SUPPLEMENTARY DATA

Novel Cell-Killing Mechanisms of Hydroxyurea and the Implication Towards Combination Therapy for the Treatment of Fungal Infections

Amanpreet Singh, Ameeta Agarwal, and Yong-jie Xu

Strain	Genotype	Source
YJ150	h ⁻ leu1-32 ura4-D18 ade6-M210 [LEU2 vector]	Lab stock
YJ1261	h ⁻ leu1-32 ura4-D18 ade6 [prom-Erg11-term-LEU2]	Lab stock
YJ760	h ⁻ ∆rad3::ura4 leu1-32 ura4-D18 ade6 [LEU2 vector]	Lab stock
APS184	h^+ $\Delta rad3$:: $ura4$ leu1-32 $ura4$ -D18 $ade6$ [prom-Erg11-term-LEU2]	This study
APS31	h? hem13-1 cds1-6his2HA leu1-32 ura4-D18 ade6 [LEU2 vector]	This Study
APS171	h? hem13-1 cds1-6his2HA leu1-32 ura4-D18 ade6 [prom-erg11-term-LEU2]	This Study
YJ1291	h ⁺ erg11-1:ura4 cds1-6his2HA leu1-32 ura4-D18 ade6 [LEU2 vector]	Lab stock
YJ1230	h ⁺ erg11-1:ura4 cds1-6his2HA leu1-32 ura4-D18 ade6 [prom-Erg11-term-LEU2]	Lab stock
SC5314	Wild-type C. albicans strain	Heitman lab
W303	<i>MATα leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15</i>	Lab stock

Table S1. List of yeast strains used in this study.

Figure S1. The dose-dependent cytotoxic effects of HU, sampangine (SMP) and the indicated antifungal drugs on wild type *S. pombe*, *S. cerevisiae* and *C. albicans* measured by the 96-well plate assay. Logarithmically growing wild type *S. pombe* (A), *S. cerevisiae* (B) and *C. albicans* (C) cells were inoculated on 96 well plates at 3000 cells/well in YE6S, YPD and SDB medium, respectively. The drugs were added to each well at the indicated concentrations in the final volume of 200 μ l. The same amounts of carriers were added as control. Cells were incubated at 30°C for 48 days. The plates were scanned in a plate reader at A600. The graphs were made from values obtained from the plate reader using Microsoft excel. Each data points are the averages of readings of three separate wells.



Figure S2. The synergistic cytotoxic effect of HU and clotrimazole in *S. pombe.* (A) Cell survival rate and the combination index (CI) values of the drug combinations 1 to 3 were determined by the 96-well plate assay and calculated using Chou-Talalay method as described in Materials and Methods. Numbers marked in bold indicate very strong synergism. (B) Graphical representation of cell-killing effect of HU, clotrimazole and various combinations of the two drugs. (C) Combination index plot. Fa values on the X-axis are the values of the cell growth inhibition at various CI values. CI values above the dash line, on the dash line, and below the dash line represent antagonistic, additive, and synergistic effect, respectively.

	HU	Clot	Cell	CI
	(mM)	(µM)	Survival	Values
	5	0	0.0647	-
2	7.5	0	0.635	-
	10	0	0.632	-
	0	0.014	0.652	-
	0	0.020	0.651	-
Ś	0	0.028	0.641	-
<i>,</i>	0	0.034	0.638	-
	0	0.043	0.614	-
	5	0.014	0.646	1.677
-	5	0.020	0.635	1.272
2	5	0.028	0.621	0.932
2	5	0.034	0.612	0.766
	5	0.043	0.378	5.43E-4
	7.5	0.014	0.579	0.160
	7.5	0.020	0.464	4.18E-3
2	7.5	0.028	0.239	2.51E-6
5	7.5	0.034	0.19	3.36E-7
	7.5	0.043	0.12	7.1E-9
	10	0.014	0.318	2.56E-5
)	10	0.020	0.21	5.04E-7
2	10	0.028	0.146	2.6E-8
)	10	0.034	0.126	8.7E-9
	10	0.043	0.107	2.7E-9



Α.

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А					В
NH	HU (mM) 1.5 3 5	Keto (μΜ) 0 0	Cell Survival 0.67 0.66 0.65	CI Values - -	120 HU 100 © 80
Keto	0 0 0 0 0	1.8 3.7 5.6 7.5 9.4	0.72 0.71 0.7 0.69 0.68	- - - - -	
Combo 1	1.5 1.5 1.5 1.5 1.5	1.8 3.7 5.6 7.5 9.4	0.616 0.216 0.089 0.079 0.083	0.055 7.3E-9 1E-11 1E-11 1E-11	C.
Combo 2	3 3 3 3 3	1.8 3.7 5.6 7.5 9.4	0.09 0.044 0.07 0.059 0.052	1E-11 1E-11 1E-11 1E-11 1E-11 1E-11	(C) Xapu Antagonism Lu 1
Combo 3	5 5 5 5 5	1.8 3.7 5.6 7.5 9.4	0.097 0.062 0.051 0.042 0.038	1E-11 1E-11 1E-11 1E-11 1E-11 1E-11	o Fa 1

Figure S3. The synergistic cytotoxic effect of HU and ketoconazole in wild type *S. pombe.* See Figure S2 for detailed description.

	HU	Fluco	Cell	CI	¹²⁰ Fluco
	(mM)	(µM)	Survival	Values	100
	5	0	0.629	-	
Η	7.5	0	0.628	-	
	10	0	0.627	-	
	0	9.7	0.63	-	
0	0	13.0	0.62	-	S 40 -
Fuc	0	16.3	0.619	-	
	0	19.5	0.618	-	20
	0	22.8	0.615	-	0 3
-	5	9.7	0.62	0.653	Increasing Drug Conc.
po	5	13.0	0.4	1.33E-6	5 5
m	5	16.3	0.31	4.7E-9	C
Ŭ	5	19.5	0.22	1E-11	
	5	22.8	0.14	1E-11	2
	7.5	9.7	0.15	1E-11	
0	7.5	13.0	0.1	1E-11	$\overline{\mathbf{U}}$
ž	7.5	16.3	0.07	1E-11	a O Antagonism
Ů	7.5	19.5	0.07	1E-11	
	7.5	22.8	0.07	1E-11	
~	10	9.7	0.11	1E-11	
õ	10	13.0	0.08	1E-11	de l
mt	10	16.3	0.06	1E-11	Ő
Ö	10	19.5	0.05	1E-11	
	10	22.8	0.05	1E-11	0 Fa 1

Figure S4. The synergistic cytotoxic effect of HU and fluconazole in wild type S. pombe.

В

А					В
	HU	Terb	Cell	Cl	- 120
	(mM)	(µM)	Survival	values	100
_	1.5	0	0.654	-	
Ч	3	0	0.643	-	80
	5	0	0.632	-	
	0	0.045	0.652	-	
0	0	0.060	0.648	-	
erk	0	0.068	0.646	-	
-	0	0.076	0.645	-	
	0	0.091	0.644	-	
	1.5	0.045	0.635	0.595	Increasing Drug Conc.
0	1.5	0.060	0.565	9.76E-3	
nbc	1.5	0.068	0.516	7.31E-4	C
20	1.5	0.076	0.445	1.91E-5	L
0	1.5	0.091	0.195	1E-11	2
	3	0.045	0.0414	1E-11	-
0 2	3	0.060	0.0429	1E-11	
nbe	3	0.068	0.1013	1E-11	ex
Q	3	0.076	0.0728	1E-11	Antagonism
Ŭ	3	0.091	0.0709	1E-11	
	5	0.045	0.073	1E-11	Synergism
0 3	5	0.060	0.06	1E-11	iq o
nbí	5	0.068	0.077	1E-11	G
Cor	5	0.076	0.065	1E-11	$\tilde{\mathbf{v}}$
0	5	0.091	0.056	1E-11	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
			1		- Гd

Figure S5. The synergistic cytotoxic effect of HU and terbinafine in wild type S. pombe.

Figure S6. The synergistic cytotoxic effect of HU and Sampangine in wild type *S. cerevisiae*. The experiment was carried out similar to that in *S. pombe* as described in Figure S2.

В

A				
	HU	SMP	Cell	CI
	(mM)	(µM)	Survival	Values
	100	0	0.435	-
	150	0	0.42	-
H	200	0	0.345	-
	225	0	0.335	-
	250	0	0.17	-
	0	4.0	0.62	-
0	0	8.0	0.595	-
N S	0	17.0	0.53	-
01	0	25.0	0.51	-
	0	35.0	0.36	-
	100	4.0	0.445	1.020
01	100	8.0	0.43	1.083
gm	100	17.0	0.37	0.948
0	100	25.0	0.08	0.120
	100	35.0	0.005	0.009
	150	4.0	0.425	1.301
0 2	150	8.0	0.38	1.129
qu	150	17.0	0.24	0.600
<u>b</u>	150	25.0	0.001	0.003
-	150	35.0	0.001	0.003
	200	4.0	0.31	1.060
03	200	8.0	0.26	0.854
qm	200	17.0	0.135	0.407
S	200	25.0	0.0001	0.001
	200	35.0	0.0001	0.001
	225	4.0	0.305	1.164
004	225	8.0	0.33	1.323
mp	225	17.0	0.075	0.252
ů	225	25.0	0.0001	0.001
	225	35.0	0.0001	0.001
10	250	4.0	0.2	0.772
200	250	8.0	0.17	0.647
m¢	250	17.0	0.03	0.118
S	250	25.0	0.0001	0.001
	250	35.0	0.0001	0.001



Increasing Drug Conc.

С



А					В		
	HU	Terb	Cell	CI			
	(mM)	(µM)	Survival	Values		100	
	50	0	0.64	_			
	75	0	0.6	-		80	
F	100	0	0.55	-	%)	60	
	150	0	0.49	-	ival	00	1
	200	0	0.41	-	urv	40	
	0	3.0	0.72	-	ell S		
0	0	6.0	0.75	-	U	20	1
Tert	0	9.0	0.69	-			
•	0	12.0	0.69	-		0	
	0	15.0	0.67	-	_		Increas
	50	3.0	0.5	0.389			
Ő	50	6.0	0.48	0.345			
mk	50	9.0	0.46	0.306			
U	50	12.0	0.43	0.255	C		
	50	15.0	0.44	0.272			
	75	3.0	0.48	0.515		2	ŀ
0	75	6.0	0.41	0.336			
mk	75	9.0	0.38	0.278	Ð		
Ö	75	12.0	0.41	0.336) Xe	-	
	75	15.0	0.33	0.200	. pu		Antagonism
~	100	3.0	0.42	0.476	on	1	
00	100	6.0	0.39	0.395	lati		Synergism
ank M	100	9.0	0.39	0.395	bir		
S	100	12.0	0.36	0.326	omo		୦୦ଁବୃତ୍ତି
	100	15.0	0.35	0.305	. 0	0	00° 0°
	150	3.0	0.38	0.556		0	0
04	150	6.0	0.33	0.400			
a de	150	9.0	0.31	0.348			
S	150	12.0	0.24	0.205			
	150	15.0	0.2	0.144			
	200	3.0	0.32	0.498			
0 5	200	6.0	0.27	0.347			
dm	200	9.0	0.19	0.175			
C	200	12.0	0.16	0.128			
	200	15.0	0.14	0.101			

Figure S7. The synergistic cytotoxic effect of HU and terbinafine in wild type S. cerevisiae.

Terb

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Increasing Drug Conc.

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↓ HU

А					В
	HU (mM)	ltra (µM)	Cell Survival	CI Values	100
ΟH	50 75 100 150 200	0 0 0 0 0	0.64 0.6 0.59 0.5 0.45	- - - -	HU HU HU HU HU HU HU HU HU HU HU HU HU H
ltra	0 0 0 0 0	1.0 2.0 4.0 5.0 7.0	0.61 0.55 0.46 0.47 0.47	- - - -	20 20 0 10 10 20 10 20 10 20 10 20 10 20 10 20 10 20 10 20 10 20 10 10 10 10 10 10 10 10 10 1
Combo 1	50 50 50 50 50	1.0 2.0 4.0 5.0 7.0	0.27 0.14 0.09 0.08 0.06	0.082 0.019 0.008 0.007 0.004	C
Combo 2	75 75 75 75 75 75	1.0 2.0 4.0 5.0 7.0	0.15 0.1 0.07 0.05 0.04	0.026 0.012 0.006 0.003 0.002	2. () Xa
Combo 3	100 100 100 100 100	1.0 2.0 4.0 5.0 7.0	0.14 0.11 0.07 0.05 0.04	0.029 0.019 0.008 0.004 0.002	Antagonism L 1 Synergism L O L O L O L O L O L O L O L O
Combo 4	150 150 150 150 150	1.0 2.0 4.0 5.0 7.0	0.12 0.1 0.07 0.05 0.03	0.030 0.022 0.011 0.006 0.002	0
Combo 5	200 200 200 200	1.0 2.0 4.0 5.0	0.1 0.09 0.07 0.04	0.028 0.023 0.014 0.005	

Figure S8. The synergistic cytotoxic effect of HU and itraconazole in wild type S. cerevisiae.

200

7.0

0.02

0.001

А					В
	HU (mM)	Keto (µM)	Cell Survival	CI Values	100 Keto
Η	50 75 100 150 200	0 0 0 0 0	0.67 0.61 0.57 0.53 0.44	- - - -	Cell Survival (%)
Keto	0 0 0 0 0	1.8 3.7 5.6 7.5 9.4	0.68 0.67 0.59 0.58 0.50	- - - -	20 4 5 Increasing Drug Conc.
Combo 1	50 50 50 50 50 50	1.8 3.7 5.6 7.5 9.4	0.32 0.16 0.12 0.09 0.08	0.126 0.031 0.019 0.011 0.010	С
Combo 2	75 75 75 75 75 75	1.8 3.7 5.6 7.5 9.4	0.25 0.14 0.1 0.07 0.06	0.099 0.033 0.018 0.010 0.008	2 (T) xəp Antagonism
Combo 3	100 100 100 100 100	1.8 3.7 5.6 7.5 9.4	0.2 0.14 0.09 0.07 0.04	0.080 0.042 0.019 0.012 0.005	Comparison Comparison
Combo 4	150 150 150 150 150	1.8 3.7 5.6 7.5 9.4	0.25 0.14 0.1 0.07 0.05	0.185 0.061 0.034 0.018 0.010	0 Fa
Combo 5	200 200 200 200	1.8 3.7 5.6 7.5	0.24 0.13 0.09 0.06	0.223 0.069 0.036 0.018	

Figure S9. The synergistic cytotoxic effect of HU and ketoconazole in wild type S. cerevisiae.

200

9.4

0.03

0.005

1

Figure S10. The synergistic cell-killing effect of HU and clotrimazole on wild type S. *cerevisiae*. The experiment was carried out similar to that in S. *pombe* as described in Figure S2. D is the time course study of the combination of 200 mM HU and 14 μ M clotrimazole when

used alone or in combination (HU+Clot).

	HU	Clot	Cell	CI
	(mM)	(µM)	Survival	Values
	50	0	0.645	-
_	75	0	0.605	-
Ŧ	100	0	0.56	-
	150	0	0.515	-
	200	0	0.415	-
	0	2.0	0.59	-
Ļ	0	5.0	0.55	-
8	0	8.0	0.55	-
	0	11.0	0.575	-
	0	14.0	0.55	-
	50	2.0	0.335	0.123
0	50	5.0	0.285	0.085
gm	50	8.0	0.305	0.099
ပိ	50	11.0	0.29	0.089
	50	14.0	0.245	0.062
	75	2.0	0.29	0.133
õ	75	5.0	0.29	0.133
and the second	75	8.0	0.295	0.138
Ŭ	75 75	11.0	0.27	0.114
	75	14.0	0.26	0.105
m	100	2.0	0.285	0.171
8	100	5.0	0.25	0.129
mc	100	8.0	0.195	0.078
Ŭ	100	14.0	0.2	0.082
	100		0.15	0.075
4	150	2.0	0.26	0.211
q	150	5.0 9.0	0.2	0.124
Mo	150	11 0	0.10	0.081
0	150	14.0	0.165	0.086
	200	20	0.10	0 1 2 5
Ъ	200	2.0 5 0	0.10	0.135
q	200	8.0	0.145	0.090
on	200	11.0	0.12	0.064
0	200	14.0	0.125	0.069



А

Figure S11. The synergistic cytotoxic effect of HU and fluconazole in wild type S. cerevisiae. D is the time course study of the combination of 200 mM HU and 16.3 μ M fluconazole when used alone or in combination (HU+Fluco).

А					В		
ОН	HU (mM) 50 75 100 150 200 0 0	Fluco (μM) 0 0 0 0 0 3.2 6.5	Cell Survival 0.64 0.57 0.52 0.45 0.73 0.72	CI Values - - - - - - - -	Cell Survival (%)	100 · 80 · 60 · 40 ·	
Fuce	0 0 0	9.7 13.0 16.3	0.69 0.64 0.59		C C	0	l Increasin
Combo 1	50 50 50 50 50	3.2 6.5 9.7 13.0 16.3	0.53 0.44 0.27 0.13 0.09	0.456 0.262 0.067 0.013 0.006	x (Cl)	2	-
Combo 2	75 75 75 75 75	3.2 6.5 9.7 13.0 16.3	0.54 0.43 0.35 0.16 0.13	0.697 0.338 0.192 0.029 0.019	ombination Inde	1	Antagonism Synergism o Q & &
Combo 3	100 100 100 100 100	3.2 6.5 9.7 13.0 16.3	0.54 0.42 0.28 0.15 0.11	0.902 0.403 0.135 0.033 0.018	D	0	<u>. යුතුල ලි</u> 0
Combo 4	150 150 150 150 150	3.2 6.5 9.7 13.0 16.3	0.48 0.42 0.32 0.2 0.11	0.857 0.581 0.277 0.091 0.026	urvival (%)	100 - 80 - 60 -	FI
Combo 5	200 200 200 200 200 200	3.2 6.5 9.7 13.0 16.3	0.43 0.38 0.31 0.15 0.08	0.792 0.566 0.334 0.065 0.019	Cell S	40 - 20 - 0 - 0	8 16



Figure S12. Time course study of the synergistic cytotoxic effects of HU in combination with SMP, itraconazole or ketoconazole in *S. cerevisiae*. Logarithmically growing wild type *S. cerevisiae* was inoculated in YPD medium on 96 well plates at 3000 cells/well. 250 mM HU and 35 μ M SMP (A), 200 mM HU and 7 μ M itraconazole (B), or 200 mM HU and 9.4 μ M ketoconazole (C) were added to the cell cultures either alone or in drug combinations. Plates were incubated at 30°C and scanned at the indicated time points.



Figure S13. The synergistic cell-killing effect of HU and itraconazole on wild type C. *albicans*. D is the time course study of the combination of 200 mM HU and 7 μ M itraconazole when used alone or in combination.



Figure S14. The synergistic cytotoxic effect of HU and clotrimazole on wild type C.

albicans. D is the time course study of the combination of 200 mM HU and 14 μ M clotrimazole when used alone or in combination.



Figure S15. The synergistic cell-killing effect of HU and terbinafine on wild type C.

albicans. The experiment was carried out similar to that in S. pombe as described in Figure S2. D is the time course study of the combination of 200 mM HU and 15 μ M terbinafine when used alone or in combination.

A					В
	HU (mM)	Terb (µM)	Cell Survival	CI Values	100 Terb
Η	50 75 100 150 200	0 0 0 0	0.61 0.545 0.53 0.435 0.36	- - - -	HU HU HU HU HU HU HU
Terb	0 0 0 0	3.0 6.0 9.0 12.0 15.0	0.66 0.425 0.37 0.255 0.175	- - -	o Increasing Drug Conc.
Combo 1	50 50 50 50 50	3.0 6.0 9.0 12.0 15.0	0.575 0.465 0.305 0.21 0.105	1.507 1.471 1.098 0.931 0.593	2 xap
Combo 2	75 75 75 75 75	3.0 6.0 9.0 12.0 15.0	0.55 0.465 0.255 0.145 0.055	1.682 1.677 0.944 0.666 0.345	opting intergenism intergeni
Combo 3	100 100 100 100 100	3.0 6.0 9.0 12.0 15.0	0.44 0.235 0.12 0.06 0.045	1.205 0.669 0.445 0.306 0.296	$0 \stackrel{ }{\frown} \cdot \cdot$
Combo 4	150 150 150 150 150	3.0 6.0 9.0 12.0 15.0	0.425 0.22 0.14 0.055 0.025	1.453 0.703 0.558 0.293 0.185	80 (%) Ferb
Combo 5	200 200 200 200 200	3.0 6.0 9.0 12.0 15.0	0.235 0.12 0.065 0.025 0.005	0.624 0.380 0.277 0.153 0.052	\overline{U}

Figure S16. The significant synergistic cell-killing effect of Sampangine and clotrimazole on wild type C. albicans. The experiment was carried out similar to that in S. pombe as described in Figure S2.

А					В
SMP	SMP (μM) 64.0 75.0 86.0 96.0	Clot (μM) 0 0 0 0	Cell Survival 0.79 0.78 0.75 0.79	CI Values - - -	120 100 (%) 80 (%) 80 60 100 Clot
Clot	0 0 0 0 0	2.0 5.0 8.0 11.0 14.0	0.54 0.51 0.53 0.529 0.525	- - - -	
Combo 1	64.0 64.0 64.0 64.0 64.0	2.0 5.0 8.0 11.0 14.0	0.5 0.424 0.378 0.269 0.238	1.93E-3 2.53E-5 7.06E-6 2.51E-7 8.44E-8	C
Combo 2	75.0 75.0 75.0 75.0 75.0	2.0 5.0 8.0 11.0 14.0	0.459 0.353 0.321 0.318 0.204	7.62E-5 4.02E-6 1.55E-6 1.41E-6 2.64E-8	2 _ A generation of the second s
Combo 3	86.0 86.0 86.0 86.0 86.0	2.0 5.0 8.0 11.0 14.0	0.393 0.403 0.186 0.188 0.09	1.43E-5 1.89E-5 1.41E-8 1.54E-8 1E-10	Antagonism iter 1 Synergism O
Combo 4	96.0 96.0 96.0 96.0 96.0	2.0 5.0 8.0 11.0 14.0	0.357 0.133 0.09 0.079 0.096	5.81E-6 1.2E-9 1E-10 1E-11 1E-10	$0 \begin{array}{c} 0 \\ 0 \\ 0 \\ Fa \end{array} $



Figure S17. The significant synergistic cell-killing effect of Sampangine and ketoconazole on wild type *C. albicans*.