PhD Project Proposal

Funder details	
Studentship funded by:	MRC iCASE award (Astrazeneca)
Project details	
Project title:	**Project title:** Folate-Degrader Conjugates (FDCs) for Tumour-specific Targeting
Supervisory team	
Primary Supervisor:	Prof. Zoran Rankovic (ICR)
Associate Supervisor(s):	Dr. Kashif Khan (AstraZeneca) Dr. Luke Masterson (AstraZeneca) Dr. George Saunders (Astrazeneca) Dr. John Caldwell (ICR)
Secondary Supervisor:	Dr. Udai Banerji (ICR)
Divisional affiliation	
Primary Division:	CCDD
Primary Team:	TPD
Site:	Sutton

Project background

Targeted protein degradation (TPD) is a novel and rapidly emerging drug discovery paradigm that provides opportunities for tackling currently intractable oncoproteins and to deliver breakthrough therapies. The TPD paradigm includes two main approaches focusing on molecules with a similar proteasome-dependent mechanism of action, namely, proteolysis targeting chimeras (PROTACs)[1], and molecular glue degraders (MGDs).[2] MGDs are small molecules capable of binding to an E3 ligase and altering its surface and specificity, leading to the recruitment, ubiquitination, and subsequent degradation of substrates that are normally not targeted by the ligase alone (neosubstrates). Therefore, this approach offers an unprecedented opportunity to degrade currently intractable and undruggable targets.

We previously described the discovery of SJ6986, a potent, selective and orally bioavailable GSPT molecular glue degrader, which markedly decreased ALL tumour burden in multiple PDX models in vivo.[3,4] However, as GSPT is a widely expressed essential protein, on-target toxicity may limit its therapeutic potential.

Here we will develop GSPT-targeting folate conjugates (**Fig.1**). Folate (FOL) conjugation is a well-established method for targeted delivery of drugs to cancer cells, as normal tissues exhibit relatively low expression of folate receptor (FOLR) while it's highly expressed in many human cancers, including multiple myeloma, lymphoma, and non-small cell lung cancer.[5]

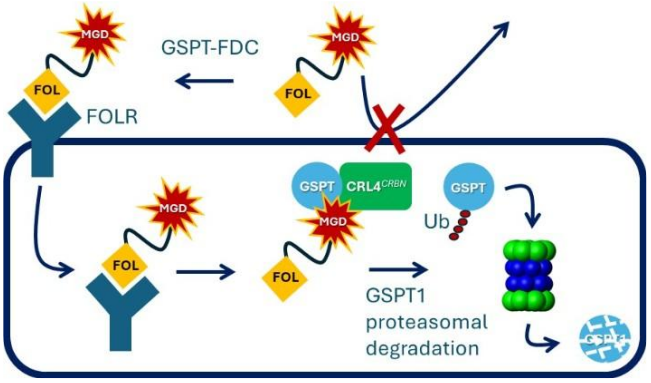

Figure 1. The GSPT-FDC mechanism of action.

We will use X-ray structure and SAR information, as well as the reported SAR to develop non-cleavable GSPT-Folate Degrader Conjugates (GSPT-FDCs), and evaluate their efficacy and toxicity, in a range of FOLR positive and negative cell lines. We hypothesize that the combination of FDC tumour specific accumulation with degrader catalytic mode of action will produce advanced GSPT degraders with high tumour-specific potency and wider therapeutic window than corresponding GSPT-MGDs.

If successful, this study would lay foundations for exploring the concept more broadly with alternative neosubstrates and delivery mechanisms. For example, a similar tumour overexpression pattern has been reported for SLC19A1, the reduced folate carrier 1 (RFC1), which is involved in transport of antifolate drugs like methotrexate, MTX.[6] Consequently, MTX could be used in a similar fashion to develop tumour-targeting GSPT conjugates. Furthermore, this tumour-specific drug delivery approach could be extended to include other CRBN neosubstrates, such as CK1α, as well as PROTAC degraders.[7,8]

Project aims

The ultimate objective of this project is to demonstrate that Folate Degrader Conjugates (FDCs) retain potency while displaying greater in vitro therapeutic window in respect to normal noncancerous cells when compared to the corresponding Molecular Glue Degraders (MGDs).

Aim 1: Conjugate design and synthesis. The SAR of MGDs reported in the literature will be explored to establish optimal positions and vectors for the linker attachments. A library of around 50 FOL-conjugates will be synthesized.

Aim 2: FDC library cytotoxicity profiling. The effect of FDCs on viability of FOLRα-expressing cancer cells and FOLRα null/low expressing cells will be established, to identify the most potent FDCs with the best in vitro therapeutic window.

Aim 3: FDC vs MGD comparison. Selected FDCs and parent MGDs will be tested in a panel of cancer and normal cells to compare their cytotoxicity profiles.

Research proposal

Aim 1: FDC library design and synthesis.

Aim1a: We will explore SAR around several GSPT MGDs reported in the literature to establish the most optimal degraders and exit vectors for developing FDCs. We will start with the GSPT-MGD disclosed in the patent by Orum Therapeutics covering the development of GSPT Antibody Conjugates (ADC).[9] Data reported in the patent clearly points towards the para-position of the MGD distal phenyl ring as the one suitable for the linker attachment (**Fig2**). We will initially synthesise analogues with several linkers to explore the tolerance of the substitutions in the para- and meta- positions. The compounds will be tested for GSPT degradation in HiBiT assay, developed in HEK293 cell line that was engineered by CRISPR/Cas9 to express endogenous GSPT1 with a HiBiT tag, which when coupled with LgBiT expression forms NanoBiT, a bioluminescent protein that can be used for kinetic studies of protein degradation.[10] The GSPT1 HiBiT assay is fully developed and available at the CPD.

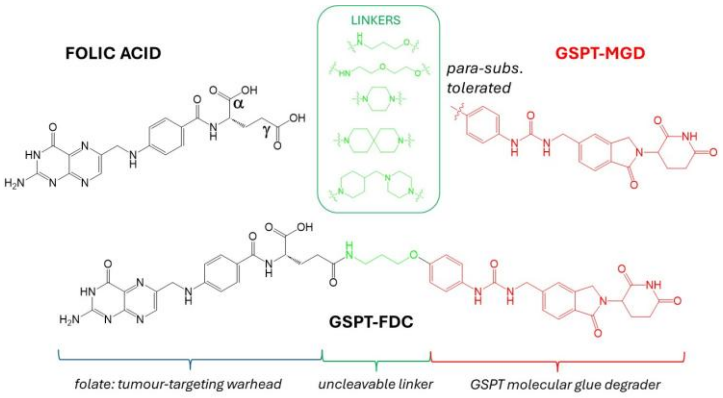

Figure 2. GSPT-FDC Design.

Aim 1b: Most optimal degraders and attachment points identified in Aim 1b will be used to synthesize a library of up to 50 FDCs. All linkers will be designed as low passive permeability and connected to Folic Acid through a γ-carboxylate amide bond using standard peptide coupling reaction conditions, to produce FDCs in 20-50mg quantity and over 95% purity.

Aim 2: FDC library cytotoxicity profiling. The GSPT-FDCs synthesised in Aim 1 will be tested for their effect on the viability of high and low FOLRα-expressing cells using the CellTiter-Glo assay (CTG). The panel of FOLRα-expressing cancer cells will include OV90 (ovary cancer), MM1S (multiple myeloma), HeLa (cervical cancer), and T47D (breast cancer). Immunoblotting after ligand competition experiments using lenalidomide and folic acid will be carried out in OV90 cell line to demonstrate that the GSPT degradation is CRBN and folate dependent. For noncancerous normal cell lines with low FOLR1 expression, we will use human fibroblast cells (HFF-1), human normal kidney epithelia cells (HK2), and peripheral blood mononuclear cells (PBMC). The in vitro therapeutic index is defined as an average fold difference between IC_{50} values in the high and low FOLRα-expressing cells (CTG). Several iterations of the synthesis (Aim 1) and testing (Aim 2) may be carried out to identify the GSPT FDC with the desired overall profile.

Aim 3: FDC vs MGD comparison. The GSPT-FDC from Aim 2 with the best overall profile will be compared with the corresponding GSPT-MGD in the panel of high and low FOLRα-expressing (CTG assays).

Our aim is to demonstrate FDC improved therapeutic index at least 10-fold over the corresponding MGD, while retaining the similar cytotoxicity against the relevant FOLRα-overexpressing cancer cells.

The best FDCs will also be evaluated for their physicochemical and pharmacokinetic properties to establish their suitability for potential future in vivo studies.

Literature references

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(4) Chang, Y.; Keramatnia, F.; Ghate, P. S.; Nishiguchi, G.; Gao, Q.; Iacobucci, I.; Yang, L.; Chepyala, D.; Mishra, A.; High, A. A.; Goto, H.; Akahane, K.; Peng, J.; Yang, J. J.; Fischer, M.; Rankovic, Z.; Mullighan, C. G. The Orally Bioavailable GSPT1/2 Degrader SJ6986 Exhibits in Vivo Efficacy in Acute Lymphoblastic Leukemia. *Blood Journal* **2023**, blood.2022017813. https://doi.org/10.1182/blood.2022017813.

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Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants: Organic Chemistry

Intended learning outcomes:

- Expand organic chemistry knowledge and experimental skills
- Learn to use modern automation and liquid handling systems
- Develop chemical biology knowledge skills, e.g. perform screening by routine biological assays such as fluorescence polarization (FP), and CellTiter-Glo (CTG) assays.
- Develop writing and oral reporting skills
- Develop knowledge of Targeted Protein Degradation (TPD)

Advertising details

Project suitable for a student with a background in:

☐ Biological Sciences

☐ Physics or Engineering

☒ Chemistry

☐ Maths, Statistics or Epidemiology

☐ Computer Science