Supplementary Information

1. Supplementary Materials and Methods

1.1. Materials and Reagents

Methods have been previously described [1,17,18,20]. Recombinant human IFN-γ, TNF-α, and IL-1β were from R&D Systems (Minneapolis, MN, USA). Compounds and sources are listed in Supplemental Table S2. Primary antibodies are as follows: CD87/uPAR, Clone VIM5 (BD/Pharm); CD62E/E-selectin, Clone ENA1 (HyCult Biotechnology BV); HLA-DR, Clone G46-6 (L243) (BD/Pharm); CD54/ICAM, Clone 84H10 (Millipore); CXCL8/IL-8, Clone 6217 (R&D Systems); CCL2/MCP-1, Clone 5D3-F7 (BD/Pharm); CXCL9/MIG, Clone B8-11 (BD/Pharm); CD142/TF, Clone TF9-10-H10 (Cal Biochem); CD141/TM, Clone 1A4 (BD/Pharm); VCAM-1, Clone 51-10C9 (BD/Pharm).

1.2. Cell Culture

Primary human umbilical vein endothelial cells (HUVEC) were obtained from Cell Applications, Inc., San Diego, CA, USA, cultured in EGM-2 medium (Lonza Corp., Walkersville, MD, USA) containing supplements provided by the manufacturer and 2% heat-inactivated fetal bovine serum (Hyclone, Logan, UT, USA), and subcultured with 0.05% trypsin with 0.53 mM EDTA (Mediatech, Herndon, VA, USA) as described by the manufacturer. Cells from pooled donors (4–5) were utilized at passage ≤4. All primary human cells utilized in this work were obtained under protocols that were reviewed by Institutional Review Board(s) (IRB) that operate in accordance with the requirement of EPA Regulation 40 CFR 26 and HHS Regulation 45 CFR 46 of the US Federal Government for the protection of human research subjects.

Experiments were performed by culturing HUVEC to confluence in microwell plates (Costar, Corning, Tewksbury, MA, USA), adding compounds at the indicated concentrations followed by a cocktail of cytokines (IL-1β, 1 ng/mL; TNF-α, 5 ng/mL; and IFN-γ, 100 ng/mL) for 24 h (BioMAP 3C system). Compounds were prepared in DMSO from 1000× stock solutions, or in PBS from stock solutions, added 1 h before stimulation of the cells, and were present during the whole 24 h stimulation period. Final DMSO concentration was ≤0.2%. Colchicine, 1.1 μM, and wells without stimulation, as well as 6 or more vehicle control wells were included as controls on each plate. For proliferation assays, compounds are added to individual cell types at subconfluence and SRB is measured at 48 h.

1.3. Cell-Based Enzyme-Linked Immuno Assay ELISA

Cell-based ELISAs were carried out as follows. Following cell culture, supernatants were removed from plates and plates were dried overnight, then blocked, and incubated with primary antibodies or isotype control antibodies (0.01–0.5 μg/mL) for 1 h. Specific antibodies used for ELISA are listed in Materials. After washing, plates were incubated with a peroxidase-conjugated anti-mouse IgG secondary antibody or a biotin-conjugated anti-mouse IgG antibody for 1 h followed by streptavidin-horseradish peroxidase for 30 min. Plates were washed and developed with 3,3',5,5'-Tetramethylbenzidine, TMB substrate (Clinical Science Products, Mansfield, MA, USA) and the absorbance (OD) was read at 450 nm (subtracting the background absorbance at 650 nm) with a Molecular Devices SpectraMAX 190 plate reader (Molecular Devices, Sunnyvale, CA, USA). Overtly adverse effects of compounds on

cells were determined by measuring alterations in total protein using SRB staining. The SRB assay was performed by staining cells with 0.1% sulforhodamine B after fixation with 10% trichloroacetic acid, and reading wells at 560 nm. Overtly cytotoxic concentrations were identified as those where the $\log_{10}$ ratio of SRB levels were <−0.3 (representing a ~50% reduction in total protein in the well).

1.4. Experimental Formats, Assay Acceptance Criteria and Quality Control

Compounds were tested in large batches over a period of years in standardized formats. Multiple compounds were tested on each microwell plate at 4 or more concentrations in singlicate. Colchicine, 1.1 μM, and wells without stimulation were included as positive controls on each plate. The negative control, DMSO or in some cases PBS, was tested in 6–8 wells on each plate.

The BioMAP platform generates multi-parameter data sets for each compound tested. Assays are plate-based and performance is assessed by positive and negative controls for each assay system. For the BioMAP 3C system, positive controls include the non-stimulated condition, without addition of cytokines (non-stim), and a positive control test agent (colchicine). Multiple endpoints are evaluated in each experiment (at least 7 of the biomarker endpoints listed in Materials and Reagents above). Data acceptance criteria are based on plate performance (%CV of negative controls), and the performance of positive controls across assays with a comparison to historical controls. The performance of each BioMAP system in a given experiment is evaluated using the Pearson statistic for the positive control, calculated individually for each assay compared to the positive control reference dataset. This provides a multi-parameter signature for assay acceptance that ensures that the cell culture has been stimulated appropriately. This test, the QA/QC Pearson Test, is performed by first establishing the 1% false negative Pearson cutoff from the positive reference dataset. The process is iteration through each profile in the positive control reference dataset, calculating Pearson value between this profile and the mean of the rest of the profiles in the dataset, so the number of Pearson values calculated is the number of profiles in the reference dataset. The Pearson at the one percentile of all Pearson values calculated is the 1% false negative Pearson cutoff. If the Pearson between a new positive control profile and the mean of positive control reference profiles exceeds this 1% false negative Pearson cutoff, then these plates pass the test. Assays are accepted when the positive control passes the Pearson test and 95% of plates have %CV < 20%. All data shown are from experiments that pass these assay acceptance criteria.

Across experiments, OD (450–650) values for TF were 0.41 ± 0.14 (SD) for the stimulated condition and 0.22 ± 0.07 for the non-stimulated condition (control antibody OD values were 0.082 ± 0.027). The $\log_{10}$ ratio for non-stim condition was −0.30 ± 0.01.

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1.5. Statistical Analysis

Measurement values for each biomarker endpoint in a treated sample are divided by the mean value from at least six vehicle control samples (from the same plate) to generate a ratio. All ratios were then $\log_{10}$

transformed. Significance prediction envelopes were calculated for historical controls (95% or 99%). All ratios are then $\log_{10}$ transformed. Significance prediction envelopes are calculated from historical negative control samples tested (e.g., 95%). Given the control distribution for each system-readout combination, the significance of an individual readout ratio can be computed from the empirical distribution by taking the 95th percentile, or 99th, or 99.9th, e.g., as compared to the control ratios. The 95% significance envelope of controls is ±0.058 for TF. Compounds tested in screening mode were defined as increasing TF in the BioMAP 3C system if they had $\log_{10}$ ratio values for TF levels of ≥0.12 (a 30% effect size and outside the 99% significance envelope for historical controls) at two or more concentrations.

1.6. Data Sources

Data used in the analyses include previously published as well as new data [1,20]. Table 1 indicates the source of data for each test agent.