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**METHODS**

**Cytokine Production, pDC Activation, and Type I IFN Gene Signature**

We obtained PBMCs from healthy donors via the MedImmune clinic internal blood donor program or leukopaks (Hemacare, Los Angeles, CA, USA) in accordance with MedImmune IRB guidelines. PBMCs were isolated using CPT™ heparin tubes (BD Biosciences, San Jose, CA, USA). Negative selection (STEMCELL Technologies, Vancouver, BC, Canada) was used to purify pDCs from PBMCs. The purity of the enriched cells (>95% pure) was confirmed by flow cytometry.

**ADCC and CDC Assays**

Antibody-dependent cell-mediated cytotoxicity (ADCC) assays were conducted with freshly isolated PBMCs. Cells were incubated with serially diluted antibodies and 150 ng/mL of recombinant human IL-2 (R&D Systems) for 20 hours at 37°C and 5% CO2. ADCC was assessed by quantifying DAPI+ CD22+ B cells using flow cytometry. For complement-dependent cytotoxicity (CDC) assays, serially diluted antibodies were prepared in RPMI medium (Invitrogen) or non-heat inactivated human serum (Sigma-Aldrich, St. Louis, MO, USA). Daudi target cells were prepared in non-heat inactivated media, and 35,000 cells were transferred to appropriate wells. Plated cells were then incubated with serially diluted antibodies for 4–5 hours at 37°C and 5% CO2. Heat-inactivated serum in parallel assays was used as a negative control for CDC and to inactivate compliment in the assays. After incubation, cell viability was assessed with CellTiterGlo® (Promega) and luminescence assessed on an Envision plate reader (Perkin Elmer, Waltham, MA, USA). Percentage CDC with anifrolumab or control antibody was determined in comparison to a maximum control, and we completed nonlinear regression analysis using Prism 6 software.

**Figure S1. The Anti-H3K1 Antibody Binds to IFNAR1 and Does Not Compete with Anifrolumab**



Binding of A488-labeled anifrolumab and A594-labeled anti-H3K1 antibody to IFNAR1, and APC-conjugated anti-CD11b antibody to the membrane marker, CD11b, in monocytes imaged by confocal microscopy. (A) The anti-H3K1 antibody binds to cell surface IFNAR1 at 37°C. (B) The anti-H3K1 antibody does not compete with anifrolumab for IFNAR1 binding at 4°C. (C) Anifrolumab elicits IFNAR1 internalization at 37°C, markedly diminishing cell surface IFNAR1 availability for subsequent binding by the anti-H3K1 antibody.

APC, allophycocyanin; CD11b, cluster of differentiation molecule 11b; IFNAR1, type I interferon receptor subunit 1.

**Figure S2.** **Recombinant IFN-α2 and CpG-A–Stimulated and DNA-IC–Stimulated pDC Media Induce HEK293-ISRE Reporter Activity**



HEK293-ISRE reporter cell luciferase activity stimulated for 18 hours with (A) 100, 1,000, 10,000, or 100,000 U/mL of recombinant IFN-α2, and 1:10, 1:100, or 1:1,000 dilutions of (B) CpG-A–stimulated pDC media or (C) DNA-IC–stimulated pDC media. Data are from one representative experiment of three replicate experiments.

IC, immune complex; IFN, interferon; ISRE, IFN-stimulated response element; pDC, plasmacytoid dendritic cell.

**Table S1. Anifrolumab Inhibits ISRE Reporter Activity in Response to   
Recombinant IFN-α2 and CpG-A–Stimulated and DNA-IC–Stimulated pDC Media**

|  |  |
| --- | --- |
| Type I IFN source | Anifrolumab IC50 (nM) |
| IFN-α2, U/mL  100  1,000  10,000  100,000 | 0.149  0.528  3.048  9.748 |
| CpG-A–stimulated pDC media, dilution  1:1,000  1:100  1:10 | 0.021  0.075  0.721 |
| DNA-IC–stimulated pDC media, dilution  1:1,000  1:100  1:10 | 0.060  0.113  0.214 |

Average of three biological replicate experiments.

IC, immune complex; IC50, half-maximal inhibitory concentration; IFN, interferon; ISRE, IFN-stimulated response element; pDC, plasmacytoid dendritic cell.

**Figure S3. Anifrolumab Inhibits the 21-Gene Interferon Gene Signature**



Heat map demonstrating Log2-fold change in expression of individual genes in the 21-gene interferon gene signature of CpG-A–stimulated PBMCs with and without preincubation with anifrolumab or isotype control antibody. Results are displayed compared with untreated control samples not stimulated with CpG-A for each of three representative donors.

**Figure S4.** **Reduced Binding of Anifrolumab to Fcγ Receptor Proteins**



Binding of recombinant FcγR1, FcγR2a, FcγR2b, and FcγR3a-158F to anifrolumab was measured on a BIAcore T200 instrument (Uppsala, Sweden). Parental molecule or anifrolumab were immobilized at high density onto separate flow cells on a CM4 sensor chip using standard amine coupling chemistry. Each FcγR, as well as an appropriate negative control protein (ovalbumin), was prepared at 1 mM using HBS-EP instrument buffer injected over both the IgG and reference cell surfaces. Binding data were collected for 50 minutes. Sensorgram overlays were generated using Prism 6 software (Graph Pad), and the results are representative of three independent assessments. The parental wild-type antibody bound all FcγR proteins tested. Binding to these proteins by anifrolumab was reduced compared with the parental antibody.