Supplementary Information for:

A Novel Use of Gentamicin in the ROS-Mediated Sensitization of NCI-H460 Lung Cancer Cells to Various Anticancer Agents

Michael F. Cuccarese[1], Amit Singh[2], Mansoor Amiji[2], George A. O'Doherty[1]
[1]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA, USA [2]Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, USA

Figure S1. SI for figures 1 and 2 showing full dose-response relationship for all drugs with GEN against H460

Figure S2. SI for figure 3 showing full dose-response relationship for all drugs with GEN against A549

Figure S3. Bradford data for normalization of GSH total content data:

Figure S4. Chou-Talalay results for combination index and dose reduction index

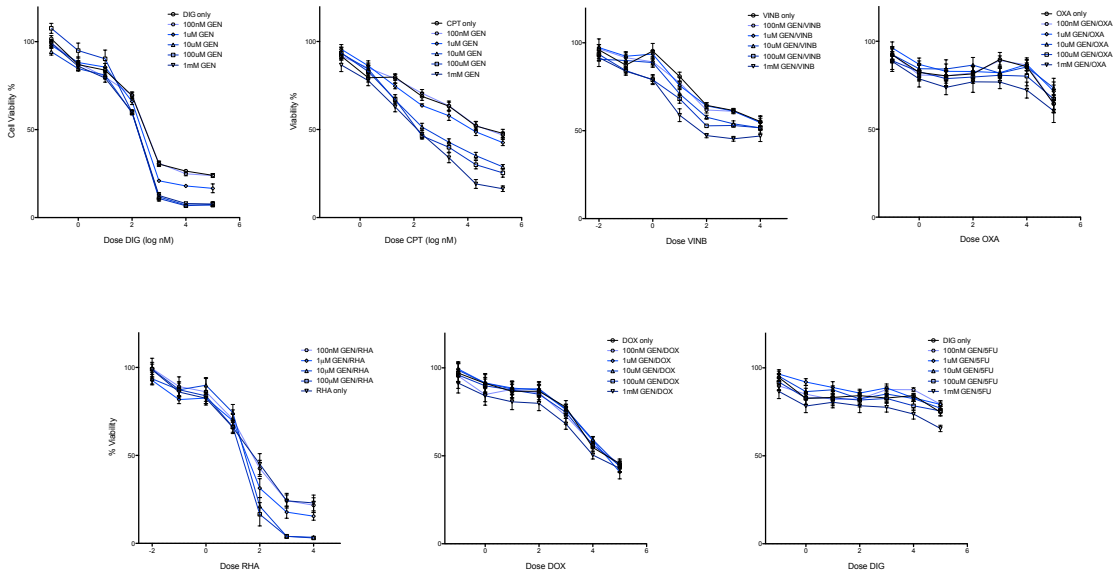


Figure S1: H460 cells were seeded in full media in 96-well plates at a density of 5000 cells per well. Drug dilutions were performed in serum-free RPMI-1640 (SFM). After seeding for 18 h, cells were dosed with GEN (5 concentrations, log 10) or SFM for 24 h. Cells were then dosed with an anticancer agent (7 concentrations, log 10) across all GEN concentrations. Plates were then incubated for 48 h (H460) at 37 °C. All treatments were performed in duplicate on each plate and repeated for a total of 3 independent experiments. Cell viability was measured via MTT assay.

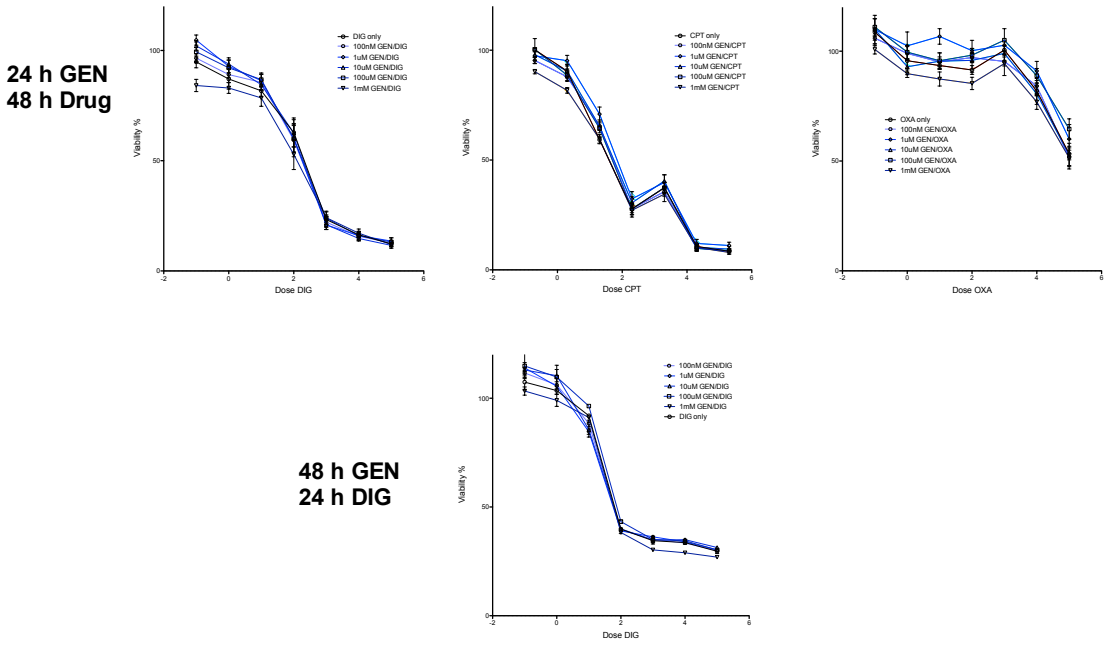
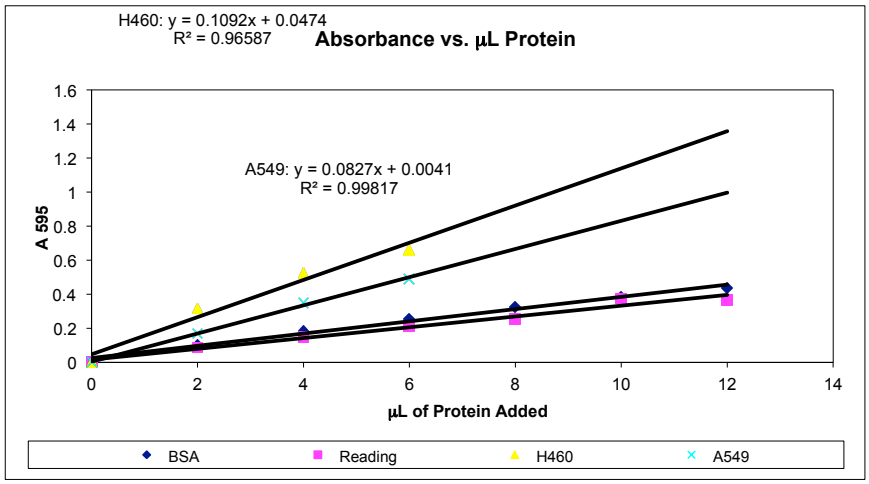


Figure S2: A549 cells were seeded in full media in 96-well plates at a density of 5000 cells per well. Drug dilutions were performed in serum-free RPMI-1640 (SFM). After seeding for 18 h, cells were dosed with GEN (5 concentrations, log 10) or SFM for 24 h. Cells were then dosed with an anticancer agent (7 concentrations, log 10) across all GEN concentrations. Plates were then incubated for 48 or 24 h at 37 °C. All treatments were performed in duplicate on each plate and repeated for a total of 3 independent experiments. Cell viability was measured via MTT assay.

Sample	µL added	BSA	Reading	H460	A549
0 (Blank	0	0	0	0	0
1	2	1.02E-01	0.087634	0.31638	0.16926
2	4	0.18449	0.14845	0.52500	0.34778
3	6	0.25745	0.21174	0.65837	0.49204
4	8	0.32538	0.25749		
5	10	0.38262	0.37446		
6	12	0.43626	0.36404		



	BSA	a835	H460	A549
slope	0.0359	0.0317	0.1092	0.0827
prot. conc (mg/mL)	2.5	2.20752	7.60446	5.75905

GSH multiplication factor to normalize A549 = 7.60446/5.75905

Figure S3. 20 µL protein lysate was saved prior to deproteination. A standard Bradford assay with BSA was performed and 3 concentrations of lysate from A549 and H460 were fit to the curve. A multiplication factor was obtained and applied to GSH concentrations to normalize to total protein.

GEN-control	
10	0.04512087
100	0.15186228
1000	0.18520397
10000	0.2413655
100000	0.20159742
1000000	0.23261723
10000000	0.47207563

DIG-control	
0.1	-0.016581
1	0.13053337
10	0.16188853
100	0.30610922
1000	0.69567094
10000	0.73592984
100000	0.75961536

DIG	response	GEN
10	0.20102685	100
100	0.33938142	1000
1000	0.88214994	10000
10000	0.93271456	100000
100000	0.92278646	1000000

CPT-control	
0.2	0.08314558
2	0.20566211
20	0.20212555
200	0.31179764
2000	0.36729596
20000	0.48022989
200000	0.52188262

CPT	response	GEN
20	0.21055359	100
200	0.3637664	1000
2000	0.57223025	10000
20000	0.69967119	100000
200000	0.83621799	1000000

VINB-control	
0.1	0.04006567
1	0.12679205
10	0.04697752
100	0.19185957
1000	0.35566272
10000	0.3847626
100000	0.44792583

VINB	response	GEN
10	0.1079921	100
100	0.2431485	1000
1000	0.42281262	10000
10000	0.47017392	100000
100000	0.53032037	1000000

OXA-control	
0.1	0.07713108
1	0.17660383
10	0.1964471
100	0.18563585
1000	0.10367252
10000	0.14506397
100000	0.35413545

OXA	response	GEN
10	0.19636327	100
100	0.17199645	1000
1000	0.18110818	10000
10000	0.19839518	100000
100000	0.3962095	1000000

DOX-control	
0.01	0.0296544
0.1	0.08838475
1	0.13318455
10	0.13273792
100	0.22317147
1000	0.45267068
10000	0.5447609

DOX	response	GEN
1	0.12211542	100
10	0.12545432	1000
100	0.23384925	10000
1000	0.43859288	100000
10000	0.57097308	1000000

5FU-control	
0.1	0.05530177
1	0.17355263
10	0.16810843
100	0.15784553
1000	0.17123482
10000	0.15759145
100000	0.2522089

5FU	response	GEN
10	0.17962918	100
100	0.14343102	1000
1000	0.14870497	10000
10000	0.21569243	100000
100000	0.34427717	1000000

CI

DIG	CPT	VINB	OXA	DOX	5FU
1.042	0.14	2.04E+04	0.012	2686.589	0.156
1.111	0.012	0.108	6.828	4074.291	810.194
0.003	0.001	0.056	16.646	2.118	1486.899
0.005	0	0.27	11.848	0.457	0.855
0.077	9.37E-06	1.143	4.28E-07	0.577	3.35E-05

DRI

DIG	CPT	VINB	OXA	DOX	5FU
0.969	7.267	3.259	535.998	2.151	102.192
0.9	81.919	9.288	1.363	0.309	0.009
324.06	1820.339	17.989	0.488	0.478	0.006
202.477	6429.166	3.709	0.536	2.189	38.288
12.975	1.07E+05	0.875	3.77E+06	1.732	5.85E+06

Figure S4. Calcusyn input and output data for all doses. Average cytotoxicity for 3 independent experiments were entered into Calcusyn (Cambridge, UK) to generate a combination index (CI) and dose reduction index (DRI) for each drug/dose combination.