## POS0251 TRANSCRIPTOMIC CHANGES INDUCED BY MAVRILIMUMAB VERSUS TOCILIZUMAB IN EX-VIVO CULTURED ARTERIES FROM PATIENTS WITH GIANT-CELL ARTERITIS

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**Background:** Giant cell arteritis (GCA) is a chronic disease, and affected patients suffer from relapses and glucocorticoid (GC)-related toxicity. Targeted therapies are emerging with the aim of achieving better disease control and reducing GC exposure. Blocking IL-6 receptor with tocilizumab has been a major advance in the treatment of GCA. However, approximately 40% of patients treated with tocilizumab in combination with GCs experience a flare or tocilizumab-related adverse event. Blocking GM-CSF receptor $\alpha$ with mavrilimumab significantly reduced risk of relapse and improved sustained remission at week 26 vs placebo in a Phase 2 trial. Not all patients satisfactorily respond to any therapy, indicating heterogeneity in leading pathogenic pathways among patients. For these reasons, it is crucial to understand the specific impact of targeted therapies on vascular lesions.

**Objectives:** In this study we investigated transcriptomic changes induced by tocilizumab or mavrilimumab in *ex-vivo* cultured arteries from patients with GCA.

**Methods:** Temporal artery sections obtained for diagnostic purposes from 11 patients with histopathologically-confirmed GCA and 3 controls were cultured *ex-vivo* and exposed to placebo, mavrilimumab, or tocilizumab (both at 20 µg/mL) for 5 days. Of 11 GCA donors, 2 had received no treatment prior to biopsy, 2 had received a single prednisone (60 mg) dose, 1 had received 2 daily doses, and the remaining 6 had extended treatment; in prednisone-treated patients, mean (SEM) treatment duration was 17.9 ±8.7 days. A separate cohort of patients (consisting of five newly diagnosed patients with GCA, age- and sex-matched with the previous cohort) was used to validate 7 transcripts by real time PCR. Genes were selected for validation based on high level of expression and differential expression with each treatment. All samples were homogenized, and total RNA was extracted with TRIzol reagent. 100 ng of RNA per sample were processed with Nanostring Inflammation gene expression assay (256 transcripts) and hybridized using nCounter Prep Station. Barcode counts from nCounter Digital Analyzer were processed with nSolver 4.0 Software. Normalised data were analyzed using R Studio 4.0.5 and IBM SPSS 22.0, and paired Wilcoxon tests were applied individually to each treatment comparison group for each analysed gene. One µg of RNA per sample from the validation cohort was retrotranscribed; subsequent real time PCRs were normalised against endogenous control GUSb and analysed using SDS 2.3 software.

**Results:** 67 out of 250 transcripts were differentially expressed between arteries from GCA patients and arteries from control patients (all placebo-treated). Of those, only 9 transcripts remained significant after correction for multiple comparisons, with a false discovery rate ≤0.05. 81 transcripts were differentially expressed in at least one comparison across groups (Figure 1A). 15 transcripts were lower, and 6 were higher in the mavrilimumab group vs placebo; 3 transcripts were lower, and 2 were higher in the tocilizumab group vs placebo. Most changes elicited between treatments were unique, but CXCL-1 was common (Figure 1B). None remained significant after correction for multiple comparisons. The effects of mavrilimumab and tocilizumab on GNAS, CXCL1, IL8, IL2, IRF3, MRC1 and BCL6 expression by Nanostring were consistent with the effect assessed using real time PCR in the separate validation cohort (Figure 1C).

**Conclusion:** Mavrilimumab and tocilizumab have a different transcriptomic impact on cultured arteries from patients with GCA, with some overlapping effects, although differential effects may have been attenuated by prior GC use. A better understanding of the impact of targeted therapies on vascular inflammation is needed to improve treatment options for patients with GCA.

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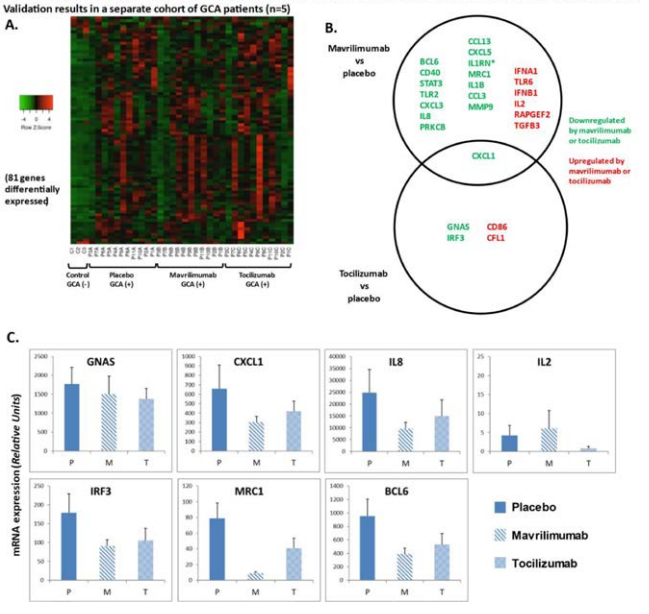
Figure 1. (A) Heat map of differential expression of 81 genes in GCA (-) control and GCA (+) treated with placebo, mavrilimumab, or tocilizumab. (B) Venn diagram of genes differentially expressed in mavrilimumab versus placebo and tocilizumab versus placebo. (C) Validation results in a separate cohort of GCA patients (n=5)




## POS0252 MYOFIBROBLASTS MAINTAIN TH1 AND TC1 POLARIZATIONS IN GIANT CELL ARTERITIS

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**Background:** Giant cell arteritis (GCA) is a large-vessel vasculitis mainly involving the aorta and cranial arteries. It is the most frequent vasculitis in adults over 50 years. When they are stimulated by interferon-gamma (IFN-$\gamma$), vascular smooth muscle cells (VSMC) contribute to GCA pathogenesis by producing chemokines triggering the recruitment of pro-inflammatory T cells and monocytes (1).

**Objectives:** Current knowledge about the interaction between resident cells of the vascular wall (VSMC, myofibroblasts [MF]) and immune cells is limited. The aim of our research was to better characterize the interactions between VSMC, MF and T cells in GCA.

**Methods:** Fresh fragments of temporal artery biopsies (TAB) performed at Dijon university hospital (France) were prospectively sent to our research unit. Fresh sections of positive and negative TAB were fixed and embedded in optimal cutting temperature OCT and stored at -80°C. Then, cryostat sections were fixed, permeabilized, blocked and incubated with primary antibodies (anti-alpha smooth muscle actin [$\alpha$-SMA], anti-myosin heavy chain 11 [MHC11], anti-Desmin, anti CD90, anti-CD45, anti-HLA-DR, anti-phospho STAT1 [pSTAT1] and anti-pSTAT3) and secondary antibodies for confocal microscopy analyses. Fresh sections of healthy TAB were embedded in MATRIGEL and covered by DMEM to obtain vascular cells in culture. Cells were treated with trypsina-EDTA between each passage. Vascular cells were used after 4-7 doubling passages. Cells were analyzed by immunofluorescence, flow cytometry and RT-PCR and their proliferation was evaluated by impedancemetry (iCELLigence system). Peripheral blood

mononuclear cells (PBMC) and vascular cells thus obtained were co-cultured for 7 days in different conditions. Vascular cells were cultured in the presence or absence of IFN-γ and tumor necrosis factor alpha (TNF-α) or interleukin-6 (IL-6) and soluble receptor of IL-6 for 72 hours. When cells reached confluence, they were cultured alone or with allogenic PBMC activated with anti-CD3/CD28 microbeads. After 7 days of culture, cells were separated with a treatment with EDTA and studied by flow cytometry.

**Results:** Confocal microscopy analyses of GCA arteries showed that neointima was mainly composed of myofibroblasts (MF) ($\alpha$-SMA$^+$Desmin$^+$MHC11$^{low}$CD90$^+$) in contact with CD45$^+$ cells and that MF expressed HLA-DR, the phosphorylated form of STAT1 (pSTAT1) and in a lesser extent pSTAT3, strongly suggesting the activation of the IFN-γ signaling pathway rather than the IL-6 pathway. The phenotype of cultured vascular cells isolated from fresh TAB was consistent with MF. When MF were exposed to IFN-γ and TNF-α *in vitro*, their proliferation capacity decreased and their levels of expression of HLA-DR and CD86 increased (median fluorescence intensity [MFI] from 0 to 57 [p=0.03] and from 34 to 103 [p=0.03], respectively). In addition, co-cultures of MF and activated PBMC revealed that MF maintained the polarization of T cells into Th1 and Tc1 cells ($p \leq 0.001$) and to a lesser extent into Th17 and Tc17 cells (p=0.03). This effect was even more significant when MF were previously exposed to IFN-γ and TNF-α but not when they were exposed to IL-6.

**Conclusion:** Our results show that myofibroblasts are present in the neointima of GCA patients and that these MF activate signaling pathways indicative of IFN-γ exposure. Moreover, these MF, especially when exposed to IFN-γ, maintain the polarization of T cells into Th1 and Tc1 cells, which contributes to amplify the production of IFN-γ and thus initiate a pro-inflammatory amplification loop that likely participates in vascular inflammation and remodelling.

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POS0253

## PERSONALIZED RISK EVALUATION FOR OUTCOME PREDICTION IN ANCA ASSOCIATED VASCULITIS (AAV) USING LATENT CLASS ANALYSIS AND MACHINE LEARNING.

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**Background:** ANCA associated vasculitides (AAV) are a heterogeneous group of rare diseases with unknown etiology. In the most severe cases AAV can lead to end stage kidney disease or death. Since etiology and detailed pathogenesis of AAV is not known, the prediction of disease outcome at the time of diagnosis is challenging. Thus, there is an unmet need for tools to identify patients with the highest risk of organ dysfunction and death and apply effective personalized therapy.

**Objectives:** The aim of this work was to search for tools allowing outcome prediction at the time of AAV diagnosis. Early identification of patients, who are likely to develop severe organ dysfunction and death is crucial for appropriate disease management. Induction therapy in AAV relays on immunosuppressive drugs characterized by a high risk of severe side effects. Thus, their administration in high doses should be limited only to individual patients with an especially high risk of poor outcome.

**Methods:** We applied here two methods of identification of AAV patients at risk to develop severe organ dysfunction and death. First method (latent class analysis [LCA] followed by logistic regression) was meant to subcategorize patients and identify a subgroup at subjects at risk to develop chronic renal replacement therapy (CRRT) and death [1]. Second, served to assess individual poor outcome risk and was based on two machine learning (ML) classifiers, which by analyzing clinical information allow assigning computed risk for CRRT and death in an individual patient allowing to identify subjects with high risk of chronic replacement therapy (CRRT) and death. We have evaluated a number of different approaches to build the ML models (including logistic regression, support vector machines, random forests), and obtained the best results for the gradient boosting algorithm implementation called LightGBM [2]. It works as a sequential ensemble of so-called weak learners (decision trees) finally combined in a one prediction model. Both analyses were based on retrospective data from Polish national AAV registry (POLVAS) [3] including presently 565 GPA and 135 MPA patients. The parameters used were: demographic data and laboratory parameters, specific organ involvement, ANCA specificity and time between selected stages of the disease.

**Results:** LCA used on our AAV cohort identified four subphenotypes – three already previously proposed - and revealing a fourth clinically relevant subphenotype. This new subphenotype includes only GPA patients, usually diagnosed at a younger age as compared to other groups, and characterized by multiorgan involvement, high relapse rate, relatively high risk of death, but no end-stage kidney disease. Logistic regression analysis revealed significant differences in the risk of CRRT and death between those subphenotypes – the worst prognosis was found for severe MPO AAV. On the other hand, using ML approach we obtained an individual prediction model with potentially relevant clinical performance (ROC AUC of 0.85 for CRRT and 0.82 for death).

**Conclusion:** We consider results obtained encouraging. They may offer a new insight into AAV course based on data available at diagnosis, and create a solid foundation for potential clinical decision support system.

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## Vaccination against SARS-CoV-2

POS0254

## IMMUNE RESPONSE TO SARS-COV-2 INFECTION IN PATIENTS WITH RHEUMATIC MUSCULOSKELETAL DISEASES: THE MAINSTREAM STUDY

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