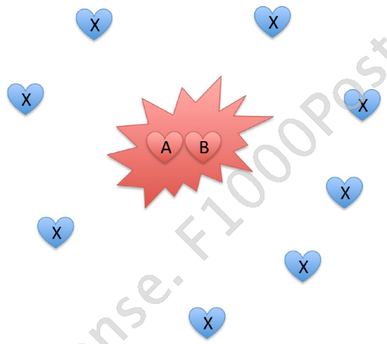
Systematic Identification of Synergistic Drug Pairs Targeting HIV



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Introduction

HIV has plagued humans for 30 years, infecting 60 million people and causing over 25 million deaths. Currently, effective cure for AIDS is expensive and suffers from serious side effects, such as lipodystrophy, hyperglycemia, pancreatitis and liver toxicity. Major benefits of combination therapy include significantly-reduced chance of evolving drug resistance, enhanced efficacy and reduced side effects. Systematic searches for synergistic drug combinations have been performed in small scale or with proprietary libraries, but there has not been a large-scale systematic screening of FDA approved drugs. Here we developed a pooled screening method named *MuSIC* (*Mu*ltiplex *S*creen for *I*nteracting *C*ompounds).

Experimental Methods

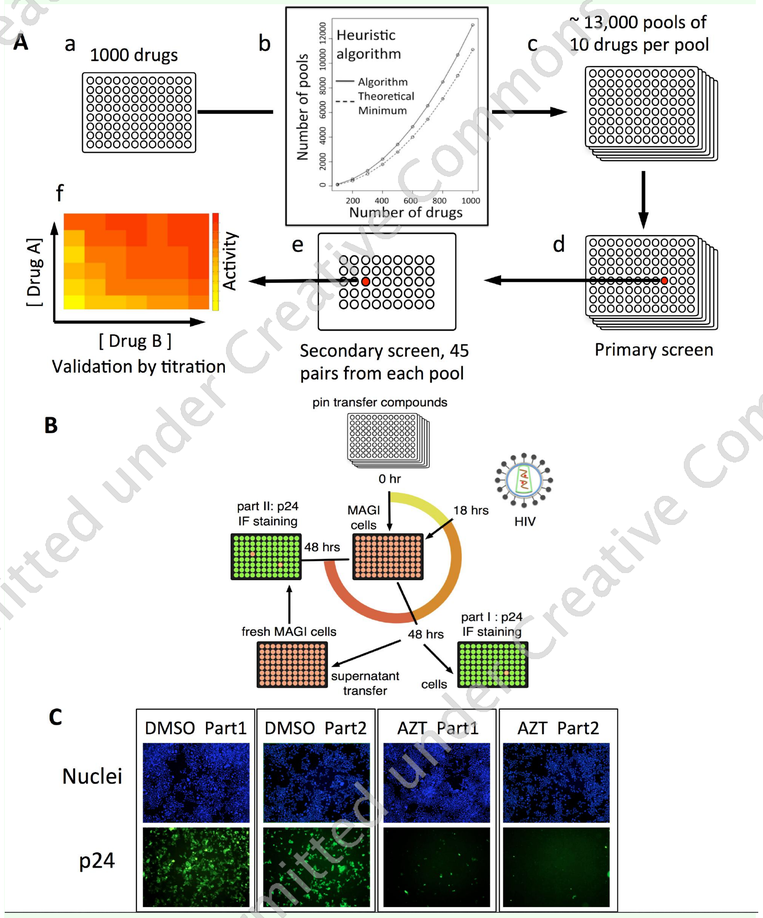


Figure 1. *MuSIC* strategy and screening assay.
(A). Protocol of the *MuSIC* screen. a,b: 1000 drugs are constructed into a pooled library with 10 drugs per well using a heuristic algorithm to ensure every pair-wise interaction is represented; c: primary screen using the pooled drug library; d: hits from primary screen are deconvoluted into pairs to construct the secondary library; e: deconvolution screen of the secondary library screen; f: hits from secondary library are validated using concentration titrations of the two drugs.
(B) The screen assay protocol: for the part one assay, cells are plated on 384-well plates overnight before drug treatment. HIV is added to the cells 18 hours after drug treatment to allow the drugs to take effect (MOI = ~0.5). Forty-eight hours after adding virus, the cells are immunostained for HIV p24 expression and imaged to quantify the percentage of cells with positive staining, indicating the infection rate. The supernatant from the part one assay is transferred to new plates with fresh cells to initiate the part two assay for quantification of newly generated virus. Forty-eight hours later, the part two plates are also stained and imaged.
(C) Part one and part two staining images of positive control (AZT) and negative control (nevirapine) used in the screen. Top row: DAPI staining of cell nuclei for the quantitation of cell number and monitoring cytotoxicity. Bottom row: p24 staining of HIV infected cells.

Abstract

Combination drug therapies play important roles in treating diseases such as cancer and AIDS. However, systematic identification of effective drug combinations has been hindered by the large combinatorial search space of interactions. Here we develop a multiplex screening method, *MuSIC* (*Mu*ltiplex *S*creening for *I*nteracting *C*ompounds), which expedites comprehensive assessment of pair-wise interactions for 1000 FDA-approved or clinically tested drugs. In this way we examined ~500,000 drug pairs and identified drugs that synergize to inhibit HIV replication. Multiple drug pairs, notably glucocorticoid and nitazoxanide, synergize by targeting different steps of the HIV life cycle. Our analysis also reveals an enrichment of anti-inflammatory drugs, i.e. glucocorticoids and NSAIDs, in the anti-HIV drug combinations. As inflammation accompanies HIV infection, our findings suggest that HIV may benefit from inflammation and inhibiting inflammation might combat HIV propagation. The *MuSIC* method is robust and can be widely applied to other disease-relevant screens to facilitate drug repurposing.

Computational Methods

- Normalize raw data by B score:

$$\text{B score} = \frac{r_{ijp}}{MAD_p}$$

r_{ijp} is the pure effect after remove the side effect and average of the plate:

$$r_{ijp} = y_{ijp} - (u_p + R_{ip} + C_{jp})$$

Y_{ijp} is the observed result; u_p is the estimated average of the plate;

R_{ip} is the estimated systematic measurement offset for row i on plate p

C_{jp} is the estimated systematic measurement column offset for column j on plate p

$$MAD_p = \text{median}\{r_{ijp} - \text{median}(r_{ijp})\}$$

- Calculate the synergistic score by S score:

$$S = \frac{u_{AB} - u_{con}}{\sqrt[2]{s_{var} \times (\frac{1}{n_{AB}} + \frac{1}{n_{con}})}}$$

Where:

u_{AB} is an estimate of W_{AB} = mean infection rate of replicates of drug pair AB
n_{AB} is number of replicates of the interested pair = 3 (typically)
u_{con} is an estimate of $W_A + W_B = u_{AX} + u_{BX} - u_{XX}$

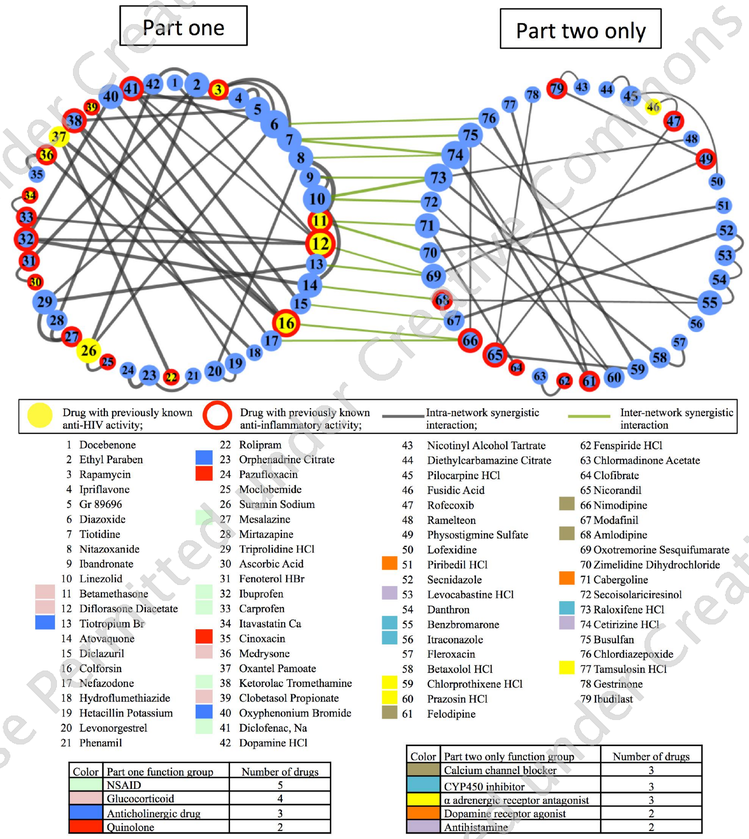
u_{AX} = trimmed mean of replicates of drug pairs of Drug A and any Drug X
u_{BX} = trimmed mean of replicates of drug pairs of Drug B and any Drug X
u_{XX} = trimmed mean of replicates of drug pairs of Drug X and Drug X
'trimmed mean' is the mean of the data excluding the top and bottom 20% to eliminate outliers

$$n_{con} = n_{AX} + n_{BX} + n_{XX}$$
$$s_{var} = \left(v_{AB} \times (n_{AB} - 1) + v_{con} \times (n_{con} - 1)\right) \div (n_{AB} + n_{con} - 2)$$

Where:

$$v_{con} = \frac{v_{AX} \times (n_{AX} - 1) + v_{BX} \times (n_{BX} - 1) + v_{XX} \times (n_{XX} - 1)}{n_{AX} + n_{BX} + n_{XX} - 3}$$

Results



Color	Part one function group	Number of drugs
	NSAID	5
	Glucocorticoid	4
	Anticholinergic drug	3
	Quinolone	2

Color	Part two only function group	Number of drugs
	Calcium channel blocker	3
	CYP450 inhibitor	3
	α adrenergic receptor antagonist	3
	Dopamine receptor agonist	2
	Antihistamine	2

Figure 2. Drug synergy network analysis reveals enrichments of drugs with known anti-HIV activity and anti-inflammatory functions.
The network of drug synergy shows drug pairs that have significant anti-viral activity and synergy (see supplemental text for the details on how the drugs were selected). Each drug is depicted by a circle with its size correlating with the number of drugs it has synergy with. Yellow circles indicates compounds with previously detected anti-HIV activity, red outer circles indicate known anti-inflammatory function. The part one network is highly enriched for drugs with previously detected anti-HIV activity ($p < 10^{-12}$) and drugs with known anti-inflammatory activity ($p < 10^{-4}$). The number in the circle is the index of the drug with the drug name shown in the list below. The line linking two drugs indicates synergy with the width of the line correlating with the strength of the synergy, the wider the line, the stronger the synergy. The green lines linking the two networks represent synergistic interactions between the two networks. The color blocks designate the functional groups that have more than one drug represented in each network. The names of the functional groups and the number of drugs belonging to each functional group are shown in the two tables below.

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