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Key message: Detection of IFNαs proteins secreted by cells from JDM patients opens new perspective for drug discovery.

Dear Editor,

A type 1 interferon (IFN) gene signature has been previously demonstrated in the peripheral blood and muscle of patients with juvenile dermatomyositis (JDM), correlating with disease activity scores. However, direct measurement of interferon alpha (IFNα) protein in samples from patients has remained a challenge until recently. We addressed this limitation by optimising an ultrasensitive single molecule array (Simoa) digital enzyme-linked immunosorbent assay (ELISA) utilizing high affinity autoantibodies isolated from APECED patients that recognize all human interferon-α species (1). Using this technology, we were able to detect and quantify serum IFNα in the blood of JDM patients (2), and the blood, cerebrospinal fluid , cell supernatant and tissues from patients with other complex and monogenic interferonopathies (2,3). Interestingly, the median serum concentration of IFN□□was at 56fg/ml in JDM patients, almost 100 times below the limit of detection of classical anti IFNα ELISAs.

In the present study, we evaluated the ability of anti-inflammatory drugs, particularly the TBK1 inhibitor BX795, to control IFNα secretion from cells of JDM patients. Previous studies have shown that TBK1 inhibition controls disease activity in a mouse model of systemic lupus erythematosus (SLE), and interferon signalling in fibroblasts from lupus patients (4) and PBMCs isolated from patients with gain-of-function of STING (3). TBK1 inhibitors are currently in preclinical evaluation for their use in inflammatory diseases such as SLE.

We first demonstrated that our Simoa assay allowed the detection of IFNα secretion by unstimulated peripheral blood mononuclear cells (PBMCs) isolated from JDM patients: culture of frozen PBMCs from all 7 patients tested demonstrated elevated levels of IFNα (median 1319 fg/ml [range: 211.7-5940 fg/ml]) in comparison to controls (median 0.0056 fg/ml [0.0004-45.05 fg/ml]; p=0.0025 (Figure 1A). In order to evaluate the ability of TBK1 to control IFNα secretion by PBMCs isolated from JDM

patients, we cultured PBMCs overnight with or without 2µM of BX795. Inhibition of TBK1 by BX795 resulted in a strong decrease of spontaneous secretion of IFNα in all tested patients (Figure 1B). As in our previous study on cells from STING-mutated patients(3), BX795 did not affect cell viability at this concentration (Figure 1C). We have also previously shown that myogenic precursor cells (MPCs) from JDM patients can produce type I-IFN (5). This may be explained in part by the over expression of nucleic acid sensors, including MDA5 (6)(Figure 1D). Thus, in order to mimic disease-related inflammation, we stimulated MPCs-derived from JDM patients by transfection of the MDA5 ligand poly I:C. After 24 hours, IFNα was measured in the supernatant by Simoa, and cell viability was evaluated by ATP measurement. Upon stimulation, MPCs from 11 of 15 patients demonstrated increased levels of IFNα (median non-stimulated 0.20 fg/ml [0.005-1.9 fg/ml]; median stimulated 1.03 fg/ml [0.015-137.8 fg/ml] p=0.017 (Figure 1E, 1F). IFNα production by MPCs under poly I:C stimulation was decreased by treatment with BX795 (median 1.03 fg/ml [0.015-137.8 fg/ml]; median 0.36 fg/ml [0.003-4.69 fg/ml] p=0.007 (Figure 1F) with no or minor effect on MPCs viability (Figure.1G).

The increased sensitivity to detect IFNα protein by Simoa technology has allowed us to reveal that circulating leukocytes from JDM patients demonstrate spontaneous *ex vivo* production of IFNα, which can be controlled by TBK1 inhibition. Similarly, we were able to measure, for the first time, the production of IFNα by stimulated primary MPCs of JDM patients, and to evaluate the inhibition of this secretion by a TBK1 inhibitor. As such, our data demonstrate that it is now possible to use IFNα secretion as a read-out to evaluate the modulation of interferon by JDM patient-derived material, and to interrogate the effect of potential therapeutic agents. Thus, we envision that Simoa technology will inform drug discovery and personalised approaches to therapy. In another hand, how type I IFNs are involved in JDM pathogenesis remain an open question. Notably, the sources of the interferon in JDM remain unknown, as does the impact of chronic exposure to interferon, even at very low levels, on endothelial and muscular cells within the tissues. The increased sensitivity to detect IFNα proteins by

Simoa should help to identify the interferon producing cells and paves the way for a better understanding of JDM physiopathology.

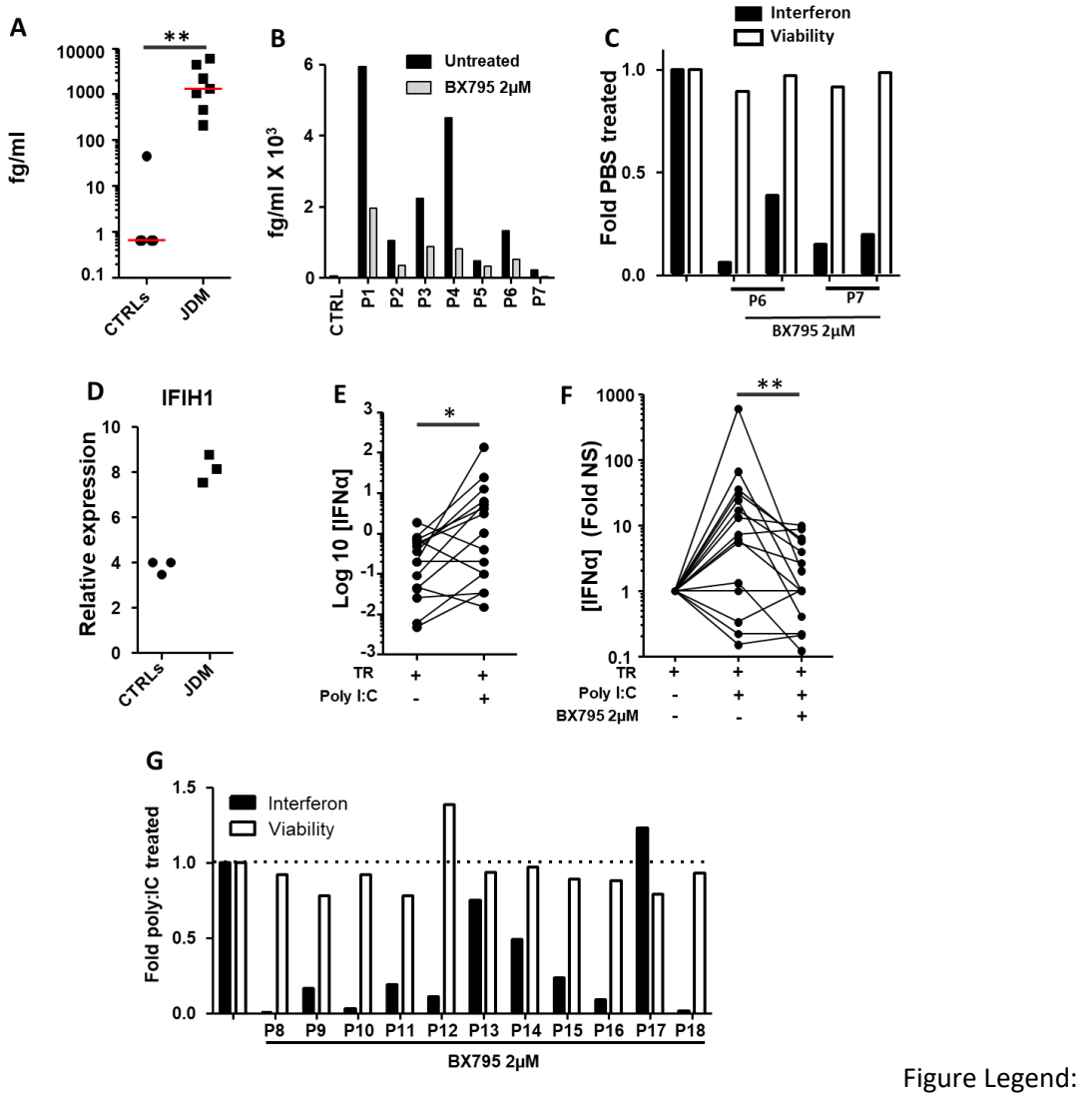

Figure Legend:

Figure 1:TBK1 inhibition blocks IFNα secretion by JDM primary cells. (A) IFNα secreted by PBMCs of 7 JDM patients can be detected by Simoa. **(B)** Treatment with a TBK1 inhibitor strongly reduces IFNα secretion. **(C)** Evaluation of TBK1 inhibition on cell survival. Results from 2 independent experiments on PBMCs from P6 and P7 show no toxic effect. **(D)** MPCs from JDM patients express more IFIH1 transcript than controls. **(E)** Stimulation of patient MPCs with poly I:C induced secretion of IFNα that can be measured by Simoa. **(F)** TBK1 inhibition controls Poly I:C induced IFNα secretion by MPCs,**(G)** with no or minor effect on MPCs viability. TR : transfection reagent; Wilcoxon matched-pairs rank test: * <0,05; **<0,01

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Conflicts of interest:

The authors have no conflicts of interest to disclose.

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