possibly reflecting mechanism of action of E6011, since the CD16⁺ monocytes highly express CX3CR1.

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Disclosure of Interests: Tomohiro Yamada Employee of: KAN Research Institute, Inc., Jungo Kakuta Employee of: KAN Research Institute, Inc., Eri Fusao-ka-Nishioka Employee of: KAN Research Institute, Inc., Jun-ichi Ito Employee of: EISAI, Nobuyuki Yasuda Employee of: KAN Research Institute, Inc., Tetsu Kawano: None declared, Toshio Imai: None declared

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FRI0010

GM-CSFR PATHWAY IS IMPLICATED IN PATHOGENIC INFLAMMATORY MECHANISMS IN GIANT CELL ARTERITIS

M. C. Cid¹, S. Muralidharan², M. Corbera-Bellalta¹, G. Espigol-Frigole¹, J. Marco Hernandez¹, A. Denuc³, R. Rios-Garcés¹, N. Terrades-García¹, J. F. Paolini², A. D'andrea². ¹*Vasculitis Research Unit, Hospital Clinic, University of Barcelona, IDIBAPS, Barcelona, Spain*; ²*Kiniksa Pharmaceuticals Corp., Lexington, MA, United States of America*; ³*HCB-IDIBAPS Biobank, Barcelona, Spain*

Background: Giant Cell Arteritis (GCA) is characterized by inflammation of large and medium arteries. Classic symptoms include headaches, malaise and, in severe cases, blindness and aortic aneurysms. Corticosteroids (CS) are the first line of treatment. Relapsing disease patients undergo multiple courses of CS therapy increasing their CS exposure and toxicity. A significant unmet need for disease-modifying CS-sparing therapy remains in GCA as the efficacy of current treatment options, including tocilizumab have limitations.

We have previously reported elevated expression of granulocyte-macrophage colony stimulating factor (GM-CSF) pathway transcriptomic signature in GCA vessels. GM-CSF may contribute to underlying disease mechanisms by regulating inflammatory macrophages, dendritic cells (DCs) and T helper (T_H1/T_H17) cells which are involved in GCA pathogenesis. GM-CSF produced by T cells¹ can promote polarization of inflammatory macrophages² and recruitment and differentiation of monocytes into inflammatory DCs² that can in turn recruit T cells and stimulate T_H1/T_H17 differentiation creating a feedback loop. GM-CSF may also exert direct effects on angiogenesis³ and vessel wall remodeling⁴.

Objectives: To demonstrate the contributing role of GM-CSF pathway to inflammation in GCA arteries.

Methods: Immunostaining was used to examine expression of GM-CSF and GM-CSF-Rα proteins in temporal artery biopsies (TABs) from GCA and controls (patients with suspected but not confirmed GCA and a negative TAB). Costaining with cell markers such as CD31, CD3, and CD68 allowed visualization of cells expressing GM-CSF and GM-CSF-Rα. Expression of GM-CSF pathway molecules such as phospho-JAK2 and PU.1 proteins was detected by immunohistochemical staining of GCA and control TABs. *Ex vivo* cultured GCA arteries treated (10 each) with mavrilimumab (anti-GM-CSF-Rα) or placebo for 5 days were assayed for gene expression by qPCR, and culture supernatants were analyzed by ELISA.

Results: Endothelial cells and macrophages were the main cell types expressing GM-CSF and GM-CSF-Rα. Increased expression of phospho-JAK2 (activated signaling molecule) and nuclear-localized PU.1 (transcription factor) in GCA TABs compared to controls indicated the presence of active GM-CSF signaling pathway in GCA.

Inhibition of PU.1 mRNA expression in *ex vivo* cultures of GCA arteries treated with mavrilimumab indicated blockade of GM-CSFR signaling pathway. Mavrilimumab induced decrease in mRNA expression of key cell type markers including DC and macrophage activation markers CD83 and HLA-DRA, monocyte markers CD14 and CD16, T cell marker CD3ε, and B cell marker CD20 in these GCA artery cultures. Expression of inflammatory T_H1/T_H17 factors IFNγ (mRNA), TNFα, CXCL10 (IFNγ-stimulated chemokine) and IL-6 (mRNA and protein) was also inhibited by mavrilimumab in GCA artery cultures.

Conclusion: Increased GM-CSF, GM-CSF-Rα, and downstream pathway-associated protein levels in GCA biopsies were consistent with previously-observed increased transcriptome signature. Expression of genes associated with inflammatory cells was suppressed by mavrilimumab in cultured GCA arteries. These data implicate the GM-CSF pathway in GCA pathophysiology and increase confidence in rationale for targeting the GM-CSF pathway in GCA.

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FRI0011

DEVELOPMENT OF A HIGH-DIMENSIONAL FLOW CYTOMETRY PANEL TO ANALYSE NATURAL KILLER CELLS IN SLE

N. Fewings¹, S. Schibeci¹, F. McKay^{1,2}, S. Swaminathan^{1,2,3,4}, M. W. Lin^{2,3,4}.

¹*The Westmead Institute for Medical Research, Centre for Immunology and Allergy Research, Westmead, Australia*; ²*The University of Sydney, Sydney Clinical School, Camperdown, Australia*; ³*Westmead Hospital, Department of Clinical Immunology, Westmead, Australia*; ⁴*Westmead Hospital, Department of Immunopathology, ICPMR, Westmead, Australia*

Background: Natural Killer (NK) cells are an innate immune cell type that has somewhat been overlooked in the context of systemic lupus erythematosus (SLE). SLE patients display a reduced number of NK cells with an activated phenotype and increased capacity to produce IFN-γ, decreased antibody-dependent cellular cytotoxicity (ADCC), and altered natural cytotoxicity (1). NK cell activation is determined by the integration of input from a myriad of activating and inhibitory receptors. Previously, using Nanostring® gene expression technologies, we found our SLE cohort showed decreased gene expression of a number of these receptors (KLRC2, KLR1, KLRB1, KLRF1, KLRG1, PRF1 and IL2RB) leading us to explore NK cells in SLE in more depth.

Objectives: Our aim was to develop a high-dimensional flow cytometry panel to characterise dysregulation of NK cell in SLE, with particular reference to the activating and inhibitory receptors found to be dysregulated in SLE at the gene expression level.

Methods: Markers for NK panel were selected to include canonical phenotyping/functional molecules of NK cells with a particular emphasis on receptors found to be lower in our SLE cohort's gene expression findings. NK panel was designed to minimise spectral overlap, expression and co-expression of markers was taken into consideration. Antibodies were titrated, and voltages optimised to achieve the best separation index for each of the antibodies. The 24-marker panel was run on 52 SLE patients of various disease manifestations, treatments and disease severity. 20 healthy controls were also run for comparison.

Results: A 24-marker flow cytometry panel including 19 NK cell antigens was optimised, including basic phenotype (CD3/CD56/CD16/NKp46) and NK differentiation markers (CD57/CD94), activating and inhibitory receptors (NKG2A/NKG2C/NKG2D), costimulatory receptors (CD244/CD226), transcription factors (Eomes/Tbet) and effector molecules (granzyme/perforin). Immunophenotypic high-parameter analysis of SLE and control samples is in progress and results will be presented.

Conclusion: Our development of a high-dimensional immunophenotypic panel allows identification of changes in NK cells in SLE including antigen expression levels, subset percentages and potentially of novel subsets. This panel will be used to investigate NK cell changes with disease course/activity, therapeutic response, and to discover potential drug targets for SLE.

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