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.

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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.
mechanism. Moreover, we show that 20A significantly reduces tumour growth in both in vitro and in vivo cancer models used.

To gain further insight into the molecular mechanisms involved in 20A cytotoxicity, we performed transcriptomic analysis which revealed functional enrichment in DDR and autophagy pathways upon 20A treatment. We confirmed these data by western blot and found that ATM is required for 20A-induced autophagy. Finally, disruption of both DDR and autophagy compromised senescence onset and increased apoptosis.

Conclusion In this study, we revealed the anti-tumour properties of this new G4L 20A in diverse in vitro and in vivo cancer models. We also show that targeting the ATM/autophagy pathway could potentiate 20A therapeutic efficiency. To our knowledge, this is the first evidence of the pivotal role of the ATM/autophagy axis in the decision between senescence and apoptosis in response to a G4L. Together, our data support further studies about 20A as a potential anti-cancer drug.

Conclusion To our knowledge, this optimised cell lysate AlphaLISA assay provides the first report and proof of concept for the interrogation of PROTAC-induced complex formation of full-length proteins with E3 ligases, which will be a valuable tool to the burgeoning PROTAC field. Consideration of assay parameters that permit optimal conditions for ternary complex formation in a POI-dependent manner allows the application of the AlphaLISA method as a screening platform. This application could profile PROTAC efficiency for a wide range of full-length, cell-derived proteins that are more relevant to the endogenous context encountered by the PROTAC intracellularly.

Poster Presentation: Experimental/Molecular Therapeutics, Pharmacogenomics

PO-449 OPTIMISATION OF AN ALPHALISA ASSAY FOR THE CHARACTERISATION OF PROTAC-INDUCED TERNARY COMPLEXES WITHIN CELL LYSATES

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Introduction Proteolysis Targeting Chimaeras (PROTACs) offer a powerful strategy to degrade cancer-associated proteins. PROTACs recruit an E3 ubiquitin ligase, such as von Hippel-Lindau (VHL), to a protein of interest (POI) to form a ternary complex. Measuring the formation of this ternary species is important for understanding PROTAC mode of action. Proximity AlphaLISA assays can be used for this purpose but have, to date, focused on using purified recombinant protein in vitro. Here, we report the development and optimisation of an AlphaLISA assay for monitoring ternary complex formation using full-length POI within cell lysates.

Material and methods AlphaLISA assays were used to profile PROTAC-induced complex formation of FLAG-tagged full-length POI in cell lysate to purified biotinylated VCB. Signal was measured from close proximity of FLAG-tagged acceptor beads to streptavidin donor beads and benchmarked against a well-characterised PROTAC, MZ1, purified His-tagged Brd4 bromodomain 2 (BD2) and biotinylated VCB. A FLAG-tagged full-length POI containing an insert of Brd4 BD2 (POI-fusion) was utilised, such that MZ1 could be employed as a tool compound for assay development.

Results and discussions Signal intensity was dependent on total lysate protein and VCB concentration, allowing for optimisation of component concentrations for maximal signal. A mild, detergent-free hypotonic cell lysis buffer gave enhanced signal over harsher buffers containing detergents. Additionally, the cell lysate cytosolic fraction provided superior signal compared to nuclear fractions and whole cell lysates, further highlighting the reliance of transient ternary complex formation on assay conditions. By utilising these optimal assay conditions determined with the POI-fusion, it is now possible to detect ternary complex formation between POI-targeting PROTACs and full-length POI.

Conclusion Two B RAF V600E and p53 WT melanoma cell lines, A375 and WM35, were treated with either trametinib or MDM2 inhibitors, or combinations.

Results and discussions The combination treatment in both A375 and WM35 showed additive to synergistic effects by Chou-Talalay median effect analysis and combination index scores. The combination treatments induced higher levels of p53 target gene transcripts (CDKN1A(p21), MDM2, BAX, BBC3(PUMA), TP5313(PIG-3)) and protein products (p21, MDM2), resulting in increased cell cycle arrest and apoptosis compared with MDM2 inhibitors alone, suggesting that trametinib synergized with MDM2 inhibitors via upregulation of p53-dependent pathways. In addition, DUSP6 phosphatase mRNA and protein were down-regulated following pERK reduction by trametinib, but did not change after p53 activation by MDM2 inhibitors. Furthermore, suppression of DUSP6 by siRNA, or inhibition with the small molecule inhibitor, BCI, potentiated the effect of MDM2 inhibitors through increased ATM-dependent p53 phosphorylation, as demonstrated by complete reversal with the ATM inhibitor, KU55933.

Conclusion Trametinib synergizes with MDM2 inhibitors through a DUSP6 mechanism in B RAF V600E and p53 WT melanoma cells. DUSP6 regulation of p53 phosphorylation via ATM, could be a therapeutic target for combination with treatments involving activation of the ATM/p53 pathway.