SHH TP53 mutated cell lines regarding proliferation and apoptosis. **Material and methods** Expression of YAP in MB was assessed in 763 medulloblastoma patients through *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 through Immunofluorescence. Verteporfin, a YAP inhibitor was purchased from Sigma Aldrich. CCK8 Proliferation assay was performed in 48 hours incubating cells with concentrations ranging from 2.5 μM to 15 μM. Apoptosis and necrosis was assessed through High content screening assay (HCS) trough Propidium Iodide and Annexin-FITC. Statistical analysis was performed through 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 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 prodrug 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.

PO-445 E7107 TREATMENT RESULTS IN ABERRANTLY SPliced TRANSCRIPTS AND PROTEIN PRODUCTS OF P53 PATHWAY GENES

1E Aptulahagho*, 2P Wallis, 3H Marr, 4S Marshall, 5E Willmore, 6J Luneu, 7Newcastle University, Northern Institute for Cancer Research, Newcastle upon Tyne, UK; 8Freeman Hospital, Department of Haematology, Newcastle upon Tyne, UK; 9City Hospitals Sunderland NHS Trust, Department of Haematology, Sunderland, UK

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 was observed in CLL samples (p = 0.5). Six out of eight cell lines were sensitive to E7107 (mean GI50 = 6±1.8 nM). The GI50 values were 60.2 and 203.5 nM for the resistant HEL and HAL-01 cells, respectively. The most frequently mutated regions of the SF3B1 gene, exons 14, 15 and 16, had no mutations detected by Sanger sequencing. There was no correlation between drug sensitivity and TP53 status.

Western immunoblot revealed a marked decrease of MDM2 protein level in all cell lines, accompanied by a reciprocal concentration-dependent increase in p53. The normal molecular weight p21 disappeared at higher doses of E7107, with concomitant appearance of a high molecular weight p21 isoform (~30 kDa) in TP53 wild-type cells. In addition, a lower molecular weight p53 isoform was seen in OCI-Ly3 cells treated with 50 nM E7107, likely due to alternative splicing. RT-PCR of p21 mRNA, spanning exons 3 to 4, revealed an additional longer transcript in OCI-Ly3 cells treated with E7107 (50 nM, 24 hours), which sequencing confirmed was due to intron retention, predicted to result in a high molecular weight protein with an alternative c-terminal sequence. RT-PCR of MDM2 mRNA spanning exons 1 to 11 detected an altered MDM2 transcript with exons 3–10 spliced out.

Conclusion E7107 treatment resulted in decreased viability of B-cell lines and primary CLL samples independently of 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.

PO-446 ANTI-TUMOUR EFFICIENCY OF 20A, A NOVEL G-QUADRUPLEX LIGAND, IN IN VITRO AND IN VIVO CANCER MODELS: ATM AND AUTOPHAGY INTERPLAY

1J Beaumarie*, 2P Ben Sadoun, 3G Labrunie, 4RN Das, 5E Richard, 6B Rosbo, 7E Darbo, 8S Croce, 9J Mergy, 10M Djavanheri-Mergy. 1Institut Bergonié, Inserm U1218, Bordeaux, France; 2Institut Européen de Chimie et Biologie, Inserm U1212, Pessac, France; 3University of Bordeaux, Animalerie A2, Bordeaux, France; 4University of Bordeaux, Centre de Bioinformatique de Bordeaux, Bordeaux, France; 5Institut Bergonié, Department of Pathology, Bordeaux, France

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