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Synergism between clofarabine and decitabine through p53R2: A pharmacodynamic drug-drug interaction modeling

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Abstract

Clofarabine (CLO), a purine nucleoside analog with promising efficacy in acute myeloid leukemia (AML), inhibits the ribonucleotide reductase, p53R2. We have shown that p53R2 mRNA is up-regulated by decitabine (DEC), another drug with promising activity in AML. We developed a pharmacodynamic model to characterize the interaction between CLO and DEC on an AML cell line and down-regulated p53R2 protein to understand its role. These results confirm a role for p53R2 in both CLO and DEC mechanism of action, demonstrate synergism between these two drugs in this AML model and support the use of this combination in a future clinical trial.

Keywords

Clofarabine; Decitabine; AML; synergy; pharmacodynamic modeling

Introduction

The treatment outcome of acute myeloid leukemia (AML) in patients 60 years old is poor, and has not improved over the last two decades [1]. Therefore new treatments are urgently needed. Clofarabine (CLO), [2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine] is a second generation nucleoside analogs approved for use in pediatric relapse or refractory acute lymphoblastic leukemia for those failing at least two prior regimens [2]. CLO exerts its effect both on dividing and non-dividing cells and ultimately disrupts mitochondrial

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Contributions: SG generated the shRNAmir clones, JDH studied cell proliferation and performed the western blots; KET, GJF, MW analyzed the data; MW, GJF, KET, AK, designed the experiments; KET, MW wrote the manuscript.

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integrity leading to apoptosis [3]. It has promising efficacy in clinical trials in AML patients 60 years old patients [4, 5]. At a dose of 40 mg/m²/day used in those trials, the median plasma CLO level was 1.5 microM (range 0.42-3.2 microM) and at the maximum tolerated dose (55 mg/m²/day), the median concentration was 19 microM (range 3-52 microM) [6]. CLO's mechanism of action involves inhibition of the ribonucleotide reductase, p53R2, an enzyme responsible for converting nucleotide diphosphates to the corresponding deoxynucleotide diphosphates [7]. Interestingly, we have shown that decitabine (DEC), another drug with promising activity in AML [8], is also associated with up-regulation of p53R2 mRNA [9]. At a dose of 15 mg/m² every 8 hours, DEC's maximum concentration (C_{max}) ranged from 64.8 to 77.0 ng/ml [10]. These concentration ranges guided us in characterizing the degree of interaction between CLO and DEC in an AML cell line.

We examined the combined effect of CLO and DEC using a noncompetitive functional interaction model [11] with an interaction parameter (Ψ). Interaction parameters may be useful in various mechanism-based models to account for the synergism or antagonism not predicted by the mechanistic expectations of the modeling scheme [11]. The estimated value of this parameter indicates the intensity of the drug-drug interaction when compared to no-interaction value.

The effects of CLO and DEC were captured in a time-dependent manner. A mechanistic drug-drug interaction model was developed, incorporating time-dependent natural cell growth and death in the system. In order to evaluate the role that p53R2 plays in this model, we down-regulated its expression with short-hairpin micro RNA (shRNAmir). These studies were designed to better understand the combined effect of CLO and DEC.

Materials and Methods

Cell culture and reagents

The human erythroleukemia cell line, HEL [purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany)], carries the JAK2 mutation V617F and served as a model system. HEL cells and their derivatives were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (all from Life Technologies, Grand Island, NY) and cultured in 5% CO₂ at 37°C. CLO and DEC were purchased from Sigma (St. Lois, MO).

The following antibodies were used: polyclonal p53R2 (Abcam, Cambridge, MA) and monoclonal Hsc70 (Santa Cruz Biotechnology, Santa Cruz, CA).

Generation of p53R2 knock-down (KD) and control [p53R2 non-silenced (NS)] cells

The Expression ArrestTM pGIPZ lentiviral shRNAmir system was purchased from Open Biosystems (Huntsville, AL). Lentiviral packaging plasmids (Didier Trono Laboratory) pCMV-dR8.74 and pMD2.G and p53R2 shRNAmir or non-silenced (NS) shRNAmir pGIPZ plasmids were transfected into HEK293T packaging cells using LipofectamineTM 2000. Two days following transfection, supernatant containing lentivirus was filtered and polybrene (Sigma) was added to a final concentration of 8 µg/mL. Infection was carried out at 25°C for 1 hour, while undergoing centrifugation at 1000 × g in 6-well plates. Cells were sorted according to green fluorescent protein (GFP) expression using the FACS VantageTM (BD Bioscience, San Jose, CA). High GFP expressing cells were then propagated. Following clone selection based on p53R2 protein down-regulation, two clones were selected (HEL-p53R2KD and HEL-NS) and used in all future experiments.

Interaction assays of CLO and DEC on HEL-p53R2KD and HEL-NS cell lines

In order to assess the *in vitro* effects of combining CLO and DEC and elucidate their mechanism of action the two cells lines, HEL-p53R2KD and HEL-NS, were used. Cells were initially exposed for 24, 48, 72 and 96 hours to single agents CLO and DEC. Cell viability was determined by trypan blue dye (Life Technologies) exclusion assay, as previously described [11]. All experiments were conducted at least in triplicates. The Hill function was fitted to each concentration-response curve for each drug as a single agent and individual IC_{50} s, concentrations resulting in 50% inhibition of cell growth, were determined. Five (5) combination ratios of the IC_{50} (CLO/DEC: 1:1, 1:4, 4:1, 1.5:3, 3:1.5) were characterized.

Western blots

Western blots were conducted as previously described [12]. The p53R2 protein levels were evaluated by densitometry using Quantity One software (Bio-Rad, Hercules, CA). The p53R2 protein levels were normalized to the internal control, Hsc70.

Pharmacodynamic Modeling

The synergistic activity of CLO and DEC on HEL-p53R2KD and HEL-NS cells was determined in a step-wise manner. First, single agent exposure data were fit to a simple inhibitory I_{max} Pharmacodynamic (PD) model (Equation 1). The individual PD parameters, including: IC_{50} , I_{max} , and Hill's constant (γ), a measure of the steepness of slope, were determined using WinNonlin (Pharsight Corporation Version 5.3) for both HEL-p53R2 and HEL-NS cells at 24-, 48-, 72-, and 96-hour exposure to CLO and DEC.

Equation 1. Inhibitory I_{max} model

$$Effect = E_0 - (I_{max} \times C^\gamma / IC_{50}^\gamma + C^\gamma)$$

To quantify the synergy or interaction of CLO and DEC, a simplified PD synergy model was developed using ADAPT 5(BMSR) [13]. This modified version of a PD interaction model that was originally developed by Ariens and Simonis [14] and more recently adapted by Jusko *et al* [11, 15, 16] was used to model the data upon exposure to the combination of CLO and DEC as followed:

Equation 2. Inhibitory Interaction Model.

$$Effect = 100 \times \left(1 - \frac{I_{max_{CLO}} * (CLO / (\psi \times IC_{50_{CLO}})^{\gamma_{CLO}}) + I_{max_{DEC}} * (DEC / (\psi \times IC_{50_{DEC}})^{\gamma_{DEC}}) + I_{max_{CLO}} + I_{max_{DEC}} - I_{max_{CLO}} * I_{max_{DEC}}}{1 + CLO / (\psi \times IC_{50_{CLO}})^{\gamma_{CLO}} + DEC / (\psi \times IC_{50_{DEC}})^{\gamma_{DEC}} + CLO / (\psi \times IC_{50_{CLO}})^{\gamma_{CLO}} + DEC / (\psi \times IC_{50_{DEC}})^{\gamma_{DEC}}} \right)$$

$I_{max_{CLO}}$ or $I_{max_{DEC}}$ are the maximal inhibitory responses achieved for each drug, CLO or DEC, respectively and are the actual drug concentrations that elicit a specific effect; $IC_{50_{CLO}}$ or $IC_{50_{DEC}}$ represent the concentration producing 50% inhibitory response as a single agent. The interaction symbol, termed ψ (Ψ), characterizes the interaction of CLO with DEC (Equation 2). Individual PD parameters estimated from the inhibitory I_{max} model upon single agent exposure (Equation 1) were all fixed leaving a single parameter in the expression, Ψ , which was estimated (Equation 2). When fitting individual exposure data to the inhibitory I_{max} model it is important to minimize the standard deviation associated with each mean parameter estimate as these I_{max} model estimated parameters are fixed in the interaction model. I_{max} represents the maximal inhibitory responses achieved for each drug. The degree of interaction between the two drugs of interest was determined by the following

criteria: when Ψ equals 1, there is no interaction or the interaction between the two drugs may be additive; a Ψ less than 1 is representative of synergy; and a Ψ greater than 1 indicates antagonism. Three dimensional, concentration-concentration-effects plots were generated in SigmaPlot to represent the interaction surface.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism5 (GraphPad Software, San Diego, CA, USA). Comparisons were assessed using analysis of variance (ANOVA) Bonferroni's multiple comparisons test to evaluate differences in parameter estimates across cell type and time of exposure. P-values less than 0.05 were considered statistically significant.

Results

Single-drug effect on survival

Exposure of HEL-p53R2KD and HEL-NS cells to single agent CLO and DEC were fitted to Inhibitory sigmoidal I_{max} model for 24, 48, 72, and 96-hour time points. At 24-hour exposure of CLO or DEC maximal inhibition was not achieved in either HEL-p53R2KD or HEL-NS cell lines and thus PD parameters could not be determined (Figures 1, 2). A large difference in I_{max} was observed between 24-hours to 96-hours with both CLO and DEC (Figures 1, 2). By 48-hours both cell lines achieved approximately 35-45% inhibition upon exposure to single agents, mean PD parameters were determined with coefficient of variation (CV%) (Figure 1, Table 1) and nonlinear regression fitting was performed using ADAPT 5 (BMSR); by 72-hrs greater than 50% inhibition was observed for both CLO and DEC. CLO demonstrated a time dependent effect on HEL-NS and p53R2KD as measured by a 9-fold higher IC₅₀ at 48-hr compared with 72- and 96-hr incubations; on HEL-p53R2KD CLO demonstrated a 90-fold higher IC₅₀ at 72- and 96-hr incubations compared with the 48-hr incubation (Table 1); this indicates that the potency of these compounds increased with the duration of CLO exposure. CLO potency was similar between HEL-p53R2KD and HEL-NS cell lines at 48-hours, IC₅₀=95.01, and IC₅₀=95.0, respectively (Table 1). Potency of DEC was much lower than CLO in both HEL-NS and p53R2KD cell lines at all incubation times, suggesting that higher concentrations of DEC are required to elicit a similar response as observed with CLO at each incubation time. Maximal inhibition of cell growth, as measured by viability, was observed by 96-hours in both cell lines. The Hill coefficient, a measure of the steepness of slope, for p53R2KD cells exposed to CLO demonstrated significant differences from 48-hour incubation to 72-96 hour ($p<0.05$). Greater variability was observed in DEC Hill coefficient from 24-96 hours in both HEL-p53R2KD and HEL-NS this may be explained by too few concentrations in the middle portion of the curve.

Drug-drug interaction on survival

A model-fitted approach was employed to determine the extent of PD interaction observed using various concentration-combinations of CLO and DEC. Upon fitting the single agent and combination exposure data to the synergy model (Equation 2), a Ψ was estimated for HEL-p53R2KD and HEL-NS cells at 48, 72, and 96-hour exposure (Figure 3, Table 2). All model estimated Psi values are expressed as mean $\pm$ SD. All combinations of CLO and DEC in both cell lines were synergistic ($\Psi<1$) (Table 2) at 48 and 72-hours, indicating a degree of synergy between CLO and DEC in HEL-p53R2KD and HEL-NS. Three dimensional concentration-effect surfaces were generated for CLO and DEC at each incubation time and are represented by a colored mesh surface. The interaction surface of CLO and DEC from 48-72-hour graphically depicts this interaction (Figure 3a, b, c, d). At 96-hours exposure the degree of synergy observed with the combination shifts towards a possible additive or

slightly antagonistic effect for HEL-NS and HEL-p53R2KD ($\Psi=1.03\pm0.02$ and $\Psi=0.75\pm0.23$, respectively) (Figure 3e, f) suggesting that exposure-time to combination CLO and DEC may play a role in mechanisms of resistance. Additionally, it is clear that significant cell killing was observed at 96-hours of exposure; the Ψ at this exposure may indicate that lower concentration combination ratios are required to fully characterize the degree of this interaction for distinguishing the contributing effects of each drug.

Interestingly, maximal cell killing was observed at high concentrations of CLO and DEC in HEL-NS cells (Figure 3a, c) and CLO contributed to a greater extent to the degree of synergy in HEL-p53R2KD cells at 48- and 72-hour exposure (Figure 3b, d). The combination of CLO and DEC exhibited an additive or slightly antagonistic response in the HEL-NS and HEL-p53R2KD at 96-hour exposure (Table 2, Figure 3e, f) suggesting that high CLO concentration led to maximal cell killing and DEC had minimal contribution to cell killing and may have led to the development of resistance to DEC by 96-hour exposure.

Drug-drug interaction on protein expression

Western blot analyses were conducted to evaluate the amount of p53R2 protein expression in both HEL-NS and HEL-p53R2KD cell lines at 48 hours exposure to single agent and combination agent exposures (Figures 4, 5, 6) due to the optimal cell survival at that time point; HSc70 was included as an internal control. As mentioned above, cell killing exceeded 50% beyond 48 hours thus making western blot analyses unreliable for measurement beyond 48 hours. The upper p53R2 bands (Figure 5) were quantified in both cell types upon single agent exposure. The 48-hour CLO exposure to HEL-NS and HEL-p53R2KD were both fit to an inhibitory Imax model (Figure 4, upper panel). Similarly DEC exposure to HEL-NS was fit to an inhibitory Imax model, while DEC exposure to HEL-p53R2KD was fit to a stimulatory Emax model as inhibition of p53R2 was not observed in this cell line (Figure 4, lower panel), rather a modest up-regulation of p53R2 protein expression as compared with baseline internal control, specifically at higher concentrations. There is a clear effect of greater down-regulation of p53R2 protein in HEL-NS compared with HEL-p53R2KD upon exposure to CLO (Figure 4, upper panel) and DEC (Figure 4, lower panel); the IC_{50} in HEL-NS was two-fold lower compared with HEL-p53R2KD cell line for both CLO and DEC (Table 3). Similarly, greater down-regulation of p53R2 protein was observed in HEL-NS compared with HEL-p53R2KD following exposure to DEC as demonstrated by two-fold lower IC_{50} in HEL-NS compared with HEL-p53R2KD. An example of the Western blots generated from the single drug effects and the combination exposures are shown in Figure 5. Please note that the lower band was not quantifiable. Surface plots demonstrate a difference in protein expression between the upper bands of HEL-p53R2KD and HEL-NS; this was confirmed with a paired T-test demonstrating a significant difference in expression of p53R2 as measured by densitometry in the upper band, $p=0.0016$ (Figure 6a, b). In the HEL-NS cell line a similar level of p53R2 expression was observed across all CLO/DEC concentration combinations (Figure 5). In the HEL-p53R2KD cell line a clear difference in p53R2 expression was observed; at the 50:50 (CLO:DEC) ratio, p53R2 expression was significantly less ($p<0.05$) than at other concentration combination ratios (Figure 5). CLO is known to inhibit p53R2, in the cell line with down-regulated p53R2 (HEL-p53R2KD) the highest level of p53R2 expression was observed in combination ratio with greatest DEC concentration. This suggests that the increased p53R2 expression observed in HEL-p53R2KD may be the result of DEC-P binding to DNA (Figure 7). The PD interaction model was applied to these data to evaluate the synergistic activity of these drugs on p53R2 expression in HEL-NS and HEL-p53R2 cell lines, the estimated Ψ was 0.57 (0.03) and 0.82 (0.02), respectively. HEL-p53R2 showed impaired p53R2 expression compared with HEL-NS; however, the combinations tested demonstrated synergistic activity on protein expression in both cell lines. The lower Ψ observed in HEL-NS suggests that the

combination of CLO and DEC leads to greater down-regulation of p53R2 protein expression as compared to the HEL-p53R2KD cell line (Table 4).

Proposed model

We have described a PD model that quantifies the degree of interaction between CLO and DEC in the AML cell line as synergistic at 48- and 72-hours. Down-regulating p53R2 protein, in the HEL-p53R2KD cell line, requires lower concentrations of DEC combined with high CLO concentrations to achieve maximal kill, suggesting that p53R2 may be involved in both CLO and DEC mechanism of action (Figure 7).

Discussion

In the present study we have developed and applied PD models to study the hypothesis that CLO and DEC will be synergistic in AML. Characterizing the extent of their interaction in an *in vitro* system is the first step in our understanding how to combine these two agents for AML patients. The results of these analyses demonstrate that CLO and DEC have a synergistic effect in both HEL-NS and HEL-p53R2KD cells. The observed down-regulation or lower levels of expression of p53R2 protein in HEL-p53R2KD compared to HEL-NS upon exposure to the combination CLO and DEC suggests that p53R2 may be a major mechanism involved in both CLO and DEC action. It has been suggested that DEC up-regulates p53R2 expression [9] and these data support this as we have demonstrated that DEC in combination with CLO up-regulates p53R2 protein expression in HEL-NS and down-regulates its expression in HEL-p53R2KD. To the best of our knowledge this is the first demonstration of the effect of CLO and DEC combination in AML using a PD model and characterizing the extent of the interaction with a PD metric, Ψ . This analysis of the effects of CLO and DEC is limited to HEL-NS and HEL-p53R2KD cell lines; but this model shows that the combination of CLO and DEC is synergistic in a relevant AML cell line; furthermore, we have demonstrated the mechanistic potential of this combination through the investigation of the role of p53R2 to support this combination in future clinical trials in AML patients.

The p53R2 densitometry results indicate that the mechanism of CLO and DEC cell inhibition is dependent of drug concentrations. Densitometry confirmed a clear down-regulation of p53R2 protein expression in HEL-p53R2KD compared to HEL-NS suggesting DEC induced apoptosis is the result of up-regulation of p53R2 protein; this was confirmed by evaluation of p53R2 expression in a PD interaction model to characterize the level of expression in both cell lines.

There are several challenges extrapolating these *in vitro* studies to a clinical model; translating drug concentrations and exposure time from *in vitro* tests to a clinical setting may be unreliable, however, the clear degree of synergy observed at 48- and 72-hours suggests that this combination would be successful in a patient when plasma concentrations of CLO and DEC are maintained in patients that are comparable to the cell line evaluated herein. A limitation to the use of this *in vitro* system is that it does not predict or capture the toxic effects of the combination that might be observed in a patient. To date, no clinical pharmacokinetic drug-drug interaction studies have been conducted with CLO and DEC to assess likelihood for toxicity; however, on the basis of the *in vitro* studies reported in the literature, cytochrome P450 inhibitors and inducers are unlikely to affect the metabolism of these compounds. Therefore, it is not expected that this combination will directly induce or inhibit the major drug metabolizing cytochrome P450 pathways. Despite several challenges inherent in translational research, our results support the idea that CLO and DEC combination may have a role in the treatment of AML patients and may warrant a clinical trial.

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Abbreviations

AML	Acute Myeloid Leukemia
PD	Pharmacodynamic
IC50	Concentration achieving 50% inhibition of cell growth
Emax	maximal response
gamma (γ)	measure of the steepness of slope
psi (Ψ)	interaction term

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Figure 1. Predicted IC₅₀ for clofarabine (CLO) in HEL-NS and HEL-p53R2KD cell lines at 24, 48, 72, and 96 hours. Full lines, HEL-NS, Dashed lines, HEL-p53R2KD; red, 24 hours exposure; blue, 48 hours exposure; purple, 72 hours exposure; black, 96 hours exposure.

Figure 2. Predicted IC_{50} for decitabine (DEC) in HEL-NS and HEL-p53R2KD cell lines at 24, 48, 72, and 96 hours. Full lines, HEL-NS, Dashed lines, HEL-p53R2KD; red, 24 hours exposure; blue, 48 hours exposure; purple, 72 hours exposure; black, 96 hours exposure.

a

b

Figure 3.

In vitro exposure of HEL-NS (a,c,e) and HEL-p53R2KD (b,d,f) to clofarabine (CLO) and decitabine (DEC) at 48-hours (a,b), 72-hour (c,d) and 96-hour (e,f) demonstrate a clear degree of synergy. HEL-NS and HEL-p53R2KD cells were exposed to CLO and DEC at escalating doses. Following 48-, 72-, and 96-hours drug exposure, cell viability was determined. Cell viability response is represented as a surface for combination of CLO and DEC. A color scheme is used to reflect cell kill, 100% cell viability is represented by brighter colors (orange and yellow) and progression to 0% is represented by dark blue. Actual individual viability measurements are represented by black dots.

Figure 4. Predicted IC₅₀ for a, clofarabine (CLO) and b, decitabine (DEC) in HEL-NS and HEL-p53R2KD cell lines. Full line with blue symbols, HEL-p53R2KD; dashed line with red symbols, HEL-NS.

Figure 5.

A representative dose dependent effect of clofarabine (CLO) and decitabine (DEC) on p53R2 protein levels after single (A and B) and dual (C) drug exposure for 48 hours. HSc70 served as an internal loading control.

Figure 6.

Expression of p53R2 protein was determined by densitometry in upper band (a) HEL-NS (b) HEL-p53R2KD cells. Varying ratio of combination exposure did impact expression of p53R2 in both cell lines. A color scheme is used to reflect protein expression, 100% protein expression is represented by brighter colors (orange and yellow) and progression to 0% is represented by dark blue. Actual individual protein expression measurements are represented by black dots. Paired T test revealed a significant difference in protein expression in upper band between HEL-p53R2KD and HEL-NS, $p=0.0016$, level of significance set as $p<0.05$.

Figure 7.

Proposed mechanism of action for clofarabine (CLO) and decitabine (DEC). CLO and DEC undergo phosphorylation (P) by deoxycytidine kinase. CLO-P leads to an inhibition of p53R2 ultimately decreasing deoxynucleoside triphosphate leading to apoptosis by inhibition of DNA synthesis and repair. DEC-P is incorporated into DNA and can either act as a hypomethylating agent leading to apoptosis or can induce p53R2 expression. Abbreviations: CLO, clofarabine; CLO-P, phosphorylated CLO; dCK, deoxycytidine kinase; dNTP, deoxynucleotide triphosphate;

Estimated parameters for single agent [clofarabine (CLO) and decitabine (DEC)] exposure on HEL and HEL-p53R2KD cells, IC50 represents the concentration resulting in 50% inhibition of cells, and gamma (γ), also known as Hill's constant, represents the steepness of slope. Concentrations are expressed in nM.

Table 1

Cell Line	Drug	Imax (%)	IC ₅₀ (nM)		γ	
			Mean	CV%	Mean	CV%
HEL-NS	CLO					
	48 hours	64.1	95.00	0.4475	1.0	Fixed
	72 hours	82.3	11.42	0.1981	0.27	0.08
	96 hours	100.0	10.0	0.1217	0.59	0.23
HEL-p53R2KD	CLO					
	48 hours	60.8	95.01	0.5592	1.0	Fixed
	72 hours	77.4	1.53	0.0576	0.14	0.068
	96 hours	98.1	1.0	0.0174	0.28	0.069
HEL-NS	DEC					
	48 hours	44.6	142.65	0.77137	1.69	0.0144
	72hours	66.8	344.1	5.038	0.68	0.0049
	96 hours	92.9	79.92	0.1786	0.82	0.0029
HEL-p53R2KD	DEC					
	48 hours	32.5	224.31	1.0397	1.03	Fixed
	72 hours	63.7	88.17	0.3772	0.72	0.0039
	96 hours	84.2	100.15	0.2288	1.24	0.00124

Table 2

Cell Viability: Estimated mean (standard deviation, sd) interaction parameter (Ψ) upon exposure to combination therapy.

Time	Cell Line	Ψ	
		Mean	SD
48hours	HEL-NS	0.42	0.05
48hours	HEL-p53R2KD	0.39	0.1
72hours	HEL-NS	0.68	0.026
72hours	HEL-p53R2KD	0.64	0.001
96hours	HEL-NS	1.03	0.02
96hours	HEL-p53R2KD	0.75	0.23

Table 3

Estimated parameters for single agent [clofarabine (CLO) and decitabine (DEC)] exposure on HEL and HEL-p53R2KD p53R2 protein expression, IC₅₀ represents the concentration resulting in 50% inhibition p53R2 expression, and gamma (γ), also known as Hill's constant, represents the steepness of slope. Concentrations are expressed in nM.

Cell Line/Single Agent Exposure	Imax (%)	IC ₅₀ (nM)	γ			
HEL-NS		Mean	CV%			
		Mean	CV%			
	CLO	85.2	106.7	0.34	0.57	0.08
	DEC	33.6	341.5	0.074	1.15	0.086
HEL-p53R2KD						
	CLO	70.6	234.2	0.22	0.47	0.04
	DEC	13.4	550.6	0.1	0.014	0.6

Table 4

p53R2 Protein expression at 48hr: Estimated mean (standard deviation, sd) interaction parameter (Ψ) upon exposure to combination therapy.

Cell Line	Ψ	
	Mean	SD
HEL-NS	0.57	0.03
HEL-p53R2KD	0.82	0.02