA (mtDNA) and protein expression were evaluated by qPCR and western blotting respectively.

**Results and discussions** Using a metabolic fluxes analyses, we showed a significant reduction of the mitochondrial oxygen consumption rate (OCR) and glycolysis-related extracellular acidification rate (ECAR) in MCF-7 cultured in HG as compared in LG-medium. According with these results, MCF-7 in HG displayed lower mtDNA amount and increased ROS level. Furthermore, the analysis of stemness markers revealed a significant upregulation of Nanog, Lin28 and Myc thus suggesting an increased stem-like phenotype due to growth in HG.

**Conclusion** Overall our results indicate that glucose may foster breast cancer progression promoting stem cell-like phenotype strongly affecting the metabolic profile in MCF-7 cell line. Further investigations are ongoing to define the mechanism underlying the switch towards an undifferentiated state to be exploited as therapeutic target.

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**PO-214 COMBINATION OF MOLECULAR HYDROGEN (H2) AND 5-FUOROURACIL (5-FU) IN CANCER TREATMENT**

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**Introduction** Oxidative stress is clearly recognised as involved in cancer development, as H2 is clearly recognised as a patent