novel, weighted ranking system was developed to semi-quantitatively score the extremely heterogeneous JAM-A membranous staining in gastric tissues; ultimately ranking tissues as low, moderate or high JAM-A expression. JAM-A scores were also correlated with HER2 status in each section. In parallel, a panel of gastro-oesophageal cell lines was used to investigate potential molecular relationships between JAM-A and HER2. Finally, HER2 protein expression was examined following transient JAM-A gene silencing in the gastro-oesophageal junction cancer cell line ESO26.

**Results and discussions** The pilot study revealed that high JAM-A expression significantly correlated with HER2 positivity, and noted a novel staining pattern for JAM-A in diffuse gastric cancers. Molecular studies showed that JAM-A gene silencing reduced HER2 protein expression in gastric cancer cells. These data suggest that JAM-A may act as a novel upstream regulator of HER2 in gastro-oesophageal cancer cells, and warrant further investigation. Current studies are focussed on elucidating the mechanisms underpinning regulation of HER2 expression by JAM-A.

**Conclusion** Promising preliminary indications that JAM-A plays a role in regulating HER2 expression in non-breast cancers may suggest it as both an unfavourable biomarker, as well as a novel putative pharmacological target for HER2-positive gastro-oesophageal cancers.

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**A POTENTIAL ROLE FOR HSP90 IN HER2-DRIVEN BREAST CANCER (BC)**

Introduction HER2 (amplified in 30% of BC) is involved in the activation of many pathways and its function is regulated by HSP90. Thus, HSP90 co-targeting is emerging as a potential molecular target for HER2-directed BC therapy.

**Material and methods** We analysed HER2 and HSP90 expression in a panel of BC cell lines, including MCF7 cells stably transfected with a constitutively active HER2. HER2/HSP90 expression and growth inhibition were monitored over time upon exposure to trastuzumab (T) and docetaxel (D), in the presence or absence of HSP90 silencing. We also retrospectively evaluated a series of 24 locally advanced/operable BC patients (pts) who underwent neoadjuvant T+D for HSP90 expression and correlated it with pathological complete response (pCR).

**Results and discussions** In the BC cell lines analysed there was no clear-cut correlation between HSP90 and HER2 expression. HER2 transfection into MCF7 cells increased HSP90 mRNA and protein expression; however, treatment with T further increased HSP90 levels. Conversely D increased HER2, but did not affect HSP90, expression. In HER2+BC cell lines, simultaneous T+D combination resulted in synergistic growth inhibition in vitro, while their staggered combination, particularly T followed by D, did not afford synergistic effects. Effects of simultaneous and staggered treatments on HSP90 and HER2 expression were analysed by WB: HER2 expression decreased in the simultaneous and staggered combination (D followed by T), while HSP90 expression did not change upon combined treatment. The effects of HSP90 silencing and overexpression on functional response to T+D are being analysed in HER2+BC models: preliminary results indicate that HSP90 silencing in HER2+BC decreases the therapeutic synergism of the simultaneous T+D combination.

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**LOSS OF SOX9 EXPRESSION IS A PREDICTIVE MARKER OF RELAPSE IN GASTRIC CANCER**

**Introduction** Gastric cancer is one of the most frequent tumours and the third leading cause of cancer-related death worldwide. The investigation of new biomarkers that can predict patient outcome more accurately and allow better treatment and follow-up decisions is of crucial importance. The transcription protein SRY-box 9 (SOX9) is a member of the high-mobility-group box class DNA-binding proteins. SOX9 is an important regulator of cell-fate decisions in embryogenesis and adulthood, playing critical roles in differentiation and proliferation, also in the gastrointestinal tract. SOX9 has been correlated to tumour behaviour in different tissues, including in gastric cancer, nevertheless with contradictory results. In this work we sought to ascertain the relevance of SOX9 transcription factor as a prognostic marker in gastric cancer.

**Material and methods** SOX9 expression was analysed by immunohistochemistry in a series of 333 cases of gastric adenocarcinoma, and its association with clinico-pathological and follow-up data was evaluated. A second gastric cancer validation cohort consisted of 354 cases from the cancer genome atlas (TCGA), showing high versus low SOX9 expression.
PO-503
THE COHESIN STROMAL ANTIGEN 1 (SA-1) MODULATES COLONIC AND COLORECTAL CANCER (CRC) STEM CELLS: MECHANISM FOR RACIAL DISPARITIES

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Introduction CRC disproportionately impacts African-Americans (incidence and mortality increased by ~25% and ~50%, respectively). While mechanisms remain unclear, Vogelstein posited that the number of stem cell divisions determine CRC risk Science 2015. CRC stem cells may impact mortality via chemoresistance. LGR5, aldehyde dehydrogenase (ALDH1a3) and DCAMKL1 are markers of both intestinal and CRC stem cells. We have noted loss of SA-1 (a chromatin remodeler) occurred during colonic field carcinogenesis was markedly accentuated in Blacks (Cancer Prev Res 2016) via specific SNPs (Neoplasia 2018). SA-1 loss was also associated with poorer CRC prognosis. We hypothesised that SA-1 loss leads to stem cell induction and hence CRC disparities.

Material and methods Rectal biopsies were obtained from endoscopically normal mucosa from ~200 patients undergoing screening colonoscopy with an IRB approved protocol. SA-1 was assessed by RT-PCR normalised to β-actin. We modulated SA-1 in human CRC cell line HT29 and tested efficacy of chemotherapy 5 fluorouracil (5-FU) and oxaliplatin via annexin V apoptosis assay.

Results and discussions Adenoma-harbouring subjects had ~50% increase in LGR5, ALD1a3 and DCAMKL1 (p<0.05) with concomitant suppression of SA-1. Causality was indicated by demonstrating that SiRNA SA-1 knockdowns (KD) in HT29 cells caused stem cell marker induction (LGR5=380%, ALD1a3=30% and DCAMKL1=85%, p<0.05). SA-1 overexpression resulted in reciprocal effects downregulation of all 3 stem cell markers. Functionally, SA-1 KD suppressed 5-FU and oxaliplatin induced apoptosis by 56% and 72% respectively versus scramble vector (p<0.0001).

Underscoring racial disparities relevance was the observation that Blacks have a 31% greater SA1 loss vs. Whites (p<0.0004) which mirrored a 35% higher upregulation in LGR5 and ALD1a3 (p<0.05). CRISPR editing of RKO cells to have the SNP rs34149860 (found only in Blacks) resulted in SA1 loss (41% loss, p<0.005) and concomitant ~45% LGR5 and ALD1a3 upregulation (p<0.05).

Conclusion This novel finding that the proneoplastic effects of SA-1 loss may be transduced through intestinal/colonic stem cell (CRC incidence) and also augmenting CRC stem cells resulting in chemoresistance (CRC mortality). Future studies may mitigate CRC disparities in Blacks through development of effective biomarkers and therapeutic.

PO-504
EXPEL: A NOVEL NON-DESTRUCTIVE METHOD FOR MINING SOLUBLE TUMOUR BIOMARKERS

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Introduction The search for biomarkers able to detect and evaluate disease such as cancer at an early stage, or to predict resistance and response to therapies, has been and remains a major challenge. Despite very important progresses in all fields of omics technologies, the success of discovery of clinically valuable biomarkers is surprisingly disappointing. Difficult mining of secreted proteins in biological fluids poses the first major hurdle, mainly because the concentration of interesting proteins in serum or urine is generally very low. The second key limitation in the field is the inaccessibility of tissue specimens from early lesions. Those are routinely required in their integrity for the complete histological evaluation in the clinical routine, leaving no residual material for research.

Material and methods We have developed a simple and original proximal tissue fluid mining method we named EXPEL. It enables efficient extraction of soluble biomarkers while conserving the tissue intact for subsequent pathological analysis. Importantly, the EXPEL method will not only allow the researchers to access human tissues that are very difficult to obtain, but for the first time, scientists and clinicians can share the same material for both experimental research and routine clinical analysis.

Results and discussions We hypothesised that subjecting tissue biopsies to cycles of low-pressure pulses under mild hypotonic conditions would allow a rapid extrusion of interstitial fluid containing the biomarkers of interest, while preserving the morphology and antigenicity of the sample for subsequent pathological investigation.

To test the value of the EXPEL method we have applied our procedure to a series of primary colorectal tumours (CRC) and liver metastasis samples (CRC-LM). This proof-of-principle study demonstrates the validity of EXPEL-extruded fluid as unique starting material for the most advanced OMICs methodologies such as proteomic, genomic, metabolic, while showing no disadvantage for routine clinical and pathological investigations.

Conclusion Our method enables, for the first time, both clinicians and scientists to explore identical clinical material regardless of its origin and size, which has a major positive impact on translation to the clinic.

PO-505
PROGNOSTIC IMPACT OF KRAS SPLICING IN MICROSATELLITE STABLE COLORECTAL CANCER

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Introduction Mutations in the KRAS oncogene represent one of the most common genetic alterations in colorectal cancer