Colony forming cell (CFC) assays predict for increased clinical neutropenia with combination therapies

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INTRODUCTION

Combination therapies are being increasingly employed to treat diseases where single agents do not have the desired clinical benefit. There are a number of publications that cite the benefit of combination therapy over monotherapy in preventing disease progression (Orlowski et al, 2007) or in obtaining an early objective response (Min et al, 2007), but this type of treatment often comes with increased toxicity. Studies have shown that neutropenia increases significantly when certain drugs are used together in combination therapies. Although increased toxicities may be predicted with some drug combinations (i.e. chemotherapeutics), there have been reports of unexpected myelosuppression/neutropenia caused by combining certain drugs including Serotnin Reuptake Inhibitors (SSRIs) and Linezolid, (Hachem et al. 2003). We previously used hematopoietic colony forming cell (CFC) assays to rank the in vitro toxicity caused by a panel of therapeutic tyrosine kinase inhibitors and were able to correlate the IC_{50} values obtained from these in vitro studies with the level of clinical neutropenia caused by the same agents , as reported in the literature. The aim of the current study was to assess if the CFC assay could also be useful at predicting the increased toxicity of drug combinations relative to the toxicity caused by the relevant single agents.

MATERIALS AND METHODS

Six Drugs were selected for testing based upon their different target and disease specifications as well as reported differences in their clinical toxicity profiles. The compounds were tested both alone and in the following paired combinations:

- Imatinib and Hydroxyurea
- Linezolid and Fluoxetine
- Doxorubicin and Velcade

Bone marrow cells from normal donors (n=3) were mixed with the single compounds listed above (extended concentration range) in ColonyGel™ 1102 complete methylcellulose-based medium (ReachBio, Seattle WA) and plated in 35 mm dishes (three replicates per concentration). The cultures were incubated in a humidified incubator at 37ºC, 5% CO_2. CFU-GM were enumerated on day 14 and IC_{50} values were determined for each drug. The drugs were then tested in the combination pairs indicated above, using the same assays system and again using marrow from multiple donors to evaluate additional toxicity using a matrix design (Figure 1). The percentage of CFU-GM inhibition was determined by comparing colony number in the treatment conditions to those in the solvent control cultures.

RESULTS

Individual IC_{50} values ranged from 0.012 µM (Velcade) to 109 µM (Linezolid), Table 1. Where two compounds were combined together at their IC_{50} equivalent values, there was an additional inhibition of CFU-GM: 84 % inhibition with Imatinib and Hydroxyurea in combination (see Table 3 for percent inhibition at multiple concentrations of both drugs), 75 % inhibition with Fluoxetine and Linezolid in combination (see Table 4 for percent inhibition at multiple concentrations of both drugs) and 69 % inhibition with Velcade and Doxorubicin in combination (see Table 5 for percent inhibition at multiple concentrations of both drugs).

TABLE 1 IC_{50} VALUES OF INDIVIDUAL COMPOUNDS

IC_{50} VALUES

DRUG	IC_{50} VALUE (µM)
Bortezomib	0.012
Doxorubicin	0.028
Imatinib	2.6
Hydroxyurea	31
Linezolid	109
Fluoxetine	27

TABLE 2 MATRIX TEMPLATE

COMPOUND 2 \ COMPOUND 1	30 µM	10 µM	3 µM	1 µM	0.3 µM	0 µM
200 µM						
50 µM						
10 µM						
5 µM						
1 µM						
0 µM						

Matrices can be set up with any compounds and at appropriate concentrations for each one.

TABLE 3 EFFECT OF IMATINIB AND HYDROXYUREA ALONE AND IN COMBINATION ON CFU-GM INHIBITION

HYDROXYUREA \ IMATINIB	30 µM	10 µM	3 µM	1 µM	0.3 µM	0 µM
200 µM	100%	100%	100%	100%	100%	100%
50 µM	98%	98%	92%	86%	84%	76%
10 µM	96%	92%	76%	44%	42%	42%
5 µM	96%	88%	70%	36%	18%	8%
1 µM	96%	84%	74%	10%	12%	4%
0 µM	94%	86%	64%	20%	0%	

The IC_{50} for Imatininb is 2.6 µM and for hydroxurea is 31 µM. We have highlighted the closest concentrations to these, showing CFU-GM inhibition and also the inhibition at the combined concentrations.

TABLE 4 EFFECT OF FLUOXETINE AND LINEZOLID ALONE AND IN COMBINATION ON CFU-GM INHIBITION

LINEZOLID \ FLUOXETINE	144 µM	36 µM	12 µM	4 µM	1 µM	0 µM
148 µM	100%	85%	83%	83%	76%	78%
120 µM	100%	80%	71%	66%	59%	59%
100 µM	100%	83%	71%	58%	42%	41%
50 µM	100%	83%	58%	48%	25%	20%
25 µM	100%	69%	47%	22%	14%	3%
0 µM	100%	64%	31%	17%	7%	

The IC_{50} for Fluoxetine is 27 µM and for Linezolid is 109 µM. We have highlighted the closest concentrations to these, showing CFU-GM inhibition and also the inhibition at the combined concentrations.

TABLE 5 EFFECT OF DOXORUBICIN AND BORTEZOMIB (VELCADE) ALONE AND IN COMBINATION ON CFU-GM INHIBITION

VELCADE (BORTEZOMIB) \ DOXORUBICIN	0.1 µM	0.03 µM	0.01 µM	0.003 µM	0.001 µM	0 µM
0.1 µM	100%	100%	100%	100%	100%	100%
0.03 µM	100%	100%	100%	100%	100%	100%
0.01 µM	99%	69%	44%	31%	18%	42%
0.003 µM	98%	47%	18%	4%	-1%	27%
0.001 µM	98%	31%	1%	2%	-5%	-4%
0 µM	96%	65%	24%	2%	1%	

The IC_{50} for Doxorubicin is 0.028 µM and for Bortezomib (Velcade) is 0.012 µM. We have highlighted the closest concentrations to these, showing CFU-GM inhibition and also the inhibition at the combined concentrations.

CONCLUSIONS

- The CFU-GM assay can be used to test single agents or multiple agents.
- The CFU-GM assay may be employed to evaluate drug combinations, where there is a concern of increased toxicity.
- Since clinical trials rarely involve treatment of naïve patients, this assay may facilitate an understanding of how the toxicity of new candidate compounds might be affected by the background of current standard of care drugs.
- CFC assays using this matrix design also allow for the discovery of compounds which may exert a protective effect on a known toxic compound (Abstracts 1819 and 2179).
- These assays could also be used to predict toxicity that may not be expected with specific drug combinations.