O-1 *Novel Insights*

Plasmablasts producing inhibitory autoantibodies against muscarinic receptor 3 in salivary glands of Sjögren's syndrome patients

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Introduction. Sjögren's syndrome (SS) is a chronic inflammatory disease of autoimmune origin characterized primarily by dysfunction of exocrine glands mainly lacrimal and salivary glands leading to dry eyes (Xeropthalmia) and dry mouth (Xerostomia) respectively. The hallmark of the disease is the presence of lymphocytic infiltrates in salivary glands and autoantibodies against intracellular antigen Ro and La. Anti-Muscarinic receptor 3 autoantibodies is another controversial set of antibodies prevalent in SS patients. Muscarinic receptor 3 (M3R) is a GPCR present on the acinar cells of the salivary glands and is known to exert a secretory function in exocrine glands. The extracellular portion of M3R consists of three extracellular domains known to play a role in interaction with acetylcholine. We hypothesized that antibodies derived from the plasmablasts in the salivary glands of SS patients binds and inhibits the M3 receptor activity and leads to primary symptoms of dry eyes and dry mouth.
Methods. Recombinant Monoclonal Antibodies Production (MAbs): Patients having dry eyes and dry mouth as prime symptoms were evaluated and classified into SS (Sjögren's) and DNMCs (Controls) according to AECG Criteria. Single cell suspension samples were prepared from the labial salivary glands obtained following biopsy. Plasmablasts (antibody secreting cells) having CD3- CD4- CD8- CD19+ $CD27^{high}$ $CD38^{high}$ IgG+ were sorted out by fluorescence-activated single cell sorting. Human recombinant monoclonal antibodies were produced from plasmablasts by sequencing the V, D, J and CDRs regions of heavy and light chains amplified by RT PCR and nested PCR, cloned into a vector, transfected into a human cell line (HEK) and expressed. IgGs were purified by beads method and confirmed by an immunoblot. The MAbs produced from SS and DNMCs were 51 and 18 respectively.
Muscarinic Receptor ELISA and Functional Assay. M3R ELISAs were done using multiple antigenic peptides of 2nd (a.a. 213-228) and 3rd (a.a. 514-527) extracellular domains of M3R. FRET-based *In-Vitro* functional assays **(FA)** were done on chimeric CHO-K1 NFAT-M3R bla cells engineered to express human M3R along with a reporter gene under control of NFAT response element. CHO-K1 cells were incubated with mAbs of SS and DNMCs (100µg/ml) with or without muscarinic agonist (carbachol) at EC80 dose to test the Inhibitory/Stimulatory potential (antagonist /Agonist assay) respectively.
Results. SS patients MAbs were highly reactive to 2nd as well as 3rd ECDs of M3R when compared to DNMCs. Significant high O.D. (>3.5) values were obtained from MAbs from SS. In SS group, 9/51 MAbs were positive to 2nd ECD and 5/51 were positive for 3rd ECD. None of the MAbs from DNMCs were positive for 2nd ECD however 2 of the MAbs were positive for 3rd ECD (Cutt Off 2 S.D. above DNMC mean O.D.). Six of the MAbs showed inhibition in the functional assay (Cuttoff >2 S.D. mean inhibition of DNMC). 4/6 MAbs positive for the assay were also positive for M3R ELISA. None of these antibodies were found to be stimulatory.
Conclusion. Our studies showed a high reactivity of monoclonal antibodies from SS patients to 2nd and 3rd ECDs that also showed high inhibition of M3R activity in in-vitro cell based M3R functional assays. A decrease in M3R activity could possibly result in salivary and lacrimal dysfunction. Our future studies will involve the passive transfer of M3R reactive monoclonal antibodies to mice for observing this inhibitory effect *in-vivo* on salivation.

O-2 *Novel Insights*

Uncharacterized X-linked protein CXorf21 provides molecular explanation for sex-bias in SLE

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Background. Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) are complex autoimmune disorder related by B cell hyperactivity resulting in autoantibody and cytokine production. Approximately 90% of patients are female. We have produced data showing an X-chromosome gene dose effect or simply the number of X chromosomes increases susceptibility. Therefore, our objective is to functionally describe the protein encoded by the gene Chromosome X open reading frame 21 *(CXorf21),* which escapes X inactivation and is a lupus risk gene, to uncover any role this protein may have in the pathogenesis or susceptibility to SS and SLE. Publically available data predict CXorf21 is a dehydrogenase/reductase expressed almost exclusively in B cells and myeloid lineage antigen-presenting cells. Additional studies show that CXorf21 directly interacts with another SS/SLE-associated risk allele, Slc15A4. SLC15a4, a pH-sensitive lysosomal proton-oligopeptide co-transporter; and, is necessary for lysosomal antigen processing, TLR7- and NOD1-mediated cytokine as well as antibody production in dendritic cells and B cells.
Methods. We used quantitative real-time PCR, Western blot protein analysis, immunofluorescence, pHrodo™ and Lysosensor™ assays, as well as, *in vitro* CRISPR-Cas9 knockdown experiments to examine the role of CXorf21 in monocytes and B cell immunity.
Results. Our *in vitro* qPCR data confirm CXorf21 expression in CD19+ B cells and monocytes. Preliminary immunofluorescence studies show that CXorf21 is a cytosolic rather than nuclear protein. Our data show that CXorf21 basal gene and protein expression is elevated more than 1.5-fold in female primary monocytes compared to male cells. Additionally, we found that following a time- and concentration-dependent activation by TLR7 (Imiquimod [2µM]) or NOD1 (C12-iE-DAP [20µM]) agonists, resulted in TLR7 and CXorf21 expression increase 3- and 5-fold, respectively. Successful knockdown of CXorf21, using CXorf21-specific gRNA (CRISPR-Cas9), abrogated this effect. Initial pHrodo™ [20µg/ml] and Lysosensor™ [10µM] lysosomal experiments revealed that healthy male primary monocytes trend toward a higher pH, while healthy female monocytes lysosomes had a lower pH, and knockdown of CXorf21 protein resulted in an increased lysosomal pH in female monocytes, and no change in the male sample. Furthermore, treatment with hydroxychloroquine (HCQ) increased lysosomal pH in female cells.
Conclusion. CXorf21 is over-expressed in female immune cells compared to male cells, and is involved in a sex-dependent dimorphic response to activation through TLR7 or NOD1. In addition, lysosomal pH is regulated by CXorf21 is a sexually dimorphic manner. We propose that CXorf21 maintains the lysosome pH environment necessary for monocyte and B cell immune response. Thus, sexual dimorphic expression of CXorf21, based on escape of X chromosome inactivation, skews (auto) antigen processing and immune response by women compared to men. CXorf21 may be a major contributor to disease pathogenesis, and sex bias of the diseases based on an X chromosome dosage effect.

O-3 *Novel Insights*

Fatigue in Sjögren's syndrome: a search for biomarkers and treatment targets

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Background. Fatigue is a major complaint in primary Sjögren's syndrome (pSS) affecting up to 70% of pSS patients and is associated with a poor quality of life (1-6). The biological basis of fatigue is largely unknown. Therefore, it is important to identify pathways underlying or regulating fatigue. Here we use a proteomics approach to try to identify biomarkers and possible treatment targets. The aptamer-based SOMAscan technology, which is optimized for protein biomarker discovery, detects over 1300 proteins simultaneously in a sample of 65uL.
Methods. SomaLogic SOMAscan 1.3k assay was used to analyze protein expression in serum samples of 65 pSS patients and 20 healthy controls. Fatigue was measured using the multiple fatigue inventory questionnaire. Instem/Omniviz software was used to identify proteins predictive for fatigue. Expression of serum proteins was further validated using ELISA.
Results. Fourteen proteins were significantly differentially expressed in fatigued compared to non-fatigued pSS patients. Proteins include several complement factors (C4b, C3b, C3d, C3 and the C1 inhibitor, SERPING1), enolases (alpha and gamma), SNAP25, IL-36, BMP6 and UCH-L1. For three of the proteins ELISAs (alpha-, gamma-enolase and IL-36) were available and they showed a good correlation with the SOMAscan data.
Conclusion. Using SOMAscan technology we were able to identify several proteins which were expressed higher in fatigued pSS patients compared to non-fatigued pSS patients. Most of these proteins are involved in cell death or survival and acute phase responses. Several of these proteins have previously been shown to cross the blood-brain barrier, are associated with different diseases involving the brain or have been associated with fatigue in different diseases. Therefore, these proteins could also be relevant in the context of fatigue in pSS.
Funding. The study was supported by a grant of the Dutch Arthritis Foundation (RF14-3-404).

References

1. CHAMPEY J, CORRUBLE E, GOTTENBERG J-E, BUHL C, MEYER T, CAUDMONT C *et al*.: Quality of life and psychological status in patients with primary Sjögren's syndrome and sicca symptoms without autoimmune features. *Arthritis Care Res* 2006; 55(3):451-57.
2. SEGAL B, THOMAS W, ROGERS T, LEON JM, HUGHES P, PATEL D *et al*.: Prevalence, Severity and Predictors of Fatigue in Primary Sjögren's Syndrome. *Arthritis Rheum* 2008; 59(12):1780-87.
3. HALDORSEN K, BJELLAND I, BOLSTAD AI, JONSSON R, BRUN JG: A five-year prospective study of fatigue in primary Sjögren's syndrome. *Arthritis Res Ther* 2011; 13(5): 1-8.
4. THEANDER L, STRÖMBECK B, MANDL T, THEANDER E: Sleepiness or fatigue? Can we detect treatable causes of tiredness in primary Sjögren's syndrome? *Rheumatology* 2010; 49(6): 1177-83.
5. KARAGEORGAS T, FRAGIOUDAKI S, NEZOS A, KARAISKOS D, MOUTSOPOULOS HM, MAVRAGANI CP: Fatigue in Primary Sjögren's Syndrome: Clinical, Laboratory, Psychometric, and Biologic Associations. *Arthritis Care Res* 2016; 68(1): 123-31.
6. OVERMAN CL, KOOL MB, DA SILVA JAP, GEENEN R: The prevalence of severe fatigue in rheumatic diseases: an international study. *Clin Rheumatol* 2016; 35(2): 409-15.

O-4 *Novel Insights*

Improvement in fatigue following a multidisciplinary, biopsychosocial intervention: data from 50 primary Sjögren's syndrome patients

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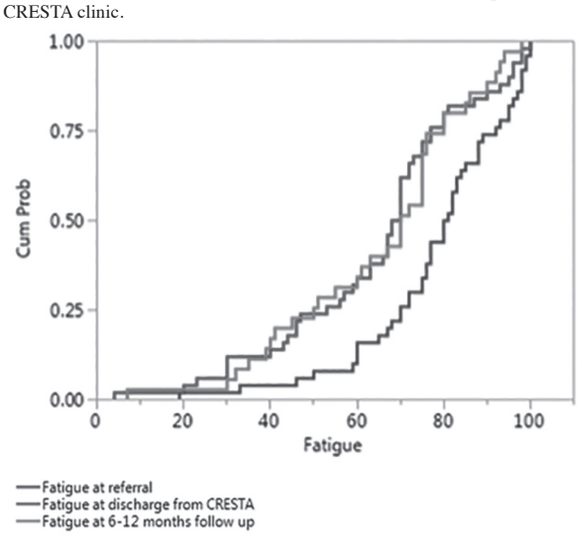
Background. The Newcastle CRESTA Fatigue clinic is a UK National Health Service multidisciplinary clinic, established in 2013 to support people with the symptom of fatigue alongside a physical health condition. Local primary Sjögren's syndrome (PSS) patients experiencing fatigue, are offered a referral to this clinic by their rheumatology clinician.
A medical consultant and an occupational therapist assess all new PSS patients to the CRESTA Fatigue clinic. The medical clinician identifies reversible causes of fatigue including; autonomic dysfunction, untreated comorbidities and reviews medications. The occupational therapist coordinates therapy interventions, ensuring these are tailored according to the needs of the patient. Therapy interventions include occupational therapy (activity management), physiotherapy (core strengthening exercises), health psychology, cognitive behavioural therapy for insomnia or a combination of therapies.
Methods. Patient outcomes are collected routinely at each rheumatology out-patient clinic visit. We compared fatigue (visual analogue scale 0-100) at referral, discharge and at 6-12 months following discharge from the CRESTA Fatigue clinic for the first PSS patient cohort (n=50) accessing the CRESTA Fatigue clinic using a Wilcoxon signed rank paired analysis. Next, we entered other baseline clinical data (age, disease activity (ESSDAI), dryness (1-10), pain (1-10), depression and anxiety scores (Hospital Anxiety and Depression Scale) into a multivariate analysis to identify factors which predict improvements in fatigue following the intervention.
Results. Each patient had a median of 8.5 (IQR 10.25) clinic appointments. Fatigue scores improved from a mean of 78.4 to 65 and were maintained at 6-12 months follow-up. These results were statistically significant (p<0.001) and clinically meaningful[1]. High pain and low anxiety scores at baseline predicted greater improvements in fatigue following the intervention (p<0.05).
Conclusion. A tailored multidisciplinary fatigue intervention has improved fatigue severity in this PSS patient group. These findings demonstrate the clinical effectiveness of interdisciplinary care for fatigue management in PSS.

References

1. GEORGE A, POPE JE: The minimally important difference (MID) for patient-reported outcomes including pain, fatigue, sleep and the health assessment questionnaire disability index (HAQ-DI) in primary Sjögren's syndrome. *Clin Exp Rheumatol* 2011; 29(2): 248-53.

Fatigue scores at baseline, discharge and 6-12 months follow-up from the CRESTA clinic.



O-5 *Novel Insights*

A novel brief questionnaire to screen dry eye patients for Sjögren's syndrome

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Purpose. To develop a screening tool based on questionnaire responses from the Sjögren's International Clinical Collaborative Alliance (SICCA) cohort that will serve as a sensitive and specific screening method for ophthalmologists to identify dry eye patients with a high likelihood of having Sjögren's Syndrome (SS).
Methods. A cross-sectional study was performed utilizing the baseline data from the SICCA study, which included the SS status of each participant. SS status was defined using the 2016 American College of Rheumatology-European League Against Rheumatism classification criteria.[1] Participants were limited to those patients with dry eye symptoms who were self-referred or referred by an ophthalmologist (n=1053). Potential predictors of SS were selected from the baseline questionnaire based on which organ systems may be affected by SS which included the following categories: 1) General/Physical/Emotional Health (21 questions); 2) reproductive and hormonal history (2 questions); 3) symptoms affecting the mouth (9 questions); 4) symptoms affecting the eyes (22); 5) medical history (17 questions), and 6) systems review (16 questions). Univariate and multivariate logistic regression models were used to identify symptoms that were useful for distinguishing SS cases from non-cases. Odds ratios and 95% confidence intervals (95% CI) from logistic regression models were used to estimate magnitude of the association between the presence of a specific symptom and SS. Area under the ROC curve (AUC) was used to summarize the ability of a specific symptom or combination of symptoms for distinguishing SS cases from non-cases.
Results. Using a univariate analysis, a set of 11 questions out of 87 were identified that could distinguish dry eye patients with SS (n=467) or without SS (n=586). Through backward variable selection, 4 questions were found to be independently associated with a higher likelihood of having SS in a multivariate logistic regression model. These questions included: 1) How often do you have excessive tearing? [none of the time = OR 3.63 (1.86-7.07)]; 2) Are you able to produce tears? [no=OR 2.23 (1.65-3.01)]; 3) Can you eat a cracker without drinking a fluid or liquid? [no=OR 1.37 (1.02-1.84)]; and 4) Is your mouth dry when eating a meal? [yes=OR 1.53 (1.14-2.05)]. The AUC from a multivariate logistic regression model using these 4 symptoms as predictors was 0.69 (95% CI 0.66-0.72). The question "Does your mouth feel dry?" was not helpful in distinguishing the two groups (7.5% in SS, 11.1% in controls).
Conclusions. We found that in a subset of dry eye patients from the SICCA study, the common screening question "Does your mouth feel dry?" was not useful in distinguishing those with SS from those without SS. We identified 4 questions that can be used to screen dry eye patients for having a high likelihood of having SS. To our knowledge, this is the first evidence-based screening tool for ophthalmologists that could be used to identify dry eye patients requiring systemic SS evaluations earlier, thereby facilitating diagnosis and leading to better clinical outcomes.

References

1. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjogren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol* 2017; 69(1): 35-45.

O-6 *Novel Insights*

Interleukin-27 regulates the magnitude of the ectopic germinal centre response in a virus-induced murine model of sialadenitis

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Background. Ectopic lymphoid structures (ELS), leukocyte aggregates forming in chronically inflamed tissues, develop in the salivary glands (SG) of 30% of Sjögren's Syndrome (SS) patients. In SS, ELS play an active role in disease progression and are typically associated with a more aggressive disease and development of MALT lymphoma. Interleukin-27 (IL-27) is prominently associated with the negative control of adaptive immunity, and in particular with the suppression of Th17-type responses.
In order to elucidate the role of IL-27 in the control of lymphoid neogenesis and its functional relationship with aberrant IL-17 production, we used a murine model of inducible ELS, where the local administration of a replication-deficient adenovirus (AdV) triggers the formation of ectopic germinal centres in salivary glands.
Methods. A single administration of AdV was delivered by cannulation directly into the SG of wild-type (WT) and IL-27R-deficient (Il27ra-/-) mice. For IL-17A blockade, an anti-mouse IL-17A antibody or IgG control was administered systemically. ELS development and peripheral immune responses were temporally tracked by immuno-histopathology, flow cytometry, and real-time qPCR.
Results. AdV cannulation induced an early upregulation of IL-27 and IL-27R in WT mice SG, which was mirrored by an increase in the infiltration of IL-27-producing T, B and NK cells. AdV-challenged Il27ra-/- mice developed exacerbated salivary gland inflammation, and by day-19 post AdV challenge developed larger and more abundant ELS as compared to WT mice. Moreover, Il27ra-/- mice displayed a heightened expression of homeostatic cytokines, chemokines and their corresponding receptors that are required for lymphoid neogenesis (*e.g.*, Cxcl13, Ccl19 and Ltb). IL-27R-deficient mice also displayed elevated markers of functional germinal centre responses (*e.g.*, activation-induced deaminase, AID). Underpinning the exaggerated development of ELS in Il27ra-/- mice was the preferential expansion of IL-17-producing T helper (Th)17 cells, which was linked to a reduction in the Th1 cell population. This was confirmed using a neutralising antibody to IL-17A, which resulted in a reduction in the size of ELS as determined by immunofluorescence detection of T and B-cell involvement. The inhibition of ELS development by anti-IL-17A treatment was also reflected by the reduced expression of lymphoid chemokines and AID. Notably, the infiltration of IL-22-producing $CD4^+$ cells, a key effector population involved in ELS formation, was also reduced in anti-IL-17-treated Il27ra-/- but not WT mice.
Conclusions. Here we show that IL-27 has a non-redundant inhibitory role in the regulation of the magnitude of ectopic germinal centre responses in inflamed SG. In the absence of a regulatory IL-27 signal, an exaggerated Th17 cell response was linked to dysregulated ELS size and activity. These findings provide new insights into the mechanisms governing ELS formation and highlight the role of IL-27 as an endogenous inhibitor of lymphoid neogenesis, which could be exploited for therapeutic purposes in SS.

O-7 *Mechanisms of Mucosal Dryness*

MiR-143-3p targets calcium-transporting ATPase sarcoplasmic reticulum isoform 2b (SERCA2b), ryanodine receptor 2 (RyR2) and adenylyl cyclase 9 (AC9) contributing to the loss of epithelial cell homeostasis in Sjögren's syndrome

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Background. Sjögren's syndrome (SS) is a systemic autoimmune disease that mainly affects salivary and lacrimal glands. SS pathogenesis is unknown, though it has been shown that both immune-independent and immune-dependent mechanisms are involved. Numerous studies have shown that epigenetic factors contribute to both mechanisms. MicroRNAs

(miRNAs) are endogenous small noncoding RNA molecules that regulate the expression of target genes through translational repression of mRNAs. Transcriptomic profiling studies in minor SGs of SS patients performed by our group showed a significant upregulation of miR-142-3p in SS. MiR-142-3p is known to play a role in regulating T cell development and its overexpression may lead to loss of self-tolerance. Other studies have shown that miR-142-3p regulates the expression of proteins such as AC9. Very little is known about the role of this miRNA in epithelial cells. The purpose of this study was to identify and validate target genes downregulated by miR-142-3p in epithelial cells and to evaluate its role in the loss of epithelial cell homeostasis observed in SS.

Methods. Structured search for target genes of miR-142-3p involved in salivary gland physiology was performed with TargetScan Release 7.1. SERCA2b, RyR2 and AC9 were selected for further validation and functional analysis. Binding of the miRNA was confirmed by luciferase reporter assays in HSG cell lines and human-derived primary epithelial cells. The mRNA and protein levels of SERCA2b, RyR2 and AC9 were determined by qPCR and Western blot, respectively. To investigate the cell-specific distribution of miR-142-3p in relation to the expression levels of SERCA2b, RYR2, and AC9 in SG biopsies and miR-142- 3p-transfected cells, a double fluorescent *in situ* hybridization was performed. Ca2+ signaling and cAMP levels were measured using fluorescent sensor upon carbachol and isoproterenol stimulation, respectively.

Results. We showed that miR-142-3p binds to the 3' untranslated region of SERCA2b and RyR2. Additionally, miR-142-3p-transfected cells showed a significant decrease in mRNA and protein levels, as well as a decrease in fluorescent intensity of SERCA2b, RyR2 and AC9 by *in situ* staining. MiR-142-3p in the pSS SGs was localized in both acinar cells and inflammatory cells, but the fluorescent intensity was higher in inflammatory cells. Importantly, functional assays showed that overexpression of miR-142-3p restricted cAMP production and altered calcium signaling upon isoproterenol and carbachol stimulation, respectively.

Conclusions. This study is the first to validate SERCA2b and RyR2 as direct binding targets of miR-142-3p in epithelial cells. The observed downstream changes associated with these novel molecular targets identified a novel mechanism that can lead to salivary gland dysfunction by altering calcium signaling pathways. These results also complement studies that show loss of epithelial cell homeostasis in SS, by identifying a molecular mechanism that leads to dysregulated calcium signaling.

O-8 *Mechanisms of Mucosal Dryness*

Senescence of salivary gland stem cells in primary Sjögren's syndrome: the cause of persistent hyposalivation?

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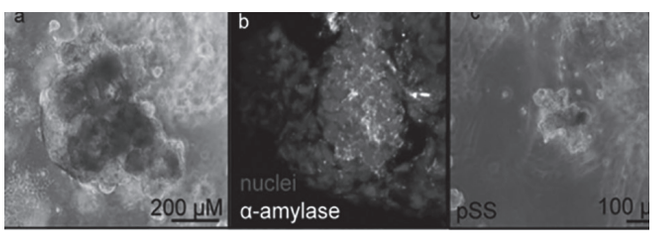
Background. Hyposalivation is frequently observed in primary Sjögren's syndrome (pSS), resulting from dysfunction of the parenchymal tissue of the salivary gland (SG). This dysfunction is often presumed to be due to lymphocytic infiltration. SG homeostasis is controlled by salivary gland stem cells (SGSCs), which differentiate and proliferate from the basal striated duct niche, through the intercalated ducts and into eventual saliva-producing acinar cells. Given the non-functional nature of SGs in pSS, we sought to investigate the regenerative capacity of SGSCs in pSS and probe reasons behind the persistent state of hyposalivation.

Methods. SGSCs were isolated from parotid SG biopsies of healthy control (HC), incomplete and complete pSS patients and exposed to a self-renewal assay to assess proliferation ability. Mini gland formation assays were performed to ascertain differentiation potential. Extracted DNA from SGSCs was analyzed for telomere length using STELA. To assess the effect of an inflammatory environment on SGSCs, self-renewal assays were performed with added IFNα, TNF-α and IL-6. Immunostaining for p16 (senescence marker) and CD24 (stem cell marker) was performed on incomplete pSS and HC tissue respectively. CD24 expression and cell cycle were analyzed in cytokine-exposed SGSC cultures by flow cytometry.

Results. SGSC yield from pSS biopsies was five-fold lower than from HC biopsies. pSS-SGSCs were capable of significantly lower degrees of proliferation, a classical characteristic of stem cells, when subjected to our self-renewal assay. Single cell-derived mini glands containing amylase expressing acinar cells could be generated from HC-SGSCs, but not from pSS- SGSCs (Fig. 1a-c). Telomeres in pSS-SGSCs were significantly shorter than HC-SGSCs, suggesting that SGSCs in pSS are senescent. Exposure of SGSCs to a IFNα, TNF-α and IL-6 cytokine cocktail resulted in increased organoid forming efficiency, followed by decrease, compared to HCs. SGSC cultures contain a mixture of basal striated duct (BSD) and intercalated duct (ID) cells. Proinflammatory cytokine exposure induced a decrease in BSD cell number and an increase in ID cell number, and expression of senescence-associated genes in SGSC. Significantly more p16+ cells, indicative of senescence, were found in incomplete pSS tissue sections, compared to HC and complete pSS. p16+ cells were localized to ID cells. These data suggest that proinflammatory cytokines can modulate SGSC dynamics and potentially induce their senescence and exhaustion.

Conclusion. We suggest for the first time that persistent hyposalivation in pSS is not per se caused by lymphocytic infiltration of the salivary glands only, but by exposure of SGSCs to pro- inflammatory cytokines. These data open the door for new therapeutic interventions for hyposalivation in pSS such as generation of new SGSCs using iPS technology, and a deeper comprehension of pSS as a disease.

Fig. 1. a) Mini salivary gland formation from healthy control SGSCs.



b) α-amylase expression in differentiated mini glands, indicative of functional acinar cells. **c**) Attempted mini gland formation from pSS-SGSCs.

O-9 *Biomarker Discovery for Diagnosis and Stratification*

The novel anti-CD40 monoclonal antibody CFZ533 modulates biomarkers relevant to disease and CD40 pathways in patients with primary Sjögren's syndrome

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Background. Primary Sjögren's syndrome (pSS) is a systemic and progressive autoimmune disease characterized by the formation of ectopic germinal centers in exocrine glands and the alteration of their secretory function. A subset of patients also develops extraglandular manifestations. CFZ533 is a novel monoclonal antibody that potently and selectively blocks CD40, a co-stimulatory pathway receptor essential for germinal center reactions and other immune mediated functions implicated in pSS pathogenesis. In a recent randomized, double-blind, placebo-controlled, multi-centric trial, patients treated with CFZ533 displayed improvement in EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), as well as in other clinical

indices, such as EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), and physician's and patient's Global Assessments. In this clinical study, we also sought to examine whether CFZ533 could mediate changes in soluble, cellular, and genomic biomarkers relevant to disease pathology and CD40 pathway inhibition.
Methods. Whole blood was collected from 32 patients in the 10 mg/kg i.v. CFZ533 or placebo-treated arms (randomized 2:1), at baseline and at predefined time points over 12 weeks, as well as over the subsequent period of 12 weeks during which placebo-treated patients were switched to 10 mg/kg i.v. CFZ533. Whole blood was profiled for the presence of leukocyte subsets and activation markers by flow cytometry, and RNA prepared from whole blood was subject to next-generation sequencing. In addition, levels of various soluble markers (B cell hyperactivity, chemokines and ANAs) were analyzed by multiplex assays or ELISA.
Results. At the cellular level, we observed a decrease in the percentage of ICAM1 expressing B cells in CFZ533 treated patients, demonstrating a pharmacodynamic effect of CFZ533 on the CD40 pathway. None of the cell types assessed by flow cytometry (B cells, T cells – CD4, CD8, CD3, Nk cells and monocytes) showed any depletion. Moreover, activation of B cells assessed using CD69 and CD23 markers was not observed upon CFZ533 treatment. At the soluble level, we observed a decrease in the germinal center-related serum biomarker, CXCL13, in CFZ533-treated patients and a trend for reduction in anti-Ro (but not anti-La) autoantibodies, despite large variability in the response. No clear differences in levels of other soluble biomarkers between CFZ533 and placebo-treated patients were observed (C3, CCL13, CCL2, CCL8, CXCL5, CXCL10, CCL22, CCL4, CCL20, CCL17 and BAFF). At the genomic level, we also observed a reduction in the expression of pro-inflammatory genes in CFZ533-treated patients. Ongoing work is evaluating the modulation of these various markers in the context of different disease activity indices.
Conclusions. In addition to evidence of clinical efficacy of CFZ533 in patients with primary Sjögren's syndrome, this work highlights the modulation of cellular, soluble, and genomic biomarkers relevant to the disease and CD40 pathways shedding light on the pathophysiology of pSS and the drug's mechanism of action.

O-10 *Biomarker Discovery for Diagnosis and Stratification*

Epigenetically quantified immune cells in salivary glands of primary Sjögren's syndrome: a novel tool that detects robust correlations of Tfh cells with immunopathology

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Background. For decades histological analysis of salivary glands has been a valuable tool in the characterization of patients with pSS and non Sjögren sicca (nSS) patients. Notably, it has helped in understanding the immunopathology of sicca patients. However, standardization of histological assessments, eg. to quantify lymphocytic foci or germinal centers is lacking, contributing to improper classification of disease and assessment of risk of lymphoma for example. Also, detailed and reproducible quantification of the heterogeneity of inflammatory cells and their contribution to immunopathology is lacking. Recent progress in epigenetics revealed that cell-specific DNA methylation profiles reliably quantify numbers of cells in blood and tissues. Our objective was to investigate whether epigenetic cell counting can serve as a novel reliable tool to quantify immune cells in salivary glands of sicca patients.
Methods. DNA was isolated from frozen tissue sections of 15 nSS, 10 incomplete SS, 25 pSS and 12 sSS patients. Bisulphite conversion of methylated DNA sites was followed by cell specific QPCR that was used to calculate the percentage of cell subsets related to the total number of cells quantified by house keeping gene expression. Percentages of epigenetically counted cells were correlated to gene expression generated by RNAseq analysis of matched salivary gland tissue and histological and clinical parameters (LFS, %IgA+ plasma cells, serum IgG, SSA positivity).
Results. Percentages of CD3 and B cells positively correlated with CD3 and CD19 RNA expression (r=0.608 p<0.0001, r=0.597 p<0.0001, resp). In addition, CD3 percentages strongly correlated with digitally quantified CD3 numbers following IHC (r=0.751, p<0.0001). Strongly increased percentages of epigenetically quantified percentages of CD3, CD4, CD8, B cells, T follicular helper (Tfh) cells and Treg cells in pSS vs nSS patients were observed (all p<0.001, CD8 p<0.01). These inflammatory cell types all strongly correlated with LFS (all at least p<0.001, CD8 p=0.014), local B cell hyperactivity (% IgA+ cells, all p<0.001, except CD8 p=0.06 and B cells, p=0.127) and systemic B cell hyperactivity (all at least p<0.01, except CD8 p=0.051). Th17 cells did not differ between nSS and pSS patients. Interestingly, CD8 T cells were significantly increased in incomplete SS patients as compared to nSS patients (p<0.05). Interestingly, percentages of Tfh cells correlated with CXCL13 (r=0.789, p<0.0001), IL7R, CXCR5 and ICOS RNA expression (all p≤0.001) and were strongly associated with autoimmunity (SSA positivity, p<0.001) and CD21-expressing FDCs in LSG (r=0.825, p=0.0006).
Conclusions. Epigenetic cell counting is a promising novel tool to reproducibly and easily quantify immune cells in the inflamed labial salivary gland of sicca patients with relatively low amount of tissue needed (<1 mm^3). In view of the potential of this technique to include a huge number of (cell-specific) biomarkers we believe this opens up new standardized ways for salivary gland analysis with high relevance for patient classification, understanding of immunopathology and clinical trials.

O-11 *Biomarker Discovery for Diagnosis and Stratification*

Genetic basis and clinical evidence for two variants of primary Sjögren's syndrome with distinct outcomes

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Background. The classification of primary Sjögren's Syndrome (pSS) is based on fulfilment of internationally accepted criteria. However, patients with pSS present heterogeneous phenotypes, with varied outcomes, making optimal treatment and long-term outcome prediction challenging. In the current study we therefore aimed to identify markers for potential clinically relevant subsets of pSS.
Methods. DNA sequencing was performed of 1853 selected genetic loci including their regulatory regions, targeting 32Mb of the genome. The regions are selected to contain genes from pathways known to be involved in immunological diseases. A clinically well characterized cohort of 1016 pSS patients from Sweden and Norway, as well as 1350 Norwegian and Swedish controls were included. After quality control, 918 pSS cases, 1264 controls and 312 853 gene variants remained for analysis. Clinical data was extracted from patient records.
Results. We confirmed previous associations with the *human leukocyte antigen (HLA)* region (p=1.4E-46, OR 3.9, 95% CI [3.2-4.7]) and *Interferon Regulatory Factor 5 (IRF5)* (p=1.9E-06, OR 0.72 [0.62-0.82]), and identified two novel associations with loci containing the genes *Glutamic-Oxaloacetic Transaminase 1 (GOT-1)* (p=1.1E-06, OR 0.70 [0.61-0.81]) and *Mitogen-Activated Protein Kinase 2* (*MAP2K2*) (p=1.7E-06, OR 0.55 [0.43-0.70]). Variants in or around 80 genes in the HLA region passed an experiment-wide Bonferroni correction (p<8.7E-07), while step-wise adjustment by conditioning on the top associated variants revealed three independent signals within the HLA, two in the MHC class II and one in the MHC class I. The top variant of each independent signal was near the *HLA-DQA1*, *HLA-DRA* and *HLA Complex P5* (*HCP5*) genes, respectively. Comparing only the patients positive for Ro/SSA and/or La/SSB (SSA/

SSB) autoantibodies (n=663, 72%) to controls, we observed a strengthened association to *HLA* (p=2.2E-62, OR 6.1 [4.9-7.5]) and *IRF5* (p=7.5E-08, OR 0.66 [0.56-0.77]). Notably, there was no association with variants in the *HLA* region in patients without these autoantibodies. Based on these identified fundamental genetic differences between patients who are positive or negative for SSA/SSB autoantibodies, we stratified the patients based on autoantibody status and found significant clinical differences in SSA/SSB positive versus negative patients, including age at symptom onset (45.1 y versus 49.0 y, p=3.9E-04), age at diagnosis (51.3 y versus 56.1 y, p=8.3E-07), purpura (13.7% versus 3.1%, p<0.0001), major salivary gland swelling (32.9% versus 22.7%, p=0.0037), development of lymphoma (5.2% versus 2.3%, p=0.045) and age at lymphoma onset (53.6 y versus 73.8 y, p=3.8E-04).

Conclusions. These data suggest a genetic basis and clinical evidence for two variants of primary Sjögren's syndrome with distinct outcomes, distinguishable by genetic associations to polymorphisms in *HLA* and *IRF5* and SSA/SSB autoantibody positivity.

O-12 *Oral and Ocular Manifestations of Sjögren's Syndrome*

Primary Sjögren's syndrome and Non-Sjögren's sicca syndrome Patients; unexpected differences in symptoms and findings of dry eye disease, dry mouth, and oral health related quality of life

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Aim. To investigate signs and symptoms of dry eye disease (DED), xerostomia, and quality of life in patients with primary Sjögren's syndrome (pSS) and non-Sjögren's sicca syndrome (non-SS).

Methods. Forty-seven patients with pSS (age: 53±12), 17 non-SS patients with sicca symptoms and findings, but negative for anti-SSA and salivary gland foci (53±11) and 32 healthy control subjects (49±12) were included in this cross-sectional study. Subjects underwent extensive dry eye work-up including Ocular Surface Disease Index (OSDI) questionnaire, tear osmolarity (TO), tear film break-up time (TFBUT), Schirmer test (ST), and vital staining (VS, Oxford grading scheme). Percentage of meibomian gland loss (MGL) was analysed with meibography. Oral examinations included unstimulated whole saliva (UWS), chewing-stimulated whole saliva (SWS) and evaluation of clinical oral dryness score (CODS). The 36-Item Short Form Survey (SF-36) was used to assess general health related quality of life (GHRQoL) and expressed as physical component score (PCS) and mental component score (MCS). The Oral Health Impact Profile (OHIP-14) was used to evaluate oral health related quality of life (OHRQoL). Kruskal-Wallis H test was used for intergroup comparison. P values indicate difference between patients and healthy controls.

Parameters	Controls	pSS	p-value	Non-SS	p-value
OSDI	4.8±7.5	33.9±18.4	*p<0.001*	54.1±23	*p<0.001*
TO (mOsm/L)	319.6±15.5	327.5±21.2	*p<0.05*	318.7±17.2	*p<0.05*
TFBUT (sec)	5.4±3.3	2.2±2.3	*p<0.01*	4.2±2.2	*p=0.11*
ST (mm/sec)	16.6±10.7	5.1±4.1	*p<0.001*	11.7±6.6	*p<0.001*
VS	0.8±1.2	3.5±2.3	*p<0.001*	0.9±1.0	*p<0.001*
MGL (%)	33.7±9.1	43.2±14.5	*p<0.05*	37.6±8.7	*p=0.09*
UWS (ml/min)	0.3±0.2	0.09±0.08	*p<0.001*	0.1±0.08	*p<0.001*
SWS (ml/min)	1.5±0.7	0.7±0.8	*p<0.001*	0.9±0.4	*p<0.001*
CODS	0.6±0.9	5.0±1.9	*p<0.001*	3.7±1.9	*p<0.05*
GHRQoL - PCS	56.8±5.3	41.4±10.4	*p<0.017*	31.4±11.2	*p<0.017*
GHRQol - MCS	55.1±6.5	46.6±11.1	*p<0.007*	43.7±11.3	*p<0.01*
OHRQoL	2.7±3.1	14.5±11.2	*p<0.007*	21.8±14.1	*p<0.001*

Results. The subjective ocular score was highest in non-SS patients as compared to pSS and healthy controls. In contrast, the pSS group had more pronounced objective findings of dry eye. Similarly, the non-SS group displayed worse GHRQoL and OHRQoL, and like the ocular objective findings, patients with pSS had the most pronounced findings of oral dryness compared to non-SS and healthy controls.

Conclusion. Despite having clinically milder DED signs and less pronounced findings of oral dryness, patients with non-Sjögren´s sicca syndrome may have more subjective symptoms of ocular dryness and reduced general and oral health related quality of life as compared to patients fulfilling the classification criteria. Consequently, clinicians should pay appropriate attention to sicca patients whether they fulfil the classification criteria or not.

O-13 *Oral and Ocular Manifestations of Sjögren's Syndrome*

Impact of dry eye on prolonged reading

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Background. Patients with dry eye frequently report difficulty with reading. However, the impact of dry eye on reading has not been studied in detail. We hypothesized that the unfavorable effect of dry eye may be more pronounced as the length of reading increases. Therefore, we aimed to evaluate the impact of dry eye on both short-duration out-loud and prolonged silent reading.

Methods. This study included 116 patients with clinically significant dry eye, 39 patients with dry eye symptoms but without ocular surface staining, and 31 controls 50 years and older. Following symptom assessment using the Ocular Surface Disease Index (OSDI), objective testing of dry eye, including tear film stability studies, Schirmer's test, and ocular surface staining was performed. A short duration out-loud reading test using the International Reading Speed Test (IReST) and a previously validated sustained silent reading test using a 7200-word passage over 30 minutes were performed. Reading speed for each test was calculated as words per minute (wpm) and compared across the three groups.

Results. Patients with clinically significant dry eye read slower than controls measured with sustained silent reading test (240 vs. 272 wpm, p=0.04), but not with IReST out-loud reading test (146 vs. 153 wpm, p=0.47). Patients with dry eye symptoms only did not have significantly slower reading speed measured using either out-loud or sustained silent reading tests as compared to controls. Subjective symptoms of dry eye, particularly vision-related symptoms, were associated with significantly slower reading speed measured with the sustained silent reading test (p=0.02). Multivariable regression models demonstrated that each one point increase in corneal staining score, led to a 10 wpm decrease in reading speed measured with sustained silent reading test (95%CI=-18.0 to -2.1 wpm, p=0.01).

Conclusions. This study showed that clinically significant dry eye, particularly presence of corneal staining, has a significant negative impact on prolonged reading. Prolonged silent reading may serve as an objective, clinically relevant test to assess the actual impact of dry eye on vision-related quality of life.

O-14 *Oral and Ocular Manifestations of Sjögren's Syndrome*

Ultrasonography of major salivary glands in juvenile Sjögren's syndrome: an international multicentre study

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Background. Juvenile Sjögren's syndrome (jSS) is a rare, poorly defined and possibly underdiagnosed condition. There is little information on the use of major salivary gland ultrasonography (SGUS) in jSS.
Objectives. To characterize symptoms and clinical findings of jSS and to investigate SGUS as a diagnostic tool.
Methods. A cross-sectional multicenter study recruited patients with disease onset before 18 years from Brazil, Norway, the Netherlands, USA and Spain (n=67). Clinical examination, sialometry and SGUS of the parotid and submandibular glands were assessed
Results. The female:male ratio was 6.5:1. Ocular and oral sicca symptoms were noted in 42/67 and 48/66 patients, respectively, and 42/67 patients fulfilled the AECG/ACR-EULAR criteria. ESSPRI score for dryness correlated with time to inclusion from diagnosis (p<0.05) and from first symptom (p<0.05). Pathological SGUS findings (SGUS+) were observed in 41/67 patients. In the Europe+USA patients 26/27 had SGUS+ compared to 15/40 Brazilian patients (p<0.001). Unstimulated whole saliva levels (ml/15 min) correlated with SGUS score (0-3) for the submandibular glands (p<0.05). In the SGUS+ group, 36/41 had anti-Ro/La antibodies and in the SGUS- group, 14/26 had anti-Ro/La antibodies (p<0.01). Symptomatic treatment was registered in 12/34 SGUS+ patients compared to 17/25 SGUS- patients (p<0.05).
SGUS+ was observed in 30/54 patients with extraglandular manifestations; interestingly 24/25 SGUS- patients also presented with extraglandular manifestations (p=0.086), possibly linked to previous systemic treatment, diverse disease profiles, or data collections bias.
Conclusions. SGUS findings correlated with sicca symptoms, hyposalivation and autoantibody status, indicating a role in jSS diagnosis. Findings regard SGUS and extraglandular disease were inconclusive due to differences in records and treatment strategies.

O-15 *Oral and Ocular Manifestations of Sjögren's Syndrome*

Correlation between salivary gland ultrasonography and minor salivary gland biopsy in the identification of a more severe subset of primary Sjögren's syndrome

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Background. Minor salivary gland biopsy (MSGB) represents the key tool for the diagnosis of primary Sjögren's syndrome (pSS). Recently, salivary gland ultrasonography (SGUS) has also been proposed as a novel approach for the assessment of pSS patients. The concordance between MSG biopsy and SGUS is generally only moderate, with the latter being less sensitive. Since MSG inflammatory lesions may display variable severity apparently correlating with pSS clinical or laboratory features, in this study we aimed at exploring whether SGUS may be helpful in recognizing subsets of patients with more severe histopathological patterns and different clinical manifestations.
Methods. We enrolled newly diagnosed pSS patients undergoing a complete rheumatologic evaluation which included a MSGB. An expert pathologist assessed various histopathological parameters including: number of foci, focus score (FS) and number of germinal centre (GC) - like structures. SGUS was carried out by the same radiographer blinded to the diagnosis. The echostructure of each gland on B-mode images was graded on a 5-point scale (0–4), and a SGUS score ≥2 was defined as pathological. To assess sonographic damage, the presence of hyperechoic bands in the parenchyma and the glandular dimensions were evaluated.
Results. Out of the 78 pSS included, 40 (53.1%) presented a SGUS ≥2 and 27 (34.6%) sonographic elements of glandular damage. SGUS score correlated with both MSG FS (r=0.504, p=0.001) and the number of GC-like structure (r= 0.550, p=0.001). Patients with a SGUS score ≥2 presented a significantly higher focus score (2.9 (1.7) vs 1.6 (1.7)), a greater number of foci (4.0(2.7) vs 2.4(2.7)) and a greater number of GC-like structure (2.2(2) vs 0.5 (0.5)). A significant correlation was also observed between sonographic damage and the number of GC-like structure (r=0.502, p=0.002). Moreover, patients with echostructural abnormalities presented a higher ESSDAI (5.5 (3,4) vs (2.9 (3)), a lower salivary flow rate (1.8(1.6) vs 3.7(3.9), and a more frequent positivity for anti-Ro/SSA and Rheumatoid factor.
Conclusions. In this study we specifically investigated the correlation between SGUS and MSG histopathology. We highlighted that SGUS abnormalities were associated not only with higher MSG FS but also with additional histopathological parameters such as GC-like structure that are considered as biomarkers of disease severity. Accordingly, we also found an association between SGUS and a more active systemic disease. Overall, our results support the role of SGUS as a complementary tool in the early identification of a distinct subset of pSS patients presenting more severe histopathological lesions and a potentially more aggressive disease course.

O-16 *Systemic Manifestations, Including Lymphoma*

Infections predispose to developing primary Sjögren's syndrome

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Background. Environmental insults are believed to trigger primary Sjögren's syndrome (pSS) in genetically susceptible individuals, and infectious agents have long been suspected as etiologic factors.
This has been further supported by the discovery of an association between pSS and upregulation of the type I and II interferon pathways. In the present study, we therefore investigated the association between infections and future risk of developing pSS.
Methods. We performed a case-control study including well-characterized and validated cases with pSS (n=945) and controls from the Swedish population matched on age, sex and area of residence (n=9,048). Data including ICD10 codes were extracted from the population-based National Patient Register to identify infections occurring before the date of pSS diagnosis. Conditional logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) of the association between infections and pSS. Infections occurring in the year before pSS diagnosis were excluded to minimize the risk of reversed causality.
Results. Preceding infections were more common in pSS cases compared to controls (21% vs 12%), and were associated with an increased risk of pSS (OR 2.0, 95% CI 1.7–2.4). Infections were more prominently related to pSS positive for both Ro/SSA and La/SSB autoantibodies (OR 2.7, 95% CI 2.0–3.5), than pSS without these autoantibodies (OR 2.1, 95% CI 1.5–2.9). Stratifying the analysis by organ system infected, we observed that respiratory infections were associated with pSS (OR 2.5, 95% CI 1.9–3.4), both with and without Ro/SSA and La/SSB autoantibodies. Interestingly, preceding skin infections were only associated significantly with Ro/SSA and La/

SSB positive pSS (OR 3.2, 95% CI 1.8–5.5), and the relationship could not be established in pSS patients without such autoantibodies (OR 1.7, 95% CI 0.8–3.6). Notably, gastrointestinal infections were however not associated with an increased risk of pSS (OR 1.5, 95% CI 0.9–2.5). Considering the long time-interval that may occur between symptom onset and pSS diagnosis, we also applied models only including infections occurring at least 3 or 7 years prior to pSS diagnosis. These analyses confirmed pulmonary and skin infections as risk factors for developing pSS associated with autoantibodies, but failed to confirm an association between infections and seronegative pSS. The robustness of the observations was further tested by analyzing data among hospitalized patients only, or infections listed as primary diagnosis only, as well as correcting for previous health care consumption. Such parameter variation did not greatly influence the results.
Conclusions. We observed a significant and consistent association between infections and the subsequent development of pSS with autoantibodies, suggesting that external triggers of immunity influence the development of the disease. The risk was dependent on the location of the infection, indicating that the route of infection and/or immunoenvironment of the primarily affected organ may modulate outcome.

O-17 *Systemic Manifestations, Including Lymphoma*

Single-cell VH and VL Ig gene analysis reveal clonal relationship between IgM+ neoplastic B cells and plasmablasts in SS patients with parotid MALT lymphoma

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Background. One of the hallmark of Sjögren's syndrome (SS) is the development of ectopic lymphoid structure (ELS) in the salivary glands. ELS support functional germinal centre (GC) responses supporting *in situ* B cell activation and differentiation into autoantibodies producing plasmacells. Moreover, 5% of SS patients develop a B cell lymphoma, most commonly of the Mucosa Associated Lymphoid Tissue (MALT), which is the result of a continuous antigen driven immune response in ectopic GCs. The accumulation of genetic instability due to hypermutation processes brings to an oligo and then monoclonal proliferation with the subsequent escape of malignant clones. However, the mechanisms underlying this process are poorly understood and the capacity of neoplastic B cells to further undergo plasmablast differentiation is unknown. Here, we aimed to characterise VH and VL gene usage at single cell level in neoplastic B cells and plasmablasts from MALT lymphoma of a SS patient.
Methods. Single CD19+IgM+IgD+CD27+CD38- memory B cells and CD19+IgM-IgD-CD27+CD38+ plasmablasts were FACS sorted from a mononuclear cells suspension obtained from a SS salivary gland-MALT lymphoma cultured for 24 h. RNA was used to amplify Immunoglobulin(Ig) VH and VL genes and PCR products were cloned and expressed as recombinant monoclonal antibodies exhibiting identical specificity of the original B cells (Corsiero *et al*., ARD 2015). Ig VH-CDR3 analysis was performed using the international ImMunoGeneTics (IMGT) database.
Results. From IgM+ memory B cells, we obtained 53 individual VH sequences of which 9 were identified as unique clonotypes. From plasmablasts, we obtained 32 individual VH-mu and 20 individual VH-alpha sequences of which 6 and 20 were unique clonotypes, respectively. 45 out of 53 (84.9%) IgM+ memory B clones were a unique clonotype expressing VH1-69/D3-22/JH4 genes with identical CDR3 and amino acid sequence. 33 out of 45 (73.3%) clones (VH1-69/D3-22/JH4) were expressing Vk3-20 gene. Moreover, the same clone (VH1-69/D3-22/JH4) was found in 90.6% (29/32) of the IgM+ plasmablast population suggesting a monoclonal proliferation and differentiation towards a shared antigen. Finally, mutational load analysis revealed that the Ig VH of IgM+ memory B cells were heavily mutated supporting the concept of antigen-driven proliferation in the ELS of SS MALT-lymphoma.
Conclusions. The preferential usage of a VH1-69/JH4 segments in the majority of IgM+ memory and IgM+ plasmablast cells in SG MALT-lymphoma with same CDR3s suggests the presence of a common antigen(s) involved in the selection of malignant B cell clones and their further differentiation into plasmablasts. The identification of such antigen(s) will allow a better understanding of the mechanisms leading to lymphoma development in SS.

O-18 *Systemic Manifestations, Including Lymphoma*

Low miR200b-5p levels in minor salivary glands: A novel independent predictor of lymphoma development in patients with Sjögren's syndrome (SS)

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Background. The miRNAs of the miR-200 family are critical regulators of oncogenesis. Preliminary evidence suggested that, although not deregulated compared to sicca-controls, miR200b-5p levels are decreased in the minor salivary glands (MSGs) of SS patients with non-Hodgkin's lymphomas (NHL). Herein, we studied the expression of miR200b-5p in the MSGs of SS-associated NHLs and its predictive value for the identification of SS-patients susceptible to develop NHL.
Methods. miR200b-5p expression was investigated in MSG-tissues of SS-patients who were at (a) low-risk and didn't develop NHL during follow-up (SSwo, n=27; median follow-up time upon biopsy performance, range: 8.9-yrs, 1.33-14-yrs), (b) high-risk and diagnosed with NHL during follow-up (pre-lymphoma, SSpL, n=17; median follow-up to till lymphoma diagnosis, range: 3.67-yrs, 0.42-8.5-yrs) and (c) had NHL (n=35), as well as non-SS sialadenitis-controls (sarcoidosis and HCV-infection, 4-each). The differential miR200b-5p expression, correlations with disease features and its discriminative/predictive value were evaluated by appropriate statistical approaches.
Results. The MSG levels of miR200b-5p were significantly downregulated in SS patients who will develop or have NHL (mean relative expression±SD: 0.31±0.33 and 0.21±0.25 *vs* 0.72±0.37 and 0.95±0.84 in SSwo and sialadenitis controls, respectively). Analysis of 14 sequential paired samples from SS patients before and on lymphoma diagnosis revealed that miR200b-5p levels were reduced long before clinical onset of lymphoma and did not significantly change upon transition to lymphoma. They also correlated with several clinical, laboratory and histological features indicative of adverse outcome and lymphoma development, as well as with worst lymphoma prognosis. Furthermore, ROC analysis revealed that strongly discriminated SSpL and SSL patients from SSwo with AUC-values 0.863 and 0.986 (p<0.0001), respectively, and cut-off values 0.4156 (sensitivity=0.765, specificity=0.926) and 0.3164 (sensitivity=0.952, specificity=1), respectively. Kaplan-Meier analysis of patients split into two groups according to miR200b-5p expression levels of 0.4156 (as defined by the specificity-sensitivity analysis) revealed that patients with miR200b-5p levels ≤0.4156 had a 4.8-fold (HR:4.81, 95%-CI:3.15-6.47, p<0.0001) higher risk to develop lymphoma compared to patients with miR200b-5p levels >0.4156. Finally, multivariate analysis identified miR200b-5p as an independent predictor of lymphoma development (HR per 1-unit change: 0.10, 95%-CI:0.01-0.87, p=0.012) along with high ESSDAI (p=0.024), SGE (p=0.012), purpura (p=0.057), vasculitis (p=0.043), splenomegaly (p=0.047), cryoglobulinemia (p=0.032) and hypergammaglobulinemia (p=0.055).
Conclusions. These findings support that miR200b-5p levels in MSGs represent a novel predictive, and possibly pathogenetic mechanism-related, factor for the development of SS-associated NHL, since its expression is impaired years before lymphoma clinical onset.

O-19 *Systemic Manifestations, Including Lymphoma*

Hospitalization rates among patients with primary Sjögren's syndrome: a population-based study, 1995-2016

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Objective. To determine rates and primary discharge diagnoses of hospitalization in a cohort of patients with incident primary Sjögren's syndrome (pSS) compared to the general population.
Methods. This was a retrospective population-based cohort study focused on Olmsted County, Minnesota. The pSS cohort consisted of patients with incident pSS in the 1976-2015 period and was compared with a cohort of individuals without pSS matched 3:1 for age, sex and calendar year, randomly selected from the same population. Hospitalizations in 1995-2016 were examined. Discharge diagnoses were categorized using the Clinical Classifications Software for ICD-9-CM.
Results. A total of 385 hospitalizations occurred in the 160 patients with pSS during 1592 person-years of follow-up. Among 466 comparators, there were 899 hospitalizations during 4660 person-years of follow-up, resulting in a significantly higher rate of hospitalizations in patients with pSS (rate ratio [RR]:1.25, 95% CI:1.11-1.41). Rates of hospitalization were increased among patients with pSS for endocrine, nutritional and metabolic diseases and immunity disorders (RR:1.82, 95% CI:1.08-2.98), diseases of the musculoskeletal system and connective tissue (RR:1.49, 95% CI:1.05-2.05), and for injuries and poisoning (RR:1.46, 95% CI:1.01-2.06). While not significantly increased overall, hospitalizations for diseases of the circulatory system were significantly increased in patients with pSS aged ≥75 years (RR:1.54, 95% CI: 1.11-2.11).
Conclusions. Patients with pSS experienced higher rates of hospitalization than the general population. Hospitalizations for endocrine/metabolic disorders, diseases of the circulatory system, diseases of the musculoskeletal system and connective tissue disorders, and injuries were more common among patients with pSS than comparators.

O-20 *Glandular Outcome Measures*

The parotid gland in primary Sjögren's syndrome: comparison of ultrasound, histopathology and saliva production in the diagnostic work-up

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Background. The parotid glands are commonly involved in primary Sjögren's syndrome (pSS). Their involvement can be assessed by performing parotid gland biopsies, salivary gland ultrasound and by collecting glandular saliva. The aim of this study was to assess how ultrasound of the parotid glands (sUS) is associated with parotid histopathology and saliva production.
Methods. Consecutive patients, clinically suspected with pSS between February 2014 and September 2016, were included. All patients were over 18 years of age and underwent a full diagnostic work-up according to the ACR-EULAR criteria1, including an sUS, a parotid gland biopsy and collection of 2% citric acid stimulated parotid saliva. For sUS, the average score for hypoechogenic areas in both parotid glands was applied (range 0-3)2, with a cut-off value of ≥1.5 for sUS positivity. On 3 μm hematoxyllin & eosin (H&E) stained sections from the parotid gland biopsies, the focus score, presence of lymphoepithelial lesions (LELs) and germinal centers (GCs) were assessed. The area of lymphocytic infiltrate was calculated digitally on the CD45 stained sections. The relative increase of IgG expressing plasma cells (≥30%) was evaluated on sections dual stained for IgA and IgG. Next, sUS score was associated with focus score, percentage of infiltrate and saliva flow using Spearman's correlation coefficient (ϱ). sUS outcome was compared with plasma cell shift, LELs and GCs by calculating the percentage of absolute agreement, sensitivity and specificity.
Results. In total, 111 patients were included of whom 53 fulfilled the ACR-EULAR classification criteria for pSS. The mean time interval between sUS and the parotid gland biopsy was 2.3 months. sUS score of the parotid glands showed moderate association with focus score (ϱ=0.494, p<0.001) and percentage of lymphocytic infiltrate (ϱ=0.575, p<0.001). There was a moderate to good absolute agreement between sUS outcome and focus score (78.5%), plasma cell shift (79.8%), LELs (81.4%) and GCs (82.7%). Presence of hypoechogenic areas was not very sensitive to predict focus score (69.2%), plasma cell shift (45.8%), LELs (61.5%) or GCs (34.6%). Interestingly, almost all of the patients with <25% presence of hypoechogenic areas in the glandular parenchyma, considered as sUS negative and corresponding to an sUS score of <1.5, were also negative for GCs (98.7%). A substantial amount of these latter patients did not have a focus score (81.4%), plasma cell shift (90.7%) or LELs (87.8%). There was a fair reversed association between sUS score and stimulated parotid saliva flow (ϱ=-0.259, p=0.07).
Conclusions. This is the first study that makes a detailed comparison between parotid sUS, histopathology and salivary secretion. We found a stronger association between sUS and histopathology than between sUS and parotid secretion. Especially specificity of sUS increases when results are compared to plasma cell shift, LELs and GCs, instead of focus score. Thus, not only the focus score should be assessed, but also the other aspects of parotid gland biopsies should be taken into account.

References

1. SHIBOSKI *et al*.: *Ann Rheum Dis* 2017; 76: 9-16
2. MOSSEL *et al*.: *Ann Rheum Dis* 2017: Accepted for Publication

O-21 *Novel Therapeutic Targets*

Targeting T-cell trafficking in a murine model of Sjögren's syndrome

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Background. Salivary glands of primary Sjögren's syndrome (pSS) are characterised by complex leukocyte infiltration organised into tertiary lymphoid structures (TLS). The mechanisms regulating leukocyte trafficking into the inflamed salivary glands are poorly described, but dysregulated T-cell recruitment during inflammation is believed to contribute to disease onset and chronicity. We recently described a homeostatic pathway in which a B cell- derived peptide (PEPITEM), secreted in response to adiponectin, regulates T-cell trafficking during inflammation via sphingosyne 1 phospate activity on endothelial cells (1). Loss of this pathway by downregulation of adiponectin receptor on circulating B cells has been demonstrated in type 1 diabetes and rheumatoid arthritis, suggesting a potential role for PEPITEM in the pathogenesis of autoimmune diseases and indicating a role for adiponectin receptor as biomarker in autoimmune diseases (1). We aimed to investigate the efficacy of PEPITEM as an inhibitor of T-cell trafficking in an inducible animal model of salivary gland inflammation that mimics the histological features of pSS and to investigate the potential translatability of this pathway in patients with pSS.
Methods. Submandibular salivary glands of C57BL/6 mice were intra-ductally cannulated with luciferase-encoding replication-deficient adenovirus to induce TLS formation as previously described (2). Mice were administered daily either with PBS or PEPITEM by intraperitoneal injection from day 0, and their salivary glands dissected at day 5 post cannulation. T-cell infiltration into salivary glands was assessed using a combination of flow cytometry, immunofluorescence and qRT-PCR.
Results. B cells in sera from cannulated animals express lower levels of both adiponectin receptors 1 and 2 in comparison with non-inflamed control

mice. In cannulated animals treated with PEPITEM, histological analysis of salivary glands revealed fewer, as well as less aggregated, infiltrating T cells. Both $CD4^+$ and $CD8^+$ numbers were significantly lower in the salivary glands of PEPITEM-treated animals. Furthermore, administration of PEPITEM also decreased mRNA transcripts for lymphotoxin beta, IL-7, lymphoid chemokines (CCL19 and CXCL13) and T cell chemokine receptor CCR7, cytokines and chemokines known to regulate ectopic lymphoneogenesis in pSS. Human samples of pSS are currently being assessed to validate the relevance of this pathway in pSS.

Conclusion. These results demonstrate that administration of exogenous PEPITEM can reduce T-cell influx into salivary glands. This may represent a rescue of the homeostatic regulation of leukocyte trafficking, which is disrupted in inflammation. Our work suggests that PEPITEM should be considered to address the therapeutic needs in chronic inflammatory conditions and that the detection of decreased levels of adiponectin receptor could be used as biomarkers in pSS.

References

1. CHIMEN, MCGETTRICK *et al*.: *Nat Med* 2015
2. BOMBARDIERI, BARONE *et al*.: JI. 2012

O-22 *Novel Therapeutic Targets*

Stromal cells in tertiary lymphoid structures: a novel pathogenic paradigm and therapeutic target in Sjögren's syndrome

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Background/Objective. Tertiary lymphoid structures (TLS) are accumulations of lymphoid cells that share similar cellular compartments, organization and function as secondary lymphoid organs (SLOs).TLS provide a local hub for maturation and proliferation of auto-reactive B-cells and can also contribute to expansion of malignant B-cell clones. TLS that form within salivary glands (SGs) of Sjögren's syndrome (SS) patients are clearly associated with poor disease outcome, autoantibody production and lymphoma development. Despite clear evidence for B-cell contribution in SS pathogenesis, B-cell depletion has shown poor outcome in clinical trials; suggesting that targeting lymphocytes might not be sufficient to eradicate disease. Moreover, it has been shown that pathogenic microenvironment of SGs is responsible for disease resistance to treatment and relapse. Others and we have shown in TLS-associated diseases, resident-stromal cells can undergo changes to acquire features of SLO-stromal cells. However, the mechanisms regulating these lymphoid-like stromal cells (LLSCs) are not clear.

Methods. Inducible SS mouse model of TLS formation by retrograde cannulation of SGs with a replication-deficient adenovirus was used. Cannulated SGs of C57BL/6 (wildtype;WT) and knockout mice (IL-4Rα–/–, IL-13–/–, IL-22–/–, IL22Rα–/–, LTβR–/–, RAG2–/–) and SG biopsies from SS patients were analysed by immunofluorescence, flow cytometry and RT-PCR.

Table I. Difference (and 95% Confidence Interval) versus randomized placebo group in various outcome measures after treatment with 10 mg/kg iv CFZ533.

	at Week 8	at Week 12
ESSDAI	-3.12 (-6.14, 0.05)	-5.64 (-10.58, -1.02
ESSPRI	-0.10 (-1.60, 1.40)	-0.83 (-2.22, 0.57)
MFI General Fatigue	-1.49 (-3.65, 0.66)	-2.87 (-5.56, -0.17)
MFI Mental Fatigue	-0.15 (-2.73, 2.44)	-2.39 (-5.14, 0.37)
MFI Physical Fatigue	-1.00 (-3.22, 1.22)	-2.41 (-5.13, 0.32)
MFI Reduced Activity	1.77 (-0.28, 3.81)	-0.85 (-2.88, 1.18)
MFI Reduced Motivation	0.83 (-1.58, 3.24)	-0.36 (-3.50, 2.79)
MFI Total	-0.37 (-9.42, 8.69)	-9.15 (-20.25, 1.96)
Patient VAS	-12.09 (-29.38, 5.19)	-8.45 (-26.08, 9.19)
Physician VAS	-8.18 (-17.57, 1.22)	-13.72 (-23.41, -4.04)
SF-36 Mental	-4.44 (-11.28, 2.40)	1.25 (-5.66, 8.16)
SF-36 Physical	2.50 (-3.11, 8.10)	3.85 (-1.84, 9.53)

Results. Our work in the inducible TLS formation model in both WT and knock-out mice and *in vitro* experiments revealed acquisition of lymphoid phenotype by non-activated resident stroma is a multistep process, fundamentally different from signals responsible in SLO. We showed early during TLS formation, LLSC priming [*i.e*. up-regulation of SLO-associated stromal markers: gp38 and adhesion molecules (ICAM-1/VCAM-1)] is mediated by IL-13 via IL-4Rα engagement on quiescent tissue-resident fibroblasts. Expansion/proliferation of these activated LLSCs requires IL-22R/IL-22 signalling. Impairment in any of these stromal induction/proliferation pathways resulted in defective TLS formation. Finally, we demonstrated LTβR and lymphocytes are required to maintain long-term secretion of lymphoid chemokines/cytokines from LLSCs for lymphocyte retention, survival, organization and generation of humoral response. Taking advantage of *in vivo* targeted-deletion of LLSCs, we confirmed that integrity of LLSCs is critical for TLS assembly, organization and disease persistence in SGs. Observational studies in SS SGs confirmed presence of LLSCs in human disease and engagement of pathways demonstrated in the animal model.

Conclusion. Our data highlight previously unappreciated pathogenic role for stromal cells in context of SS. It demonstrates that activated lymphocytes and local stromal cells cooperate in an amplificatory loop to induce TLS and are responsible for disease chronicity and persistence. We propose that treating LLSCs either directly or via modulation of the signals identified in this study could be used in combination with leukocyte depletion to increase therapeutic activity of these compounds in clinical trials.

O-23 *Clinical Trial Design and Emerging Treatments for Sjögren's Syndrome*

Patient and investigator reported outcomes suggest improvements upon treatment with the novel anti-CD40 monoclonal antibody CFZ533 in patients with primary Sjögren's syndrome: a phase iia double-blind, placebo–controlled randomized trial

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Background/Purpose. Primary Sjögren's syndrome (pSS) is a systemic, progressive autoimmune disease characterized by formation of ectopic germinal centers in exocrine glands and secretory gland dysfunction. A subset of patients also develops extraglandular manifestations. CFZ533 is a novel monoclonal antibody that potently and selectively blocks CD40, a co-stimulatory pathway receptor essential for germinal center reactions and other immune mediated functions implicated in pSS pathogenesis. We conducted a randomized, double–blind, placebo-controlled, multi-centric, partial cross-over Phase IIa Proof of Concept (PoC) study to evaluate the safety, tolerability and efficacy of CFZ533 infusions in patients with pSS.

Methods. Clinically active (EULAR Sjögren's Syndrome Disease Activity Index [ESSDAI]≥6) pSS patients were randomized to 10 mg/kg *i.v.* CFZ533 or placebo (2:1,) over 12 weeks in Period 1. Four additional doses 10 mg/kg i.v. CFZ533, respectively, were administered in an open label extension (Period 2) for 12 weeks. Key outcomes included safety and efficacy (as measured by changes in ESSDAI) after 12 weeks treatment. Patient reported outcomes included EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), Multi-dimensional Fatigue Inventory (MFI), Patient's Global Assessment, SF-36. Physician's Global Assessment was also monitored.

Results. Thirty- two patients were enrolled 21 received 10 mg/kg *i.v.* CFZ533 and 11 placebo. Overall, CFZ533 was safe and well tolerated, and the majority of AEs were mild or moderate. There was a single SAE (atrial fibrillation) in the 10 mg/kg *i.v* group, which was unrelated to study drug. Improvements in ESSPRI, MFI, and Patient's Global Assessment were observed (Table I). Physicians's VAS showed statistically significant decreases (Table I). Overall, these changes were in line with the improvements seen in the ESSDAI which demonstrated a statistically and clinically significant decrease after 12 weeks of treatment as compared to placebo (ΔESSDAI=5.64 (95% CI=1.02 – 10.58).

Conclusion. In this proof of concept study, testing a blocking, non-depleting anti-CD40 antibody for the first time in primary Sjögren's syndrome, results suggest that CFZ533 may offer a new treatment modality in clinically active pSS. A new dose ranging trial trying a single infusion followed by subcutaneous injections or subcutaneous injections is in progress.

Disclosure: A.S. Papas, Biogen, GSK **A.M. Farag**, None **B. Fisher**, Novartis, Roche, Virtualscopics, 5; **M. Zeher**, None; **W.F. Ng**, Pfizer, UCB, MedImmune, Takeda and Sanofi, 5; **M. Bombardieri**, GSK, Amgen/MedImmune and UCB, 5; **M. Posch**, None; **T. Daikeler**, None;**B. Bannert**, None; **A.J. Kivitz**, Sanofi, Pfizer, Roche, and UCB, 5; **S.E. Carsons**, None; **D.A. Isenberg**, EMD Serono, Inc, 5; **F. Barone**, None; **S. Bowman**, AstraZeneca/Meddimmune, BMS, Celgene, Eli Lilly, Glenmark, GSK, MTPharma, Novartis, Ono, Takeda, UCB, XLT Bio., 5; **P. Espie**, Novartis Pharmaceutical Corporation, 3; **G. Wieczorek**, Novartis Pharmaceutical Corporation, 3; **P. Moulin**, Novartis Pharmaceutical Corporation, 3; **D. Floch**, Novartis Pharmaceutical Corporation, 3; **C. Dupuy**, Novartis Pharmaceutical Corporation, 3; **X. Ren**, Novartis Pharmaceutical Corporation, 3; **P. Faerber**, Novartis Pharmaceutical Corporation, 3; **A.M. Wright**, Novartis Pharmaceutical Corporation, 3; **H.U. Hockey**, Novartis Pharmaceutical Corporation, 3; **M. Rotte**, Novartis Pharmaceutical Corporation, 3; **J. S. Rush**, Novartis Pharmaceutical Corporation, 3; **P. Gergely**, Novartis Pharmaceutical Corporation, 3.

O-24 *Clinical Trial Design and Emerging Treatments for Sjögren's Syndrome*

Efficacy and safety of low-dose interleukin-2 therapy in active Sjögren's syndrome

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Background. Primary Sjögren's syndrome (pSS) is a chronic, systemic inflammatory autoimmune disease associated with imbalances among $CD4^+$ T and B cells. Current treatment strategy for typical pSS is primarily symptomatic. Part of the immune imbalance in SS lies in impaired Treg and effector T cell functions. Our previous studies have shown that low dose IL-2 can restore the balance between effector and regulatory $CD4^+$ T cells. We hypothesized that low-dose IL-2 can be used to treat SS by selectively expanding regulatory $CD4^+$ T (Treg) cells in both the salivary glands and circulation.
Methods. We conducted a randomized, double-blinded, placebo-controlled clinical trial in 60 patients with active SS. Patients were randomized at 1:1 ratio to receive either IL-2 (n=30) or placebo group (n=30) and followed up for 24 weeks. In the IL-2 group, three cycles of rhIL-2 were administered subcutaneously at a dose of 1 million IU every other day for 2 weeks, followed by a 2-week break. The effect of treatment was assessed by comparing clinical and laboratory data at baseline and every 2 weeks thereafter until week 12. At that point, clinical and laboratory parameters were assessed every 4 weeks from week 12 to week 24. The primary end points were the ESSPRI, Sjögren's Syndrome Disease Activity Index (ESSDAI) and safety at week 12 and 24. Secondary end points were the effects of the therapy on Treg, Th17, Tfh and Breg cells.
Results. For all 57 patients, disease activity as measuring using the ESSPRI and ESSDAI decreased significantly with IL-2 treatment both at week 12 and week 24. Compared with placebo, immunological analysis revealed that low-dose rhIL-2 administration was associated with selective expansion of Treg and Breg cells ($p<0.001$) and decreasing Th17 and Tfh cells ($p<0.001$). At week 12, resolution of clinical activity present at baseline was observed in multiple manifestations in patients with IL-2 treatment, including fatigue (12/22), leukopenia (18/18) and arthritis (5/8). Laboratory parameters showed reductions of anti-SSA and anti-SSB titres ($p<0.001$) as well as reduction in IgG levels and ESR ($p<0.005$). No severe adverse events were observed. Immunological analysis revealed that low-dose rhIL-2 administration was associated with selective expansion of Treg and Breg cells ($p<0.001$) and conversely with the reductions of Tfh and Th17 cells ($p=< 0.001$). To study tissue inflammation in SS, NOD mice were subcutaneously administrated with low does IL-2. Immunized mice were analysed 8 weeks later. Inflammation scores decreased significantly in IL-2 treated mice and this was associated with increased numbers of Foxp3+T cell in both the circulation and salivary glands.
Conclusions. Low-dose IL-2 was effective and well tolerated in active SS. The effect was associated with extension of Treg cell in both circulatory and inflammatory tissue. (ClinicalTrials.gov number, NCT02464319).

O-25 *Novel Insights*

Decreased levels of the Cysteine protease inhibitor, Cystatin C, may contribute to the increased activity of Cathepsin S and an altered protein profile in tears of Sjögren's syndrome patients

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Background. We have previously shown that Cathepsins S (CTSS) activity is increased in the tears of Sjögren's Syndrome (SS) patients compared to patients with other autoimmune rheumatic diseases as well as non-autoimmune dry eye, and that this increased activity may serve as a biomarker for SS. Here we further explore the relationship between tear CTSS activity and the tear levels of its endogenous inhibitor, Cystatin C (Cys C), and the abundant tear proteins, lactoferrin (LF) and secretory IgA (sIgA).
Methods. We first determined the presence of Cys C in tears and lacrimal glands in 12-week male non obese diabetic (NOD) mice, a model of SS dacryoadenitis, and in healthy age- matched BALB/c control mice, using Western Blot (WB), immunofluorescence (IF) and RT-PCR. The ability of CTSS to degrade Cys C and LF in tears collected from SS patients and healthy controls was determined by spiking tears with recombinant Cys C, LF and CTSS followed by analysis by WB. To evaluate if changes in these tear proteins can create a panel of biomarkers for SS, we recruited a total of 156 female subjects: 33 with SS; 33 with rheumatoid arthritis (RA); 31 with other autoimmune diseases (OAD); 35 with non-autoimmune dry eye (DE) and 24 healthy controls (HC). Tears were collected by a basic tear secretion test and analyzed for CTSS activity using a biochemical assay and Cys C, LF and sIgA levels by ELISA.
Results. WB analysis showed that Cys C levels in NOD mouse tears and LG lysates were reduced to 41% ($p<0.001$) and 68% ($p<0.0001$) compared to control, respectively. IF of lacrimal gland confirmed weaker expression of Cys C in NOD mouse than in BALB/c mice. However, there was no difference in gene expression. In SS patient tears spiked with recombinant CTSS, we observed a 33% increased degradation of both spiked LF and Cys C ($p<0.05$). However, in tears from healthy controls or in PBS, both spiked with CTSS, there was no significant degradation of either LF nor Cys C. Median CTSS activity was significantly higher in SS patient tears than in all other groups ($p<0.0001$ for all): 5.5-fold vs RA; 7.2-fold vs OAD; 6.2-fold vs DE and 21-fold vs HC. Median Cys C, LF and sIgA were significantly reduced in SS patient tears compared to all other groups. Cys C was reduced by 3.7-fold vs RA, 5.3-fold vs OAD, 3.8-fold vs DE and 7-fold vs HC. LF was reduced by 5.0-fold vs RA, 6.9-fold vs OAD, 9.7- fold vs DE, and 7.6-fold vs HC. Secretory IgA was reduced 6.0-fold vs RA, 4.9-fold vs OAD, 8.6-fold vs DE and 6.5-fold vs HC ($p<0.0001$ for all comparisons except for Cys C SS vs DE, $p=0.0002$).
Conclusion. Lacrimal gland lysates and tears of male NOD mice exhibit reduced levels of the CTSS inhibitor Cys C. Furthermore, tears of SS patients exhibit a similar imbalance between CTSS and its inhibitor, suggesting reduced levels of Cys C may contribute to increased CTSS activity. This in turn may contribute to the degradation of other tear proteins including Cys C itself, LF and possibly sIgA. Tear CTSS activity is confirmed here as a putative biomarker of SS in a second independent cohort distinct from our previous studies. Our results suggest that measurements of tear biomarkers may be beneficial in the diagnostic workup of dry eye or autoimmune disease when SS is suspected.

O-26 *Novel Insights*

Increased mTORC1 activity in salivary gland B cells and T cells from Sjögren's syndrome patients: mTOR inhibition as a novel therapeutic strategy to inhibit immunopathology

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Background. Hallmark features of primary Sjögren's syndrome (pSS) are lymphocytic infiltration in the salivary glands and B cell hyperactivity, including presence of autoantibodies, aberrant presence of B cells and plasma cells in the salivary glands, elevated serum IgG levels and increased risk of lymphoma development. The mTOR pathway is essential for cell growth, survival and proliferation of both B cells and T cells. mTOR inhibition is effective in immune suppression in transplant patients and in treatment of B cell lymphomas. Interestingly, in a murine pSS model mTOR targeting inhibited lymphocytic infiltration in the lacrimal gland. However, mTOR activation and its inhibition in B cells and T cells has not been studied in pSS patients.
Methods. The expression of mTOR pathway-related genes *(MTOR, RPTOR, RICTOR, DEPTOR, AKT1, IGF1R, IGF1, PTEN*) was measured in purified peripheral blood B cells and monocytes from pSS patients (n=12), non-Sjögren's sicca patients (nSS, n=17) and healthy controls (HC, n=9). Gene expression was scrutinized for correlation with clinical parameters: lymphocytic focus scores (LFS), anti-SSA/SSB auto-antibodies, ESSDAI, ESSPRI, serum IgG levels, ESR. Immunofluorescence was performed on pSS (n=12) and nSS (n=6) salivary gland tissue co-localizing CD20 and CD3 with phosphorylated S6 kinase (pS6 representing kinase activity downstream of mTORC1). Next, inhibition of the mTORC1 pathway by rapamycin (pS6 suppression) and its effect on of B and T cell proliferation and IgG production by mTOR inhibition were studied *in vitro* using PBMC stimulated with anti-IgM or a combination of superantigen (SEB) and TLR9 ligand (CpGC).
Results. *RPTOR* and *IGF1R* expression were significantly decreased in circulating B cells from pSS patients (p=0.02 and p<0.01, respectively) and correlated with serum IgG levels (r=-0.43, p=0.02, and r=-0.40, p=0.03). We did not observe significant differences in any of the studied genes in monocytes or T cells. In the salivary glands of pSS patients immunofluorescent co-localization showed presence of large numbers of T and B cells with mTORC1 activity (pS6) at the lymphocytic loci as compared to non-Sjögren's sicca patients (4±2 vs 49±34 and 6±4 vs 24±19 35% positive T and B cells in nSS vs pSS, respectively, both p<0.01). *In vitro* activation of PBMCs by SEB/TLR9L resulted in phosphorylation of S6, T and B cell proliferation, IFN-γ and IgG production in both HC and pSS. Inhibition of mTOR by rapamycin reduced B cell proliferation (80.8±9.9 vs 19.1±15.8%, p<0.001), T cell proliferation (74±9 vs 52±25%, p<0.001), IgG+ B cells (40±15 vs 11±6%, p=0.001), IgG production (800±322 vs 389±70 ng/mL, p=0.001) and IFN-γ production (3.5±0.9 vs 2.9±1.4 ng/mL, p=0.002).
Conclusion. pSS patients display decreased mTORC1 activity in peripheral blood B cells correlating with B cell hyperactivity and increased mTORC1 activity in salivary gland T and B cells. Stimulated T and B cell activity associated with increased mTOR activity is robustly inhibited by rapamycin *in vitro*, indicating that mTOR inhibition might represent a novel therapeutic strategy for pSS.

O-27 *Novel Insights*

Multiple functional roles of an EBV microRNA in Sjögren's syndrome pathogenesis: from alterations of calcium signaling to activation of IFN

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Background. Sjögren's syndrome (SS) is an autoimmune disorder primarily targeting the salivary and lacrimal glands, resulting in dry eyes (xeropthalmia) and dry mouth (xerostomia). In the salivary glands, the reduction or complete loss of saliva secretion has been attributed to acinar cell dysfunction. Although the underlying mechanisms of this dysfunction are unclear, it has been reported that more than 55% of SS patients display high activity of type I and II interferon (IFN) responses. We have previously reported that the EBV microRNA ebv-miR-BART13-3p is significantly elevated in the salivary glands of SS patients and targets both the stromal interacting molecule, STIM1, a primary regulator of the store-operated Ca^{2+} entry (SOCE) pathway and the water channel AQP5, both critical components of saliva formation. The objective of this project was to examine if ebv-miR-BART13-3p also plays a role in immune-dependent mechanisms in SS.
Methods. We have previously established primary human salivary gland epithelial cell cultures (phSG) that either retain highly proliferative growth or display a differentiated acinar-like phenotype when maintained in low calcium (0.05 mM) or high calcium (1.2 mM) growth medium, respectively. Whole transcriptome RNA-Seq was performed to evaluate the effects of ebv-miR-BART13-3p transfected in phSG cells. Quantification by RT-PCR and Western blotting were performed to monitor the expression of target genes at transcript and protein levels, respectively. Co-immunoprecipitation was used to study the interaction between ebv-miR-BART13-3p and protein complexes in phSG cells. *In situ* hybridization (ISH) was performed to study the localization of miRNAs in human minor SG biopsies of healthy controls and SS patients.
Results. We identified a novel role of ebv-miR-BART13-3p in the activation of IFN signature in phSG. Whole transcriptome analysis revealed the involvement of IFN signaling and antigen presentation pathways in ebv-miR-BART13-3p-transfected phSG cells. Transfection of ebv-miR-BART13-3p in phSG cells not only up-regulated MX1 expression but also induced IFN-β synthesis and secretion. Knockdown of MDA5, MAVS and RIG-I diminished the MX1 expression induced by this miRNA. Knockdown of AGO2 also reduced MX1 induction suggesting a canonical role of this miRNA in IFN signaling induction. Co-immunoprecipitation confirmed the direct binding between ebv-miR-BART13-3p to RIG-I and to AGO2. Mutation and deletion of ebv-miR-BART13-3p showed that its length and sequence are both critical for the activation of IFN signaling as deletion of one or two nucleotides at either 5'- or 3'-end abolished the MX1 expression.
Conclusions. Our findings revealed that ebv-miR-BART13-3p associates with RIG-I and AGO2 contributing to the pathogenesis of SS by inducing IFN-β expression by directly binding to RIG-I and through canonical miRNA functions by associating with AGO2. These results might have identified a novel potential target for the treatment of Sjögren's syndrome.

O-28 *Novel Insights*

Identifying outcomes important to patients with Sjögren's syndrome

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Background. To promote evidence-based management of Sjögren's Syndrome (SS), it is crucial that the most important outcomes are consistently reported in research. 'Core outcome sets', which refer to the minimum set of outcomes that should be reported in clinical trials within a disease area, can promote such consistency. But, core outcome sets must include outcomes relevant to all stakeholders, including patients.
Methods. We first identified all outcomes reported in at least 10% of Cochrane systematic reviews and clinical trials related to dry eye. We identified patients with dry eye (including SS and other conditions) around the world through Dry Eye Zone®. Patients are rating the importance of each outcome from 0 (not important) to 10 (very important) using a two-round anonymous online Delphi survey. We are classifying outcomes as "important" if ≥75% of patients assigned a rating of 6 or more in Round 2. We also are providing patients the opportunity to suggest and rate additional outcomes.
Results. We identified 18 outcomes, including symptoms, signs, laboratory measurements, safety outcomes, and other outcomes (Table I). Out of 618 patients with dry eye who completed Round 1 of the Delphi survey, 106 patients (17%) from 12 countries had been diagnosed with SS. Patients with SS were predominantly female (81%), 50 years of age or older (79%), white (91%), non-Hispanic (93%), living in the US (86%), and had been diagnosed with dry eye >5 years ago (69%) (Table II). At the Symposium, we will present the final results from this survey. We will also describe additional strides towards developing core outcome sets for dry eye, such as multi-stakeholder engagement and consensus development.

Table I. Eighteen outcomes that have been reported in at least 10% of Cochrane systematic reviews and clinical trials related to dry eye.

	Category of outcome	Outcome
1	Symptoms	Patient's overall assessment of ocular surface symptoms
2		ocular burning/stinging
3		Ocular foreign body sensation
4		Ocular dryness
5		Ocular discomfort
6		Dryness of the mouth
7	Signs/Clinical testing	Ocular surface staining
8		Conjunctival staining
9		Corneal staining
10		Conjunctival hyperemia
11		Tear production/volume
12		Tear film stability
13		Visual acuity
14	Laboratory measurements	Conjunctival impression cytology
15		Salivary flow
16	Safety outcomes	Adverse events (ocular)
17		Adverse events (non-ocular)
18	Other outcomes	Artificial tear use

Table II. Characteristics of 106 respondeents to Round 1 of our Delphi survey of patients with dry eye due to Sjögren's Syndrome.

Characteristics	Patients who completed Delphi Round 1 (N=106) n	(%)
Age categories (years)		
20-29	2	(2)
30-39	5	(5)
40-49	13	(12)
50-59	24	(23)
60-69	38	(36)
70-79	19	(18)
80 or above	2	(2)
Prefer not to answer	3	(3)
Gender		
Female	84	(79)
Male	19	(18)
Other	1	(1)
Prefer not to answer	2	(2)
Race		
White	96	(91)
Black or African American	4	(2)
American Indian or Alaskan Native	1	(1)
Asian	2	(2)
Native Hawaiian or Pacific Islander	0	(0)
Other	2	(2)
Prefer not to answer	1	(1)
Ethnicity		
Hispanic	5	(4)
Non-Hispanic	98	(93)
Not sure	0	(0)
Prefer not to answer	3	(3)
Country of current residence		
Australia	1	(1)
Canada	8	(6)
United Kingdom	4	(3)
United States	89	(86)
Other	3	(3)
Prefer not to answer	1	(1)
Time since first diagnosed with dry eye		
Less than 1 year	1	(1)
1-2 years	9	(9)
3-5 years	20	(19)
6-10 years	30	(28)
More than 10 years	44	(41)
Can't remember	2	(1)
Prefer not to answer	0	(0)

Conclusion. By rigorously surveying large numbers of patients around the world, we are identifying outcomes that matter to patients with SS. This process will feed into core outcome set development, a much-needed step to promote consistency in outcome measurement and reporting in research for SS.

O-29 *Late-Breaking Abstracts*

Dental implants in patients with primary Sjögren's syndrome – preliminary results from a prospective controlled clinical study

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Background. Few data exist regarding survival and success rate of dental implants in patients with primary Sjögren's syndrome (PSS). Although a previous study suggest lower success rate for dental implants we hypothesize that dental implants have similar survival and success rate in PSS as healthy controls.

The purpose of the present study is to present the study protocol in progress and preliminary results of a study evaluating the long term survival and success rate of dental implants in patients with PSS as compared to healthy matched controls.

Methods. Study patients must fulfill the Copenhagen Criteria and/or the US-EU criteria for PSS, miss at least one tooth and have sufficient bone volume for a single implant insertion without bone augmentation.

Recruitment of PSS patients has been done via own existing data bases on PSS patients as well as repeated national announcements in the Danish Dental Journal. We anticipate including 50 consecutive patients with PSS. For each PSS patient, an age, gender, and tooth-type-matched healthy control patient is enrolled. Fifty control patients are planned. A Straumann Bone Level Roxolid® implant is inserted and allowed to heal for 3 months. Similar procedures for PSS patients and control patients are applied. After 3 months, the suprastructure is mounted and the patient is recalled for baseline examination. At baseline (0 years) and after 1, 3, and 5 years, biological (marginal bone level, inflammation etc.), technical (fractures, loosening's etc.) and esthetic (Copenhagen Index score) assessments will be performed. This research protocol (ID:H-3-2014-032) was approved by the Research Ethics Committee of the Capital Region of Denmark.

Results. We have contacted 290 patients with PSS (telephone screening) and screened 47 patients with clinical and radiographic examination. Of these 21 (7 %) fulfilled the enrollment criteria.

At December 1st , 2017, we have enrolled and inserted implants in these 21 PSS patients (mean age 57 years). Similarly, we have enrolled 20 control patients (mean age 57 years). The PSS patients had implants inserted in molar- (n=10), premolar- (n=9) and incisor regions (n=2). The control patients had implants inserted in molar- (n=14) and premolar regions (n=5) and incisor regions (n=1). Implant length ranged from 8 to 12 mm, diameter: 4.1 mm (n=18) and 4.8 mm (n=23). 16 PSS patients and 13 controls have reached the baseline examination. 6 PSS patients and 4 controls have reached 1 year examination. All had stable successful implants.

Conclusions. Although PSS may lead to loss of teeth due to decay, it has been a time consuming process to identify and recruit PSS patients, as only 7% of the screened patients fulfilled the inclusion criteria. All 41 inserted implants have osseointegrated successfully and crowns have been mounted as planned. The preliminary results up to 1 year suggest that implants can be inserted successfully in patients with PSS, but more PPS patients and longer follow-up visits (5-10 years) are needed to properly evaluate the therapeutic option.

This Study was supported by a Grant from the ITI Foundation (International Team for Implantology). Grant no. 1036_2014.

O-30 *Late-Breaking Abstracts*

Hydroxychloroquine treatment downregulates systemic interferon activation in primary Sjögren's syndrome in the JOQUER randomized clinical trial

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Background. Hydroxychloroquine (HCQ) is the most frequently prescribed immunosuppressant for primary Sjögren's syndrome (pSS). Evidence regarding the efficacy of HCQ however is limited. The JOQUER randomized placebo-controlled trial studied the efficacy of 24 week HCQ treatment in pSS patients (1). HCQ-treated pSS patients did not improve symptoms of dryness, pain or fatigue (collected by ESSPRI questionnaires) or disease activity as measured by ESSDAI compared to placebo treated patients. Interferon (IFN) type I activation and upregulation of the TLR7 pathway is present in a subgroup of pSS patients (2, 3). *In vitro* studies showed that HCQ inhibits this pathway (4). Here we investigated:
1) if subclassification of patients based upon the expression of IFN signature genes showed an improvement on ESSDAI and ESSPRI with HCQ in the IFN positive patient group compared to the IFN negative patients and the placebo group.
2) the effect of HCQ treatment on expression levels of IFN inducing pathways and IFN stimulated genes (ISGs) in peripheral blood mononuclear cells (PBMCs) of patients enrolled in the JOQUER trial.
Methods. ESSDAI and ESSPRI questionnaires were obtained at baseline and after 24 weeks of treatment. Systemic IFN activation was determined in whole blood RNA samples (paxgene) of 77 pSS patients at baseline and after 24 weeks of treatment with HCQ or placebo. Systemic IFN activation was assessed by determining three modular IFN scores, previously described by Chiche et al. (2014): M1.2 (activation of IFN type I only), M3.4 (activation of IFN type I predominantly) and M5.12 scores (activation of IFN type I and IFN type II). Additionally, gene expression of the IFN inducing TLR7,9 pathway and its downstream signaling molecule MyD88 was determined. Furthermore expression levels of the IFN-inducible cytosolic RNA-sensing sensors (IFIH1, DDX58, DHX58, PKR) and DNA-sensing receptors (IFI16 and ZBP1) were assessed.
Results. Stratification based on IFN score did not reveal differences in changes between HCQ and placebo of ESSDAI and ESSPRI scores. However, HCQ treatment did significantly lower modular systemic IFN scores of M1.2, M3.4 and M5.12. Furthermore, mRNA expression levels of TLR9 and MyD88 and the downstream IFN-inducible cytosolic RNA-sensing sensors (IFIH1, DDX58, DHX58, PKR) and cytosolic DNA-sensing receptors (IFI16 and ZBP1) were downregulated in HCQ treated patients compared to placebo treated controls. mRNA levels of TLR7 were not reduced upon HCQ treatment.
Conclusion. Subclassification in IFN positive and IFN negative patients did not reveal an improvement of ESSPRI or ESSDAI scores with HCQ in either subgroup. Treatment with HCQ for 24 weeks decreased systemic IFN activation and reduced expression of IFN-inducible RNA and DNA sensors in the cytosol showing that HCQ affected the known pathways in the pSS patients. These data suggests the involvement of other pathways than the IFN pathway in the induction of dryness, pain and fatigue in pSS.
Funding The study was supported by a grant of the Dutch Arthritis Foundation (RF14-3-404) and French ministry of health (PHRC 2007).

References

1. GOTTENBERG J, RAVAUD P, PUÉCHAL X *et al.*: Effects of hydroxychloroquine on symptomatic improvement in primary Sjögren syndrome: The joquer randomized clinical trial. *JAMA* 2014; 312(3): 249-58.
2. BRKIC Z, MARIA NI, VAN HELDEN-MEEUWSEN CG, VAN DE MERWE JP, VAN DAELE PL, DALM VA *et al.*: Prevalence of interferon type I signature in CD14 monocytes of patients with Sjögren's syndrome and association with disease activity and BAFF gene expression. *Ann Rheum Dis* 2013 2013; 72(5): 728-35.
3. MARIA NI, STEENWIJK EC, IJPMA AS, VAN HELDEN-MEEUWSEN CG, VOGELSANG P, BEUMER W *et al.*: Contrasting expression pattern of RNA-sensing receptors TLR7, RIG-I and MDA5 in interferon-positive and interferon-negative patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2016.
4. KUŽNIK A, BENČINA M, ŠVAJGER U, JERAS M, ROZMAN B, JERALA R: Mechanism of Endosomal TLR Inhibition by Antimalarial Drugs and Imidazoquinolines. *J Immunol* 2011; 186(8): 4794-804.

O-31 *Late-Breaking Abstracts*

Characterization of salivary gland hypofunction associated with immune checkpoint inhibitor therapy

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Introduction. The advent of immune checkpoint inhibitor (ICI; *e.g.*, pembrolizumab, nivolumab, ipilimumab) biologic agents has significantly advanced the field of cancer therapeutics. However, administration of these antibodies can trigger immune-related adverse events (irAEs), including salivary gland hypofunction and xerostomia, which has been previously reported but insufficiently characterized. Herein, we describe the clinical phenotype of the largest cohort of patients to date who experienced dry mouth associated with ICI. Our report illustrates evidence of a novel mechanism of salivary gland hypofunction providing insight into its pathogenesis and helping to inform the clinical management of this irAE.
Methods. Patients with ICI-related dry mouth underwent comprehensive medical and dental evaluations at the NIH Salivary Gland Dysfunction Unit. Whole unstimulated and glandular (parotid and submandibular/sublingal) unstimulated and citric-acid stimulated salivary flows were measured. Minor salivary gland biopsies (MSG) were obtained for histopathology, immunohistochemistry, RNA sequencing, primary tissue culture, and for measuring functional (*e.g.*, volume change) and immunological changes (*e.g.*, fluorescence-activated cell sorting, FACS).
Results. Sixteen patients were evaluated an average of 10 weeks (range: 2-37 weeks) after the onset of xerostomia. Each patient had received one or more ICI agents to treat melanoma (n=10), respiratory papillomatosis (n=3), prostate adenocarcinoma, non-small cell lung carcinoma, or gastroesophageal adenocarcinoma, (n=1 each). All subjects reported "dry mouth" and 15/16 (94%) subjects had objective salivary hypofunction as measured by whole unstimulated saliva flow (mean: 0.67±0.69 mL/15 min, range: 0-2.49mL/15 min, ref range >1.5 mL/15 min). Salivary ultrasonography revealed consistent, albeit mild, changes in the major glands. Histopathologic changes in MSG biopsies included mild-to-moderate chronic sialadenitis with fibrosis and atrophy of the terminal acini, acinar disruption, mucin congestion, and mucin extravasation; 5 cases (31%) exhibited lymphocytic aggregates (focus score ≥1). Functional studies on MSG *ex vivo* demonstrated deficits in carbachol-stimulated volume change and impaired calcium release and influx indicative of profound secretory deficits. Scanning electron microscopy of MSG revealed disrupted cell-cell contacts between salivary epithelial cells and marked congestion of the secretory granules. RNA sequencing showed enrichment of neuronal and immune pathways but down-regulation of protein translation pathways. FACS on enzymatically dispersed MSG demonstrated infiltration of cytotoxic T cells exhibiting high expression of PD-1 and elevated cytokine secretion in response to phorbol myristate acetate (PMA) and ionomycin (ION). Immunohistochemical staining to characterize the immune cell infiltration in the MSG was also performed.
Conclusions. We demonstrate that ICIs can elicit profound negative effects on salivary secretion, which, like other irAEs, is expected to increase in incidence with the rising use of these drugs. Our data suggest that the mechanism of salivary gland hypofunction is distinct from other diseases affecting the salivary glands (*e.g.*, Sjögrens syndrome). We propose that ICI therapy may break immune tolerance locally leading to activation of cytotoxic T cells, cytolysis, and cytokine secretion. Because of the exquisite sensitivity of the salivary glands to injury, we hypothesize that promptly preventing the activation or function of these resident or infiltrating immune cells locally may prevent long-term sequelae of ICI-induced salivary hypofunction.