Specific inhibition of Btk blocks pathogenic plasma cell signatures and myeloid cell-associated kidney damage in IFNα-driven lupus nephritis

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Conflict of interest statement

All authors are or were employed by Genentech Inc. at the time of the study and hold equity in the Roche Group. In addition, the research in this manuscript was fully funded by Genentech Inc.

Supplemental Methods

SelectScreen® profiling service

The vendor (Invitrogen-Life Technologies) offered two types of kinase activity assays, a peptide phosphorylation assay (Z'-LYTE®) and an ADP quantitation assay (Adapta®), as well as a kinase ATP-site competitive binding assay (LanthaScreen®). For kinases that were inhibited close to or >50% by 1 μM G-744, the vendor carried out 10-point inhibitor titrations using the same assays in order to determine the concentrations of G-744 that caused 50% inhibition (IC_{50}). In Table 1, kinases were run in Z'-LYTE activity assay format except EphA7 and SLK which were run in LanthaScreen binding assay format. The ATP concentrations used in the activity assays were typically within 2-fold of the experimentally determined apparent Michaelis constant (K_m) value for each kinase, whereas the competitive binding tracer concentrations used in the binding assays were generally within 3-fold of the experimentally determined dissociation constant (K_d) values.

p100/p52 ratio by Western blot

Purified murine B cells were pre-incubated with G-744 for 60 min and stimulated with BAFF (500 ng/ml) at 37°C for 16 hours. Cell lysates were prepared using RIPA buffer (Thermo Scientific). Proteins from lysates were separated on 10% SDS-PAGE gels and transferred onto Nitrocellulose membrane (Millipore) by standard techniques. Membranes were blocked and then probed with anti-NFκB2

(p100/p52) rabbit polyclonal (Cell Signaling) and anti-p38 mouse monoclonal Ab as loading control followed by goat anti-rabbit IgG IRDye800CW and goat anti-mouse IgG AlexaFluor680 (Licor Biosciences). Signals were detected with an Odyssey Infrared Imager (Li-Cor Biotechnology) and analyzed with the manufacturer's software.

B cell subset analyses in mice

For assessment of B cell populations in C57/BL6 mice, splenocytes were harvested 7 days after twice daily (BID) oral drug treatment and analyzed by flow cytometry. Antibodies used for these analyses were murine reactive CD21-FITC, B220-FITC, CD3-PerCPCy5.5, CD11b-PerCPCy5.5, Gr1-PerCPCy5.5, B220-PerCP, CD23-PECy7, B220-AlexFluor700, IgD-BV510 (all from BD Bioscience); IgM-Dylight649 (Jackson ImmunoResearch Laboratories Inc); CD93-PE (eBioscience).

In vitro whole blood pBTK assays

Heparinized human whole blood or murine whole blood was treated with G- 744 at 37^{o}C as indicated in 0.4%DMSO for 6hr or 1h, respectively. Blood was lysed with 2X lysis buffer (Cell Signaling Technology) containing protease (Roche Applied Science) and phosphatase inhibitors (cocktail II and III) (Sigma). Phosphorylation of Btk at the Y223 residue was evaluated using Meso Scale Discovery (MSD) technology as described for purified B cells.

In vitro whole blood FcεRI/CD63 assay

Whole blood CD63 assays were performed using the Basotest (Orpegen Pharma) according to the manufacturer's instructions.

Spontaneous NZB/W1 lupus models

NZB/W_F1 hybrids are genetically predisposed to develop severe lupus-like phenotypes comparable to that seen in lupus patients including hemolytic anemia, proteinuria, and progressive IC glomerulonephritis (1). They spontaneously develop anti-nuclear autoantibodies at 16 to 20 weeks, LN between 20 and 44 weeks of age and death from end stage renal disease occurs between 36 and 54 weeks of age. At 32 weeks of age mice were treated as indicated with vehicle or drugs.

Serum antibody ELISAs

Total Ig (IgG, IgA, IgM) autoantibodies against mouse nuclear antigens were quantified by mouse anti-nuclear antigens Ig's ELISA kit from Alpha Diagnostic International, according to the manufacture's protocol.

Serum IgG against dsDNA (but not ssDNA) were detected by ELISA using calf thymus DNA coated plates (Sigma) and detection with HRP-conjugated goat anti-mouse IgG antibody. Pooled sera from aged or young NZB/W_F1 mice served as controls. The absorbance of the negative control was multiplied by a factor of three to yield a cut-off. The log titer for each sample was calculated as the log of

the dilution needed to reach the absorbanceof the cut-off point. A value of 3 means a 1:1000 dilution was required to reach this point. Given the minimum sample dilution of 1/25, the minimum quantifiable log titer value was 1.4.

Isotype IgM and IgG3 autoantibodies against dsDNA were detected by ELISA using mouse anti-dsDNA IgM and anti-dsDNA IgG3 ELISA kits from Alpha Diagnostic International. Serum samples were diluted in low non-specific binding sample diluent (1:50 fold dilution for anti-dsDNA IgM and 1:35 fold dilution for anti-dsDNA IgG3) and ELISAs were performed according to the manufacturer's protocol. These assays did not detect anti-ssDNA antibodies.

Total Ig (IgG, IgA, IgM) autoantibodies against Histone were quantified by ELISA (Alpha Diagnostic International).

Serum total Ig isotypes (IgM, IgG1, IgG2a, IgG2b, IgG3) were detected by Luminex using Mouse Immunoglobulin Isotyping Magnetic Panel (EMD Millipore). Serum samples were diluted 1:10000 fold in assay buffer and simultaneous detection of Igs was performed according the manufacturer's protocol.

Flow cytometry

Cells were incubated with anti-CD16/CD32 (Fc block, clone 2.4G2; BD Bioscience) plus 2% normal mouse and normal rat serum and stained with various combinations of the following Abs in FACS buffer (PBS containing 2% FBS, 2 mM EDTA, and 0.1% NaN3) for 30 min at 4^{o}C. Murine reactive CD38-FITC, CD44-FITC, B220-FITC, CD69-PE, CD86-PE, CD138-PE, CD3-

PerCPCy5.5, CD5-PerCPCy5.5, B220-PerCP, ICOS-PECy7, CD4-PB, B220-PB, B220-AlexFluor700, IgD-BV510, CD8-APC, Ki67-APC (all from BD Bioscience); IgM-Dylight649 (Jackson ImmunoResearch Laboratories Inc); IgG2a FITC, IgG2b-PECy7, IgG1-APCCy7 (all from Southern Biotech). Human plasmablasts were stained with human-reactive anti-CD20 FITC or anti-CD38 APC (BD Biosciences) mAbs. Isotype-matched mAbs were used as control. Dead cells were excluded with Sytox Blue (Life Technologies) or propidium iodide (Sigma). Samples were analyzed by flow cytometry using a LSR II or FACS Calibur instrument (BD Biosciences). Cell subsets were identified as described in the figure legends.

Histological Staining and Scoring

Four-micron tissue sections were evaluated. Kidney sections were stained with Periodic acid–Schiff (PAS) for evaluation of glomerulonephritis, and hematoxylin and eosin (H&E) for evaluation of periarteritis by light microscopy. Glomerulonephritis severity scoring was on a 4 point semiquantitative scale: “0” – normal or mild global lesions in <50% of glomeruli, “1” - global lesions in >50% of glomeruli, <20% of which are severe (defined as >1 segment with <3 patent capillaries), “2” - global lesions in >50% of glomeruli, 20-80% of which are severe, and “3” - >80% of glomeruli with severe global lesions. Periarteritis severity scoring was on a 4 point semiquantitative scale: “0” – no significant inflammation, “1” – sparse or focal periarterial inflammatory infiltrates, “2” – occasional

moderate sized non-circumferential perivascular inflammatory infiltrates, and “3” – frequent large perivascular inflammatory infiltrates, often circumferential. Glomerular and tubular immunoglobulin fluorescence signals were separately scored in a blinded fashion using a visual semiquantitative 4-point scale: “0” – negative; “1” – low/infrequent signal; “2” – moderately frequent and/or intense signal; “3” – high/frequent signal.

Splenic GC recognized as well-circumscribed clusters of at least 5 PNA^+ cells were enumerated on one whole splenic section per animal. To evaluate splenic B cell follicular structure, 5 μm acetone-fixed sections obtained from frozen spleen were subjected to sequential 3-color immunofluorescence using biotinylated rat anti-mouse CD4 antibody (Clone RM4-5, Pharmingen), streptavidin-Alexafluor 546 for 30 min (Life Technologies), rat anti-mouse MOMA-1-FITC (AbD Serotec), and goat anti-mouse IgM-AMCA (Vector Labs).

RNA sequencing and differential expression analyses

RNA from frozen spleen and kidney were isolated by disrupting the tissues in RLT buffer using a tissue lyser and then extracting RNA using the RNeasy mini kit (Qiagen) including the on-column DNase digestion. Total RNA concentration and RNA integrity of samples was determined using the NanoDrop 8000 (Thermo Scientific) and the Fragment Analyzer with High Sensitivity RNA kit (Advanced Analytical), respectively.

Libraries were made from 1 µg total spleen or kidney RNA each using the TruSeq RNA Sample Preparation Kit v2 (Illumina). Generated libraries were amplified with 8 cycles of PCR. The size of the libraries was confirmed using a Fragment Analyzer and High Sensitivity DNA NGS kit (Advanced Analytical) and their concentration was determined by a qPCR-based method using the KAPA Library quantification kit. The libraries were multiplexed and sequenced on HiSeq2500 (Illumina) using manufacturer's recommendations. The raw RNA-sequencing reads and normalized data are available from GEO under accession number GSE72410.

Bioinformatic analyses were performed using custom scripts written in the R programming language (http://r-project.org), using packages from the Bioconductor project (http://bioconductor.org). Demultiplexed RNA sequencing reads were processed using the HTSeqGenie package (version 3.14.2). Reads were aligned to the reference mouse genome sequence (NCBI Build 37) using the GSNAP algorithm (2) version 2013-10-10. Uniquely aligned read pairs that fell within exons were tallied to estimate gene expression levels. For inclusion, genes were required to have >10 reads in ≥4 samples.

To determine differential expression of genes, we used the DESeq2 algorithm to fit a negative binomial generalized linear model, using the default parameters. To control for multiplicity of testing, p-values were adjusted using the Benjamini-Hochberg method. For heat maps, the count data were transformed using the variance-stabilizing transformation implemented in DESeq2. Genes were

centered and scaled to unit variance, and hierarchical clustering was performed using Euclidean distance and Ward linkage.

QT PCR using Fluidigm

RNA from murine blood was isolated using Trizol and further purified using the RNEasy mini kit (Qiagen). cDNA synthesis was performed on 200 ng total-RNA using an iScript cDNA synthesis kit (Biorad). Gene-specific pre-amplification was performed (Applied Biosystems) of 117 genes including housekeeping genes HPRT1, b-Actin, RPL19 and GAPDH (Supplemental Table 2). RT-PCR was performed using the BioMark 96.96 Dynamic Arrays (Fluidigm Corporation) using the manufacturer's protocol. Data were collected using the BioMark Data Collection Software and CT values were obtained using the BioMark RT-PCR Analysis Software (V.2.1.1, Fluidigm). The relative abundance (dCt) to RPL19 (the most stable housekeeping gene) was calculated: 2log-(average Ct gene - average Ct RPL19). For statistical analyses, values below the lower limit of detection were set to be 1 Ct lower than the lowest recorded value.

All statistical analyses were performed using custom scripts written in the R programming language. To identify genes affected by Btk inhibition, we used the limma R package to fit a linear model for each gene, calculating the fold change between baseline (8 month old NZB/W_F1) samples to one or three month post drug treatment samples. Genes were considered to be significantly affected by

treatment if they were decreased by at least 1.5-fold compared to 36-week old mice, with an adjusted p-value less than 0.05.

Survival assays with differentiated plasmablast and plasma cells

Memory B cells were differentiated using CpG or R848, IL-2, IL-10, IL-15, IL-6, IFNα for 4 days followed by culturing the cells in IL-2, IL-10, IL-15, IL-6, IFNα and IL-21 for 10 days. Cells were sorted on day 14 using anti-CD38 and anti-CD138 antibodies to detect plasmablasts and plasma cells, respectively. Sorted plasma cells and plasmablasts were then treated with either DMSO or G-744 at IC_{90} concentration (650 nM) for 48 h and cell survival was assessed by flow cytomtery using Annexin and Propidium Iodide.

Btk RNA expression in B cell-lineage cells

Total RNA from FACS-sorted B cell subsets (naïve, memory and plasmablasts) were isolated with the RNeasy kit using on-column DNase I digestion (QIAGEN) followed by cDNA synthesis using iScript cDNA sysnthesis kit (Bio-rad laboratories). Taqman quantitative RT-PCR was then performed, according to the manufacturer's instructions (Applied Biosystems) and run using a 7900HT Fast Real Time PCR system (Applied Biosystems). The samples were run in triplicate reactions and were analyzed for expression of Btk and results were normalized to HPRT1. The values were plotted as Btk expression 2(-dCT) normalized to HPRT1.

Supplemental Tables

Supplemental Table 1

Invitrogen kinase selectivity (% Inhibition at 1 μM [IC_{50}, nM])

Kinase	G-7744	P505-15	RN486	Ibrutinib	PF-112	Kinase	G-7744	P505-15	RN486	Ibrutinib	PF-112
Abl	-1	15	42	39	-18	EphA7	83 [548]	24	9	18	-3
ACVR1B	-5	-3	1	0	6	EphA8	1	14	9	48	11
ACVR2B		1	7	2	-1	EphB1	4	25	6	7	4
AKT1	4	1	2	8	6	EphB2	1				
AKT2	2	1	24	3	7	EphB3	1	-1	3	3	1
AKT3	6					EphB4	0				
ALK2	-1	-2	14	14	-5	ErbB2	54 [1750]	6	-11	91 [32.7]	-7
Arg	4					ErbB4	0	22	14	93 [2.7]	97
ARK5	14	69	10	2	-13	ERK1	7				
ASK1	14	5	4	5	0	ERK2	-1	27	0	11	9
Aurora_A	3	9	14	45	32	FAK	3	61	5	-3	8
Aurora_B	6	-2	7	18	4	FAK2	2	59 [587]			
Aurora_C	5					Fer	9				
Axl	3	25	5	9	6	Fes	4	55	13	5	3
B-Raf	21	18	5	59	11	FGFR1	9	-3	54	53	40
Blk	-3	75	50	102 [<0.5]	54	FGFR2	14				
BMPR1A		0	14	-1	1	FGFR3	7	10	35	31	22
Bmx	49 [>3333]	46	89 [220]	99 [1.63]	96	FGFR3(K650E)	15				
Brk	1	14	38	100 [16.5]	92	FGFR4	-2	4	29	22	14
BrSK1	3	10	5	1	5	Fgr	53 [>1111]	78	100 [70.8]	103 [2.63]	53
BTK	95 [1.77]	69 [616]	101 [3.56]	101 [0.55]	99 [1.92]	Flt1	2	1	16	22	6
CaMKI	8	57	10	-7	19	Flt3	-6	61 [792]	24	86	25
CaMKI_delta	8	25	12	8	5	Flt3(D835Y)	7				
CamKII_alpha	-7	10	4	-2	-1	Flt4	13	16	56	69	27
CaMKII_beta	12	9	0	13	6	Frk	9	57	37	88 [44.1]	19
CamKII_delta	3					Fyn	6	53 [1010]			
CamKIV	-5	33	3	8	2	GCK	8	78 [275]			
CAMKK1	4	-6	-3	-12	1	GRK2	14	1	1	-5	0
CAMKK2	-7	3	8	-1	1	GRK3	16	1	3	-5	-4
CDK1/cyclinB	1	24	7	6	-2	GRK4	-4				
CDK2/cyclinA	-4	60 [789]	-1	2	3	GRK5	-5	2	-1	-1	-1
CDK5/p25	2	50	1	2	4	GRK6	-1	8	9	1	-2
CDK5/p35	3					GRK7	3				
CDK7/cyclinH	-11	34	5	-20	5	GSK3_alpha	4	19	7	15	7
CDK8/cyclinC	-1	2	9	14	6	GSK3_beta	-5	12	-2	-1	3
CDK9/cyclinK	11					Haspin	-1				
CDK9/cyclinT1	11	26	-7	1	-14	Hck	6	72		98 [27.6]	
CHK1	1	9	13	2	3	HIPK1	-2	1	0	0	1
CHK2	1	48	-4	12	10	HIPK2	-4	3	6	3	4
CK1_alpha1	6	5	5	2	3	HIPK4	11	8	4	5	5
CK1_delta	0	46	3	8	5	Hyl	4	-2	1	-1	-5
CK1_epsilon1	1	27	2	32	6	IGF1R	3	60 [611]	15	8	4
CK1_gamma1	5	2	5	-3	4	IKK_alpha	-5	4	16	-16	3
CK1_gamma2	6	10	4	2	2	IKK_beta	15	5	6	13	2
CK1_gamma3	-1					IKK_epsilon	9	0	4	4	6
CK2_alpha1	3	1	6	2	1	InsR	6	31	7	9	0
CK2_alpha2	4					IRAK1	-4	40	1	1	-5
CLK1	7	30	5	-4	6	IRAK4	6	43	-9	14	-10
CLK2	4	53	10	-2	2	IRR	2	28	10	14	2
CLK3	4	-1	7	5	8	Itk	6	8	16	81 [218]	-4
CLK4		78	13	6	-5	JAK1	-2	43	-4	-2	-1
Cot	16	39	10	30	4	JAK2	-5	39	2	1	-2
CSF1R	6	69 [372]	24	55	6	JAK3	-4	41	-7	80 [240]	-4
CSK	4	11	22	98 [39.5]	34	JNK1_alpha1	1	28	13	9	10
DAPK1	1	47	-29	27	13	JNK2	12	5	16	5	3
DCAMKL2	3	-1	6	7	5	JNK3	8	25	13	5	5
DDR1		-3	3	2	4	KDR	7	7	32	66	27
DDR2	1					KHS1	7	26	10	17	12
DMPK	-4	66	7	-8	-1	Kit	-11	6	2	18	-2
DNA-PK		49	3	11	9	Kit(T670I)	0				
DRAK1	-5	6	8	4	4	Kit(V654A)	-7				
DYRK1A	5	4	1	4	5	Lck	17	66 [494]	86 [396]	98 [3.64]	74
DYRK1B	2					LIMK1	9	2	47	41	-2
DYRK3	6	79	23	4	1	LIMK2	6				
DYRK4	2	3	0	-1	-1	LRRK2	-1	71 [203]	3	-3	-16
eEF-2K	2	6	1	-3	11	LRRK2(G2019S)	-15				
EGFR	17	26	3	89 [23.2]	11	LTK	4	43	-7	6	-3
EGFR(L858R)	23					Lyn	7	81 [215]	50	97 [15.8]	62
EGFR(T790M,L858R)		79 [274]	18	26	12	LynB	18				
EphA1	3	42	10	30	11	MAP4K4	4	76	7	12	25
EphA2	16	13				MAPKAPK2	-5	-1	15	8	6
EphA3	-7	12	-1	12	2	MAPKAPK3	4	3	40	1	6
EphA4	3					MARK1	1	9	7	9	5
EphA5	1					MARK2	10				

Supplemental Table 1 (continued)

Invitrogen kinase selectivity (% Inhibition at 1 μM [IC_{50}, nM])

Kinase	G-7744	P505-15	RN486	Ibrutinib	PF-112	Kinase	G-7744	P505-15	RN486	Ibrutinib	PF-112
MARK3	8	33	8	7	5	PKC_alpha	5	61	5	7	10
MARK4	3					PKC_beta1	4	-2	6	18	11
MEK1	0	20	4	40	58	PKC_beta2	-1				
MEK2	5					PKC_delta	1	7	39	5	-2
MEK3	2	39	11	6	1	PKC_epsilon	1	18	21	4	3
MEKK2	-2	12	6	35	28	PKC_eta	20	9	-2	7	6
MEKK3	3					PKC_gamma	-1				
MELK	5	16	8	-1	-5	PKC_iota	6				
Mer	1	36	6	31	5	PKC_theta	-5	3	13	6	6
Met	9	8	2	12	9	PKC_zeta	10	5	-5	11	5
Met(1250T)	5					PKD1	2	59 [820]	11	11	15
Mink1	11	56	0	21	26	PKD2	0				
MKK6	11	5	9	-1	-2	PKD3	7				
MKK6(S207E,T211E)	-4					PKG1_alpha	4	5	5	4	2
MKNK1		8	-3	10	-2	PKG2	2				
MKNK2		5	4	18	3	PLK1	4	0	4	7	2
MLK1	-3	70	-6	2	-2	PLK2	21	2	6	3	-1
MLK2	-5	31	6	10	3	PLK3	-15	17	10	16	5
MLK3	-4					PRAK	3	2	7	1	4
MRCK_alpha	6	5	-9	3	-4	PRK1	1	10	13	11	6
MRCK_beta	15					PRKAA1	1	27	7	6	2
MSK1	2	5	9	1	7	PRKAA2	5				
MSK2	3					PrKX	1	8	3	4	2
MSSK1	9	2	3	3	-2	RAF1(Y340D,Y341D)	10	4	11	56	1
MST1	-4	24	-1	22	11	Ret	14	57	32	87	54
MST2	23	24	-7	16	7	RIPK2	1	7	7	99 [16]	85
MST3	3	4	5	14	20	ROCK1	1	22	-1	5	1
MST4	7	17	10	16	12	ROCK2	1	6	-3	-1	2
mTOR	-4	10	2	28	7	Ron	11	2	20	4	0
MuSK	7	43	55	-12	2	Ros	7	50	7	-1	3
MYLK(smMLCK)	-4	9	3	-3	1	Rse	4	31	10	7	5
MYLK2(skMLCK)	14					Rsk1	-1	16	6	5	1
MYLK3(caMLCK)	-8	26	13	6	13	Rsk2	1	15	30	-7	1
NEK1	5	2	6	-6	10	Rsk3	3	12	9	7	3
NEK2	4					Rsk4	9				
NEK4	2	6	10	4	1	SGK1	-11	4	-7	4	6
NEK6	4	0	3	5	8	SGK2	3	-2	10	-1	2
NEK7	1					SGK3	5	10	28	-1	3
NEK9	-1	-4	0	2	-1	SIK2	4	49	9	0	5
NIK	-3					SLK	4	9	8	6	4
NLK	-10	-5	6	5	17	SPHK1	5	16	17	9	0
p38_alpha	3	7				SPHK2	-1				
p38_alpha(direct)	-2	1	-3	6	10	Src	39	70 [349]	99 [122]	98 [26.1]	45
p38_beta	6	2	8	8	22	Src_N1	21				
p38_delta	5	7	6	14	3	Srm	-3	-3	33	105 [5.78]	15
p38_gamma	11	5	1	2	5	SRPK1	5	3	0	3	3
p70S6K	5	14	4	-1	-3	SRPK2	-3	8			
PAK1	9	10	-1	9	5	STK16	-3	-1	-2	2	3
PAK2	6					STK33	2	29	6	14	3
PAK3	-3	4	16	19	6	Syk	0	99 [1.31]	8	2	15
PAK4	-6	97 [62.9]	5	6	3	TAK1-TAB1	1	35	8	2	2
PAK6	-7	35	10	2	2	TAO1	8	9	-10	-1	-5
PAK7	4					TAO3	1				
PASK	21	79	8	1	1	TBK1	4	3	8	8	5
PDGFR_a	5	23	3	50	4	TEC	15	16	90 [283]	100 [10.2]	91
PDGFR_a(D842V)	0					TGFBR1		-3	7	32	7
PDGFR_a(T674I)	-4					Tie2	45	7	-5	57	30
PDGFR_a(V561D)	6					TNK2	0	16	40	86 [167]	4
PDGFR_b	3	20				TrkA	11	68 [309]	30	24	19
PDK1	19					TrkB	8	47	10	11	6
PDK1(direct)	14	6	3	3	2	TrkC	-8				
PhK_gamma1	6	9	-5	3	3	TSSK1	5	83	3	4	7
PhK_gamma2	-1	19	3	4	2	TSSK2	2				
PI3K-A	14	-2	-4	5	4	TTK	-3	26	8	10	13
PI3K-D	-5					TXK		51	7	93 [2.52]	76
PI3K-G	-1	-10	1	-9	-9	TYK2	3	30	6	2	3
PI3KC2a	0					WEE1	-4	-6	4	1	1
PI3KC2b	-3					WNK2	2	43	9	10	10
PI3KC3_hVPS34	0					Yes	8	65 [553]	81 [751]	99 [4.37]	62
PI4Ka	-5					YSK1	-13	5	10	6	4
PI4Kb	-4					ZAK	-1	-3	10	27	4
PIM1	2	68	-4	12	1	ZAP-70	3	10	4	13	-1
PIM2	1					ZIPK	-6	16	-1	5	-3
PKA	-3	19	-1	4	2						
						% Inhibition	<50	50-80	≥80	Not Tested	

Invitrogen kinase selectivity. The selectivity of G-744, P505-15, RN486, Ibrutinib and PF-122 (3-6) was defined at 1 μM against a panel of active recombinant human kinases as indicated using the SelectScreen® profiling service and the individual and average % inhibition values were reported. G-744, RN486, Ibrutinib and P505-15 titrations were carried out in order to determine the inhibitor concentration that causes 50% inhibition (IC_{50}) for kinases that were inhibited close to or greater than 50% by 1 μM of the respective inhibitor. The ATP concentrations used in most of the activity assays were within 2-fold of the experimentally determined apparent Michaelis constant (K_m) value for each kinase, whereas the competitive binding tracer concentrations used in the binding assays were generally within 3-fold of the experimentally determined dissociation constant (K_d) values.

Supplemental Table 2: Probes used for the RT-PCR analyses from whole blood of the spontaneous NZB/W_F1 lupus model presented in Supplemental Fig.6.

No	Gene	Probe	No	Gene	Probe	No	Gene	Probe	No	Gene	Probe
1	AID	Mm01184115_m1	31	CX3CR1	Mm02620111_s1	61	IL15	Mm00434210_m1	91	NFKBIZ	Mm00600522_m1
2	ANXA7	Mm00477557_m1	32	CXCL10	Mm00445235_m1	62	IL1b	Mm00434228_m1	92	nudE	Mm00481033_m1
3	ATF3	Mm00476032_m1	33	CXCL13	Mm04214185_s1	63	IL2	Mm00434256_m1	93	Oas2	Mm00460961_m1
4	b-actin	Mm01205647_g1	34	Egr2	Mm00456650_m1	64	IL21	Mm00517640_m1	94	Oas3	Mm00460944_m1
5	BAFF	Mm00446347_m1	35	Egr3	Mm00516979_m1	65	IL21R	Mm00600319_m1	95	PAX5	Mm00435501_m1
6	Bank1	Mm01317729_m1	36	ESPL1	Mm01313921_m1	66	IL24	Mm00474102_m1	96	Pla2g4c	Mm01195718_m1
7	BASP1	Mm02344032_s1	37	Factor B	Mm00433918_g1	67	IL27	Mm00461162_m1	97	PRDM1	Mm00476128_m1
8	Blk	Mm00432077_m1	38	FAM129A	Mm00452065_m1	68	IL3	Mm00439631_m1	98	PTPN1	Mm00448427_m1
9	BLNK	Mm01197846_m1	39	Fcgr3	Mm00438882_m1	69	IL4	Mm00445259_m1	99	PTPN22	Mm00501246_m1
10	BMP2K	Mm00499813_m1	40	FCRL5	Mm00806273_m1	70	IL5	Mm00439646_m1	100	RAB21	Mm00804090_m1
11	Btk	Mm00442712_m1	41	FoxA3	Mm00484714_m1	71	IL6	Mm00446190_m1	101	RGS1	Mm00450170_m1
12	C17orf49	Mm04243238_m1	42	FYB	Mm01304022_m1	72	IL8	Mm04208137_m1	102	RPL19	Mm02601633_g1
13	CCDC28B	Mm00508352_g1	43	GAPDH	Mm99999915_g1	73	IRAK2	Mm01184677_m1	103	SGK1	Mm00441387_g1
14	CCL1	Mm00441236_m1	44	GBP1	Mm00657086_m1	74	IRAK4	Mm00459443_m1	104	SLC15a4	Mm00505709_m1
15	CCL22	Mm00436439_m1	45	GSTP1	Mm04213618_gH	75	Irf4	Mm00516431_m1	105	SOCS1	Mm01342740_g1
16	CCL3	Mm00441259_g1	46	HCK	Mm01241463_m1	76	IRF5	Mm00496477_m1	106	STAT1	Mm00439531_m1
17	CCL4	Mm00443111_m1	47	HERC5	Mm01341950_m1	77	IRF7	Mn00516791_g1	107	STAT4	Mm00448890_m1
18	CD11b	Mm00434455_m1	48	HPRT1	Mm01545399_m1	78	IRF8	Mm00492567_m1	108	STAT6	Mm01160477_m1
19	CD16	Mm00438882_m1	49	ICAM1	Mm00516023_m1	79	JUN	Mm00495062_s1	109	Syk	Mm01333032_m1
20	CD19	Mm00515420_m1	50	IFNAR1	Mm00439544_m1	80	Lyn	Mm01217488_m1	110	TANK	Mm00495541_m1
21	CD38	Mm01220906_m1	51	IFNb1	Mm00439552_S1	81	MAP3K8	Mm00432637_m1	111	Tec	Mm00443230_m1
22	CD40	Mm00441891_m1	52	IFNg	Mm01168134_m1	82	MARCKS	Mm02524303_s1	112	TMCC3	Mm00468135_m1
23	CD64	Mm00438874_m1	53	Ighg1	Mm01742100_s1	83	MCP1	Mm00441242_m1	113	TNFa	Mm00443260_g1
24	CD69	Mm01183378_m1	54	Ighm	Mm01718955_g1	84	MMP1	Mm00439491_m1	114	TNFRSF21	Mm00446361_m1
25	CD79A	Mm00432423_m1	55	Igj	Mm00461780_m1	85	MMP14	Mm00485054_m1	115	TRAF1	Mm00493827_m1
26	CD86	Mm00444543_m1	56	IGSF6	Mm00459510_m1	86	MMP2	Mm00439498_m1	116	TRAF6	Mm00493836_m1
27	CLIP2	Mm00515608_m1	57	IL-1RA	Mm00446186_m1	87	Mx1	Mm00487796_m1	117	ZC3H12A	Mm00462535_g1
28	CMPK2	Mm00469582_m1	58	IL10	Mm00439614_m1	88	NCF2	Mm00726636_s1			
29	CSF1	Mm00432686_m1	59	IL12b (p40)	Mm00434174_m1	89	NFKBIA	Mm00477800_g1			
30	CSFR1	Mm01266652_m1	60	IL13RA1	Mm00446726_m1	90	NFkBIE	Mm01269649_m1			