Methods The SNQ tri-peptide from i2 was engineered into *H pylori* strain 60190 (VacA s1/i1/m1) to make strain 60190/SNQ. The vacuolating activities of 60190/SNQ and wild type strains were compared by incubating AGS cells with broth culture supernatants, and counting vacuolated cells in random fields by microscopy. To allow further structure-function characterisation of i-region variants, we modified a recombinant VacA system[2] by expressing VacA p33 and p55 subunits in *Escherichia coli* and purifying both under denaturing conditions by metal affinity chromatography, then refolding by stepwise dialysis. Recombinant VacA was tested for vacuolating activity and previously described effects on Jurkat T-cells.[3]

Results Introduction of the i2-specific SNQ insertion into i1 VacA in the *H pylori* 60190 strain resulted in a 1.8-fold decrease in native toxin vacuolating activity on AGS cells lines (pH pylori—they induced extensive vacuolation of gastric epithelial cell lines at concentrations down to 0.2 μg/ml. The recombinant toxin also inhibited proliferation of, and IL-2 production by Jurkat cells. Engineering the SNQ insertion into recombinant i1 VacA resulted in modest reductions in the stability of the toxin.

Conclusion A naturally-occurring three amino acid insertion in the VacA i-region renders the toxin less active in vitro. We speculate that this small insertion is a major contributor to the reduced association with cancer of strains with type i2 VacA. Recombinant VacA toxin is highly active on both epithelial and T cells and can be genetically modified to explore VacA structure-function relationships. We aim to use this system to further characterise the structure and function of i-region variants.

Competing interests None declared.

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PWE-172 FACTORS INFLUENCING MALNUTRITION AFTER CURATIVE GASTRECTOMY FOR GASTRIC CARCINOMA

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Introduction Patients with gastric carcinoma often suffer from malnutrition after curative gastrectomy. It is difficult to identify patients who require further nutritional support as part of their long-term post-operative care because factors influencing weight loss are unknown, and it is also inefficient to directly evaluate the nutritional status of every patient using available screening tools. This study aims to identify factors influencing weight loss after curative gastrectomy, which can be used to efficiently identify at-risk patients to improve outcomes.

Methods 163 patients (71.8% of those eligible) who underwent curative gastrectomy from 2000 to 2010 at the National University Hospital were identified from a prospectively maintained database. Factors predicting severe malnutrition at 6 months post-gastrectomy (defined as ≥10% loss of pre-operative weight), and factors influencing gain/plateau vs further weight loss over the following 6 months in the patients who were malnourished at 6 months post-gastrectomy were identified with multiple logistic regression.

Results Severe malnutrition was present in 52.8% (n=86) at 6 months post-gastrectomy. For these patients, those who had anaemia (OR 3.662, p=0.006), gastrointestinal bleeding (OR 2.532; p=0.038) or weight loss (OR 1.052; p=0.010) at presentation, histopathologies of poorly and moderately differentiated tumours (OR 3.632 and 1.757 respectively; p=0.029), adjuvant therapy (OR 8.464; p=0.000), and those requiring step-down care upon discharge (OR 5.739; p=0.021) were at increased risk of weight loss. However, male gender (OR 0.288; p=0.012), vomiting at presentation (OR 0.219; p=0.014), and neo-adjuvant therapy (OR 0.225; p=0.027) were associated with decreased risk. Age, race and operation length were not significant factors at 6 months. Of the patients who were malnourished at 6 months, 54.9% (n=39) developed further weight loss over the subsequent 6 months. Pre-operative total lymphocyte count (OR 2.141; p=0.033) and tumour size (OR 1.235; p=0.054) were associated with increased risk. Age, gender and race were not significant factors at 12 months.

Conclusion Malnutrition is a significant problem post-gastrectomy. More nutritional care should be provided to patients presenting with acute presentation, poor tumour biology and those requiring adjuvant therapy and step-down care as these are risk factors at 6 months. At 1 year, patients who initially had larger tumour sizes and higher pre-operative total lymphocyte counts are at greater risk of continued weight loss, and should receive further nutritional support as part of their long-term post-operative care.

Competing interests None declared.

PWE-173 INCREASED CCL20 AND CCR6+ REGULATORY T-CELL RESPONSES IN THE *HELICOBACTER PYLORI* INFECTED HUMAN GASTRIC MUCOSA

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Introduction *Helicobacter pylori* (*Hp*) infection may result in peptic ulcers or gastric cancer. Disease risk is associated with more virulent strains, such as those with the *cag* pathogenicity island (*cag*PAI), which induce higher levels of gastritis. Disease is also associated with an insufficient anti-inflammatory regulatory T-cell (Treg) response to *Hp*. Preliminary studies of circulating T-cell adhesion molecule expression highlighted an increased frequency of $CCR6^+$ Tregs in the blood of *Hp*+ patients. This chemokine receptor is expressed by both T-helper 17 (Th17) cells and Tregs. It has been demonstrated that the *Hp*-infected human gastric mucosa contains significantly raised concentrations of the CCR6 ligand, CCL20. We hypothesised that CCL20 may play a role in the migration of Tregs to the infected mucosa, and therefore aimed to investigate the mechanisms by which CCL20 expression is induced by *Hp,* and to quantify and characterise gastric mucosal CCR6+ Tregs.

Methods 24 *Hp*+ and 34 *Hp*− patients attending the Queen's Medical Centre, Nottingham, donated gastric biopsies and peripheral blood with informed consent and ethics approval. Isolated CD4 cells were stained for Treg markers ($CD25^{hi}$, $FOXP3^+$, $CD127^{lo}$) and CCR6, prior to analysis by flow cytometry. Gastric epithelial cell lines (AGS, MKN28 and MKN45) were cultured with *Hp* (Wild type, and the following null mutants: Δ*cagA*, Δ*cagE*, Δ*vacA* and Δ*slt*) at a range of multiplicities of infection (MOI), with or without chemical inhibitors, for up to 48 h. CCL20 in culture supernatants was quantified by ELISA.

Results CCL20 levels were threefold higher in biopsies from *Hp*+ patients than Hp− patients (p=0.015). >80% of Tregs extracted from gastric biopsies were $CCR6^+$, and 3.5-fold higher numbers of Tregs were present in samples from infected compared to uninfected patients (p=0.050). twofold higher proportions of Tregs in the peripheral blood of *Hp*+ patients were $CCR6^+$ (p=0.021). In cell lines, *cag*PAI+ *Hp* strains induced a dose-dependent increase in