bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

1 # Anti-inflammatory properties of chemical probes in human whole
2 # blood: focus on prostaglandin $E_2$ production

3 **Filip Bergqvist[a,b,*], Yvonne Sundström[a,b], Mingmei Shang[a,b], Iva Gunnarsson[a], Ingrid**
4 **Lundberg[a], Michael Sundström[a,b], Per-Johan Jakobsson[a,b], Louise Berg[a,b,*]**

5 [a]Division of Rheumatology, Department of Medicine, Solna, Karolinska Institutet, and Karolinska
6 University Hospital, SE-17176, Stockholm, Sweden
7 [b]The Structural Genomic Consortium (SGC), Karolinska Institutet, Stockholm, Sweden

8 *Corresponding authors. Division of Rheumatology, Department of Medicine, Solna, Karolinska
9 Institutet, and Karolinska University Hospital, SE-17176, Stockholm, Sweden
10 E-mail address: Bergqvist.Filip@gmail.com (F. Bergqvist), Louise.Berg@ki.se (L. Berg)

11 **Abstract**
12 We screened 57 chemical probes, high-quality tool compounds, and relevant clinically used drugs to
13 investigate their effect on pro-inflammatory prostaglandin $E_2$ ($PGE_2$) production and interleukin-8 (IL-
14 8) secretion in human whole blood. Freshly drawn blood from healthy volunteers and patients with
15 systemic lupus erythematosus (SLE) or dermatomyositis was incubated with compounds at 0.1 or 1 μM
16 and treated with lipopolysaccharide (LPS, 10 μg/mL) to induce a pro-inflammatory condition. Plasma
17 was collected after 24 hours for lipid profiling using liquid chromatography tandem mass spectrometry
18 (LC-MS/MS) and IL-8 quantification using enzyme-linked immunosorbent assay (ELISA). Each
19 compound was tested in at least four donors at one concentration based on prior knowledge of binding
20 affinities and *in vitro* activity. Our screening suggested that PD0325901 (MEK-1/2 inhibitor), trametinib
21 (MEK-1/2 inhibitor), and selumetinib (MEK-1 inhibitor) decreased while tofacitinib (JAK inhibitor)
22 increased $PGE_2$ production. These findings were validated by concentration-response experiment in two
23 donors. Moreover, the tested MEK inhibitors decreased thromboxane $B_2$ ($TXB_2$) production and IL-8
24 secretion. We also investigated the lysophophatidylcholine (LPC) profile in plasma from treated whole
25 blood as these lipids are potentially important mediators in inflammation, and we did not observe any
26 changes in LPC profiles. Collectively, we deployed a semi-high throughput and robust methodology to
27 investigate anti-inflammatory properties of new chemical probes.

28 ***Highlights***
29 - Inhibitors for MEK decreased $PGE_2$ and $TXB_2$ production
30 - Inhibitors for MEK and ERK decreased IL-8 secretion
31 - JAK inhibitor tofacitinib increased $PGE_2$ and $TXB_2$ production

32 **Keywords:** prostaglandin $E_2$, whole blood assay, interleukin-8, inflammation, drug screen

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

# Introduction

Inflammation is a highly controlled immune response to eliminate the cause of tissue injury or infection and to initiate tissue repair back to homeostasis via resolution [1, 2]. However, inflammation is not always terminated. Unresolved inflammation causes persistent pain, tissue degeneration, and loss of function. In particular, inflammatory responses drive many autoimmune diseases [3] and inflammation is a hallmark of cancer [4]. Thus, there is a great need for new therapies that are anti-inflammatory and safe.

Prostaglandin $E_2$ ($PGE_2$) is a potent lipid mediator of inflammation and immune responses, and $PGE_2$ is a central mediator of pain, edema, and cartilage erosion typically observed in the joints of rheumatoid arthritis patients [5, 6]. In addition, $PGE_2$ is a promotor of the immunosuppressive tumor microenvironment with major impact on tumor progression [4, 7, 8]. During inflammation, $PGE_2$ is synthesized via conversion of arachidonic acid by cyclooxygenases (COX-1 and COX-2) into unstable $PGH_2$ that is further metabolized by the inducible terminal synthase microsomal prostaglandin E synthase-1 (mPGES-1) to generate $PGE_2$. Multiple non-steroidal anti-inflammatory drugs (NSAIDs) exist in clinical practice that unselectively decrease $PGE_2$ production via inhibition of COX, but these drugs are all associated with adverse effects. Hence, selective inhibition of $PGE_2$ production with small molecule inhibitors could therefore be a desirable therapeutic strategy in inflammation and cancer [9].

Interleukin-8 (IL-8) is a potent chemoattractant and activator of neutrophils. IL-8 signalling is implicated in multiple chronic inflammatory diseases [10] and cancer [11]. For example, a recent meta-analysis concluded that patients suffering from systemic lupus erythematosus (SLE) have increased levels of circulating IL-8 [12]. Patients with central neuropsychiatric SLE have increased concentration of IL-8 in cerebrospinal fluid compared to patients with non-central neuropsychiatric SLE [13]. IL-8 is also associated with renal damage and pulmonary fibrosis in SLE patients [14, 15]. Given that IL-8 is a stimulant for neutrophil activation, which plays a significant role in the pathogenesis of SLE [16], targeting IL-8 secretion or signalling could constitute a therapeutic strategy for SLE. A similar role of neutrophils and net formation has been reported in patients with dermatomyositis [17, 18]. In cancer, IL-8 is highly expressed in several types of cancer tissues [19] and serum concentration of IL-8 correlates with tumour burden [20]. The tumour-favouring actions of IL-8 include promotion of angiogenesis, increased survival of cancer stem cells, and attraction of myeloid cells that indorse the immunosuppressive tumour microenvironment [20].

In this study, we aimed to evaluate the effect of 57 chemical probes, high-quality tool compounds, and relevant control drugs on eicosanoid production and IL-8 secretion in human whole blood. A chemical probe is defined as "… a selective small-molecule modulator of a protein's function that allows the user to ask mechanistic and phenotypic questions about its molecular target in biochemical, cell-based or animal studies" [21], and these compounds follow the criteria of *in vitro* potency ($IC_{50}$ or Kd $<100$ nM),

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

high selectivity versus other protein subfamilies (>30-fold), and on-target cell activity at 1 μM. The chemical probes and other high-quality tool compounds included are mainly epigenetic modulators and kinase inhibitors that were produced in academic collaborations or donated by pharmaceutical companies within the Structural Genomic Consortium (SGC, www.thesgc.org), which aims to investigate novel targets for drug development in open science and in collaboration with the pharmaceutical industry. These inhibitors were tested here at one concentration (in triplicates, n=4-15 donors) based on previous knowledge of binding affinities and toxicity *in vitro*, as assessed using other validated assays in our laboratories (https://ultra-dd.org/tissue-platforms/cell-assay-datasets).

## Materials and methods

### Ethical approval and consent to participate

Ethical approval for this study was granted by local research ethics committee at Karolinska University hospital (Dnr 02-196) and the Regional Ethical Review Board in Stockholm (Dnr 2015/2001-31/2). Full informed consent according to the Declaration of Helsinki was obtained from all patients.

### Collection of blood

Peripheral venous blood was drawn from 10 females and 6 males, aged between 27 and 81 years. Healthy controls (n=4) and two patient groups were included: systemic lupus erythematosus (n=9) and dermatomyositis (n=3). The blood was collected in tubes containing sodium heparin (1000 U/mL).

### Inhibitors

The inhibitors (chemical probes and other high-quality tool compounds) tested here were obtained through the SGC (www.thesgc.org) and supplied by different distributers (**Supplementary Table 1**). Inhibitors and control drugs (**Supplementary Table 1**) were reconstituted at 10 mM in DMSO (D2250, Sigma-Aldrich), aliquoted in Eppendorf tubes or 96-well plates, and kept at -80°C. A fresh aliquot was used at each experiment. Diclofenac (dual COX-1/2 inhibitor) was used as positive control for inhibition of prostanoid production. LPS (L6529, Sigma-Aldrich) was reconstituted in PBS (D8537, Sigma-Aldrich) to a final concentration of 0.1 mg/mL and kept at +8°C.

### Whole blood assay

Inhibitors and vehicle control (DMSO) were diluted in PBS at room temperature with no direct light on. The treatments were prepared in 25 μL portions to U-shaped 96-well plate and 200 μL of freshly drawn heparin blood (<2 hrs at room temperature) was added to the plate. The plate was incubated at 37°C for 30 min and then 25 μL of 0.1 mg/mL LPS in PBS was added followed by pipetting up and down 3 times (final concentration of LPS was 10 μg/mL). The tested concentration for inhibitor was 0.1 or 1 μM (Supplementary Table 1). The plate was incubated for 24 hrs at 37°C and then centrifuged at 3000 g for 10 min at 4°C. Working on ice, 100 μL plasma was recovered to a new plate (for prostanoid profiling)

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

101 and from this 20 µL was transferred to a second plate (for IL-8 quantification). The plates were sealed
102 with aluminum foil and stored at -80°C.

103 **Extraction of lipids**

104 Plasma samples (80-240 µL) were thawed on ice and spiked with 50 µL deuterated internal standard
105 mix containing 17 ng 6-keto-$PGF_{1\alpha}$-d4, 8 ng $PGF_{2\alpha}$-d4, 12 ng $PGE_2$-d4, 8 ng $PGD_2$-d4, 8 ng $TXB_2$-d4,
106 and 8 ng 15-deoxy-Δ12,14$PGJ_2$-d4 (Cayman Chemical Company) prepared in 100% methanol. Protein
107 precipitation was performed by addition of 800 µL 100% methanol, followed by vortexing, and
108 centrifugation at 3000 g for 10 min at 4°C. The supernatants were collected in a new plate and evaporated
109 under vacuum for 4 hrs. The evaporated samples (100-200 µL) were diluted to 1 mL with 0.05% formic
110 acid in water and then loaded onto Oasis HLB 1cc 30mg plate (Waters Corporation, USA) that had been
111 pre-conditioned with 1 mL of 100% methanol and 1 mL of 0.05% formic acid in water. The plate was
112 washed with 10% methanol, 0.05% formic acid in water and lipids were eluted with 100% methanol.
113 The eluates were dried under vacuum over-night and stored at -20°C until reconstituted in 50 µL of 20%
114 acetonitrile in water prior to analysis with liquid chromatography tandem mass spectrometry (LC-
115 MS/MS).

116 **Lipid profiling by LC-MS/MS**

117 Lipids were quantified in negative mode with multiple reaction monitoring method, using a triple
118 quadrupole mass spectrometer (Acquity TQ detector, Waters) equipped with an Acquity H-class UPLC
119 (Waters). Eicosanoid were purchased from Cayman Chemicals and individually optimized for based on
120 precursor ion m/z, cone voltage, collision energy, and fragment ion m/z (**Supplementary Table 2**). An
121 eicosanoid mix containing all standards of interest was used to check interference in the LC-MS/MS
122 analysis. LPC(14:0) and LPC(18:0) were used to set optimal analytical parameters for quantification of
123 LPCs. Separation of lipids was performed on a 50 x 2.1 mm Acquity UPLC BEH C18 column 1.7 µm
124 (Waters) with a 12 min stepwise linear gradient (20-95%) at a flowrate of 0.6 mL/min with 0.05% formic
125 acid in acetonitrile as mobile phase B and 0.05% formic acid in water as mobile phase A. Data were
126 analyzed using MassLynx software, version 4.1, with internal standard calibration and quantification to
127 external standard curves for prostanoids. LPCs were normalized as area-% within each injection. Only
128 lipids with peaks intensities of signal-to-noise greater than 10 (S/N >10) were considered in our data
129 analysis.

130 **Quantification of IL-8**

131 IL-8 was quantified in plasma by Human IL-8 (CXCL8) ELISA development kit (3560-1H, Mabtech)
132 according to manufacturer's instructions.

133 **Statistical analyses**

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

134 Data are presented as mean±SEM if not stated otherwise. Statistical analyses were performed using
135 GraphPad Prism 6 (GraphPad Sofware). One sample t-test was used to test significant difference.
136 Statistical significance level was set to $p<0.05$.

137
# Results

138
### Development of whole blood assay

139 The whole blood assay was developed to screen for changes in multiple eicosanoids. Each eicosanoid
140 and corresponding deuterated variant were individually optimized in the LC-MS/MS analysis. A dilution
141 curve containing 6-keto $PGF_{1\alpha}$-d4, $PGE_2$-d4, $PGD_2$-d4, $PGF_{2\alpha}$-d4, $TXB_2$-d4, 15d-$PGJ_2$-d4, $LTB_4$-d4,
142 $LTC_4$-d5, $LTD_4$-d5, 5-HETE-d8, 12-HETE-d8, 15-HETE-d8, and undeuterated variants of 13-HODE,
143 RvD1, RvD2, 17-hydroxy DHA, and protectin DX was spiked into 100 µL plasma at different stages
144 throughout the extraction. A dilution curve was spiked in water at the same step. The dilution curve
145 ranged from 0.006-1.5 pmol as final amount injected on the column in the LC-MS/MS analysis. This
146 enabled us to investigate the lower limit of quantification (LLOQ), recovery efficacy, and matrix effect
147 for each eicosanoid. The LLOQ injected on column was considered as great (0.02-0.05 pmol), good
148 (0.1-0.2 pmol), or poor (0.4-1.5 pmol). Eicosanoids with great LLOQ were $PGE_2$, $PGF_{2\alpha}$, $TXB_2$, RvD1,
149 RvD2, $LTB_4$, protectin DX, and 13-HODE; good LLOQ were 6-keto $PGF_{1\alpha}$, $PGD_2$, 5-HETE, 15-HETE,
150 and $LTD_4$; poor LLOQ were 15d-$PGJ_2$, 12-HETE, 17-hydroxy DHA, and $LTC_4$. The extraction recovery
151 rates were 33-125%. The response in plasma compared to 20% acetonitrile were 52-116% due to matrix
152 effects. The estimated LLOQ in 100 µL plasma was approximately 1 ng/mL for the best performing
153 eicosanoids including $PGE_2$, $TXB_2$, $PGF_{2\alpha}$, RvD1, RvD2, and protectin DX. We can conclude that the
154 method provided similar quantitative performance in plasma for many eicosanoids.

155 LPS at 0.1-10 µg/mL increased $PGE_2$ and $TXB_2$ production in human whole blood, which are the two
156 dominant eicosanoids produced under these conditions [22]. All other eicosanoids were below the
157 LLOQ. We chose 10 µg/mL of LPS as our final concentration, yielding a robust amount of $PGE_2$ (49±4
158 ng/mL, n=5 donors) and $TXB_2$ (24±9 ng/mL, n=5 donors). The prostanoid production was completely
159 blocked using the dual COX-1/2 inhibitor diclofenac (10 µM). High concentration of DMSO (0.1%)
160 slightly decreased $PGE_2$ production by 20% (n=2 donors) while DMSO at 0.01% or 0.001% had no
161 effect. The intra-assay coefficient of variation (CV, n=20 technical replicates) was 12% and 11% for
162 $PGE_2$ and $TXB_2$, respectively. The inter-assay CV for control material (n=3 donors) was 20% for $PGE_2$
163 and 30% for $TXB_2$. This was performed on blood that was drawn, incubated, extracted, and analyzed at
164 separate occasions. The suppression in signal due to matrix effects and/or recovery efficiency varied
165 between donors and experiments, ranging from 10-70% suppression compared to signal in extracted
166 blank (mean ± SD, n=6 donors, $PGE_2$: 45±25%, $TXB_2$: 40±20%). In summary, 24 hrs incubation of
167 whole blood with 10 µg/mL LPS resulted in profound induction of the COX-1/2 products $PGE_2$ and
168 $TXB_2$ that was efficiently blocked by diclofenac at 10 µM.

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

169 **Effect on $PGE_2$ and $TXB_2$ production**

170 Our screening of inhibitors suggested that selected kinase inhibitors affected prostanoid production
171 (**Figure 1**). The strongest reduction in $PGE_2$ production was observed by MEK-1 inhibitor PD0325901
172 (31±6%, $p=0.001$, $n=4$) and MEK-1/2 inhibitor trametinib (34±7%, $p<0.0001$, $n=15$). Moderate
173 suppression in $PGE_2$ concentration was found for MEK-1/2 inhibitor selumetinib (65±9%, $p=0.02$, $n=5$),
174 ERK-1/2 inhibitor SCH772984 (76±11%, $p=0.04$, $n=13$) and p38 inhibitor skepinone-L (76±8%,
175 $p=0.01$, $n=13$). However, the tested p38 inhibitor pamapimod did not affect $PGE_2$ production. Two of
176 these compounds decreased $TXB_2$ production, namely trametinib (63±6%, $p=0.02$, $n=15$) and
177 selumetinib (74±7%, $p=0.02$, $n=5$). Diclofenac, here used as a positive control for inhibition of
178 prostanoid production, blocked the prostanoid production while selective COX-2 inhibitor NS-398
179 inhibited only $PGE_2$ production, in agreement with previously reported data for these compounds in
180 whole blood assay [23]. The JAK inhibitor tofacitinib increased both $PGE_2$ (286±51%, $p=0.01$, $n=6$)
181 and $TXB_2$ (169±20%, $p=0.02$, $n=6$) production. The IRAK-1/4 inhibitor I slightly increased the
182 concentrations of $PGE_2$ (139±15%, $p=0.04$, $n=7$) and $TXB_2$ (133±8%, $p=0.008$, $n=7$).

183 We chose to investigate the strongest observed effects in more detail by performing concentration-
184 response experiments for PD0325901, trametinib, selumetinib, and tofacitinib. All three MEK inhibitors
185 showed a concentration-dependent response on both $PGE_2$ and $TXB_2$ production while tofacitinib
186 showed a concentration-dependent response on $PGE_2$ production (**Figure 2**).

187 **Effect on IL-8 secretion**

188 In line with the effect on prostanoid production, reduction in IL-8 secretion was found for PD0325901
189 (24±9%, $p=0.03$, $n=3$), trametinib (27±5%, $p<0.0001$, $n=13$), and selumetinib (45±10%, $p=0.03$, $n=3$)
190 (**Figure 3**). Moderate reduction in IL-8 secretion was found for SCH772984 (62±9%, $p=0.002$, $n=12$)
191 and diclofenac (66±8%, $p=0.003$, $n=11$). We could also observe that tofacitinib increased IL-8 secretion
192 (225±57%, $p=0.16$, $n=3$), however not with statistical significance.

193 **Effect on LPC profile**

194 We measured LPC species within our targeted LC-MS/MS analysis. LPCs are mainly generated by
195 metabolism of membrane phosphatidylcholine by cytosolic phospholipase $A_2$ [24]. These lipids have
196 been reported to be involved in several cellular processes; sometimes with opposing effect depending
197 on degree of saturation, concentration, and biological context [25, 26]. We observed no difference in
198 total LPC or LPC profile when whole blood was treated with LPS neither did any of the tested inhibitors
199 alter the LPC profile (**Figure 4**).

## 200 Discussion

201 We have tested the inhibitory effect on prostanoid production and IL-8 secretion in human whole blood
202 for 57 high-quality inhibitors with known target specificities and *in vitro* potencies. None of the tested

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

203 epigenetic modulators, which are acting on demethylases, bromodomains, or methyltransferases,
204 affected $PGE_2$ or IL-8 concentration. Inhibition of MEK-1/2 or ERK decreased $PGE_2$ production and
205 IL-8 secretion in this assay. This effect was observed for allosteric inhibitor trametinib (MEK-1/2), non
206 ATP-competitive inhibitors PD0325901 (MEK-1) and selumetinib (MEK-1/2), and ATP-competitive
207 inhibitor SCH772984 (ERK-1/2). These kinase targets are part of the RAS/RAF/MEK/ERK signaling
208 transduction pathway, where inhibition of MEK prevents the downstream phosphorylation and
209 activation of ERK that ultimately regulates cellular responses such as survival, lipid metabolism, and
210 protein translation [27]. For example, MEK-1/2 inhibitor PD184352 decreased $PGE_2$ production in
211 melanoma cell line by decreased COX-2 expression due to inhibition of phosphorylation on ERK [28]
212 and trametinib reduced IL-8 production in melanoma cell line [29]. We found that our positive control
213 diclofenac for blocking prostanoid production decreased IL-8 secretion, which is explained by the fact
214 that $PGE_2$ stimulates IL-8 production in cultured cells [30-33]. While our study mainly focused on
215 identifying inhibitory effects, we observed that JAK inhibitor tofacitinib increased both $PGE_2$
216 production and IL-8 secretion. Tofacitinib is used to treat rheumatoid arthritis and it is known that
217 tofacitinib can increase the expression of pro-inflammatory mediators, including $PGE_2$, in macrophages
218 by acting inhibitory on the expression of anti-inflammatory IL-10 [34]. The increased formation of pro-
219 inflammatory $PGE_2$ and platelet activating thromboxane $A_2$ (as measured by stable metabolite $TXB_2$) in
220 human whole blood may be associated with the recently recognized increased risk of thromboembolism
221 associated with JAK inhibitors in treatment of rheumatoid arthritis [35]. We acknowledge that the
222 limitation of our study is the usage of one concentration per tested inhibitor. However, the used
223 concentrations were based on reported $IC_{50}$ and/or $EC_{50}$ values as well as solid experiences in our
224 laboratories using other validated assay systems (https://ultra-dd.org/index.php/tissue-platforms/cell-
225 assay-datasets). We also demonstrated in concentration-response experiments that greater inhibitory
226 effect could be achieved by increasing the concentration for the MEK inhibitors. However, this increases
227 the risk of off-target effects and/or introduction of cellular toxicity that needs to be taken into account
228 in experimental design and interpretation of results. In conclusion, we identified inhibitors for MEK or
229 ERK as anti-inflammatory hits in our human whole blood assay. Based on the suppression in $PGE_2$
230 production and IL-8 secretion, further investigation of the MEK/ERK signaling pathway may inform
231 future therapeutic strategies to treat inflammatory diseases such as SLE and dermatomyositis.

232 **Acknowledgements**

233 This work was supported by grants from Innovative Medicines Initiative (EU/EFPIA, ULTRA-DD,
234 grant no: 115766), the Swedish Research Council (grant no: 2017-02577), Stockholm County Council
235 (ALF, grant no: 20160378), The Swedish Rheumatism Association (grant no: R-755861), King Gustaf
236 V's 80 Years Foundation (grant no: n/a), and funds from Karolinska Institutet (grant no: n/a).

237 **Conflicts of interests**

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

The SGC receives funds from AbbVie, Bayer Pharma, Boehringer Ingelheim, the Canada Foundation for Innovation, the Eshelman Institute for Innovation, Genome Canada, Janssen, Merck (Darmstadt, Germany), MSD, Novartis Pharma, the Ontario Ministry of Economic Development and Innovation, Pfizer, the São Paulo Research Foundation, Takeda and the Wellcome Trust (authors: F.B., Y.S., M.M., M.S., P-J.J., and L.B.). These funders had no direct role in study conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript. P-J.J. is engaged in Gesynta Pharma AB, a company that develops anti-inflammatory drugs. I.G. and I.L. have no conflicts of interests to declare.

**Author contributions**

F.B., Y.S., M.S., P-J.J., and L.B. contributed to study conception and design. F.B., Y.S., and M.M. performed experiments. F.B. analysed data, performed statistical analysis, and drafted the manuscript. I.G. and I.L. facilitated administrative, technical, or material support. All authors critically revised and approved the final version of the manuscript.

# References

[1] C. Nathan, Points of control in inflammation, Nature, 420 (2002) 846-852.
[2] C.D. Buckley, D.W. Gilroy, C.N. Serhan, B. Stockinger, P.P. Tak, The resolution of inflammation, Nat Rev Immunol, 13 (2013) 59-66.
[3] I.B. McInnes, G. Schett, The pathogenesis of rheumatoid arthritis, The New England journal of medicine, 365 (2011) 2205-2219.
[4] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell, 144 (2011) 646-674.
[5] J. Akaogi, T. Nozaki, M. Satoh, H. Yamada, Role of PGE2 and EP receptors in the pathogenesis of rheumatoid arthritis and as a novel therapeutic strategy, Endocr Metab Immune Disord Drug Targets, 6 (2006) 383-394.
[6] M.J. Fattahi, A. Mirshafiey, Prostaglandins and rheumatoid arthritis, Arthritis, 2012 (2012) 239310.
[7] D. Wang, R.N. Dubois, Eicosanoids and cancer, Nature reviews. Cancer, 10 (2010) 181-193.
[8] E. Ricciotti, G.A. FitzGerald, Prostaglandins and inflammation, Arteriosclerosis, thrombosis, and vascular biology, 31 (2011) 986-1000.
[9] F. Bergqvist, R. Morgenstern, P.J. Jakobsson, A review on mPGES-1 inhibitors: From preclinical studies to clinical applications, Prostaglandins & other lipid mediators, (2019) 106383.
[10] R.C. Russo, C.C. Garcia, M.M. Teixeira, F.A. Amaral, The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases, Expert Rev Clin Immunol, 10 (2014) 593-619.
[11] D.J. Waugh, C. Wilson, The interleukin-8 pathway in cancer, Clinical cancer research : an official journal of the American Association for Cancer Research, 14 (2008) 6735-6741.
[12] Y.M. Mao, C.N. Zhao, L.N. Liu, Q. Wu, Y.L. Dan, D.G. Wang, H.F. Pan, Increased circulating interleukin-8 levels in systemic lupus erythematosus patients: a meta-analysis, Biomark Med, 12 (2018) 1291-1302.
[13] T. Yoshio, H. Okamoto, K. Kurasawa, Y. Dei, S. Hirohata, S. Minota, IL-6, IL-8, IP-10, MCP-1 and G-CSF are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus, Lupus, 25 (2016) 997-1003.
[14] L.C. Lit, C.K. Wong, L.S. Tam, E.K. Li, C.W. Lam, Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus, Ann Rheum Dis, 65 (2006) 209-215.
[15] A. Nielepkowicz-Gozdzinska, W. Fendler, E. Robak, L. Kulczycka-Siennicka, P. Gorski, T. Pietras, E. Brzezianska, A. Antczak, Exhaled IL-8 in systemic lupus erythematosus with and without pulmonary fibrosis, Arch Immunol Ther Exp (Warsz), 62 (2014) 231-238.

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

284 [16] M.J. Kaplan, Neutrophils in the pathogenesis and manifestations of SLE, Nature reviews.
285 Rheumatology, 7 (2011) 691-699.
286 [17] S. Zhang, X. Shu, X. Tian, F. Chen, X. Lu, G. Wang, Enhanced formation and impaired
287 degradation of neutrophil extracellular traps in dermatomyositis and polymyositis: a potential
288 contributor to interstitial lung disease complications, Clinical and experimental immunology, 177
289 (2014) 134-141.
290 [18] Y. Peng, S. Zhang, Y. Zhao, Y. Liu, B. Yan, Neutrophil extracellular traps may contribute to
291 interstitial lung disease associated with anti-MDA5 autoantibody positive dermatomyositis, Clin
292 Rheumatol, 37 (2018) 107-115.
293 [19] J.M. David, C. Dominguez, D.H. Hamilton, C. Palena, The IL-8/IL-8R Axis: A Double Agent in
294 Tumor Immune Resistance, Vaccines (Basel), 4 (2016).
295 [20] C. Alfaro, M.F. Sanmamed, M.E. Rodriguez-Ruiz, A. Teijeira, C. Onate, A. Gonzalez, M. Ponz,
296 K.A. Schalper, J.L. Perez-Gracia, I. Melero, Interleukin-8 in cancer pathogenesis, treatment and
297 follow-up, Cancer treatment reviews, 60 (2017) 24-31.
298 [21] C.H. Arrowsmith, J.E. Audia, C. Austin, J. Baell, J. Bennett, J. Blagg, C. Bountra, P.E. Brennan,
299 P.J. Brown, M.E. Bunnage, C. Buser-Doepner, R.M. Campbell, A.J. Carter, P. Cohen, R.A. Copeland,
300 B. Cravatt, J.L. Dahlin, D. Dhanak, A.M. Edwards, M. Frederiksen, S.V. Frye, N. Gray, C.E.
301 Grimshaw, D. Hepworth, T. Howe, K.V. Huber, J. Jin, S. Knapp, J.D. Kotz, R.G. Kruger, D. Lowe,
302 M.M. Mader, B. Marsden, A. Mueller-Fahrnow, S. Muller, R.C. O'Hagan, J.P. Overington, D.R.
303 Owen, S.H. Rosenberg, B. Roth, R. Ross, M. Schapira, S.L. Schreiber, B. Shoichet, M. Sundstrom, G.
304 Superti-Furga, J. Taunton, L. Toledo-Sherman, C. Walpole, M.A. Walters, T.M. Willson, P.
305 Workman, R.N. Young, W.J. Zuercher, The promise and peril of chemical probes, Nat Chem Biol, 11
306 (2015) 536-541.
307 [22] L.L. Mazaleuskaya, J.A. Lawson, X. Li, G. Grant, C. Mesaros, T. Grosser, I.A. Blair, E. Ricciotti,
308 G.A. FitzGerald, A broad-spectrum lipidomics screen of antiinflammatory drug combinations in
309 human blood, JCI Insight, 1 (2016).
310 [23] K. Larsson, J. Steinmetz, F. Bergqvist, S. Arefin, L. Spahiu, J. Wannberg, S.C. Pawelzik, R.
311 Morgenstern, P. Stenberg, K. Kublickiene, M. Korotkova, P.J. Jakobsson, Biological characterization
312 of new mPGES-1 inhibitors in preclinical models of inflammation and vascular tone, British journal of
313 pharmacology, (2019).
314 [24] J.E. Burke, E.A. Dennis, Phospholipase A2 structure/function, mechanism, and signaling, Journal
315 of lipid research, 50 Suppl (2009) S237-242.
316 [25] A. Drzazga, A. Sowinska, M. Koziolkiewicz, Lysophosphatidylcholine and
317 lysophosphatidylinosiol--novel promissing signaling molecules and their possible therapeutic activity,
318 Acta poloniae pharmaceutica, 71 (2014) 887-899.
319 [26] I. Sevastou, E. Kaffe, M.A. Mouratis, V. Aidinis, Lysoglycerophospholipids in chronic
320 inflammatory disorders: the PLA(2)/LPC and ATX/LPA axes, Biochimica et biophysica acta, 1831
321 (2013) 42-60.
322 [27] J.A. McCubrey, L.S. Steelman, W.H. Chappell, S.L. Abrams, E.W. Wong, F. Chang, B.
323 Lehmann, D.M. Terrian, M. Milella, A. Tafuri, F. Stivala, M. Libra, J. Basecke, C. Evangelisti, A.M.
324 Martelli, R.A. Franklin, Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation
325 and drug resistance, Biochimica et biophysica acta, 1773 (2007) 1263-1284.
326 [28] S. Zelenay, A.G. van der Veen, J.P. Bottcher, K.J. Snelgrove, N. Rogers, S.E. Acton, P.
327 Chakravarty, M.R. Girotti, R. Marais, S.A. Quezada, E. Sahai, C. Reis e Sousa, Cyclooxygenase-
328 Dependent Tumor Growth through Evasion of Immunity, Cell, 162 (2015) 1257-1270.
329 [29] M.L. Hartman, M. Rozanski, M. Osrodek, I. Zalesna, M. Czyz, Vemurafenib and trametinib
330 reduce expression of CTGF and IL-8 in (V600E)BRAF melanoma cells, Laboratory investigation; a
331 journal of technical methods and pathology, 97 (2017) 217-227.
332 [30] I. Venza, M. Visalli, C. Fortunato, M. Ruggeri, S. Ratone, M. Caffo, G. Caruso, C. Alafaci, F.
333 Tomasello, D. Teti, M. Venza, PGE2 induces interleukin-8 derepression in human astrocytoma
334 through coordinated DNA demethylation and histone hyperacetylation, Epigenetics, 7 (2012) 1315-
335 1330.
336 [31] S. Caristi, G. Piraino, M. Cucinotta, A. Valenti, S. Loddo, D. Teti, Prostaglandin E2 induces
337 interleukin-8 gene transcription by activating C/EBP homologous protein in human T lymphocytes,
338 The Journal of biological chemistry, 280 (2005) 14433-14442.

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

339 [32] H. Aso, S. Ito, A. Mori, M. Morioka, N. Suganuma, M. Kondo, K. Imaizumi, Y. Hasegawa,
340 Prostaglandin E2 enhances interleukin-8 production via EP4 receptor in human pulmonary
341 microvascular endothelial cells, American journal of physiology. Lung cellular and molecular
342 physiology, 302 (2012) L266-273.
343 [33] A. Agro, C. Langdon, F. Smith, C.D. Richards, Prostaglandin E2 enhances interleukin 8 (IL-8)
344 and IL-6 but inhibits GMCSF production by IL-1 stimulated human synovial fibroblasts in vitro, The
345 Journal of rheumatology, 23 (1996) 862-868.
346 [34] P. Kothari, R. Pestana, R. Mesraoua, R. Elchaki, K.M. Khan, A.J. Dannenberg, D.J. Falcone, IL-
347 6-mediated induction of matrix metalloproteinase-9 is modulated by JAK-dependent IL-10 expression
348 in macrophages, Journal of immunology, 192 (2014) 349-357.
349 [35] I.C. Scott, S.L. Hider, D.L. Scott, Thromboembolism with Janus Kinase (JAK) Inhibitors for
350 Rheumatoid Arthritis: How Real is the Risk?, Drug Saf, 41 (2018) 645-653.

351 **Figure Legends**

352 **Figure 1.** Volcano plots showing effects on $PGE_2$ (A) and $TXB_2$ (B) production in LPS-induced human
353 whole blood. The top altered conditions compared to vehicle control based on fold-change (<0.5 or >2)
354 and p-value (<0.05) are highlighted. Each inhibitor was tested in 4-15 donors. Statistical significance
355 was tested using one sample t-test (p<0.05).

356 **Figure 2.** Validation of inhibitory effect on $PGE_2$ production by MEK inhibitors in human whole blood.
357 Diclofenac at 10 µM was used as positive control. Data are presented as mean±SD of biological
358 replicates (n=2-6 per condition) from one representative experiment. The absolute $PGE_2$ production in
359 LPS control was 53.3±8.3 ng/mL. The concentration-response was tested in two donors.

360 **Figure 3.** Volcano plot showing effects on IL-8 secretion in LPS-induced human whole blood. The top
361 altered conditions compared to vehicle control based on fold-change (<0.5 or >2) and p-value (<0.05)
362 are highlighted. Each inhibitor was tested in 3-13 donors. Statistical significance was tested using one
363 sample t-test (p<0.05).

364 **Figure 4.** Effect on LPC profile in whole blood. There was no difference in total LPC (A) or LPC profile
365 (B) with LPS treatment, and none of the tested compounds affected the LPC profile (C). Each inhibitor
366 was tested in 4-15 donors.

367 **Supplementary material**

368 **Supplementary Table S1.**

369 **Supplementary Table S2.**

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

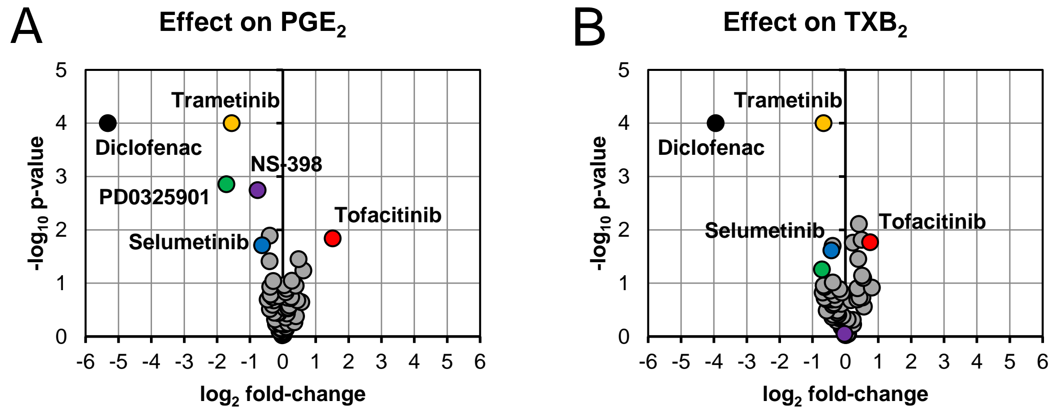


**Figure 1.**

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

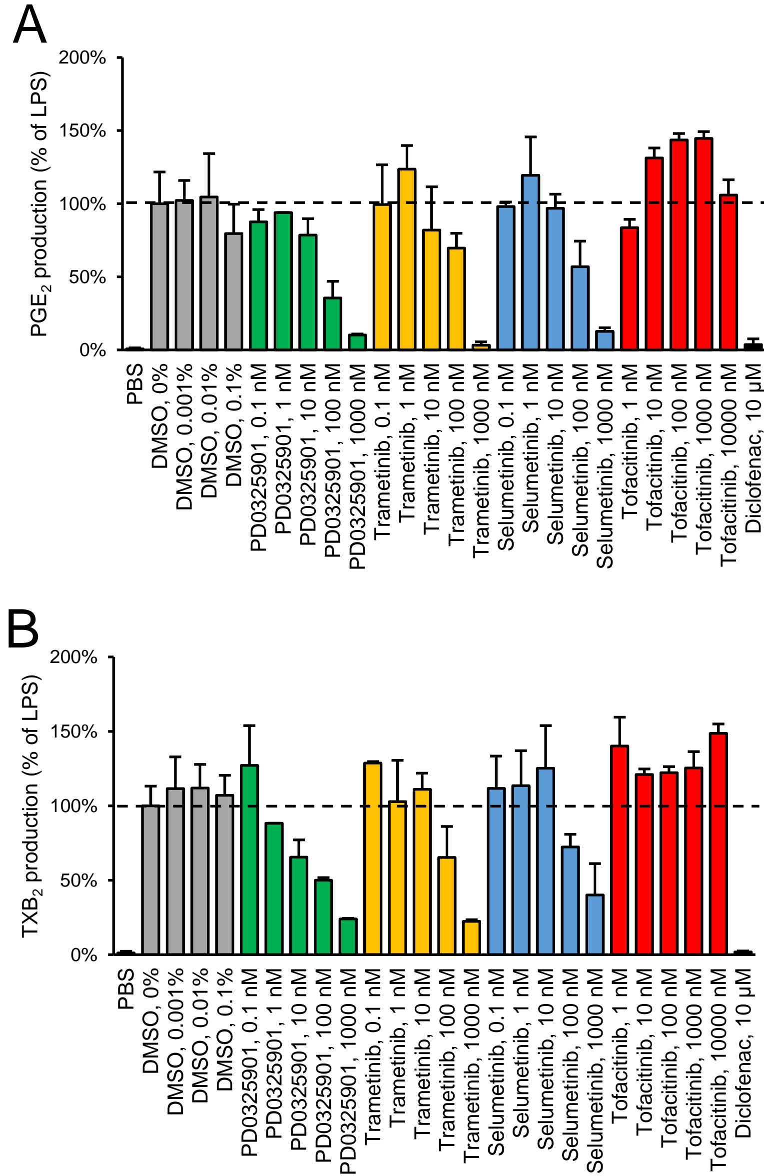


**Figure 2.**

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

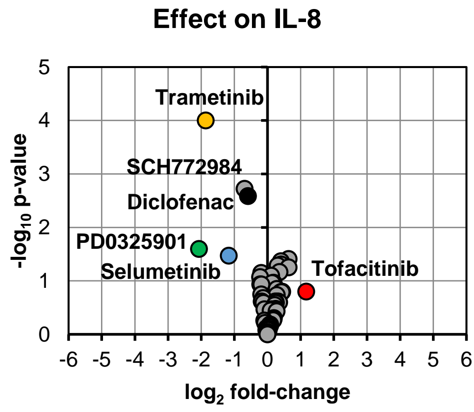


**Figure 3.**

bioRxiv preprint doi: https://doi.org/10.1101/2019.12.30.890715; this version posted December 30, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

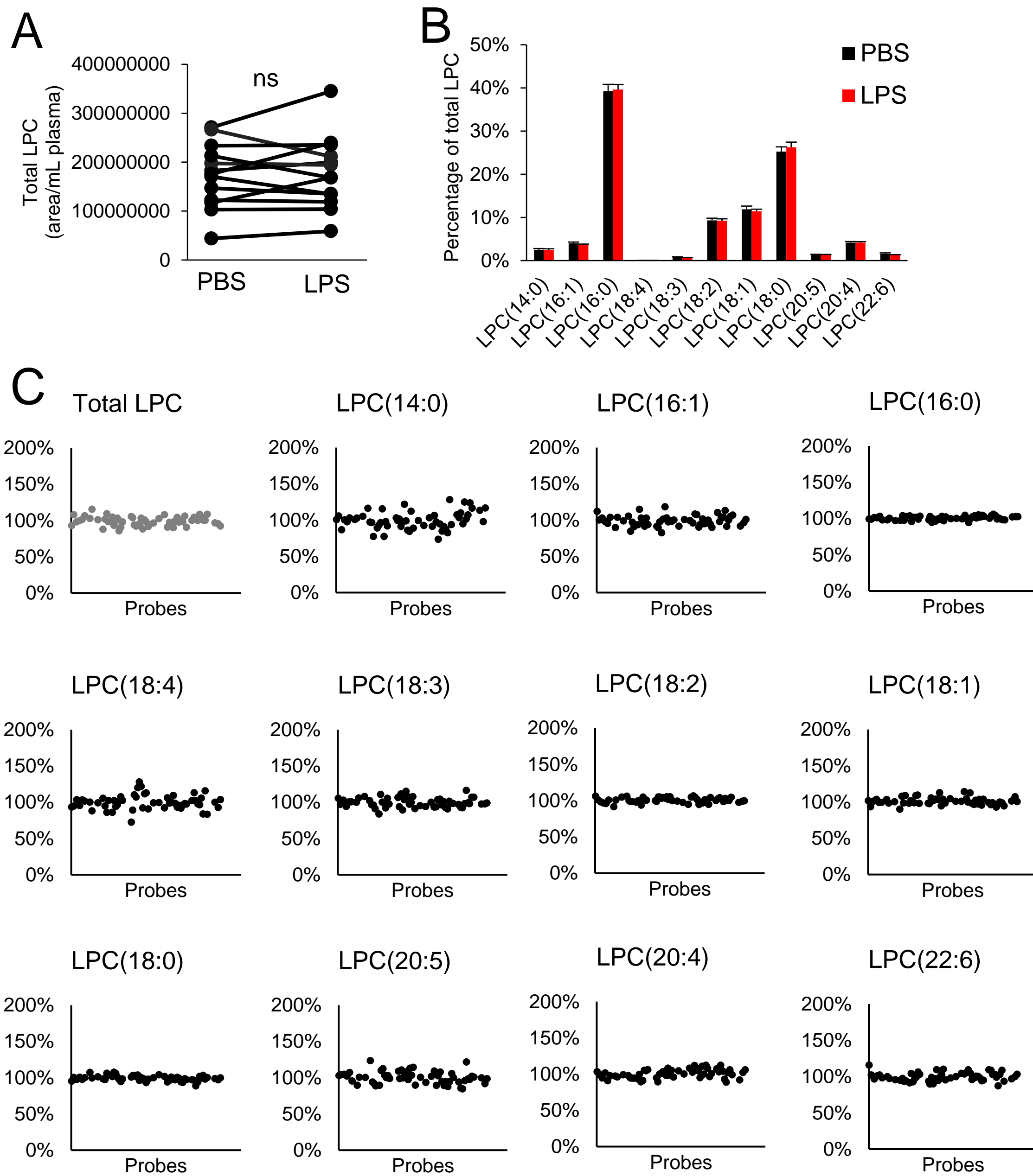


**Figure 4.**