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Combination of ATP-competitive mammalian target of rapamycin inhibitors with standard chemotherapy for colorectal cancer

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Abstract ATP-competitive mammalian target of rapamycin (mTOR) inhibitors are in early phase clinical trials. These novel targeted agents, including PP242, are mechanistically distinct from the allosteric, partial mTOR inhibitor, rapamycin. The goal of this study was to evaluate how PP242 best combines with standard chemotherapies for colorectal cancer (CRC), and which subsets of patients are most likely to benefit. The combination index for PP242 plus 5-fluorouracil, oxaliplatin, or irinotecan was determined in CRC cell lines with different mutational backgrounds. In KRAS mutant CRC cell lines, sensitivity to PP242 increases with co-mutation of PIK3CA. Mutation of p53 predicts resistance to chemotherapy, but not PP242. Efficacy of PP242 was comparable to that of standard chemotherapies over the dose range tested. Sensitivity or resistance to PP242 dictates relative synergy or antagonism, respectively, when PP242 is combined with 5-fluorouracil. The same trend exists for PP242 + oxaliplatin, but with a narrower dynamic range. Conversely potency of PP242 and the combination index for PP242 + irinotecan were unrelated, but synergy exists across all dose levels in PP242 and irinotecan sensitive, p53 wild-type cell lines. Overall, our in vitro analysis predicts that mutational status can be used to rank sensitivity to PP242 and standard chemotherapies. Single agent potency can in turn be used to predict the combination index in a drug-specific manner. Our data suggest a clinical trial to determine whether ATP-competitive mTOR inhibitors provide benefit in combination with standard chemotherapies for patients with PIK3CA mutant metastatic CRC, stratified by the presence or absence of KRAS co-mutation.

Keywords ATP-competitive · mTOR · PI3K · Combination index · Colorectal cancer

Abbreviations

mTOR	mammalian target of rapamycin
CRC	colorectal cancer
PI3K	phosphoinositide 3-kinase
PIK3CA	gene encoding the PI3K protein
EGFR	epidermal growth factor receptor
4EBP1	eukaryotic initiation factor 4E-binding protein-1
CI	combination index
Fa	fraction affected
Fu	fraction unaffected
DMSO	dimethyl sulfoxide
5FU	5-fluorouracil

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Introduction

Synthesis of the first potent, selective, and ATP-competitive mammalian target of rapamycin (mTOR) inhibitor, PP242, was reported in 2008 [1]. PP242 and other pyrimidine-based mTOR active site inhibitors have since shown promise in pre-clinical studies [2–5]. Five such inhibitors, including two analogs of PP242, are in clinical trials for treatment of cancer. These mTORC1/2 inhibitors represent a novel class of targeted agents with a different mechanism of action from the allosteric, partial mTORC1 inhibitor, rapamycin, or its analogs (rapalogs) [6]. With phase I trials underway, it is time to determine how to maximize the efficacy of ATP-competitive mTOR inhibitors, including how they can be combined with standard therapies and which patients are most likely to benefit.

The present study was conducted using human colorectal adenocarcinoma (CRC) cell lines. CRC was chosen because 1) there is a need for new agents to treat advanced stages of the disease and 2) there is a high frequency of derangements in the KRAS and/or phosphoinositide 3-kinase (PI3K) signaling pathways, upstream of mTOR. CRC is the second leading cause of cancer deaths in the United States. With distant metastases, the median 5-year survival is only 11% [7]. Approximately 40% of colorectal tumors have KRAS mutations and up to 40% have PI3K pathway alterations including PIK3CA mutations (15%) or loss of phosphatase and tensin homolog (PTEN) activity [8]. This study focuses on KRAS mutant cell lines because the demand for new therapies is greatest in this patient population, where epidermal growth factor receptor (EGFR) directed monoclonal antibodies are ineffective [9].

mTOR, a serine-threonine kinase in the PI3K-like kinase family, is a downstream node in the epidermal growth factor receptor (EGFR)➔ PI3K➔ AKT signaling pathway, and is critical for cell growth and survival [10]. mTOR is the active kinase in at least two multi-protein signaling complexes: mTORC1 and mTORC2. Activated mTORC1 regulates protein translation via phosphorylation of p70 ribosomal protein S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein-1 (4EBP1). Rapamycin is an mTORC1-specific allosteric inhibitor that blocks a subset of mTORC1 functions, principally phosphorylation of S6K. Unlike ATP-competitive mTOR inhibitors, rapamycin poorly inhibits phosphorylation of 4EBP1 and minimally affects cap-dependent translation in most cell types. As part of mTORC2, mTOR phosphorylates S473 on AKT [2, 11]. Treatment with rapamycin releases negative feedback inhibition on AKT; the AKT activation resulting from treatment with rapalogs is thought to be responsible for their poor performance in clinical trials [6, 12]. Blocking mTORC2 as well as mTORC1 disrupts AKT activation, and limits rapamycin-like feedback. Inhibition of cap-dependent translation and AKT signaling differentiate mTORC1/2 inhibitors from rapalogs. Elevated expression of mTOR and components of the mTORC1 and mTORC2 signaling complexes are found in advanced CRC and have been associated with progression and metastasis [13].

Although PP242 induces apoptosis in select cancers [14], we show that it is cytostatic in CRC cell lines. Consequently, while mTORC1/2 kinase inhibitors will be evaluated first as monotherapy, we also anticipate their testing in combination with other agents. It is unknown whether there will be an incremental benefit when an ATP-competitive mTOR inhibitor is combined with cytotoxic chemotherapy. We hypothesized that by inducing cell cycle arrest mTORC1/2 inhibitors might antagonize the effects of chemotherapeutic agents which target dividing cells. Alternatively, because AKT and other mediators of apoptosis such as mcl-1 are translationally controlled, blocking mTOR activity could sensitize cells to the apoptotic effect of cytotoxic drugs [14, 15].

Experience with EGFR inhibitors provides precedent for testing molecularly targeted therapies in combination with conventional chemotherapy. EGFR inhibitors exhibit synergy when combined with cytotoxic drugs in CRC cell lines and enhance the activity of standard therapies in xenograft models [16–18]. The best evidence for clinical benefit from the combination of an EGFR-targeted monoclonal antibody and standard therapy in any cancer type is with metastatic CRC. Improvements in response rates and progression free survival are dependent on the chemotherapy backbone [19–21]. The EGFR-targeted monoclonal antibody, cetuximab, is more active in combination with irinotecan than as monotherapy, even in irinotecan refractory disease [19]. The clinical significance of benefit from combination therapies must always take into account increases in toxicity. Moreover, patients with tumors harboring KRAS codon 12 mutations do not benefit from EGFR-targeted agents, highlighting the importance of patient selection [9, 22].

Due to the complexity of cell-signaling networks, one cannot infer that inhibiting a downstream target in a pathway will produce the same results as with an upstream inhibitor. The present study represents the first systematic evaluation of the combination of an ATP-competitive mTOR inhibitor with standard therapies for CRC. Attention was paid to tumor mutational status and class of chemotherapeutic agent. The goal of this work is to serve as a foundation for the rational design of future clinical trials.

Materials and methods

Cell culture All cell lines except SW620-R were from the American Type Tissue Collection (ATCC, Manassas, VA). Cell lines purchased more than 6 months prior to use were authenticated using the Promega Powerplex 1.2 System

(Madison, WI). Cell lines containing KRAS mutations were grown in Dulbecco's Modified Eagle Medium, 1 g/L glucose, supplemented with 10% fetal calf serum, glutamine, 10 U/ml penicillin and 100 μg/ml streptomycin. KRAS wild-type cell lines were grown in Eagle's Minimal Essential Medium (ATCC), 3 g/L glucose, supplemented as above. Cells were cultured in a 5% CO_2 atmosphere and fresh cells were used for each set of experiments.

Syngeneic SW620-R cell line The H1047R p110α expressing SW620 cell line, SW620-R, was created by retroviral infection using a pMIG-p110α plasmid as described [23]. Briefly, ecotropic p110α viral stocks were generated by transfecting pMIG-p110α plasmid DNA into the Phoenix cell line. Retroviral supernatants collected at 48, 72, 96, and 120 h were clarified by centrifugation (500 rpm, 5 min, 4°C) and stored at −80°C. Human SW620 cells were pseudotyped for infection with murine ecotropic virus by transient transfection with pcDNA3-EcoR/MCAT1. Transfection by nucleofection (Amaxa Nucleofector) was per the manufacturer's instructions for Kit T; transfected cells were plated into 6-well plates. 24 h after nucleofection with pcDNA3-EcoR/MCAT1, the SW620 cells were infected with p110α viral stocks for 12 h, switched to growth medium for 6 h, and expanded in 75 cm^2 flasks. After 3 days, the infected cells were FACS sorted for green fluorescent protein (GFP) expression. Each stable GFP expressing cell population was confirmed to contain the H1047R mutation by sequencing using primers that spanned exon junctions encompassing the inserted p110α copy.

Inhibitors PP242 was synthesized from commercially available starting materials [1]. 5FU, oxaliplatin, irinotecan, SN-38 and rapamycin were from Sigma. Ku-0063794 is commercially available.

Cell proliferation assay Cells were plated on 96 well plates at 30% confluence and then allowed to adhere overnight prior to adding drugs. Six drug concentrations were used to determine the IC_{50} (half-maximal growth inhibitory concentration). Each experiment was performed at least in triplicate. Control cells were treated with 0.1% DMSO for single drug testing; 0.2% DMSO for combination assays. 10 μl of 440 μM resazurin sodium salt was added to control wells at the initiation of drug treatment and, after 2 h, the fluorescence intensity was measured using a Safire bottom-reading fluorescent plate reader with excitation at 530 nm and emission at 590 nm. After 72 h of treatment, resazurin was added and fluorescence intensity was measured. Data was analyzed using Prism 4 software.

Combination index calculations Drug combination assays were designed according to the methods of Chou and Talalay [24, 25]. Data was analyzed using Calcusyn software (Biosoft, Cambridge, UK) to determine the Combination Index (CI) at the ED_{50}, ED_{75} and ED_{90}. Synergism is defined as a CI<0.85; a CI of 0.85–1.2 reflects additivity; and a CI>1.2 indicates antagonism. Except where noted, R^2 is >0.95 for 3–5 replicates.

Results

We determined the effect of combining PP242 with chemotherapies used to treat CRC. Three classes of cytotoxic agents were tested: 5-fluorouracil (5FU), a pyrimidine analog anti-metabolite; oxaliplatin, a platinum-based alkylating agent; irinotecan (CPT-11) and it's active metabolite, SN-38, topoisomerase I inhibitors. Drug combination assays were designed according to the methods of quantitative analysis of dose-effect relationships at equipotent drug ratios [24, 25]. To carry out this analysis, we first determined potency, defined by the IC_{50} (half maximal inhibitory concentration), of each drug to be combined.

The IC_{50} of PP242 in human CRC cell lines with different mutational backgrounds was ascertained via proliferation assays. Cell lines in Table 1 are ordered by sensitivity to PP242; a heat map represents potency of each additional compound across the tested cell lines. Cell lines where KRAS is mutant in the absence of a PIK3CA mutation were most resistant to PP242. Potency is higher in cell lines in which KRAS and PIK3CA are both mutant. The syngeneic SW620-R cell line, with an introduced PIK3CA H1047R mutation, was 8.5-fold more sensitive to PP242, as compared to SW620 parental cells. Therefore, co-mutation of PIK3CA can sensitize KRAS mutant cell lines to PP242. The relationship between KRAS and/or PIK3CA mutational status and PP242 potency was consistent in 4 additional CRC cell lines (G.S.D., manuscript submitted). The SW-48 cell line, which is wild-type for KRAS and PIK3CA but contains an activating EGFR mutation (G719S), was included as a more extreme counterpart to resistant SW620 cells. A 150-fold difference in IC_{50} exists between the SW-48 and SW620 cells.

Sensitivity rankings were recapitulated in cell lines treated with a structurally unrelated mTOR active site inhibitor, Ku-0063794, a precursor to the more potent clinical compound, AZD8055 [5, 26]. Potency of the allosteric TORC-1 inhibitor, rapamycin, does not correlate with KRAS or PIK3CA mutational status, consistent with rapamycin having a more limited impact on signal transduction. No correlation was observed between the KRAS or PIK3CA status and sensitivity to conventional chemotherapeutics, however cell lines with p53 mutations were generally more chemotherapy-resistant. By contrast, PP242 potency does not correspond with p53 status.

The Combination Index (CI) is a quantitative measure of drug interaction derived from the median-effect equation, taking into account potency, efficacy, and the shape of the dose effect curve [24, 25]. Whereas the IC_{50} measures drug

Table 1 CRC cell line mutation status; inhibitor IC_{50} (μM) and efficacy (%)

	Mutational Status			TOR Inhibitors						Standard Chemotherapy									
Cell Line	KRAS	PI3K	p53	PP242		Ku-0063794		Rapamycin		5FU		Oxaliplatin		Irinotecan		SN-38		Potency
SW-48*	WT	WT	WT	0.1	39	0.1	26	0.0002	9	2.3	64	0.3	46	5.7	31	0.002	33	Sensitive
HCT-15	G13D	E545K	S241F	0.3	60	1.3	51	0.0006	35	16.7	71	11.6	55	>10		0.6	27	↓
HCT 116	G13D	H1047R	WT	0.6	51	1.5	62	0.003	21	14	73	3	55	2.2	32	0.005	33	↓
SW620-R	G12V	H1047R	R273H	1.3	42					28	43	1.1	37			0.03	49	↓
SK-CO-1	G12V	WT	WT	2.1	41			0.0004	15	17.6	44	0.5	38			0.008	48	Resistant
SW620	G12V	WT	R273H	11	43	5	41	0.0013	10	19.5	60	1.9	47			0.02	49	
Avg. % Efficacy					*46*		*45*		*18*		*59*		*46*		*32*		*40*	

Mutational status from the Sanger Catalogue of Somatic Mutations in Cancer [35]. Reported efficacy is percent change at 4× the drug IC50, compared to vehicle control. *EGFR mutated. WT, wild-type. Hatched, not done. HCT-15 proliferation was inhibited by SN-38 but not irinotecan, suggesting that the carboxylesterase enzyme required for conversion to SN-38 was not active. Sensitivity ranges specific to each inhibitor can be found in Online Resource 1

potency, efficacy is defined as the fractional inhibition of cell growth by the drug dose. The utility of the Chou and Talalay method has been questioned when combining cytotoxic and cytostatic agents because cytostatic drugs often lead to incomplete dose-effect curves [27]. This translates into a difference in efficacy, or the efficiency of inhibiting cell proliferation. To determine whether the Chou and Talalay method is appropriate to study combinations of PP242 with chemotherapeutic agents, we compared the efficacy of PP242 with that of the cytotoxic agents used in this study. Combination assays are performed using equipotent drug ratios, at concentrations of 1/4 to 4× the IC_{50} for each drug (Fig. 1a). For this reason, the efficacy of PP242 and the cytotoxic agents was calculated at 4× the IC_{50} of each agent (Table 1). The average efficacy of PP242 was similar to that of oxaliplatin and between that of 5FU and irinotecan or SN-38. PP242 efficacy was similar in all cell lines at the maximum concentration tested (10 μM). By contrast, rapamycin is an example of a potent inhibitor, with low-nanomolar IC_{50}s, which exhibited poor efficacy at inhibiting cell growth in most CRC cell lines (Table 1). Because the efficacy of PP242 and the chemotherapeutics is similar, it is valid to use the Chou and Talalay method to study combinations of these agents.

When clinically relevant drug concentrations are unknown, the CI should be reported for a range of doses because an inhibitor combination may be synergistic at some concentrations (CI<0.85) and antagonistic (CI>1.2) at others. This is illustrated in a representative median-effect plot and isobologram for the combination of PP242 with 5FU in HCT 116 cells (Fig. 1b–c). The ED_{50} (half maximal effective dose) in drug combination experiments, derived from the upward sloping isobologram, is conceptually equivalent to the IC_{50} in proliferation assays. In Fig. 2, the CI at the ED_{50}, ED_{75}, and ED_{90} is reported because it is not known which dose of PP242-like agents will be applicable in vivo. The CI was calculated for PP242 plus 5FU, oxaliplatin, irinotecan or SN-38 in 5 cell lines, SW-48, HCT-15, HCT 116, SW620-R, and SW620, ordered by sensitivity to PP242 (Fig. 2a–c). As a control we combined mTOR active site inhibitors to confirm additivity of agents with the same target: the CI was 1.0 at the ED_{50} for PP242 + Ku-0063794 in HCT 116 cells.

The combination of PP242 with the conventional therapies was at least additive at the ED_{50} in most cell lines, but there was a trend toward antagonism at higher effective doses. In combination with 5FU, the IC_{50} of PP242 correlated with relative synergy or antagonism (Fig. 2a). The higher the sensitivity to the mTOR inhibitor as a single agent, the more likely the combination with 5FU was synergistic. The same trend exists for oxaliplatin + PP242 but with less dynamic range (Fig. 2b). Conversely, there was no relationship between sensitivity to PP242 and the CI for PP242 + irinotecan or SN38, but the combination was synergistic across all doses in the PP242 sensitive *and* p53 wild-type cell lines, SW-48 and HCT 116.

SW620 cells (KRAS mutant, PIK3CA wild-type) are resistant to PP242. In SW620 cells, the combination of PP242 with each cytotoxic agent was antagonistic at the ED_{90}. In the data presented above, we observed a correlation between mutant PIK3CA and sensitivity to single agent PP242 as well as a favorable CI with 5FU or oxaliplatin, suggesting that mutation of PIK3CA sensitizes cells to mTOR inhibition. To test this idea, we utilized the syngeneic SW620-R cell line, expressing PIK3CA H1047R. In SW620-R cells, the introduction of mutant PIK3CA sensitized cells to PP242 and reversed the antagonism between PP242 and 5FU or oxaliplatin seen at high effective doses in the parental cells. Interestingly, introduction of a PIK3CA mutation did not improve the CI for PP242 and SN-38 in SW620-R versus SW620 cells. Together, our data show that while mutation of PIK3CA sensitizes cells to PP242 and increases cooperativity of PP242 with certain chemotherapy, the choice of chemotherapeutic agent is important. HCT-15, SW620 and SW620-R cells are resistant to SN-38, possibly because they each have a mutation in the p53 DNA-binding domain, suggesting that synergy between PP242 and SN-38 may require sensitivity to both compounds as single agents. In support of this hypothesis, the combination of PP242 and SN-38 is antagonistic in SK-CO-1 cells which are p53 wild-type and SN-38 sensitive, but resistant to PP242.

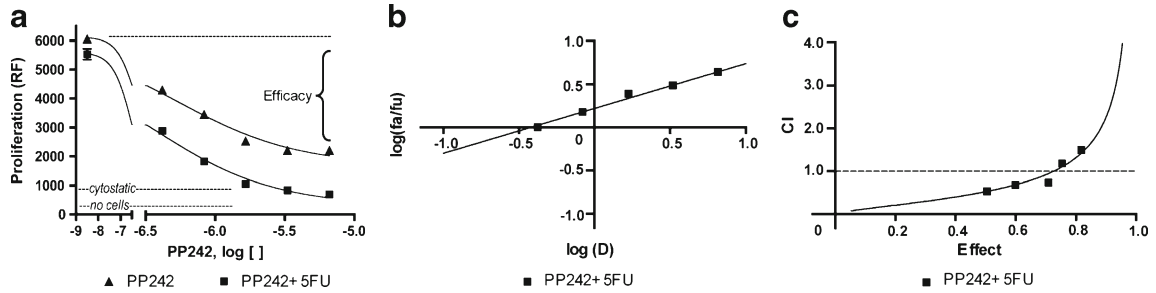

Fig. 1 Proliferation assays used to determine the Combination Index (CI) of PP242 and 5FU in HCT 116 cells (**a**). Data points are at 0, 1/2, 1/4, 1, 2, and 4× the IC_{50} of PP242 ± 5FU at equipotent concentrations (R^2=0.98). Background resazurin fluorescence (RF) is defined as *no cells*; RF upon initiation of the 72 h drug treatment is delineated as *cytostatic*. The PP242 + 5FU curve crosses the cytostatic line, indicating cytotoxicity. Median-effect plot (**b**) and isobologram (**c**) generated using Calcusyn software for the combination of PP242 with 5FU in HCT 116 cells. Fa, fraction affected; fu, fraction unaffected by the dose, D

Discussion

The present study constitutes the first systematic evaluation of the combination of an ATP-competitive mTOR inhibitor with standard chemotherapy for colorectal cancer. Clinical testing of mTORC1/2 kinase inhibitors in combination with cytotoxic chemotherapy is a natural consideration because this class of targeted agents is cytostatic in CRC cells. We hypothesized that PP242 might antagonize the activity of conventional chemotherapeutics targeting dividing cells, but that mutational status, class of chemotherapy, and effective dose could be important variables. To our knowledge, ours is the first observation of a correlation between genetic determinants of single agent potency and synergy between a molecularly targeted inhibitor and chemotherapy, as measured by the combination index. Including replicates, the work presented here encompasses over 5000 treatment data points and would not have been feasible in an animal model.

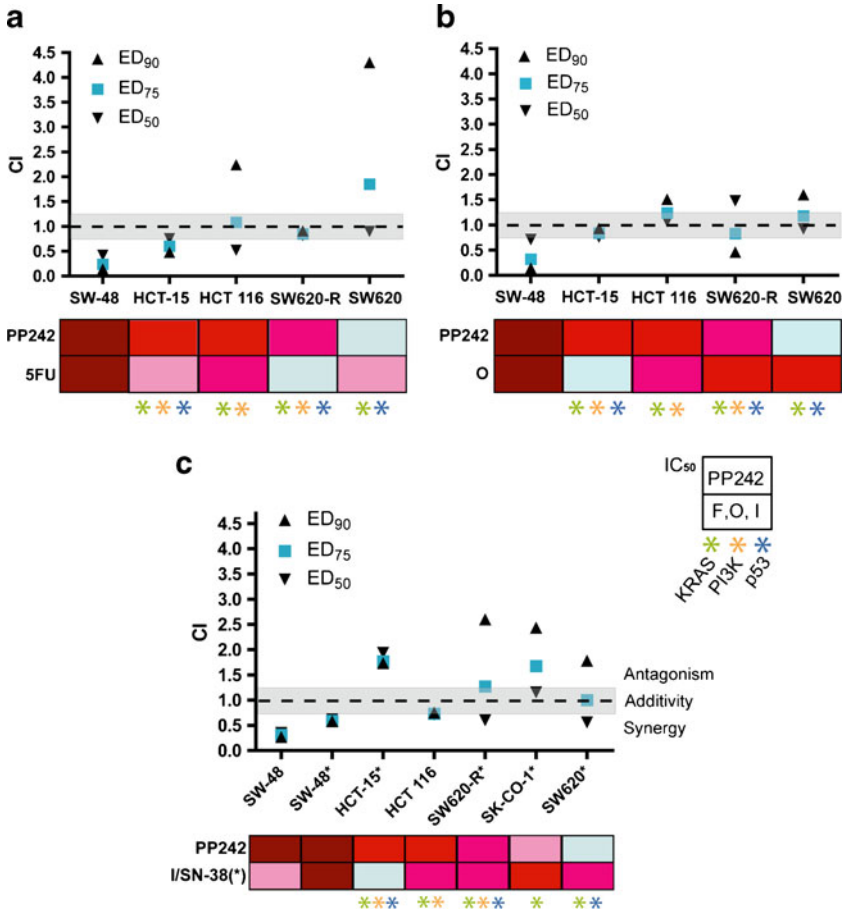

Fig. 2 Sensitivity to PP242 predicts synergy or antagonism between PP242 and 5FU or oxaliplatin but not irinotecan. Combination Index (CI) at the ED_{50}, ED_{75} and ED_{90} for PP242 plus 5FU (**a**), oxaliplatin (**b**), and irinotecan or SN38 (*) (**c**), in the indicated cell lines, ordered by decreasing sensitivity to PP242. The grey bar encompasses CI values that are nearly additive; points below the bar denote synergy and points above the bar indicate antagonism. R^2=0.97–0.99 except for PP242 + oxaliplatin in HCT 116 cells, R^2=0.91 (all 3–5 replicates). Squares below each panel encode sensitivity to PP242, upper half, or the cytotoxic agent, lower half, as defined in Table 1. Asterisks denote mutant KRAS (green), PI3K (orange) and p53 (blue)

The goal of our research is to aid the rational design of clinical trials to give ATP-competitive mTOR inhibitors and patients the best chance for success.

We focused our investigation on KRAS mutant cell lines, representing the best-defined patient population with an urgent need for new treatments. While determining single agent potency, a necessary prerequisite for the CI, we examined the relationship between mTOR inhibitor potency and KRAS/PIK3CA status. We observed that cell lines with KRAS and PI3K co-activation are sensitive to PP242, whereas cell lines with wild-type PIK3CA but mutant KRAS are more resistant. Introduction of mutant PIK3CA into KRAS mutant cells increased sensitivity to PP242 8.5-fold. PI3K and mTOR pathway activation have been linked to resistance to EGFR targeted therapies, suggesting that some patients with KRAS wild-type tumors resistant to EGFR-directed monoclonal antibodies may also benefit from mTORC1/2 inhibitors [28–30]. Roughly half of the 15% of CRC tumors with PIK3CA mutations are wild-type for KRAS, but no such cell lines were commercially available (Fig. 3).

There was no relationship between the potency of standard chemotherapies and KRAS or PIK3CA status. Mutation of p53 predicted resistance to irinotecan, but not PP242. Two studies report that p53 activity is a determinant of sensitivity to 5FU and oxaliplatin, but not to irinotecan in CRC cell lines [31, 32]. Although apparently incongruous with our data, both studies rely on an isogenic p53 knockout ($p53^{-/-}$) HCT 116 cell line that may behave differently from tumor cells with naturally occurring p53 missense mutations. Indeed, our findings are consistent with a study using an overlapping panel of CRC cell lines; there, loss of p53 activity inversely correlated with irinotecan sensitivity [33]. The impact of p53 mutations on response to chemotherapy may be more complex in patients, as recent work of ours revealed an interaction between specific p53 mutations, gender, and survival following adjuvant chemotherapy (C.E.A., manuscript submitted).

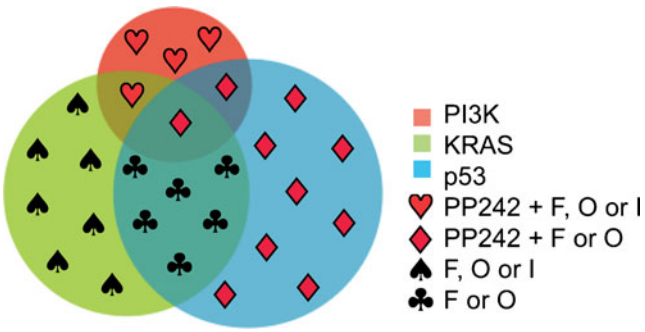

Fig. 3 Which card to play? Genotype-based treatment strategies. Approximately 16% of CRC tumors have mutant PI3K (orange); 40% have KRAS mutations (green); and 48% have p53 mutations (blue). Not shown are the 28% of patients with PI3K, KRAS and p53 wild-type tumors. Predicted best therapies: ♥, PP242 + 5FU, oxaliplatin, or irinotecan; ♦, PP242 + 5FU or oxaliplatin; ♠, 5FU, oxaliplatin, or irinotecan; ♣, 5FU or oxaliplatin

Efficacy of PP242 was comparable to that of standard chemotherapies over the dose range tested. In combination assays, we found that sensitivity to PP242 dictates relative synergy or antagonism when PP242 is combined with 5FU, and to a lesser degree, oxaliplatin. The combination of irinotecan and PP242 was synergistic across doses as long as the cell lines were sensitive to PP242 *and* irinotecan (p53 wild-type). Antagonism was rarely observed at the ED_{50} when PP242 was combined with any chemotherapeutic.

In general, for cancer therapies, high effect levels are considered more therapeutically relevant than low effect levels, but high effective doses may not be achievable in vivo [25]. One challenge to applying Chou and Talalay's method to cytostatic pathway inhibitors is that IC_{50}s of the inhibitors may exceed pharmacologically relevant concentrations [27]. This limitation is unlikely to apply to PP242. Mice have successfully been treated daily with PP242 at 100 mg per kg body weight, corresponding to a 2 mg oral PP242 dose for a 20 g mouse [14]. We have found by liquid chromatography-mass spectrometry that, 2 h after an oral dose of 2.5 mg PP242, the plasma concentration in a 25 g mouse was 5.8 μM (data not shown). A trend toward antagonism at higher effective doses was observed in several cases, however, suggesting that the maximally tolerated dose may not be most beneficial for combined mTOR inhibition and chemotherapy. This hypothesis can be tested using next generation active-site mTOR inhibitors with better pharmaceutical properties that allow prolonged dosing at lower effective doses in vivo [6].

The significance of mTOR in the carcinogenesis of metastatic CRC with specified mutations will be revealed upon testing mTORC1/2 inhibitors in clinical trials. Our data using CRC cell lines provide working hypotheses for the design of trials combining mTORC1/2 inhibitors with chemotherapy, stratified by tumor genetic profile (Fig. 3). Because we observed that PIK3CA mutation predicts cooperativity between PP242 and 5FU and oxaliplatin, we propose testing the combination of any of the ATP-competitive mTOR inhibitors in clinical development with these chemotherapeutic agents in patients with PIK3CA mutations, stratified by the presence of absence of a KRAS co-mutation. If an mTORC1/2 inhibitor is combined with irinotecan, benefit may be limited to patients with functional p53. Although the present study defines tumors with respect to PIK3CA status, a variety of alterations give rise to PI3K pathway activation, so a more comprehensive readout of pathway status may identify additional patients with tumors susceptible to combination chemotherapy including an mTORC1/2 inhibitor [34].

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Disclosures K.M.S. is a co-founder of Intellikine and serves on the Scientific Advisory Board.

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