SHH TP53 mutated cell lines regarding proliferation and apoptosis.

Material and methods
Expression of YAP in MB was assessed in 763 medulloblastoma patients trough in silico analysis utilising R2 genomic data base. It was utilised the cell lines DAOY and UW228 as TP53 mutated and ONS-76 as TP53 Wild Type. Cytoplasmatic and Nuclear detection of YAP was performed trough Immunofluorescence. Verteporfin, a YAP inhibitor was purchased from Sigma Aldrich. CCK8 Proliferation assay was perfomed in 48 hours incubating cells with concentrations ranging from 2.5 μM to 15 μM. Apoptosis and necrosis was assessed trough High content screening assay (HCS) trough Propidium Iodide and Annexin-FITC. Statistical analysis was performed trough One-way ANOVA followed by Bonferroni. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee.

Results and discussions
We have found an overexpression of YAP in MB SHH compared to other molecular subgroups and predominantly in SHH TP53 mutated subtype (alpha subtype). Cytoplasmatic and Nuclear YAP was identified in all cell lines. It was found a decrease in cell proliferation when incubated with Verteporfin. Interestingly, in 10 μM concentration, DAOY showed lower proliferation ratio (0.37) (p<0.05) compared to ONS-76 (0.47) (p<0.05). However, in UW228 it was identified a proliferation ratio of (0.88) (p<0.05). DAOY and UW228 bears distinct TP53 mutation site, which might explain its different behaviour when exposed to compound. HCS showed an increase in apoptosis and necrosis levels in DAOY and ONS-76 cell line when incubated with Verteporfin.

Conclusion
This initial screening with Verteporfin lead us to primary conclusion such as the role of YAP promoting cell proliferation and cease apoptosis. However, the biological role of YAP still remains to be explored in MB SHH TP53.
a new angle in the MTR approach, by administering an oral pro-drug of gemc, Oral Gem, to improve gemc’s therapeutic properties, but also cover patients’ quality of life.

**Material and methods** The A549 lung cancer cell line was used to establish an *in vitro* model that simulated the MTD versus the MTR conditions. Cells were cultured either in presence of a high concentration of gemc or in medium in which lower concentrations were added daily in order to study alterations in the expression of various angiogenic factors. Additionally, an *in vivo* xenografted animal model was set up to study the effects of MTR chemotherapy on tumour’s expansion, toxicity of the drug and angiogenesis.

**Results and discussions** Daily addition of gemc in A549 cells led to a decreased expression of VEGFA, a well-established angiogenic factor, compared to the high dose incubation. In NOD/SCID xenografted mice, the MTR administration of Oral Gem led to a decreased expression of VEGFA and CD31, a marker found on endothelial cells, suggesting a suppressed angiogenic profile. Finally, MTR administration of Oral Gem led to an increase in the expression levels of Thrombospondin-1, an anti-angiogenic factor, compared to MTD chemotherapy.

**Conclusion** MTR administration of Oral Gem limits the formed vessels around the tumour combining restriction of angiogenesis and vessel normalisation. In contrast, MTD chemotherapy seems to enhance the angiogenic potential around the tumour site, serving tumour’s establishment and expansion.

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**PO-445**

**E7107 TREATMENT RESULTS IN ABERRANTLY SPliced TRANSCRIPTS AND PROTEIN PRODUCTS OF P53 PATHWAY GENES**

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**Introduction** Recent studies indicate that E7107, a spliceosome inhibitor, causes altered splicing of key genes in CLL. We evaluated the effect of E7107 on cell viability and key proteins in the p53 pathway.

**Material and methods** Eight leukaemia/lymphoma cell lines and eight primary CLL samples (6 wild-type and 2 mutant for SF3B1) were exposed to E7107 (H3 Biomedicine) for 72 and 48 hours. Cell viability was assessed by XTT assay. To understand the effect of splicing modulation on key proteins in the p53 pathway, including p21 and MDM2, five B-cell lines were treated with E7107 for 24 hours.

**Results and discussions** E7107 decreased cell viability at low nanomolar concentrations in all CLL samples (mean LC50=10.5±2.0 nM; but >300 nM in two healthy PBMC controls). No correlation between drug sensitivity and SF3B1 status. This was associated with the production of an aberrant high molecular weight isoform of p21 due to intron retention, and a short isoform of MDM2 missing exons 3–10. Loss of normal MDM2 was accompanied by increased p53. Further investigation is needed to understand the contribution of abnormal isoforms to cell fate.

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**PO-446**

**ANTI-TUMOUR EFFICIENCY OF 20A, A NOVEL G-QUADRUPLEX LIGAND, IN IN VITRO AND IN VIVO CANCER MODELS: ATM AND AUTOPHAGY INTERPLAY**

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**Introduction** G-quadruplexes are non-canonical DNA structures that can be stabilised by molecules called G-quadruplex ligands (G4L). G4L were first found to target telomeres and inhibit telomerase activity in cancer cells, limiting cell proliferation and making them attractive for cancer therapy. However, it has been proposed that some G4L can exert anti-tumour properties through telomerase-independent mechanisms. In this line, we investigated the underlying molecular mechanisms and anti-tumour effects of a novel G4L (20A) in several cancer models.

**Material and methods** We assessed the *in vitro* anti-proliferative capacity of 20A in cervical carcinoma (HeLa), lung adenocarcinoma (A549), and osteosarcoma (Saos-2) cell lines by measuring cell viability, senescence and apoptosis. We used two *in vivo* cancer models: HeLa-cell subcutaneous and A549-cell lung orthotopic xenograft mouse models. Mice were treated by peri-tumoral and intraperitoneal 20A injections, respectively.

Activation of the DNA Damage Response (DDR) and autophagy pathways were assessed by western blot analysis of the DDR kinase ATM phosphorylation and the autophagic marker LC3-II accumulation, respectively. Targeted genetic depletion of ATM and autophagic genes (ATG5 and ATG7) allowed analysing their role in 20A cytotoxicity.

**Results and discussions** We found that 20A treatment causes a significant and rapid cell growth inhibition in three different types of cancer cell lines. This effect is associated with senescence induction and apoptosis through a p53-independent