**Figure 1: A salt bridge in ABL1 suggests that clinical abundance may not be predicted by the amount of drug resistance conferred.**

**(A)** A schematic of factors affecting the evolutionary dynamics of drug resistance. Without transmission, intra-tumoral variables are the only factors involved in cancer evolutionary dynamics and are limited to the host level. **(B)** ABL1 crystal structure. Ribbon diagram of secondary structure is shown. Image is zoomed in on the kinase P-loop. Loss of the E225-K247 salt bridge is associated with imatinib resistance. **(C)** Prevalence of E255K/V mutations in 6 imatinib clinical trials. A cross-trial sum is included, p-val is for chi-square test. **(D)** Imatinib IC50 curves for BCR-ABL transformed BaF3 cells. Relative viability is measured by Cell-Titer Glo relative to a DMSO control. N=3 per concentration, error bars are standard deviations. **(E)** Relative growth rates of BCR-ABL BaF3 variants. Each dot is an independent transduction and selection. N=11-14, error bars are standard deviations. **(F)** Substitution-specific counts of synonymous mutations in ABL1 from the Broad Institute ExAC data and the codon structure of E255. Indicated codons highlight the mutational path.

**Figure 2: An analytical model of stochastic dynamics identifies where survival of the likeliest can occur.**

**(A)** In a theoretical drug target gene with two potential resistance alleles, Allele A is assigned a high fitness and low probability, Allele B is assigned a low fitness and high probability. **(B)** A schematic of a general timeline for mutation and outgrowth for either allele given the assigned evolutionary profiles. In cases where both mutations occur, the first resistant clone to reach detection drives relapse. **(C)** A phase plane that is modeled across many mutation rates and effective population sizes. Color indicates whether Allele A (**dark blue**) or Allele B (**red**) is more likely to drive relapse. In regions where Allele B is more dominant, we expect mutation bias to be a primary evolutionary force. **(D)** Schematic of general leukemic cell population hierarchy. Only mutations in leukemic stem cells can form stable resistance clones, effectively limiting the population size to 105-106.

**Figure 3: An analysis of epidemiologic incidences of BCR-ABL mutations**.

**(A)** A crystal structure ribbon diagram of the ABL1 kinase domain and distribution of the 19 most prevalent BCR-ABL resistance mutants. These 19 variants account for approximately 95% of resistance mutations observed clinically in the six studies from Figure 1C. **(B)** Drug-dose response was measured by Cell-Titer Glo and is plotted in the heatmap. N=3 independent infections for all 20 cell lines (WT and mutants). The range of drug resistance conferred by these mutations is plotted in the heatmap. Each BCR-ABL BaF3 line was dosed with 11 serial dilutions of imatinib in triplicate. All cell lines are ordered by sensitivity. Raw data and code are available on GitHub. **(C)** A regression model of clinical mutation prevalence based upon the growth rates in the presence and absence of imatinib only. X-error bars are 95% binomial confidence intervals of abundance counts; Y error bars are 95% CI from LOOCV in our negative binomial regression. **(D)** A table that includes IC50s (in nM) fit from the data in Figure 3B. **(E)** Observed versus predicted plots for the regression model of clinical mutation prevalence (determined by our clinical meta-analysis**)** regressed against growth rate in the presence of drug and mutational probability (which is the final model). X-error bars are 95% binomial confidence intervals of abundance counts; Y error bars are 95% CI from LOOCV in our negative binomial regression. **(F)** Observed versus predicted plots as in **3E** for independent Sanger data set.

**Supplemental Figure 1: Analysis of IC50 measurements in BCR-ABL resistance mutants across studies.**

We analyzed the IC50 of BaF3s transduced with BCR-ABL variants as reported in other studies. The diagonal of the figure shows the histogram of IC50s for each study. The correlations are reported in the off-diagonals. In general, four studies (ours, Gozgit 2013, Zabriskie 2014, and Redaelli 2012) exhibit high cross-correlation (Pearson’s rho ≥ 0.88). The Wylie 2017 study was excluded from further analysis given its low correlation with the remaining four studies. See Methods and Supplemental Table 1 for explanation of the normalization procedure.

**Supplemental Figure 2: Construction of resistance mutation prevalence model.**

A statistical model was developed to explain the variance in clinical prevalence of BCR-ABL resistance mutations. **(A)** To construct the model, we considered three potential predictor variables: imatinib IC50 (Figure 3B), growth rate in the absence of drug (Supplemental Figure 2A), and amino acid substitution probability (Figure 3D). **(B)** The amino acid prevalence assembled from our meta-analysis of multiple clinical trials was analyzed to identify the appropriate generalized linear model to use for regression. The empirical CDF of the prevalence count data was compared to theoretical CDFs given a Poisson (left) and negative binomial distribution (right). The Poisson distribution was overdispersed, while the negative binomial distribution appeared to agree with the empirical data and so a negative binomial regression model was used. **(C)** Validation of the regression model trained on our clinical meta-analysis data set. Leave-one-out cross validation was used to estimate the test error of the N-variable model with the lowest AIC for each N. The N=1 model includes substitution bias; the N=2 model includes substitution bias and IC50; the N=3 model includes substitution bias, IC50, and growth rate in the absence of drug. The N=2 model had the lowest estimated mean square error. For a complete explanation of model construction see the Figure 3 R-markdown file on GitHub. **(D)** Validation of regression model as in **(C)** for model trained on the Sanger Institute data set.

**Figure 4: A mechanistic, stochastic model of CML evolutionary dynamics.**

**(A)** Schematic of stochastic CML evolutionary dynamic model. The initial deterministic model of 3 differential equations (shaded in **blue**) is from Fassoni et al. 201835 and is fit to phase 3 clinical data. We reformulated a stochastic version of 60 differential equations parameterized from Fassoni et al. 201835 and our clinical data. Leukemic stem cells alternate between proliferating (P-LSC) and quiescent state (Q-LSC). P-LSC give rise to differentiated leukemic cells (DLC). P-LSCs may also spawn a resistant subclone P-LSCi when dividing. The allele-specific mutational probability is given by ρi. Note that we added the ability for all 19 resistance mutations to occur, such that there are 20 sets of differential equations with 3 populations per mutant. The system is solved stochastically. **(B)** An example stochastic simulation of the model described in **3A**. **(C)** Simulation results for the stochastic model without mutation bias (uniform ρi). The Pearson correlation between observed and predicted prevalences is 0.14. **(D)** Simulation results for the stochastic model with mutation bias (allele-specific ρi). The Pearson correlation is 0.78.

**Figure 5: Mutational liabilities of second-generation BCR-ABL TKIs.**

**(A)** Power curves for nilotinib and dasatinib frontline clinical trials. It depicts the power to reach an alpha of 0.05 for the comparison of the total number of resistant mutants in imatinib versus the second line inhibitor. Labeled points indicate the power of the ENESTnd (frontline nilotinib; N=282 and 1-β=0.43) and DASISION (frontline dasatinib; N=259 and 1-β=0.54) phase 3 clinical trials. The assumed effect size was estimated from our simulation results. **(B)** Experimentally measured IC50s create measured resistance profiles for imatinib, nilotinib, and dasatinib. **(C)** Mutational liabilities (defined as the sum of conditional probabilities of resistance-conferring mutations) and predicted resistance frequencies for imatinib, nilotinib, and dasatinib.

**Figure 6: An evolution-guided approach drug design is predicted to minimize resistance prevalence**

**(A)** Resistance profiles for versions of a hypothetical drug, “maxitinib”. Maxitinib K1 targets the first through fifth most likely mutants; K2 targets the second through sixth most likely; and so on. **(B)** Maxitinib simulation results. Orange bars represent the resistance incidence of each maxitinib chemotype relative to imatinib. Green points indicate mutational liability.

**Figure 7: Multiple cases of restricted heterogeneity are expected to exhibit survival of the likeliest**

**(A)** *Top*: A spatially heterogeneous tumor with mitotically active cells in the periphery and a necrotic core. The effect is a reduction in the number of cells able to spawn a resistant subclone. *Middle*: Output of a single simulation of a three-dimensional model of tumor growth. Viable cells (sensitive - **blue**; resistant - **red**) are restricted to the tumor’s periphery, while necrotic cells (**dark gray**) comprise the tumor’s core. *Bottom*: Summary of simulation results. Along the x-axis are individual simulation results (virtual patients). The colors along the vertical indicate the composition of the tumor upon relapse for each simulation. Allele A (**dark blue**) is more resistant but less likely; Allele B (**red**) is less resistant but more likely. **(B)** *Top*: Adjuvant therapy involves surgically debulking the tumor and thus restricting the effective population size. *Middle*: Simulation results for population before resection Mpre=109 and after resection Mpost=105 (*left*) and for Mpre=1012 and Mpost=108 (*right*). Points represent specific resistance variants and ρ values are the Spearman rank correlation between frequency and degree of resistance (*top*) and frequency and probability (*bottom*). *Bottom*: Summary of simulation results for various values of Mpre and Mpost. Colors represent the difference in correlation (probability ρ –resistance ρ) for each set of parameters. **(C)** *Top*: In infectious disease, transmission bottlenecks constrain the number (and thus heterogeneity) of pathogens passed from one host to another. *Middle*: Results of a single simulation of disease transmission. In this simulation, δ=.01% of the population in one host is passed on to the next during a contact event. Nodes in **blue** are patients that never exhibit resistance; nodes in **dark blue** and **red** are patients that exhibit resistance (Allele A and B, respectively). *Bottom*: Summary of infectious disease model results. The x-axis is the bottleneck size (δ) and the y-axis is the mean resistance allele frequency across 5000 simulations for each δ value.

**Figure 8: Schematic of how the process of evolutionarily-guided drug design would proceed.**

**Supplemental Figure 3: ENU mutagenesis qualitatively identifies resistance mutations but fails to quantitatively predict abundance.**

Bradeen et al. 200662 have previously used ENU mutagenesis to nominate imatinib resistance mutations in BCR-ABL. The abundance of each mutation as predicted by the observation frequency in the mutagenesis experiment is given on the x-axis. The observed abundance of each mutation from our meta-analysis of clinical trials is given on the y-axis. The results indicate some qualitative concordance of resistance phenotypes between mutants predicted in the lab and those observed clinically. However, the *in vitro* mutagenesis experiment poorly predicts the quantitative frequency of mutations observed *in vivo*.