Supplementary Note 1. Definition of the Antagonistic Pleiotropy Index (API)

1 Problem Statement

Given a stack of n cards, each colored red (x cards), blue (y cards), or green (z cards), where $x + y + z = n$, what is the average number of draws without replacement before you draw a red and a blue? Can we produce a generalized solution for this problem as a function of x, y, and z?

2 Problem solution

Let D denote the random variable representing the number of draws it takes before we get one red card and one blue card. We want to calculate the average number of draws, or mathematically speaking, the expected value of D:

$$\mathbb{E}[D] = \sum_{k=1}^{n} k * \mathbb{P}(D = k).$$

Note that we need to draw at least two cards to get the desired result, so we do not have to consider $k = 1$. Further, we can assume without loss of generality that $x \leq y$, since the values can simply be switched otherwise. Then the largest number of draws we can have to get our result is $z + y + 1$, ie, the scenario in which every green card and blue card is drawn before we draw the first red card. Hence we can rewrite our expected value of D as

$$\mathbb{E}[D] = \sum_{k=2}^{z+y+1} k * \mathbb{P}(D = k).$$

The bulk of this problem then is to find the probability distribution on D. The best way to calculate this probability is by method of combinatorics - that is, calculating frequency probabilities by counting the total number of ways we get a desired outcome and divide by the total number of possible outcomes.

Assume for now that we have a k such that $k - 1 \geq x, y, z$. Additionally, consider specifically the case where we want to know the probability that we pull only red and green

cards before we pull a blue card on the k^{th} draw. We know that in the first $k-1$ cards, we must pull at least one red card, since in the general problem, we stop when we pull at least one red and one blue. We can pull up to $k-1$ red cards, since we have the assumption that $x \leq k-1$. Thus we must account for every possible number of red cards being pulled, $j = 1, ..., k-1$, and every possible drawing order for which this can occur. So for any fixed j, there are $\binom{k-1}{j}$ ways that in a drawing of cards, j are red. For the rest of the formula, since order matters in card drawings, we use the formula of permutations, which we denote

$$^nP_k = \frac{n!}{(n-k)!}.$$

We use permutations to caculate the probability that j red cards and $k-1-j$ green cards are drawn in the first $k-1$ drawings from a deck of n cards. Finally, the probability that a blue card is pulled on the k^{th} draw is $\frac{y}{n-k+1}$. Putting all these facts together we get that the probability of drawing all green cards and red cards for the first $k-1$ draws and then drawing a blue card is:

$$\sum_{j=1}^{k-1} \binom{k-1}{j} \frac{(^xP_j) * (^zP_{k-1-j})}{(^nP_{n-k+1})} * \frac{y}{n-k+1}.$$

Simplifying gives

$$\sum_{j=1}^{k-1} \binom{k-1}{j} \frac{(^xP_j) * (^zP_{k-1-j}) * y}{(^nP_{n-k})}.$$

Note that by symmetry, we get a similar probability formula for drawing only blue and green cards before finally drawing a red card on the k^{th} draw:

$$\sum_{j=1}^{k-1} \binom{k-1}{j} \frac{(^yP_j) * (^zP_{k-1-j}) * x}{(^nP_{n-k})}.$$

Hence, when $k-1 \geq x, y, z$, we can add these two probabilities and simplify to get a total probability of

$$\mathbb{P}(D=k) = \frac{1}{(^nP_{n-k})} \sum_{j=1}^{k-1} \binom{k-1}{j} (^zP_{k-1-j})(y * (^xP_j) + x * (^yP_j)).$$

Note we now have to consider possible scenarios for when $k-1 > x$, or $k-1 > y$, or $k-1 > z$. This can easily be resolved by inserting indicator functions $\mathbb{I}(\cdot)$, which will prevent the formula for accidentally adding in scenarios that are numerically impossible. Note that we only need them within the sum, since these scenarios are already not counted in the total possible scenarios. Hence the final formula for all k is

$$\mathbb{P}(D=k) = \frac{1}{(^nP_{n-k})} \sum_{j=1}^{k-1} \binom{k-1}{j} \mathbb{I}_{[z \geq k-1-j]} (^zP_{k-1-j})(y * (^xP_j)\mathbb{I}_{[x \geq j]} + x * (^yP_j)\mathbb{I}_{[y \geq j]}).$$

Supplementary Note 2. Validation of the API

To validate the API, we reanalyzed a published set of full-genome CRISPR/Cas9 screens conducted in 14 AML cell lines [71]. 644/18663 genes in this dataset, corresponding to 3.45% of the library, identified as AP. Next, we reasoned that if this measure of AP is able to pull out meaningful biological relationships, then strong AP genes playing cooperative roles (e.g. members of the same complex, nodes in a common pathway) should behave similarly across cell lines. To test this, we performed gene ontology analysis on the top 15% of AP genes, ranked by API (**Supplementary Fig. 1a**). Not only were the identified ontologies strongly enriched, they could also be traced back to coherent, gene-level relationships (**Supplementary Fig. 1b-f**). Similarly, we also reanalyzed a large series of RNAi-based essentiality screens performed in 398 cancer cell lines representing 20 tumor lineages [72]. After excluding genes with missing data, 6556 out of 6557 remaining genes were identified as AP, representing nearly the entire library (**Supplementary Fig. 1g**). Here too, gene ontology analysis performed on the top 15% of AP genes was able to pull out distinct ontologies, some of which have previously been shown to exhibit context-specific, pro- and anti-tumorigenic properties (**Supplementary Fig. 1h-j**).

The discrepancy in the prevalence of AP between these datasets is likely due to compositional differences in the datasets. Examining these differences can teach us how the API behaves in response to varied inputs. First, the API is sensitive to the number of contexts considered. Datasets with more contexts provide genes with more opportunities to declare themselves as AP, producing lower APIs and more AP genes (**Supplementary Fig. 1k**). Second, analyzing diverse contexts is more likely to identify AP genes than analyzing similar contexts. This point is supported by subanalysis of the study by McDonald et al. which shows that analyzing lineage-specific cohorts, opposed to cell lines selected at random, always identifies fewer AP genes (**Supplementary Fig. 1k**). Third, studies performed with curated libraries comprised of well-studied, annotated genes are more likely to be enriched for genes that are able to significantly impact cellular fitness, producing lower APIs. Finally, the strength of the API depends on the type of input; data from noisy techniques will tend to overcall genes as AP. On the basis of these considerations, it stands to reason that the study by Wang et al., which screened 14 AML cell lines with a full-genome CRISPR library, identified drastically fewer AP genes than the study by McDonald et al., which screened 398 cell line contexts across multiple distinct tissue lineages using a customized 6557-gene RNAi library.

Supplementary Note 3. KDM1A anchors a differentiation-based AP paradigm

The broad AP associations between decitabine, azacitidine, and ABT-199 described in the main text can be dissected to better understand the mechanisms that govern AP in these drug treatment contexts. Azacitidine and decitabine share H3K4 histone demethylase LSD1 (encoded by KDM1A) and its positive regulators, repressor element-1 silencing transcription factor (REST) corepressor 1 and 2 (RCOR1 and RCOR2) as sensitizers (**Fig. 2c**, **Supplementary Fig. 3a**) [73, 74]. REST corepressor 3 (RCOR3), which inhibits the methyltransferase activity of LSD1, scored as a resister to both drugs (**Fig. 2c**, **Supplementary Fig. 3a**). Conversely, our screens identified loss of LSD1, RCOR1 and RCOR2 as promoting resistance to cytarabine and ABT-199 while the loss of RCOR3 promoted sensitivity to cytarabine and ABT-199. This reciprocity is defining of an AP paradigm. To test this, we generated CRISPR-mediated LSD1-knockout derivatives (**Supplementary Fig. 3b**). LSD1-knockout cells were more sensitive to azacitidine and more resistant to cytarabine and ABT-199 (**Fig. 2d-f**), validating the AP character of LSD1. Consistent with their resistance to ABT-199, LSD1-knockout cells were also found to be less BCL-2 primed by BH3 profiling (**Supplementary Fig. 3c**). Intriguingly, we found that loss of LSD1 resulted in upregulation of the myeloid differentiation

marker CD11b (**Supplementary Fig. 3d,e**), consonant with the known roles of LSD1 in stem cell maintenance and the ability of LSD1 inhibitors to induce differentiation [75-79]. Broadly, this finding suggests that cellular differentiation is a continuum that may modify sensitivity to ABT-199, azacitidine, and cytarabine (**Supplementary Fig. 3f-i**) [80]. Further, it also suggests that the clinical efficacy of combining azacitidine and ABT-199 may be in part attributed to their non-overlapping sensitivity modifier profiles.

Supplementary Note-only references

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