POSTER SESSION

Cytotoxics (including Antimetabolites, Anthracyclin, Alkylating agents, Aurora kinases, Polo-like kinase, Topos-isomerase inhibitors, Tubulin-binding compounds)

182 (PB062)

N-1,2,3-Triazole-Isatin derivatives in lymphoma cell lines

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Background: Molecular hybrid constructs are an interesting approach to merge individual pharmacophores with different mechanisms of action, potentially decreasing side effects. The 1,2,3-triazole unit is present in many bioactive compounds and it is characterized by its ability to be stable towards hydrolysis to increase the compounds lipophilicity. Hybrids containing this pharmacophore together with isatin and its analogues have shown a wide spectrum of potential therapeutic activities, also against cancer. Burke et al. have recently reported new *N*-1,2,3-triazole-isatin hybrids with *in vitro* anti-tumor activity in solid tumor cell lines (RSC Medicinal Chemistry 2022; EP3400938). Here, we present the *in vitro* anti-lymphoma activity and structure activity relationships (SAR) of 9 *N*-1,2,3-triazole-isatin hybrids.

Methods: Anti-proliferative activity assessed by 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide (MTT) assay at 72 h. Cell cycle assessed by FACS. IC_{50} values defined as the concentrations corresponding to 50% viability inhibition.

Results: Cell lines derived from diffuse large B-cell lymphoma (DLBCL) of the germinal center B-cell-like (GCB) type (DOHH-2, VAL) and of the activated B-cell-like (ABC) type (OCI-LY-10, SU-DHL-2) were exposed to increasing concentrations of 9 chemically modified oxindole derivatives (Table 1). While 6 compounds did not show any activity at concentrations <20 μM, compounds (1a-c) were active, with IC_{50}s lower in ABC- than in GCB-DLBCL. Specifically, compound (1c), which carries a methyl group in the 5-position of the aromatic ring of the isatin scaffold, was the most active. The chiral non-racemic *N*-1,2,3-triazole-oxindole derivatives (2) did not show activity. Compounds (1a) and (1c) (10 μM; OCI-LY-10; 48, 72 h) induced an accumulation of cells in the sub-G_0 phase, suggestive of the induction of cell death, slightly higher with (1c) than with (1a), in agreement with the IC_{50}s. In terms of SAR, compounds with the free carbonyl unit in the 3-position, i.e. (1a)-(1c) gave the best results against the ABC-DLBCL cell lines. Since both (1a) and (1c) were more active than (1b), the *N*-benzyl unit might also be important in determining anti-tumor activity.

Table 1. Chemically modified oxindole derivatives and their IC_{50} values obtained in DLBCL cell lines. Compound names according to Burke *et al*., RSC Medicinal Chemistry 2022. IC_{50} values in μM.

Compound	DOHH2	VAL	OCI-LY-10	SU-DHL-2
(1a)	>20	>20	10	0.75
(1b)	>20	>20	7	15
(1c)	10	>20	5	0.75
(*S*)-(2a)	>20	>20	>20	>20
(*R*)-(2b)	>20	>20	>20	19
(*R*)-(2d)	>20	>20	>20	>20
(*S*)-(2f)	>20	>20	>20	>20
(*S*)-(2i)	>20	>20	>20	>20
(*R*)-(2j)	>20	>20	>20	>20

Conclusions: *In vitro* anti-tumor activity in ABC-DLBCL models was observed for specific *N*-1,2,3-triazole-isatin hybrids, indicating that their structures represent the starting point to design compounds with stronger activity. *AJB and FB: co-senior authors.*

Conflict of interest:
Ownership: Patent EP3400938

183 (PB063)

The antimetabolite KAT/3BP has in vitro and in vivo anti-lymphoma activity

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Background: Reprogramming of cellular metabolism is one of the hallmarks of cancer (Hanahan, 2022), thus representing an important therapeutic target. 3-bromopyruvate (3BP or KAT/3BP) is a small, highly reactive molecule formed by the bromination of pyruvate (Ko et al, 2012). The very high similarity of its structure with pyruvic acid and lactic acid is the basis of its mechanism of action as an anti-cancer agent. Indeed, 3BP enters cancer cells via monocarboxylic acid transporters and it can then inhibit glycolysis and oxidative phosphorylation process. KAT/3BP has received FDA Orphan Drug Designation for different solid tumors and is about to enter the early controlled clinical evaluation. Here, we present *in vitro* and *in vivo* assessments of KAT/3BP in lymphoma models.

Materials and Methods: Cell lines were exposed to increasing concentration of KAT/3BP by MTT assay for 72 h. Apoptosis and cell cycle were evaluated by FACS. BALB/c mice (A20 cells in the left flank; 5 mice/group) were treated (4 weeks on, 1 off) with: oral (PO) and intratumoral (IT) vehicles, PO KAT/3BP [2.5 (low), 10 mg/kg (high)], IT KAT/3BP [0.5 (low), 2 mM (high)], PO-low/IT-low, PO-high/IT-high.

Results: KAT/3BP showed dose-dependent anti-proliferative activity in cell lines derived from diffuse large B-cell lymphoma (DLBCL; n = 8) and mantle cell lymphoma (MCL; 4), with median IC50 s of 8 μM and 5.5 μM, respectively. The compound was also tested in marginal zone lymphoma (MZL; 2) cell lines and in their derivatives with acquired resistance to idelalisib (2), ibrutinib (1), and copanlisib (1). 3BP was equally active in parental and resistant cell lines. KAT/3BP (5 μM, 72 h) was able to induce strong apoptosis in cell lines (1 DLBCL, 1 MCL) already after 24 h of treatment.

An *in vivo* pilot experiment using the murine syngeneic model (A20 lymphoma cells, BALB/c mice) confirmed the *in vitro* observed anti-tumor activity. All mice in the control groups died after nearly 20 days. A tumor reduction compared to the control vehicle was observed in all the treatment groups based on slope values extrapolated by a linear regression model. In particular, PO-high KAT/3BP lead to complete tumor reduction in 3/5 mice (2 still alive at D92, 1 dead at D36). After D26, also 1 mouse in IT-low and 1 in PO-low/IT-low survived and showed reduced tumor mass. Peripheral and focal tumor necrosis was seen in the tumor in PO-high/IT-high, PO-high, and IT-high (1 each). Necrosis was more extensively observed at histology in IT-high and PO-high/IT-high groups.

Conclusions: KAT/3BP showed *in vitro* activity in MCL, DLBCL, and MZL, including models resistant to PI3 K/BTK inhibitors. *in vivo* activity was also seen in a syngeneic mouse model. KAT/3BP induced apoptosis *in vitro* and necrosis *in vivo*.

*CT, FS: Equally contributed

Conflict of interest:
Advisory Board: Gilead, AbbVie, Janssen, AstraZeneca, MSD, BMS/Celgene, Roche, Mei Pharma, Astra Zeneca,
Celltrion Healthcare, Incyte, Kite/Gilead
Corporate-sponsored Research: Celgene, Roche, Janssen, Acerta, ADC Therapeutics, Bayer AG, Cellestia, CTI Life Sciences, EMD Serono, Helsinn, ImmunoGen, Menarini Ricerche, NEOMED Therapeutics 1, Nordic Nanovector ASA, Oncology Therapeutic Development, PIQUR Therapeutics AG, Gilead, AbbVie, Janssen
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184 (PB064)

Transcriptome and computational analysis assess the anti-tubulin activity of [1,2]oxazole derivatives in lymphoma

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Background: Anti-tubulin agents are widely used in the treatment of lymphoma and are included in important chemotherapy schemes (R-CHOP, ABVD, BEACOP) as well as in the innovative therapeutic approaches utilizing antibody-drug conjugates (ADCs) such as brentuximab vedotin and polatuzumab vedotin. Recently, we have devoted our efforts to the investigation of a large family of [1,2]oxazole-based derivatives that showed anti-proliferative activity in several lymphoma models, with IC_{50} values between the low micromolar and nanomolar range. High inhibition of tubulin polymerization due to strong interactions with colchicine-binding site was confirmed by computational studies and colchicine binding to tubulin. We now present the transcriptome changes induced by the best candidate of this family (SIX2-F).

Material and methods: To unravel gene expression changes, MINO cell line was exposed to DMSO or to 100 nM of compound for 8, 12 and 24 hours and transcripts changes analyzed by RNA-Seq. The binding mode to colchicine-binding site was elucidated by X-ray crystallographic studies, using a T2R-TTL protein complex composed of two αβ-tubulin (T2) dimers, the stathmin-like protein RB3 (R) and tubulin tyrosine ligase (TTL). Each tubulin dimer in the complex contained an accessible colchicine site, termed β1 and β2. Finally, docking poses and molecular dynamics simulations (MDs) were generated towards the 4O2B PDB model.

Results: The gene expression changes caused by SIX2-F revealed a significant upregulation of genes related either to spindle and microtubule assembly and to G2/M transition. The top upregulated genes were CCNB1 (cyclin B1), CCNB2 (cyclin B2), AURKA (aurora kinase A), PLK1 (Polo-like kinase 1), CENPA (centromere protein A), CENPE (centromere protein E) and BIRC5 (survivin), while genes involved in transcription and DNA replication were downregulated. In T2R-TTL-ligand structure, a large, compound shaped difference density within the binding site was observed for SIX2-F, proving that the ligand was bound. In particular, the βT7 and αT5 loops were flipped to accommodate the compound. Docking calculations confirmed that SIX2-F well-fitted the colchicine-binding pocket (G-Score = −9.71 Kcal/mol), directing the methoxybenzyl moiety towards C241. The complex was further stabilized by means of several hydrophobic interactions with the residues A180, L242, L248, L250, L252, L255, A316, I317 and A354.

Conclusions: Transcriptome changes induced by SIX2-F in lymphoma overlapped with those reported for other microtubule inhibitors. These data, further supported by its docking poses and interactions with the crystal structure of tubulin, validated its activity as anti-tubulin agent and confirmed SIX2-F as promising candidate for further studies for the treatment of refractory lymphomas.

Conflict of interest:
Ownership: Patent

185 (PB065)

NUC-3373 is a potent TS inhibitor and induces DNA damage in NSCLC cancer cells regardless of histological subtype

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Background: Non-small cell lung cancer (NSCLC) represents >80% of lung cancer cases and is the leading cause of cancer mortality. It is comprised of three histological subtypes: adenocarcinoma (50–65%), squamous cell carcinoma (30–40%) and large cell carcinoma (5–15%). The majority of NSCLCs do not harbour actionable mutations, so treatment options include immune checkpoint inhibitors (ICIs) alone or in combination with chemotherapies. ICI/chemotherapy combinations have resulted in significant overall survival benefit compared to chemotherapy alone in both adenocarcinoma and squamous histology. The thymidylate synthase (TS) inhibitor 5-FU has limited clinical utility in NSCLC; attributed to the high levels of 5-FU degrading enzyme dihydropyrimidine dehydrogenase (DPD). Pemetrexed, also a TS inhibitor, is the backbone therapy for first- and second-line adenocarcinoma but is not recommended in squamous histology due to high basal TS expression. NUC-3373, a phosphoramidate transformation of FUDR, is resistant to breakdown by DPD and generates high intracellular levels of the active anti-cancer metabolite fluorodeoxyuridine monophosphate (FUDR-MP or FdUMP) and is a potent inhibitor of TS. We hypothesise that NUC-3373 inhibits TS and causes DNA damage in NSCLC, regardless of histological subtype.

Methods: TS protein complex formation was measured by western blot in adenocarcinoma (A549, Calu3) and squamous (Nx002, Cx140) lung cancer cell lines treated with NUC-3373, following immunocytochemistry to assess basal TS. FUDR-MP, dUMP levels (surrogate of TS inhibition) and incorporation of FdUTP into DNA (FUDR as surrogate) were assessed by LC-MS/MS. DNA damage was assessed by immunofluorescence using p-Chk1 and γ-H2AX as markers of strand breaks. 5-FU and pemetrexed were used as positive controls.

Results: Consistent with the literature, squamous cell lines had higher basal TS expression than adenocarcinoma. NUC-3373 promotes formation of TS ternary complexes (60–84% of total TS) at sub-IC_{50} doses in all lung cancer cell lines. NUC-3373 generated high intracellular levels of the TS-inhibiting metabolite FUDR-MP, resulting in increased levels of dUMP, up to 4.5- and 3.3-fold higher than 5-FU and pemetrexed at equimolar doses, respectively. NUC-3373 treatment led to FdUTP incorporation into DNA and subsequent DNA damage in both adenocarcinoma and squamous subtypes.

Conclusion: NUC-3373 is a potent inhibitor of TS in both adenocarcinoma and squamous NSCLC cells. Furthermore, NUC-3373 causes extensive DNA damage, likely owing to the high intracellular generation of FUDR-MP and incorporation of FdUTP into DNA. These data indicate that NUC-3373 may be an effective treatment for NSCLC, regardless of histological subtype.

Conflict of interest:
Corporate-sponsored Research: NuCana plc
Other Substantive Relationships: Jennifer Bré is a full-time NuCana employee. David James Harrison and Alison Louise Dickson are part-time NuCana employees.

186 (PB066)

HORMAD1 drives spindle assembly checkpoint defects and sensitivity to multiple mitotic kinases

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Background: The study aimed to identify and target effects associated with HORMAD1 expression in breast cancer cells.

Materials and methods: Isogenic cell line models with inducible HORMAD1 expression were used to identify dependencies induced by HORMAD1 expression.

Results: Expression of HORMAD1, a gene whose function is best understood in meiosis, is usually restricted to germ-line cells but becomes aberrantly expressed in 60% of triple-negative breast cancers (TNBCs), where a clearly bimodal distribution of expression is associated with signatures of genomic instability. Here, we report that HORMAD1 expression in mitotic cells leads to defects in the spindle assembly checkpoint (SAC). These defects were observed despite functional MAD2-dependent SAC activity, and were instead a consequence of an interaction with and disruption of the chromosome passenger complex, leading to decreased Aurora B signalling. HORMAD1-driven SAC defects were also associated with excessive chromosome instability and cellular sensitivity to MPS1, Aurora B and BUB1 inhibitors, all of which are of great clinical interest in TNBC.

Conclusions: Our data demonstrate that aberrant HORMAD1 expression in cancer leads to SAC defects, and highlights several therapeutic targets for a large subset of breast cancers that express HORMAD1. This may also be relevant in a wider group of high clinical need cancers that similarly demonstrate bimodal *HORMAD1*expression.

Conflict of interest:
Ownership: CJL has stock in: Tango, Ovibio, Enedra Tx., Hysplex ANJT has stock in: InBiomotion
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ANJT: Medivation AstraZeneca,
Pfizer, Vertex, Artios, Prime Oncology