PWE-013 SELECTIVE CYTOTOXICITY OF ABERRANTLY GLYCOSYLATED MUC1 BINDING-PS1 PHOTOIMMUNOCONJUGATES FOR OESOPHAGEAL ADENOCARCINOMA TARGETED PHOTODYNAMIC THERAPY IN PRIMARY AND METASTATIC DISEASE

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Introduction Targeted photodynamic therapy (PDT) has the potential to overcome current limitations of PDT agents by offering specific tumour kill with reduced side effects. We have previously shown expression of AG-MUC1, an epithelial cancer target bound by the antibody HuHMFG1, in patients with Barrett's with dysplasia, oesophageal adenocarcinoma (OA) and on OE19, an OA cell line. We have recently developed and characterised photoimmunoconjugates (PIC) of Hu-HMFG1 with the photosensitiser PS1 to specifically target PDT to OA. This is the first study to test the cytotoxic efficacy of HuHMFG1:PS1 PIC's against cancer cells in vitro.

Methods Hu-HMFG1 was conjugated with PS1 (PhotoBiotics) using recently optimised methods. Binding of the HuHMFG1:PS1 PIC to the cancer cell lines SKOV-3 (ovarian), MCF-7 (breast), OE-19 (oesophageal) and HT-29 (colon) was examined with flow cytometetry and analysed with FlowJo software. The efficacy of HMFG1:PS1 PICs on these lines were compared with equivalent free PS1 in the presence or absence of laser activation. Power was set to a clinically relevant light dose. Cell viability was measured with standard MTS assay and the plates read on an ELISA plate reader at 490 nm.

Results Flow cytometry confirmed binding of HMFG1:PS1 PIC to SKOV-3, OE19 and MCF-7 but not HT-29 cells. Cell viability counts in all plates were initially corrected for untreated plate controls and then plotted on a log scale to produce dose response curves. This confirmed significantly superior cytotoxic efficacy of HuHMFG1: PS1 over PS1 in SKOV-3 ($F=104.93$, $p=0.00051$) and OE-19 ($F=11.13$, $p=0.0125$), and a trend towards effect for MCF-7 ($F=3.13$, $p=0.116$) cells using linear regression analysis and an F test to compare treatments. HuHMFG1:PS1 did not kill HT-29 effectively over control ($p=0.84$) or differ significantly from PS1 in its efficacy ($F=0.155$, $p=0.71$) confirming cytotoxicity to be limited to HuHMFG1 expressing cells.

Conclusion This pilot study is the first to successfully demonstrate binding and potential cytotoxicity of targeting HuHMFG1:PS1 PICs against OA cells in vitro. Absence of photosensitiser effect in the negative control (HT29 cells) confirmed selective cytotoxicity of HuHMFG1:PS1 to AG-MUC1 expressing cells. We further demonstrated that HuHMFG1:PS1 can effectively kill other AG-MUC1 expressing tumours which have historically been treated with HuHMFG1 related therapies.

Competing interests None declared.

PWE-014 POLO-LIKE KINASE 1 EXPRESSION PREDICTS ANEUPLOIDY IN THE BARRETT'S METAPLASIA-DYSPLASIA-CARCINOMA SEQUENCE

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Introduction Finding an accurate and convenient biomarker for cancer progression in Barrett's (BE) is of high clinical importance. DNA ploidy abnormalities (DNA-PA) are a reliable predictor of cancer risk in BE, but measurement is expensive and scarcely available. We have shown polo-like kinase-1 (PLK1) may act as a surrogate marker of DNA-PA in oesophageal adenocarcinoma (OA) resection specimens. This study aimed to examine the potential of PLK1 in predicting DNA-PA in the metaplasia-dysplasia-OA sequence.

Methods 36 paraffin embedded oesophageal tissue specimens were selected from patients with non-dysplastic BE (NDBE, n=5), low grade dysplasia (LGD, n=5), high grade dysplasia (HGD, n=12), intramucosal cancer (IMC, n=5) and invasive OA (IOA, n=9). Sections were mounted and immunostained with 2 PLK-1 antibodies PLK1-M and PLK1-L using an automated system (BOND-MAX, Leica) for consistency. Results were reported by two independent expert pathologists blinded to patient status with the Allred scoring system, a composite of the percentage and intensity of staining.

Results Aneuploidy was present in LGD (20%), HGD (75%), IMC (20%) and IOA (56%) samples. Using linear regression analysis, Pearson coefficient was calculated for the correlation between DNA-PA and mean Allred expression scores for each PLK1 antibody. PLK1-M had a higher degree of correlation ($r^2=0.26$, $p=0.001$) then PLK1-L ($r^2=0.22$, $p=0.004$). Inter-observer analysis with linear κ scores confirmed good correlation of PLK1-M ($\kappa=0.72$, 95% CI 0.60 to 0.83) and PLK1-L ($\kappa=0.53$, 95% CI 0.38 to 0.68), but a Bland–Altman plot found pathologist 2 had a trend to score PLK-L more highly. However, intra-observer analysis confirmed both PLK1-L scores correlated with ploidy status ($r^2=0.14$, $p=0.023$ and $r^2=0.27$, $p=0.0016$). Pathologists took 90.3 s/slide (mean) to review and score with the Allred method. Using a mean Allred cut off score of 3.5, the sensitivity and specificity for the detection of aneuploidy were 81.3% and 75% for PLK1-M, 93.8% and 45% for PLK1-L.

Conclusion This pilot study demonstrated a significant correlation between Allred reporting of PLK1 staining and DNA-PA. PLK1-L appears more sensitive and PLK1-M more specific. PLK1 immunostaining is relatively inexpensive, less work intensive and is more available than current methods to predict DNA-PA. Scoring staining with Allred is rapid and reproducible. In future, PLK1 staining may provide the basis of a more durable biomarker panel to predict DNA ploidy.

Competing interests None declared.

PWE-015 HER2 EXPRESSION IN THE BARRETT'S METAPLASIA-DYSPLASIA-CARCINOMA SEQUENCE AND POTENTIAL TARGETING IN VITRO WITH THE SINGLE CHAIN ANTIBODY FRAGMENT C6.5

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Introduction There is increasing attention on the integration of targeted agents for oesophageal adenocarcinoma (OA) therapy. The most notable example of success of oesophagogastric targeted therapy was the addition of a HER2 targeting agent in the Phase III ToGA study. However, there is limited data on the expression of

HER2 in the progression from Barrett's (BE) to OA. This study aimed to clarify expression in this sequence, and to show binding of C6.5, a single chain HER2 targeting human antibody Fv fragment (scFv), to known HER2 expressing OA cell lines in vitro. Efficacy of antibody based therapies can be enhanced by scFv's which penetrate tumours more quickly and demonstrate better tumour: normal tissue specificity.

Methods 33 paraffin embedded oesophageal tissue specimens were selected from patients with squamous (n=4), non-dysplastic BE (NDBE; n=4), low grade dysplasia (LGD; n=6), high grade dysplasia (HGD; n=8) and OA (n=12). Sections were immunostained with the automated Oracle Bond system (Leica, UK) for consistency. Staining was then scored by 2 expert pathologists according to the proportion of cells in the tissue staining positively as negative (<5%), borderline (5%–10%) or positive (>10%). In phase 2, binding of C6.5 scFv to the cancer cell lines SKOV-3 (ovarian), OE-19, OE-33 (oesophageal) and HT-29 (colon) was identified with flow cytometry using a mouse secondary followed by tertiary anti-mouse FITC. Data were then analysed with FlowJo software.

Results Significant expression of HER2 (>10% cells positive according to National Guidelines) was only seen in HGD (25%) and cancer (25%) specimens. Borderline staining (5%–10%) was seen in LGD (17%), HGD (13%) and cancer (8%). All NDBE and the remaining LGD, HGD and cancer samples were negative. Flow cytometry demonstrated C6.5 binding to SKOV-3, OE19 and OE-33 cells but not HT-29 cells.

Conclusion This study demonstrated that significant HER2 expression is only seen in roughly a quarter of patients with HGD and OA and not in NDBE or LGD. We also found that the HER2 targeting scFv C6.5 binds to breast cancer and OA cell lines but not colon cancer, the negative control. HER2 targeting therapies could therefore be postulated for patients with BE with HGD and OA, and these may be enhanced with scFv's such as C6.5.

Competing interests None declared.

PWE-016 EVOLUTION OF ENDOTHERAPY FOR HIGH GRADE DYSPLASIA AND EARLY CANCER IN BARRETT'S OESOPHAGUS: COMPLETING THE AUDIT CYCLE IN A SINGLE CENTRE

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Introduction We have previously reported our outcomes for endotherapy in Barrett's oesophagus (BO) patients with high grade dysplasia (HGD) and intra-mucosal adenocarcinoma (IMAC) to this Society. Using Endoscopic Mucosal Resection (EMR) and Argon Plasma Coagulation (APC) in patients unfit for surgery, no procedure related mortality, minimal morbidity but a recurrence rate of 27% was seen.[1] We were interested to complete the audit cycle, particularly with the advent of Radiofrequency Ablation (RFA), which has become available since our initial report.

Methods The aim was to re-audit the outcomes of endotherapy for HGD and IMAC in patients with BO attending our gastroenterology unit and to compare the results to our previous audit in terms of outcome and complications. A retrospective review of patient records using specialised endoscopy and patahology databases was performed. Demographic information, diagnosis, procedural details and subsequent progress was entered into a Microsoft Access database and analysed using Microsoft Excel.

Results A total of 54 patients who underwent endotherapy from January 2005 to December 2011 were identified. The mean age of the group was 71 (range 45–89) years. There were 42 males and 12 females. The underlying diagnosis was HGD in 49 and IMAC in 5. Patients with focal raised lesions were treated by EMR, while diffuse dysplasia was treated by mucosal ablation (APC or RFA). Eight patients had EMR alone. EMR was followed by APC in six patients and RFA in 20. Five patients had EMR, APC and RFA. Ten patients had RFA and five APC alone. Six patients (11%) developed an oesophageal stricture requiring dilatation. No bleeding, perforation or procedure related mortality occurred. Over a median follow-up of 18.9 months (range 0–71), two patients (4%) developed a recurrence of their condition, defined as the reappearance of HGD or IMAC after one or more negative biopsies. Two patients (4%) had persistent disease. These four patients (7%) were referred for surgery and underwent oesophagectomy. Seven patients are still under treatment with RFA, 38 have no dysplasia, four low grade dysplasia with one lost to follow-up. During the follow-up period three patients have died, one in the post-operative period.

Conclusion In our department, endotherapy has evolved with APC being replaced by RFA, often in combination with EMR. This has resulted in a considerable improvement in patient outcomes (7 vs 27% recurrence/persistent disease), no procedure related mortality and minimal morbidity. Endotherapy is now used as the primary treatment for HGD and IMAC with surgery being reserved as a salvage procedure if no response or disease recurs.

Competing interests None declared.

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PWE-017 CAN WE USE BLOOD MARKERS INSTEAD OF ROUTINE CONTRAST SWALLOWS TO PREDICT ANASTOMOTIC DEHISCENCE AFTER OESOPHAGEAL RESECTION?

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Introduction Resectional surgery for oesophageal cancer is associated with significant risk of anastomotic dehiscence. In the era of enhanced recovery after surgery (ERAS), some anastomotic leaks may not present until after the patient is discharged home. This study aims to identify markers which may predict likelihood of anastomotic dehiscence.

Methods All patients undergoing oesophageal cancer resection from January 2001 to November 2011 were identified from the Upper GI database where data is recorded prospectively. Retrospective review of patient demographics, operation type, radiology, blood results, histology, length of stay and mortality was performed. Blood results of patients with clinical evidence of anastomotic leak and 50 matched controls were collected for the first 10 post-operative days.

Results 309 patients (median age 66 years) underwent oesophageal resection during this period. In-hospital mortality occurred in 22 patients (7.1%). 216 patients (69.9%) underwent routine contrast studies and 12 radiological leaks were identified. Two patients with radiological leaks had clinical findings suggestive of anastomotic leak, all were managed conservatively. Anastomotic leakage occurred in a further 20 patients. 13 of these should have undergone contrast swallow at day 7 according to the protocol at the time. Two had contrast swallows at day 7, which were normal, but subsequently leaked. 16 patients demonstrated clinical deterioration at days 2–7 which prompted either imaging with CT, endoscopy or surgical exploration. Two patients were never fit enough to undergo contrast study. The overall mortality in patients with leak was 5/32 (15.6%). Results of mean white cell count on days 1 to 9 are given in the Abstract PWE-017 table 1 below. There was a significant difference in white cell count from days 6 to 9 between the control group and leak group. There was a difference in mean CRP in the control group