PWE-011 ABERRANTLY GLYCOSYLATED MUC1 AS A POTENTIAL THERAPEUTIC TARGET FOR BARRETT'S WITH HIGH GRADE DYSPLASIA AND PRIMARY AND METASTATIC OESOPHAGEAL ADENOCARCINOMA

doi:10.1136/gutjnl-2012-302514d.11

[1,2]M A Butt,* [3]H Pye, [1,2]R J Haidry, [4]D Oukrif, [5]M Rashid, [1,2]M R Banks, [3]M P Deonarain, [2,4]M R Novelli, [1,2]L B Lovat. *[1]National Medical Laser Centre, University College London, London, UK; [2]University College Hospital, London, UK; [3]Recombinant Antibody Therapeutics Laboratory, Imperial College London, London, UK; [4]Research Department of Pathology, University College London, London, UK; [5]Research Department of Oncology, University College London, London, UK*

Introduction Patients with Barrett's (BE) associated oesophageal adenocarcinoma (OA), show strong expression of the mucin MUC1, but binding is not specific to dysplasia or cancer. Aberrant glycosylation of MUC1 (AG-MUC1) accompanies the development of cancer in most epithelial tumours, exposing peptides hidden on normal cells. Humanised antihuman milk-fat globule-1 (Hu-HMFG1) antibody binds one of these regions. This study assesses expression of AG-MUC1 in the squamous-metaplasia-dysplasia-OA sequence, in OA specimens with infiltrated nodes and in cancer cell lines.

Methods 34 paraffin embedded oesophageal tissue specimens were selected from patients with squamous (n=5), non-dysplastic BE (NDBE; n=3), low grade dysplasia (LGD; n=6), high grade dysplasia (HGD; n=9) and OA (n=11). 11 OA resection specimens with clear margins containing tumour and infiltrated nodes were also stained. Slides were immunostained with Hu-HMFG1 antibody and scored by an expert pathologist. Binding of HuHMFG1 to the cancer cell lines SKOV-3 (ovarian), MCF-7 (breast), OE-19, OE33 (oesophageal) and HT-29 (colon) was examined with flow cytometry using a secondary FITC conjugated antibody and analysed with FloJo.

Results AG-MUC1 was significantly expressed (>33% positively stained cells) in 22% of HGD and 36% of OA specimens. Non-significant mild staining was seen in NDBE (100%), LGD (33%), HGD (44%) and OA (64%). In 27% of OA and 43% of HGD, adjacent squamous epithelium also stained. In surgically resected OA's, 45% stained significantly for AG-MUC1 in primary tumour. Of these, 80% stained significantly in related lymph nodes. All OA resection margins were clear of significant staining. On flow cytometry, binding was noted on SKOV-3, MCF-7, OE-19 but not HT-29 or OE-33.

Conclusion This pilot study demonstrates AG-MUC1 to be upregulated in the BE metaplasia-dysplasia-OA sequence with significant staining limited to HGD and cancer. Although some squamous staining was noted, this was likely a field effect as no significant staining was noted in OA resection margins. In patients with significantly stained primary tumour, most had significant staining in infiltrated lymph nodes. Finally, with flow cytometry we identified the OA cell line, OE-19 expressed AG-MUC1 in preparation for in vitro studies. AG-MUC1 targeting with the antibody Hu-HMFG1 offers a novel strategy to target HGD and OA, including those patients presenting with metastatic disease.

Competing interests None declared.

PWE-012 IN VITRO CHARACTERISATION OF ABERRANTLY GLYCOSYLATED MUC1 BINDING-PS1 PHOTOIMMUNOCONJUGATES FOR OESOPHAGEAL ADENOCARCINOMA TARGETED PHOTODYNAMIC THERAPY IN PRIMARY AND METASTATIC DISEASE

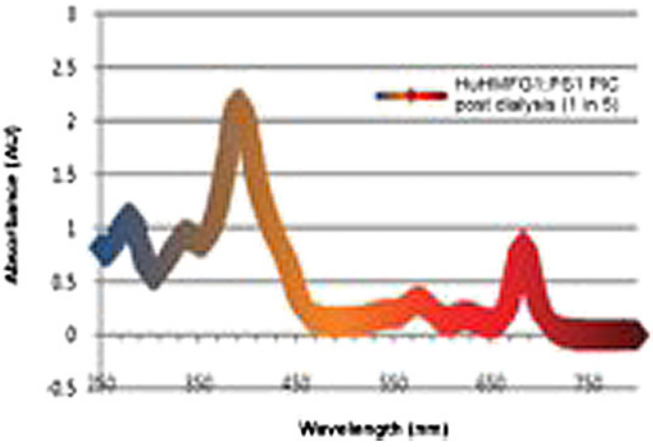
doi:10.1136/gutjnl-2012-302514d.12

[1,2]M A Butt,* [3]H Pye, [3,4]I Stamati, [4]G Yahioglu, [1,2]R J Haidry, [5]D Oukrif, [2]M R Novelli, [3,4]M P Deonarain, [1,2]L B Lovat. *[1]National Medical Laser Centre, University College London, London, UK; [2]University College Hospital, London, UK; [3]Recombinant Antibody Therapeutics Laboratory, London, UK; [4]PhotoBiotics Ltd, Imperial College London, London, UK; [5]University College London, London, UK*

Introduction Most patients with oesophageal adenocarcinoma (OA) are unfit for curative treatment but are fit for photodynamic therapy (PDT). PDT has been limited by poor tumour selectivity, poor tissue penetration of light and protracted side effects. These restrictions may be overcome with targeted PDT. We have previously demonstrated aberrantly glycosylated MUC1 (AG-MUC1), bound by humanised anti-human milk-fat globule-1 (Hu-HMFG1) antibody is significantly expressed in Barrett's epithelium (BE) with high grade dysplasia and OA. In this study, we characterise photoimmunoconjugates (PIC) of Hu-HMFG1 with the photosensitisers (PS) pyropheophorbide-α (PPa), and PS1, a water-soluble derivative of PPa activated in the near infra-red.

Methods PPa succinimidyl ester (PPA-SE) and PS1 were first synthesised (PhotoBiotics) from PPa. Both PS were then conjugated with Hu-HMFG1. The resulting PICs were dialysed, centrifuged and the supernatant and resultant pellet were resuspended in PBS and retained for spectroscopic and SDS-Page gel analysis. Fluorescence of PICs in the gels were photographed and the gels stained with Coomasie to confirm accurate loading. AIDA image analyser software was used for densitometry measurement.

Results HuHMFG1:PPa PICs, although well conjugated by fluorescent photography of SDS-page gels, were very insoluble. Further characterisation was limited to PS1 PICs. UV/Vis analysis of the soluble HuHMFG1:PS1 PIC supernatant, suitable for subsequent in vitro testing, confirmed maximum absorption at 683 nm (Abstract PWE-012 figure 1). SDS page analysis indicated up to 52% of PS1 photosensitiser was conjugated in the supernatant mixture post-dialysis. AIDA image analysis confirmed that in those antibodies conjugated, a loading ratio of PS1:HuHMFG1 of up to 7:1 was achieved.



Abstract PWE-012 Figure 1 UV/Vis spectrum of HuHMFG1:PS1 PIC.

Conclusion This pilot study is the first to successfully conjugate HuHMFG1 with the photosensitisers PPa and PS1. These PICs could potentially selectively target photosensitisers to tissues of interest preserving the surrounding architecture during PDT. HuHMFG1:PS1 PICs have shown enhanced absorption in the red spectral region which will translate clinically into deeper tissue penetration than currently licenced photosensitisers. Further experiments are needed to both optimise the conjugation protocol and purify the product to GLP standards prior to clinical studies.

Competing interests None declared.

Gut: first published as 10.1136/gutjnl-2012-302514d.12 on 28 May 2012. Downloaded from http://gut.bmj.com/ on April 24, 2024 by guest. Protected by copyright.