BIOMARKER CHANGES FOR PATIENTS WITH RHEUMATOID ARTHRITIS RECEIVING TOFACITINIB WITH METHOTREXATE OR GLUCOCORTICOIDS VS TOFACITINIB MONOTHERAPY

Julie Lee1, Xing Chen1, Weidong Zhang1, David Martin3, Craig Hyde1, Tomohiro Hirose2, Shweta Shah3, Lori Fitz1, Pfizer Inc, Cambridge, MA, United States of America; 4Pfizer Japan Inc, Tokyo, Japan; 5Pfizer Inc, Collegeville, PA, United States of America

Background: Tofacitinib is an oral Janus kinase inhibitor for the treatment of RA. Herpes zoster is more common in patients (pts) with RA vs the general population. This risk increases with tofacitinib use and appears to be further enhanced with concomitant use of csDMARDs such as methotrexate (MTX), or glucocorticoids (GC). The mechanism for these increases in risk may be linked to treatment-induced interferon (IFN) suppression, given that replication of the varicella zoster virus appears to be limited by IFN activity.

Objectives: To evaluate whether treatment of RA with tofacitinib + MTX or GC suppresses IFN pathway proteins to a greater extent than treatment with tofacitinib monotherapy.

Methods: This was a post hoc analysis of pooled data from 1 Phase (P2) Japan study [NCT00687193] and 3 P3 (ORAL Scan [NCT00847613]; ORAL Start [NCT01039688]; tofacitinib studies. Serum samples were collected at baseline (BL), Week (W)12 and/or W24 from pts with RA treated with tofacitinib 5 or 10 mg BD, given as monotherapy (Japan study; ORAL Start) or with stable doses of MTX (15–25 mg weekly for ≥6 weeks; ORAL Scan) and/or GC (≤10 mg/day prednisone or equivalent; all studies). A total of 376 proteins associated with cellular and inflammatory processes, including 6 IFN pathway proteins (CXCL10, CXCL9, CXCL11, IL-12, IFNγ and IL-20), were measured using a homogeneous solution-based assay (Olink Proseek® Multiplex Assay, Uppsala, Sweden). Changes in protein levels from BL to W12 (Japan study, ORAL Scan) and/or W24 (ORAL Scan, ORAL Start) were compared for tofacitinib monotherapy vs tofacitinib + MTX or GC using linear regression models. The dependent variable was change from BL in protein levels at W12 or W24. The independent variable was MTX or GC status. Age, gender, GC status (in MTX model) and BL protein levels and tofacitinib dose were covariates. Regressions were performed separately for each study; results for GC were combined via meta-analysis using fixed and random effects models. Significance was considered at p<0.05 after controlling for false discovery rate (FDR). To evaluate effect size, data quality control included accounting for plate/batch defects and limits of detection, and removal of samples/analytics with excessive missing data.

Results: In total, 659 serum samples were collected from 321 pts. Of the 6 IFN pathway proteins, 2 (IFNγ and IL-20) were below the limit of detection. There was no strong evidence suggesting statistical differences between tofacitinib monotherapy and tofacitinib + MTX or GC in changes in levels of the 4 detectable IFN pathway proteins (CXCL10, CXCL9, CXCL11 and IL-12) from BL to W12 and/or W24. Significant differences were observed for 2 of the 370 other proteins: MMP-1 and IL1Ra, IFNγ and IL-20), were measured using a homogeneous solution-based assay (Olink Proseek® Multiplex Assay, Uppsala, Sweden). Changes in protein levels from BL to W12 (Japan study, ORAL Scan) and/or W24 (ORAL Scan, ORAL Start) were compared for tofacitinib monotherapy vs tofacitinib + MTX or GC using linear regression models. The dependent variable was change from BL in protein levels at W12 or W24. The independent variable was MTX or GC status. Age, gender, GC status (in MTX model) and BL protein levels and tofacitinib dose were covariates. Regressions were performed separately for each study; results for GC were combined via meta-analysis using fixed and random effects models. Significance was considered at p<0.05 after controlling for false discovery rate (FDR). Data quality control included accounting for plate/batch defects and limits of detection, and removal of samples/analytics with excessive missing data.

Results: In total, 659 serum samples were collected from 321 pts. Of the 6 IFN pathway proteins, 2 (IFNγ and IL-20) were below the limit of detection. There was no strong evidence suggesting statistical differences between tofacitinib monotherapy and tofacitinib + MTX or GC in changes in levels of the 4 detectable IFN pathway proteins (CXCL10, CXCL9, CXCL11 and IL-12) from BL to W12 and/or W24. Significant differences were observed for 2 of the 370 other proteins: MMP-1 (FDR adjusted p=0.08) and IL1Ra (FDR adjusted p=0.09), where levels decreased from BL to W12 for tofacitinib + MTX to a greater extent than for tofacitinib monotherapy.

Conclusion: The results of this post hoc analysis suggest that tofacitinib + MTX or GC may not suppress circulating serum levels of IFN pathway proteins to a greater extent than tofacitinib monotherapy. Although there were differences at W12 for tofacitinib + MTX vs tofacitinib monotherapy in MMP-1 and IL1Ra, it is not yet clear whether these observations may be attributable to differences in the ethnicities of the study populations receiving these two treatment regimens (global vs Japan). Further analyses of biomarker changes with tofacitinib are ongoing.

REFERENCES


Figure 1. The absolute number of B cell, NK cell, Th1 cell, Th2 cell, Treg cell in RA patients and health controls. Data are expressed as means ±SD.
**INTERRELATIONSHIP BETWEEN NICOTINIC ACETYLCHOLINE RECEPTOR AND CYTOKINE PRODUCTION NOTED FOLLOWING T-CELL ANTIGEN RECOGNITION AND ACTIVATION**

Nicholas Mangoni 1,2,3, Kevin Hou4, Han-Shen Tae2, David Adams5, 1University Sydney-Westmead Hospital, Rheumatology, Sydney, Australia; 2The University of Sydney, Rheumatology; Camperdown, Australia; 3Westmead, Rheumatology, Westmead, Australia; 4Westmead, Rheumatology, Westmead, Sydney, Australia; 5University of Wollongong, Wollongong, Australia;

Background: T cells express muscarinic and nicotinic acetylcholine receptors (mAChRs, nAChRs) that increase intracellular Ca2+ [1] on stimulation. The expression of these receptors on macrophages and their activation by vaginal stimulation has recently been the focus for novel arthritis treatment [2].

**Objective:** Our aim in the present study was to assess the effect of various peptides, on cytokine production and nAChRs inhibition.

**Methods:** nAChR heterologous subunits were expressed in Xenopus oocytes and the inhibitory activity of various peptides at ACh-activated currents was assessed. The effect of these peptides on T-cell antigen recognition and subsequent cytokine production was assessed using a T-cell antigen presentation assay (APA).

**Results:** Briefly, the 2B4.11 murine T cell hybridoma recognizing cytochrome c as the antigen was co-cultured with the antigen presenting B cell hybridoma line LK35.2 (I-Ek bearing) and pigeon cytochrome c in the absence or presence of peptide or several nAChRs antagonists, including mecamylamine (broad nAChR antagonist), waglerin-1 (a[516a], o-bungarotoxin (o[7], Rgla (o[10]10), Vc1.1 (o[10])10) and dihydro-beta-erythroidine hydrobromide (o[8]4 and o[8]2), ELISA and real-time PCR were performed to measure cytokine protein levels and nAChRs T-cells mRNA express levels separately.

**Results:** At 10μM, peptide W32052 had modest 50-55 % inhibition of human (h) o[3]2 and hoh[10]10 nAChR subtypes, and 35% inhibition at hoh[10]10. W32052 greatly inhibited chimeric rat o[10]10 mouse (ε) 85% at 10μM. W32052 also inhibited IL-2, IL-6, TNF-α and GM-CSF production at 50μM in the APA. nAChRs antagonists, mecamylamine (100μM), Rgla (10μM), Vc1.1 (17.5μM) and dihydro-β-erythroidine hydrobromide (10μM) could decrease IL-2 production. However, waglerin-1 and o-bungarotoxin did not affect IL-2 production in the APA.

**Conclusion:** W32052, an antagonist of nAChR, inhibits cytokine production following antigen recognition suggesting that there is a close link between T-cell antigen activation, ion channel regulation mediated by AChR and cytokine production. Further experiments are in progress.

**REFERENCES**


