Supplemental Materials and Methods

Identification of target immunoglobulin light chain variable region peptides

Serum/plasma (0.5µL) was added to reducing sample buffer (50mM DTT final) and heated at 95°C for 10 min prior to electrophoresis on a 10.5-14% Criterion precast gel (Bio-Rad, Hercules, CA). The gel was subsequently stained with BioSafe colloidal Coomassie blue stain and the bands corresponding to immunoglobulin light chain excised from the gel. The excised gel band was destained with 50% acetonitrile/50mM Tris, pH 8.1 until clear. The bands are reduced with 30 mM TCEP/50mM Tris, pH 8.1 at 55ºC for 40 minutes and alkylated with 40mM iodoacetamide at room temperature for 40 minutes in the dark. Proteins are digested in-situ with 30ul (0.005µg/µL) of trypsin (Promega Corporation, Madison WI) in 20 mM Tris pH 8.1 / 0.0002% Zwittergent 3-16, at 37°C for 4 hours to overnight, followed by peptide extraction with 40ul of 2% trifluoroacetic acid and then 60ul of acetonitrile. The extracts are pooled and concentrated to less than 5ul on a SpeedVac spinning concentrator (Savant Instruments, Holbrook NY) and then brought up in 0.15% formic acid/0.05% trifluoroacetic acid for protein identification by nano-flow liquid chromatography electrospray tandem mass spectrometry (nanoLC-ESI-MS/MS) using a Orbitrap-based Mass Spectrometer (Q Exactive; Thermo Scientific, Bremen, Germany) coupled to an Eksigent nanoLC-2D HPLC system (Eksigent, Dublin, CA). The peptide digest (2µL) is loaded onto a 250nl OPTI-PAK trap (Optimize Technologies, Oregon City, OR) custom packed with Michrom Magic C8 solid phase (Michrom Bioresources, Auburn, CA). Chromatography is performed using 0.2% formic acid in both the A solvent (98%water/2%acetonitrile) and B solvent (80% acetonitrile/10% isopropanol/10%

water), and running a 5%B to 45%B gradient over 60 minutes at 350 nL/min through a Michrom Magic C18 (75µm x 150mm) packed tip capillary column. The Q Exactive mass spectrometer experiment was set to perform a full scan from 360-2000 *m/z* with resolution set at 70,000 (at 200 *m/z*) followed by MS/MS scans at 17,500 resolution (200 *m/z*) on the top fifteen $[M+2H^+]^{2+}$, $[M+3H^+]^{3+}$, and $[M+4H^+]^{4+}$ ions with a 2 Da isolation window. Dynamic exclusion was set to 45 seconds. The lock-mass option was enabled for the full scans using the ambient air ions of 371.10123 and 445.12003 Da for real time internal calibration giving < 2 ppm mass tolerances of the precursor masses.(32)

Light Chain mRNA Sequencing

Light chain proteomic derived sequences were compared to Sanger sequenced plasma cell light chain mRNA via the following method. Briefly, bone marrow aspirates were CD138 sorted to enrich for plasma cells.

mRNA Extraction Sorted cells were resuspended in 1 mL of RNAlater (Qiagen, Valencia, CA) RNA stabilizing solution. Five hundred mL of the cell suspension was resuspended in 500 mL of ice cold PBS. Cells in the resuspension were pelleted by centrifuging at 5000 RPM for six minutes at 4°C. Total RNA was extracted from the cell pellet using Trizol reagent (Life Technologies, Carlsbad, CA) and the resulting RNA was quantified using the Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA).

PCR Amplification cDNA was made from 1 µg of the total RNA using iScript cDNA kit (BioRad, Hercules, CA). Multiplex PCR reactions were configured with appropriate 5’

and 3’ primers targeting each light chain family (kappa or lambda) and the KOD Hot Start DNA polymerase kit (EMD Millipore, Billerica, MA). PCR reactions were optimized with 1 mM $MgSO_4$, 4% DMSO, 0.5 mM of a primer and 1 mL of undiluted cDNA. Amplification was performed on a GeneAmp 9900 thermocycler (Perkin Elmer, Waltham, MA) with the following conditions: 95°C for 2 minutes; 27 cycles of 95°C for 20 seconds, 60°C for 10 seconds and 72 °C for 10 seconds. Amplified products were electrophoresed on a 1.5% agarose-TAE gel and visualized using ethidium bromide (200 ng/mL). Visible bands were excised and purified using the Wizard SV gel and PCR purification kit (Promega, Madison, WI).

Sanger Sequencing Purified PCR products were directly Sanger sequenced on an ABI PRISM™ DNA Analyzer using appropriate 3’ primer and ABI PRISM™ Big Dye Terminator Cycle Sequencing chemistry.