Figure S1

Figure S1 contains scatter plots showing the relationship between different cell lines and treatments. Each plot represents a comparison of two conditions, with the p-value indicated for each.
| Time  | BRCA1 | IKKa | CHK1 | CCND1 | CDK4 | Tubulin |
|-------|-------|------|------|-------|------|---------|
| 0h    | -     | -    | -    | -     | -    | -       |
| 6h    | +     | +    | +    | +     | +    | +       |
| 6h    | -     | -    | -    | -     | -    | -       |
| 24h   | +     | +    | +    | +     | +    | +       |
| 24h   | -     | -    | -    | -     | -    | -       |
| 48h   | +     | +    | +    | +     | +    | +       |
| 48h   | -     | -    | -    | -     | -    | -       |

50nM AUY922
Serum starvation
Figure S4
Supplementary Methods

Reagents and cell lines. 17-AAG, ibritinib and AT13387 were purchased from Selleck (Cat. No. 75747-14-7, 526880 and S1163, respectively), PU-H71 [8-(6-iodobenzo[d][1.3]fioxol-5-ythio)-9-(3-(isopropylamino)propyl)-9H-purine-6-amine] was a kind gift of the Chiosis Laboratory, NVP-AUY922 (AUY922) and NVP-HSP990 (HSP990) were provided by Novartis. Bortezomib was purchased from Millennium Pharmaceuticals. Doxorubicin (Adrimedac®) was purchased from Medac, bendamustine (Levact®) from Mundipharma, ifosfamide (IFO-Cell® N) and cytarabine (ARA-Cell®) from Cellpharma, all in Germany.

The cell lines used in this study were cultured in RPMI 1640, IMDM, or DMEM (all Gibco), supplemented with 10%-20% fetal bovine serum (FBS), 2 mmol/L L-alanyl-glutamine, and 100 μg/mL penicillin-streptomycin (all Gibco). Cell line authenticity was confirmed for all lines in August 2012 (DFCI Microarray Core) and November 2014 (Eurofins Genomics, Germany) by short tandem repeat profiling.

Primary patient samples. The acquisition and use of primary specimens was approved by Dana-Farber Cancer Institute Institutional Review Board #01-206. Primary MCL cells were cultured in RPMI 1640 (Pan Biotech) with 10% FBS (Biochrom).

Immunoblotting. Western blotting was performed as previously described. Cells were lysed with RIPA buffer or cOmplete Lysis-M (Roche, Cat. No. 04719964001), supplemented with Phosphatase Inhibitors 2 and 3 (Sigma Aldrich, P5726 and P0044, respectively).

Antibodies were directed against human AKT (Cell Signaling, 4691S, and Cell Signaling, 2965), pAKT (Cell Signaling, 9271S), pAKT Ser473 (Cell Signaling, 4058), BTK (Cell Signaling, 3533), pBTK (Cell Signaling, 5082), CDK4 (Santa Cruz, sc-260, and Cell Signaling, 2906), cyclin D1 (Santa Cruz, sc-8396, and BD Pharming, 556470), Caspase3 (Cell Signaling, 9665), ERK1/2 (Cell Signaling, 9102S), pERK1/2 (Cell Signaling, 4307S, and Cell Signaling, 4377), Histone H3 (Cell Signaling, 9715), HSP70 (Cell Signaling, 4876/S), HSP90 (Cell Signaling, 4877), IκBα (Cell Signaling, 9242), IKKα (Cell Signaling, 2682), NF-κB p100/p52 (Cell
Signalining, 4882), PARP (Cell Signaling, 9542), GAPDH (Santa Cruz, sc-32233), and tubulin (Sigma Aldrich, T9026).

**Cellular reactive oxygen species (ROS) detection assay.** A DCFDA (2′,7′-dichloro fluorescein diacetate)-Cellular Reactive Oxygen Species Detection Assay Kit (Abcam Inc.) was used to measure and quantify hydroxyl, peroxyl and other ROS activity within the cell. A total of 3 × 10^5 cells per well were seeded in a 96-well plate stained with 1 μm DCFDA for 30 min at 37°C. Cells were then treated with 50 nM AUY922, 50 µM Tert-Butyl Hydrogen Peroxide (TBHP) or 0.05% DMSO in triplicates for 3h at 37°C. Fluorescence was measured by flow cytometry on Cytoflex (Beckman Coulter) with maximum excitation and emission spectra of 488 and 535 nm, respectively and analyzed with FlowJo software (Tristar).

**Comparison between serum starvation and treatment with HSP90 inhibitor.** A total of 40 x 10^6 cells were incubated under serum starvation or with 50nM AUY922 and aliquots for immunoblotting were taken after 0h, 6h, 24h, and 48h. Cells were washed in PBS and collected in RIPA lysis buffer (SigmaAldrich) containing Protease Inhibitor (SigmaAldrich). Protein concentrations were determined by the BCA method (Thermo Fisher Scientific) and equal amounts were loaded onto precast 4–12% NuPAGE gels (Invitrogen). Antibodies used were directed against Tubulin (Sigma Aldrich), CCND1 (Santa Cruz), CDK4, IKKα, CHK1 (Cell Signaling) and BRCA1 (EMD Millipore).

**In vivo models.** MAVER1 and Z-138 cells were inoculated subcutaneously into 6-week old CB17.Cg-Prkdc<sup>scid</sup>Lyst<sup>bg</sup>/Crl (SCID/beige) mice (1x10^7 cells per mouse in Matrigel basement membrane matrix (Becton Dickinson)). Upon evidence of measurable engraftment, mice were randomized to receive either AUY922 (50 mg/kg by tail vein injection thrice weekly) or vehicle. Mice were sacrificed when measureable tumor size exceeded 2,000 mm^3 or they developed hind limb paralysis or became moribund.

The PDX was derived from a 68 year-old man who was diagnosed with MCL with peripheral blood, bone marrow, and splenic involvement. Cytogenetic analysis revealed a complex karyotype with 41~42,X,Y, add(4)(q23), add(7)(q22), 8, add(8)(q24.3), 9, t(11;14)(q13;q32), -
13,-17,+mar[cp9]/46,XY[11], including t(11;14), deletion of P53, and monosomy 13. He had an excellent initial response to 6 cycles of bendamustine and rituximab but relapsed within 3 months of completion. He had no response to single agent rituximab or bortezomib. He received one cycle of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy with a good disease response but intolerable toxicity and was then switched to ibrutinib without response, at which time the sample was obtained for xenografting. He passed away from disease progression complicated by enterococcal bacteremia 16 months after his initial diagnosis.

Peripheral blood from the patient was xenografted by tail-vein injection of 12.4 million total peripheral blood mononuclear cells into a NSG mouse. The transplanted mouse became moribund after 28 days. 4.7 million splenocytes harvested from that mouse were secondarily transplanted into 6 NSG mice. Representative flow cytometry of these splenocytes demonstrating a clonal population of CD19+CD20+CD5+, kappa restricted B cells consistent with involvement by MCL is shown in Figure S5. After 18 days, mice were randomized to receive either AUY922 (50mg/kg by tail vein injection thrice weekly) or vehicle. ¹⁸F-FLT PET was performed after the third treatment of either AUY922 or vehicle. Mice were euthanized and immunoblotting of the spleens was performed.

**CT-PET imaging.** At the indicated time-points, 3 mice from each cohort were anesthetized through sevoflurane/air inhalation and scanned on a dedicated small animal CT-PET scanner (Inveon Multimodality System, Siemens Medical Solutions USA Inc.). Low dose CT scans were first acquired (80 kVp, 0.5 mA, 220 degree rotation, 600 ms per degree exposure time, 80 μm reconstruction pixel size) for anatomical reference and to provide guidance for the delineation of selected tissue volume of interest (VOI). Following a bolus intravenous injection of approximately 14.8 MBq of ¹⁸F-FLT, static PET emission scans were then acquired in list-mode format over 10 min (60 min post-radiotracer injection) and corrected for decay and dead time. The acquired data were then sorted into 0.5 mm sinogram bins and 1 time frame for image reconstruction using ordered subset expectation maximization in 3 dimensions followed by MAP reconstruction (OSEM3D/MAP; 256x256x159 matrix size, 0.43x0.43x0.80 mm3 pixel size, 4 OSEM3D iterations, 18 MAP iterations, b=0.1 optimized for uniform resolution, FWHM 1.29
mm). The PET data analysis was performed with Siemens Inveon Research Workplace software. The radioactivity concentrations within selected tissues were obtained from mean voxel intensity values within the VOI and then converted to megabecquerels per milliliter using the calibration factor determined for the Inveon PET system. These values were then divided by the administered activity in megabecquerels and animal body weight to obtain an image VOI-derived standardized uptake value (SUV). To achieve better reproducibility, quantitative PET data were assessed using the maximum SUV values (SUV\textsubscript{max}). All PET images are maximum intensity projections (MIP) of the 10 min static scan, with the mice in prone position.

Supplementary Legends

**Figure S1.** Correlation plots of protein levels between Mino or JeKo-1 parental and Mino or JeKo-1 transduced with either WT (Mino: i and iv; JeKo-1: vii and x) or C481S BTK (Mino: ii and v; Jeko-1: viii and xi) treated with either DMSO or AUY922, respectively, for all proteins affected by log 2 fold $> 0.5$ in at least one cell line upon treatment with AUY922. Correlation plots of protein levels between Mino and JeKo-1 transduced with WT or C481S BTK (Mino: iii and vi; JeKo-1: ix and xii) treated with either DMSO or AUY922, respectively

**Figure S2.** HSP90 inhibition and protein kinases in MCL lines. The protein kinases whose abundance is reduced by log 2 fold $> 0.5$ in response to HSP90 inhibition with AUY922 in 80% of MCL lines tests are marked in the dendrogram of the human kinome.

**Figure S3.** Serum starvation of MAVER-1 cells or treatment with AUY922 for the indicated time and immunoblotting for the indicated targets.

**Figure S4.** Chemical structures of 17-AAG and newer-generation HSP90 inhibitors.
Figure S5. Flow cytometry of splenocytes collected from an NSG mouse 28 days after injection of primary patient MCL.

Table S1. Raw data for protein levels for over 8000 proteins for each cell line. D=DMSO; A=AUY922; Mino1 and JeKo1 refer to parental cell lines; transfected cell lines with BTK WT and BTK C481S are indicated by BTK wt and BTK CS, respectively; ptAA 2I0 = PDX treated with vehicle; ptAA 2H1=PDX treated with AUY922.

Table S2. Number of proteins in single and overlapping categories.

Table S3. MCL cell line proteins found to be altered by a log2 fold change of > 0.5 in the presence of AUY922. Proteins highlighted in yellow show disconcordance between parental and transduced lines.

Table S4. 50% inhibitory concentrations for 10 primary MCL samples treated for 72 hours with the indicated agents. Peripheral blood mononuclear cells (PBMCs) from two normal controls (Al, Se) are also included. MCL, mantle cell lymphoma; n/a, not available.

Table S5. PDX proteins found to be altered by a log2 fold change of > 0.5 in the presence of AUY922.