cytotoxic than the parent PBD it releases. The N10-benzyl and SEM control molecules were significantly less cytotoxic in both NTR+ and NTR- A2780 cells, with IC_{50} values ranging from 1.9 to 3.3 mM. In preliminary *in vivo* experiments, the N10-(*p*-nitrobenzylcarbamate) prodrug was evaluated in a nude mouse human tumour xenograft model implanted with A2780 CMV-NTR cells. A clear response to the prodrug was observed at non-toxic doses.

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A potent PBD-heterocyclic polyamide conjugate targeting an ICB2 transcription factor binding site

F. Brucoli[1], R.M. Hawkins[1], G. Wells[1], T.C. Jenkins[2], T. Ellis[3], M. Kotecha[4], D. Hochhauser[4], J.A. Hartley[4], P.W. Howard[3], D.E. Thurston[1]. [1]*The School of Pharmacy (University of London), Pharmaceutical & Biological Chemistry, London, United Kingdom;* [2]*University of Greenwich, School of Science, Kent, United Kingdom;* [3]*Spirogen Ltd, London, United Kingdom;* [4]*University College London, Cancer Research UK Drug-DNA Interactions Research Group, London, United Kingdom*

The binding of Nuclear Factor Y (NF-Y), a ubiquitous CCAAT-binding transcription factor, to five inverted CCAAT boxes (ICBs) within the promoter region of DNA topoisomerase IIa (topo IIa) results in control of cell proliferation. The regulation of NF-Y/topo IIa interactions by small molecules is of interest in relation to both the development of novel anticancer agents and also chemical tools and probes for use in cancer biology experiments. In this context, we have recently demonstrated that the pyrrolo[2,1-*c*][1,4]benzodiazepines (PBD) C8-*bis*-pyrrole conjugate, GWL-78, can interact specifically at CCAAT-box sites and block NF-Y binding (Kotecha, M. *et al*, *Molecular Cancer Therapeutics*, 7, 1319–1328, 2008).

To further explore this property of GWL-78, and to try to improve both selectivity and potency, a third heterocycle has now been added to the GWL-78 C8-side chain to produce examples of pyrrolo[2,1-*c*]-[1,4]benzodiazepines (PBD) C8-*tris*-heterocyclic conjugates which should span a slightly longer region of DNA upon interaction within the minor groove and offer additional molecular interactions (*e.g.,* hydrogen bonds, electrostatic interactions etc) that may further stabilize the adduct and modify sequence selectivity. On this basis, a library of fourteen PBD C8-*tris*-heterocyclic conjugates was synthesized by attaching a PBD capping unit to pre-constructed tri-heterocyclic polyamides comprising of a combination of pyrrole, imidazole and thiazole heterocycles assembled in a combinatorial fashion. The effect of the composition and length of the C8-heterocyclic polyamide side-chains on DNA binding was evaluated using a number of biophysical and cellular methods. Binding affinity was measured using a calf thymus DNA thermal denaturation assay, and sequence selectivity was evaluated using DNase I footprinting. Their ability to disrupt interaction of the NF-Y transcription factor with its cognate binding site was measured using an EMSA assay, and cytotoxicity was evaluated in the NCI 60 cell line panel.

One conjugate, RMH-41 (Py-Py-Im-PBD; Figure 1), which had the highest DNA binding affinity, also exhibited the ability to inhibit NF-Y transcription factor binding and had significant selective cytotoxicity in human tumour cells. Interestingly, the results of the footprinting experiments showed that, of all the novel conjugates evaluated, RMH-41 appeared to discriminate between two of the ICBs studied, binding to the ICB2 site at a lower concentration compared to that required to bind to ICB1.

In conclusion, the ability of low molecular weight ligands such as RMH-41 to recognize predetermined DNA sequences and prevent endogenous transcription factors from binding could be successfully exploited to modulate transcription and block cancer cell proliferation as part of a therapeutic strategy.

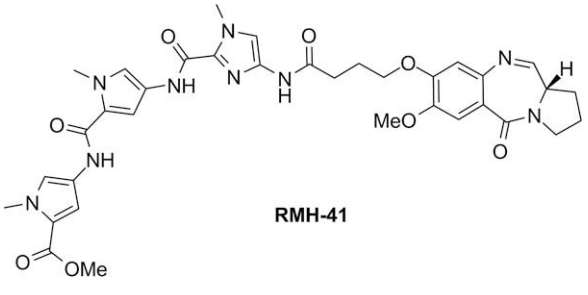

Figure 1. Structure of RMH-41

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siRNA targeting of thymidylate synthase and thymidine kinase for anti-cancer therapy

C. Di Cresce[1], P.J. Ferguson[2], R. Figueredo[2], M.D. Vincent[2], J. Koropatnick[2]. [1]*The University of Western Ontario, Microbiology and Immunology, London Ontario, Canada;* [2]*Lawson Health Research Institute, Cancer Research Laboratory Program, London Ontario, Canada*

Thymidylate synthase (TS) is the only *de novo* source of thymidylate (dTMP) for DNA synthesis and repair. Cytosolic thymidine kinase 1 (TK1) and mitochondrial TK2 are salvage pathways for producing dTMP. TS and TKs are often upregulated in human tumors, suggesting a role for both in malignancy. We have previously shown that antisense oligodeoxynucleotides (ODNs) targeting TS, as single agents, inhibit human tumor cell growth *in vitro* and *in vivo*. In addition, anti-TS ODNs and small interfering RNAs (siRNAs) enhance tumor cell growth inhibition by TS-targeting drugs. TS mRNA is a good target for development of antisense anticancer drugs. However, we hypothesize that when TS enzyme activity is inhibited, the ability of TKs to generate dTMP may mediate resistance to TS-protein targeting drugs.

To determine whether antisense to TK has potential therapeutic benefit, siRNAs targeting TK1 or TK2 were used *in* vitro in 3 different protocols against cultured tumor cell lines: (1) on their own; (2) in combination with TS siRNA; (3) in combination with TS siRNA and the anti-TS drugs 5-fluorodeoxyuridine (5FUdR) or pemetrexed. siRNAs targeting TS or TK1 or TK2, as single agents and in combination with each other, decreased TS or TK mRNA by more than 85% in human cervical carcinoma (HeLa) and human breast carcinoma (MCF7) cell lines (5 nM siRNA, 24 h post-transfection) and decreased TS and TK1 protein. siRNAs targeting TS, TK1 or TK2 did not independently decrease HeLa cell proliferation but did decrease TS, TK1 and TK2 mRNA and TS and TK1 protein levels (5 nM siRNA, 24 and 96 h post-transfection). The capacity of each siRNA to downregulate its target mRNA was unaffected by combination treatment with other siRNAs. HeLa cell TK2 protein after TK2 siRNA treatment was not measured. Treatment with TS siRNA sensitized HeLa cells to 5FUdR by approximately 50% and to pemetrexed by approximately 34% compared to non-targeting control siRNA. siRNA targeting TK1 or TK2 alone did not enhance tumor cell sensitivity to 5FUdR. In support of the concept that TK activity can reduce the ability of antisense TS to sensitize human tumor cells to TS-targeting drugs, we report that simultaneous treatment with TK2 siRNA and TS siRNA enhanced sensitivity to 5FUdR by approximately 25%, and adding TK1 siRNA to TS siRNA enhanced sensitivity to pemetrexed by approximately 20%, beyond the sensitization induced by TS siRNA alone. Enhanced sensitization to 5FUdR and pemetrexed by targeting both TS and TK with siRNAs suggests that the TK salvage pathways are potential targets for anticancer therapies. These data support the hypothesis that combined antisense targeting of TS and TK1/TK2 is more effective than either siRNA used alone to sensitize tumor cells to the effects of TS-targeting chemotherapeutic drugs.

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Observation of the reversibility of formation of a pyrrolobenzodiazepine (PBD) covalent DNA adduct using HPLC/MS and CD spectroscopy

K.M. Rahman[1], C.H. James[1], D.E. Thurston[1]. [1]*School of Pharmacy (University of London), Gene Targeted Drug Design Research Group, London, United Kingdom*

It has been previously demonstrated that exposure of a pyrrolobenzodiazepine (PBD) DNA adduct to heat and/or acidic conditions leads to the loss of up to 70% of covalently-bound ligand. For example, Hurley and co-workers have reported that anthramycin cleaves from DNA after heating in the presence of TFA, with simultaneous oxidative formation of a C11a-C1-dehydro product which is non-electrophilic at the N10-C11 position and so cannot re-react with DNA. Therefore, in principle, given the relatively labile nature of the aminal bond formed between PBDs and DNA, an adduct formed from a non-oxidizable PBD should be reversible upon exposure to conditions such as heat or low pH, although this has not been previously demonstrated. The PBD conjugate GWL-78, which is not prone to such oxidation, comprises a C-ring-unsubstituted PBD attached to a methyl ester terminated *bis*-(*N*-methylpyrrole) unit *via* a four-carbon linker between the C8-oxygen of the PBD A-ring and the N-terminus of one pyrrole unit. Using HPLC/MS and CD methodologies to monitor the interaction of GWL-78 with short oligonucleotides, we have now demonstrated for the first time that such reversibility occurs.

A GWL-78/DNA adduct was initially formed and characterized by HPLC/MS and CD. It was then heated to 90ºC at a rate of 10ºC/min which resulted