risk and increased severity of Crohn’s disease progression. However, the biological mechanisms responsible of the underlined smoking effects on UC progression remain largely elusive. Several studies have demonstrated the anti-inflammatory action of cholinergic agonists, such as the main tobacco alkaloid nicotine. The contrasting results observed in clinical studies addressing the role of nicotine in UC opened new questions on the molecular mechanisms at the base of the intrinsic nicotinic anti-inflammatory activity and the possible involvement of other tobacco alkaloids in the observed reduced ulcerative colitis disease risk, progression and relapse rate in smokers. In the present study we aimed to investigate the potential preventive anti-inflammatory activity of a tobacco alkaloid (Alkaloid #1), structurally similar to nicotine, in a murine model of intestinal inflammation.

**Methods:** UC-like symptoms were induced in C57BL/6 male mice (n = 14) by 3.5% DSS administration for 7 days. Mice were then treated with nicotine and Alkaloid #1 at 2 different concentrations in drinking water for a total of 21 days (14 days pre-DSS + 7 days during DSS). During the treatment animals were evaluated for progression and severity of UC symptoms.

**Results:** Daily observations (body weight, intestinal bleeding, hematochezia, etc.) and cytokine analysis revealed a protective alkaloid-related effect. Although no differences observed on the histological analysis of colons, alkaloid-treated mice showed a reduced body weight loss, intestinal bleeding and hematochezia. Further molecular analysis are ongoing to investigate the molecular mechanisms implicated in the disease progression and amelioration.

**Conclusions:** Further molecular analysis are ongoing to investigate the molecular mechanisms implicated in the disease progression and amelioration.

**P032 has been withdrawn.**

**P033**  
**The efficacy of tonsil-derived mesenchymal stem cells conditioned medium in chronic colitis model**

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**Background:** Tonsil-derived mesenchymal stem cells (TMSCs) obtained from tonsillecetomy have many advantages such as a short doubling time, high differentiation capacity, and immune modulatory activity. In previous studies, intraperitoneal administration of TMSMC in DSS-induced acute and chronic colitis animal models have shown results of improvement in disease activity index and down regulation of histological grading and pro-inflammatory cytokine expression levels. However, the TMSCs were not observed to migrate to the inflamed intestine in vivo. These results are presumed to be due to the paracrine effect of TMSCs. In this study, we tried to verify the therapeutic effect of TMSCs conditioned medium (TMSCS-CM) in the mouse model of DSS-induced chronic colitis.

**Methods:** In vitro, immunosuppressive effects of TMSCs-CM were confirmed by splenocyte immunosuppression assay. C57BL/6 mouse splenocytes were stimulated with mitogen such as LPS or PMA/Ionomycine, and then cultured in TMSCs-CM or co-cultured with TMSC. After 24 h, proliferation of splenocytes were measured using the cck-8 kit. In vivo, eight-week-old C57BL/6 mice were randomly assigned into four groups: normal, colitis, TMSC, and TMSC-CM groups. Chronic Colitis was induced by oral administration of 1.5% dextran sulfate sodium (DSS) for 5 days followed by 5 days of drinking water continuously for three cycles. TMSC (1x106/500 μl) and TMSMC-CM (500 μl) were administered via intraperitoneal injection four times and 12 times. The severity of the colitis was assessed by measuring the disease activity index (DAI), colon length, histologic grading, and cytokine levels.

**Results:** The splenocyte stimulated by LPS showed decreased proliferation when co-cultured with TMSMC (3.18 ± 0.07 vs. 2.03 ± 0.12, mean ± standard error mean, control group vs. p < 0.0008, ANOVA), and cultured in TMSMC-CM (3.18 ± 0.07 vs. 1.81 ± 0.06, p < 0.0001). Proliferation was significantly reduced by the number of TMSMC (p = 0.0035) and TMSMC-CM concentration (p = 0.0028). In a chronic colitis animal model injected TMSMC [X4] or TMSMC-CM [X12], reduction of DAI (3.25 ± 0.25 vs. 1.50 ± 0.28 vs. 1.30 ± 0.28, control vs. TMSMC vs. TMSMC-CM group, p = 0.0038), increased of weight gain (1.65 ± 2.88 vs. 5.08 ± 3.31 vs. 6.00 ± 0.52, p = 0.4562, p = 0.1888, respectively), and recovery of colon length (61.4 ± 2.82 vs. 70.0 ± 2.74 vs. 72.60 ± 2.68, p = 0.0602, p = 0.0205 respectively) was observed at day 30.

**Conclusions:** In the DSS induced chronic colitis animal model, the administration of TMSMC as well as TMSMC-CM showed almost the same effect on improvement of inflammation. Therefore, we suggest the use TMSMC-CM for IBD treatment utilising paracrine factors of TMSC without any cell transplantation.

**P034**  
**Validation of a novel xenograft mouse model for intestinal fistulas**

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**Background:** Fistulas are a frequent, relapsing complication in Crohn’s disease (CD), affecting up to 50% of patients. Surgical resection is regularly required, as medical treatment outcome with conventional drugs is often insufficient. Previously, we demonstrated that epithelial-to-mesenchymal transition (EMT) plays a critical role for fistula development in CD patients. We found particular cytokines and their receptors upregulated along the tracts, supporting fistula development via the stimulation of EMT. Despite some progress in understanding the pathogenesis, there is still an urgent need for more effective medical treatments for CD fistulas. Due to a lack of a reliable in vivo model, new drug developments are complicated. Here, we validated a promising new human gut xenograft (XGR) mouse model of intestinal fistulas, clearly resembling the human situation.

**Methods:** 12–18 weeks (w) old human foetal small intestine was transplanted subcutaneously onto the backs of SCID mice. After 12–16w, ~15% of the mature xenografts spontaneously developed enterocutaneous fistulas. Using systemic LPS treatment followed by mild skin irritation adjacent to the transplant, we established
a reproducible model system, resulting in enterocutaneous fistulas 2–4 w later. XGR samples were analysed by immunohistochemistry staining (IHC) for EMT-, immune cell- and cell death markers.

**Results:** IHC staining of the XGR fistulas showed similar expression patterns for various EMT markers (e.g., SNAIL1) like in human CD fistula samples. The overexpression of the mesenchymal marker alpha-smooth muscle actin confirmed the hypothesis that EMT plays a critical role for the fistula development in the XGR mouse model, too. Moreover, collagen staining showed that the inflammatory regions were associated with fibrosis suggesting extracellular matrix remodelling. The inflammatory response up- and downstream to the XGR fistula tracts mainly consisted of human CD45+ cells, but only very few murine CD45+ cells. Further characterisation revealed CD4+ T cells as predominant cell type in the fistulating samples. Also strong expression levels of human CD68+ cells were found in the XGR fistulas. The overexpression of TNFα in the XGR fistulas samples emphasizes the importance of this mouse model, since this most likely represents a novel platform for the evaluation of new therapies for CD fistulas in vivo. Positive TUNEL and cleaved caspase-3 IHC staining in the XGR fistula samples suggest apoptosis playing a role here, too.

**Conclusions:** Our data demonstrate that the in vivo model recapitulates both morphologically and mechanistically, human CD-associated fistulas. Establishing this novel in vivo platform, could improve identifying unique treatment targets and help to evaluate new therapies.

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**P035**

**Serum markers predict outcome to ustekinumab in patients with refractory Crohn's disease and provide insights in the mechanism of action**

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**Background:** Ustekinumab (UST), targeting the IL-12/23 shared p40 subunit, was recently approved by FDA and EMA for treatment of moderate-to-severe Crohn’s disease (CD). The aim of this study was to identify potential predictive immunological biomarkers for response, which may guide treatment strategies with ustekinumab.

**Methods:** Serum samples of 36 CD patients (73% female, median disease duration 15.9 years), all refractory to anti-TNF therapy and vedolizumab and with baseline endoscopic active disease, were prospectively collected prior to UST initiation. Patients received UST 6 mg/kg IV at induction, with subcutaneous UST 90 mg q8w thereafter. Endoscopic response was assessed at week 24, and defined as ≥50% SES-CD decrease. Proteomic analysis (OLINK) was performed on baseline serum samples. Additionally, inflamed ileal (n = 10) and colonic (n = 17) biopsies, prior to UST therapy, were collected. Mucosal total RNA was isolated, and next-generation sequencing performed. Differentially gene expression was evaluated by DESeq R package.

**Results:** Patients with (n = 7) and without (n = 29) endoscopic response at week 24 had a similar baseline inflammatory burden, reflected by similar median faecal calprotectin (1800 vs. 1721 µg/g, p = 0.22), C-reactive protein (20.3 vs. 9.4 mg/L, p = 0.36) and IL-6 (p = 0.37, fold change (FC)=1.06) before start of UST. Baseline endoscopic activity was much higher in patients responding to UST, compared with non-responders (median SES-CD 21 vs. 13, p < 0.001). Several proteins significantly correlated with baseline SES-CD, but only one protein, CD40 (r = 0.87, p = 0.05), also significantly differed between responders and non-responders before UST initiation (p = 0.029 with corresponding FC 1.46). At baseline, CCL11 also varied between responders and non-responders (p = 0.06, FC 1.45), but did not correlate with baseline SES-CD (p = 0.97). ROC-statistics showed a significant area under the curve (81.5%, p = 0.011) for prediction of response based on the combination of both. On mucosal level, a non-significant increase in both CD40 and CD40L could be observed in colonic biopsies of responders at baseline (FC 1.6 and 1.5, respectively). Ileal biopsies also expressed increased CD40L in responders (FC 2.0).

**Conclusions:** Two potential predictive biomarkers for response to UST were identified, which need validation in larger and independent cohorts. Because it has been shown that CD40/CD40L-triggering of dendritic cells induces expression of high levels of IL-23 and not IL-12, low CD40 levels in non-responders suggest another mechanism, apart from the IL-12/23 pathway, driving inflammation in these patients. These findings may aid in individualised selection of biological agents in Crohn’s disease, and provide mechanisms of primary (non-)response to UST.

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**P036**

**Faecalibacterium prausnitzii produces butyrate to maintain Th17/Treg balance and to ameliorate colorectal colitis by inhibiting histone deacetylase 1**

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**Background:** Inflammatory bowel disease (IBD)-associated dysbiosis is characterised by a loss of Faecalibacterium prausnitzii, whose supernatant exerts an anti-inflammatory effect. However, the anti-inflammatory substances in F. prausnitzii supernatant and the mechanism in ameliorating colitis in IBD have not yet been fully investigated.

**Methods:** Experimental colitis models were induced and evaluated by clinical examination and histopathology. Levels of cytokines and ratio of T cells were detected by enzyme-linked immunosorbent assay and flow cytometry analysis, respectively. F. prausnitzii supernatant was separated by microporous resins. After extraction, the substances in supernatant were identified by gas chromatography-mass spectrometer. T cell differentiation assay was conducted in vitro. Changes in signalling pathways were examined by western blot, immunohistochemistry and immunofluorescent staining.

**Results:** We found that the supernatant of F. prausnitzii could regulate T helper 17 cells (Th17) and regulatory T cells (Treg) differentiation. Then, we identified that butyrate produced by F. prausnitzii that played the anti-inflammatory effects by inhibiting interleukin (IL)-6/ signal transducer and activator of transcription three (STAT3)/IL-17/interleukin (IL)-17 receptor (IL-17R) and signal transducer and activator of transcription five (STAT5)/signal transducer and activator of transcription four (STAT4) pathway. Finally, we demonstrated the target of butyrate was histone deacetylase 1 (HDAC1).