Supplementary Materials for

Measuring competitive exclusion in non–small cell lung cancer

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Supplementary Information

Evolutionary Game Assay

To quantify the dynamics in our in vitro environments, we used the experimental game assay developed by Kaznatcheev et al. (30).

Tracking individual subclones in heterotypic cultures: To track differential growth dynamics of two populations in the same culture, each population was transduced with a vector encoding a different heritable fluorescent protein. For this experiment, the resistant and sensitive cells were made to stably express mCherry and EGFP respectively. The expression of these proteins was linked to nuclear localization signal (NLS) repeats for localization of the fluorescent signal into each cell’s nuclei. This increases resolution and accuracy of cell number counts at higher confluency. Once plated together in heterotypic culture, each subclone could be tracked through time in their respective fluorescent channel using time-lapse microscopy systems (Fig 1A).

Translating image information into growth rates: Cell number counts were extracted from each fluorescent image at each time point throughout the time series. Exponential growth rates were determined via semi-log regression of change in cell number against change in time (hours) using the Theil-sen estimator (Fig 1B).

Fitness functions - growth as a function of population composition: To find the dependence of fitness on the frequency of subclonal interaction, least squares regressions were performed on the growth rate against the initial proportion of sensitive in each well (Fig 1C). This regression was weighted against the inverse of the errors ($\frac{1}{\sigma^2}$) associated with each growth rate. The resulting linear equations describe growth ($\hat{w}_S$, $\hat{w}_R$) as a function of the initial proportion of the opposing subclone (in our case, $p$ represents the sensitive proportion of the population, or $\frac{N_S}{N_T}$, where $N_S$ is the sensitive population size and $N_T$ is the total population size):

$$\hat{w}_S = Ap + B(1 - p)$$  \hspace{1cm} (S1)

$$\hat{w}_R = Cp + D(1 - p)$$  \hspace{1cm} (S2)

Game theoretical payoff matrix: To clearly represent the fitness outcome of specific interactions, payoff matrices corresponding to each of the different conditions can be derived from the resulting fitness functions (Fig 1D). For example, the fitness outcome of sensitive cells interacting with one another occurs when $p = 1$, which translates to $\hat{w}_S = A$. Similarly, the fitness outcome of when sensitive interacts with resistant occurs when $p = 0$, which translates
to \( \hat{w}_S = B \).

\[
\begin{pmatrix}
  S \\
  R
\end{pmatrix}
\begin{pmatrix}
  A & B \\
  C & D
\end{pmatrix}
\]

(S3)

For more details on the game assay, see Kaznatcheev et al. (30) for method and Kaznatcheev (34) for interpretation.
Studying the effect of density dependence on monoculture growth rates

Our findings of frequency dependent changes in growth rate in co-culture could, in theory, have been affected by pure density dependent effects. To tease apart these two effects, we performed a monoculture experiment over a range of initial seeding densities following a 2-fold dilution pattern ranging from 3,000 cells/well to 187 cells/well.

In Supplementary Figure S1, we show that both the sensitive and resistant strains have a slight positive density-dependence to their growth rate. However, based on our game-assay data, the resistant (red) population would have to have a large negative density-dependence for density effects to be responsible for our observed game phenotype. This is because as the resistant population gets smaller (approaches a $p = 1$ fraction), we observed an increase in growth rate, the opposite of the density effect we observed. Experiments in the main draft were plated at approximately 1500 cells and thus closely match in initial and final cell counts here.

![Graph showing growth rate vs. Initial cell population](image)

**Figure S1:** Monoculture density-dependence does not explain the observed co-culture dynamics. Monoculture sensitive (blue) and resistant (red) populations were plated in two fold dilutions ranging from approximately 200 to 3,000 initial populations. These monoculture measurements indicate a slightly positive effect of density on growth for both sensitive and resistant populations.
Growth curves suggest pure exponential growth fit is insufficient in co-culture.

Here we confirm the effect of ecological dynamics on the growth of the population through an alternate method. Using a large and diverse collection of initial co-culture frequencies grown in DMSO, we fit each growth curve with an exponential that assumes the growth rates are simply what was measured in mono-culture. Put another way, we assume there are no ecological interactions Supplementary Figure S2. We can observe by eye that the exponential fit is best in the case of no ecological interactions (that is, monoculture growth). To confirm this we plot the residuals for each plot (Observed cell count at t=80h - Predicted cell count at t=80h via no ecological interactions) Supplementary Figure S3. We see the residuals are minimized in the case of monoculture experiments (initial resistant fraction of 0.0 or 1.0.) and largest in co-culture. In addition, the residuals measured are all positive, suggesting the ecological interactions are spurring greater growth than predicted by monoculture alone, which matches the positive growth effect on co-culture we observe in the main draft.

Figure S2: Monoculture growth rates are bad predictors of co-culture growth curves. Raw cell count over time (growth curves) for a collection of varied initial resistance fraction ranging from 0.0 to 1.0. Blue line on each plot represents a predicted growth curve based on monoculture growth rates (or an absence of ecological interactions).
Figure S3: Residuals are maximized under co-culture and minimized under monoculture. For each initial fraction, we plot the difference between the observed cell count at 80h and the predicted cell count at 80h assuming no ecological interactions (and thus monoculture growth rate).

**Raw data from competition experiments**

For ease of visualization, we left out the entirety of the raw data in our main text exposition. For transparency, we include a visualization of those data here. In Supplementary Figure S4, we show all the raw count data (ln-transformed) for the above density-dependence experiment. This is highly representative of data collected throughout the manuscript. In addition, we show the best fit regressions, showing the subsection of the data used in the analysis (represented by the red lines).

In Supplementary Figure S5, we then show the raw data underlying the fitted trend lines from Figure 3 of the manuscript. These raw data were excluded from the main manuscript, as to not clutter the visual with too many overlapping error bars.
Figure S4: Typical experimental growth curves for a game assay experiment. Blue dots represent raw count data plotted on a log scale. Red best-fit regressions show the range of data used in the analysis. These data in particular are taken from the monoculture density-dependence experiment leading to Figure S1.

Figure S5: Raw data underlying fitted trend lines from Figure 3. A. Raw data underlying Figure 3B trend lines at 0.25µM gefitinib. B. Raw data underlying Figure 3B trend lines at 0.50µM gefitinib. C. Raw data underlying Figure 3B trend lines at 1.00µM gefitinib. D. Raw data underlying Figure 3B trend lines at 4.00µM gefitinib.
Gene expression profiling

In order to study gene expression patterns, we plot log scale fold-change values and associated \( p \)-values in phred scale, in **Supplementary Figure S6**, which quantifies the gene expression patterns and assesses differential regulation between sensitive and resistant populations. We show that in addition to multiple possibly relevant genes, *EGFR, KRAS* and *MET* show differential regulation suggestive of possible resistance associated mechanisms as given in main text.

Figure S6: Differential gene expression analysis showing down/up regulation of genes in KEGG NSCLC pathway. Utilizing *edgeR* we normalized the raw counts by trimmed mean of m-values (TMM) and quantified statistical differences between sensitive and resistant populations where *EGFR* showed decreased expression in-contrast *KRAS, MET* genes showed increased expression.