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The PARP inhibitor ABT-888 potentiates darbazine-induced cell death in carcinoids

Yash Somnay, BS[1], **Sam Lubner, MD**[2], **Harpreet Gill**[1], **Jon Blake Matsumura**[1], and **Herbert Chen, MD FACS**[1,2]

[1]Endocrine Surgery Research Laboratories, Department of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI

[2]Division of Hematology and Medical Oncology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison WI

[2]Department of Surgery, University of Alabama-Birmingham, Birmingham, AL

Abstract

Monoagent DNA-alkylating chemotherapies like dacarbazine are among a paucity of medical treatments for advanced carcinoid tumors, but are limited by host toxicity and intrinsic chemoresistance through the base excision repair (BER) pathway via poly (ADP-ribose) polymerase (PARP). Hence, inhibitors of PARP may potentiate DNA-damaging agents by blocking BER and DNA restoration. We show that the PARP inhibitor ABT-888 (Veliparib) enhances the cytotoxic effects of dacarbazine in carcinoids. Two human carcinoid cell lines (BON and H727) treated with a combination of ABT-888 and dacarbazine resulted in synergistic growth inhibition signified by combination indices <1 on the Chou-Talalay scale. ABT-888 administered prior to varying dacarbazine doses promoted the suppression of neuroendocrine biomarkers of malignancy ASCL1 and CgA, shown by Western analysis. ATM phosphorylation and $p21^{Waf1/Cip1}$ activation, indicative of DNA damage, were increased by ABT-888 when combined with dacarbazine treatment, suggesting BER pathway attenuation by ABT-888. PE Annexin V/7-AAD staining and sorting revealed a profound induction of apoptosis following combination treatment, which was further confirmed by increased PARP cleavage. These results demonstrate that ABT-888 synergizes dacarbazine treatment in carcinoids. Therefore, ABT-888 may help treat carcinoids unresponsive or refractory to mainstay therapies.

ALL CORRESPONDENCE TO: Herbert Chen MD FACS, 1808 7th Avenue, South Suite 502, Birmingham, AL 35233, USA. Tel: + 1 205.934.3333, herbchen@uab.edu.

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INTRODUCTION

Carcinoids are heterogenous neuroendocrine tumors (NETs) that arise from the body's enterochromaffin cells and vary in anatomical site. They are most commonly found in the gastrointestinal tract, with the small intestine being the site of highest occurrence, followed by those of the lung, comprising about 2% of all bronchopulmonary tract tumors[1, 2]. Carcinoids can occur either sporadically or as part of hereditary syndromes, but are collectively rare with an age-adjusted incidence of about 2–4 per every 100,000 people[1, 3, 4]. Interestingly, the incidence of small bowel and bronchopulmonary carcinoids appears to have increased over a recent five-decade period per pan-SEER reports, possibly from improvements in surveillance or secondary to an evolving disease. A significant portion of carcinoids present with distant metastases of which about half have primaries of unknown origin[4]. In fact, carcinoids are among the most prominent causes of isolated hepatic metastasis, second only to colorectal cancer.

Surgical resection can offer definitive cure in the absence of metastatic disease, though no current data exists supporting adjuvant systemic therapies. Cytoreductive resection may be considered when intent is palliative and complete resection is not possible. In such cases, chemotherapies such as somatostatin analogs have been demonstrated to abate hormonal symptoms and also improve time-to-progression[5]. Cytotoxic agents are typically reserved for tumors with high proliferative indices (Ki-67≥5%), historically involving combined 5-fluorouracil or doxorubicin with the alkylating agent streptozocin[6, 7]. An alternative alkylating agent known as dacarbazine along with its oral, less toxic formulation temozolomide have also shown moderate activity in advanced NETs in addition to melanoma and glioma[8–12]. Notably, prospective studies of dacarbazine-inclusive polychemotherapy have demonstrated limited efficacy among carcinoids relative to other NETs[13–17]. Dacarbazine exerts its effect by methylating the O^6-guanine position of its target DNA thereby causing mismatch repair and eventual cell death. More commonly, however, dacarbazine methylates the N^7-guanine and N^3-adenine position, comprising 70% and 9% of adducts respectively, and which may be removed by base excision repair (BER) pathway[18]. Hence, through its ability to restore DNA to its normal state, robust BER activities have been associated with dacarbazine resistance[18, 19].

The BER pathway is carried out by the enzyme poly (ADP-ribose) polymerase (PARP), a nick-sensing enzyme that recruits BER complex proteins to double stranded DNA break sites to initiate repair following base excision of, for instance, N^7 and N^3 adducts[20]. Inhibitors of PARP have been thus developed with the intent of circumventing dacarbazine resistance by blocking BER and promoting N^7 and N^3 methylation-induced cell death[20, 21]. This approach has been successfully demonstrated in malignancies of pulmonary, colonic, glial and hematopoetic origin both *in vivo* and *in vitro*[22–27]. A novel PARP inhibitor ABT-888, also known by its trade name Veliparib, has been shown to potentiate DNA-damaging chemotherapies in advanced solid tumors[28–32]. Importantly, studies have supported the use of ABT-888 in combination with alkylating agents like dacarbazine and its sister drug temozolomide across a spectrum of cancers including glioblastoma, leukemia, hepatocellular carcinoma, and metastatic melanoma with recent corroboratory clinical investigations[33–43].

Given the clinical refractoriness of carcinoids to dacarbazine-based therapies and mounting evidence regarding resistance mechanisms implicating DNA-damage responses, we sought to investigate the *in vitro* interaction between ABT-888 and dacarbazine in carcinoid cell lines.

MATERIALS AND METHODS

Cell culture

Human gastrointestinal carcinoid cells (BON), were gifted by Drs. Courtney M. Townsend, Jr. of the University of Texas Medical Branch (Galveston, TX, USA) and B. Mark Evers of the University of Kentucky (Lexington, KY, USA). Human bronchopulmonary carcinoid (H727) cells were purchased from the American Type Culture Collection (Manassas, VA, USA). BON and H727 cells were grown in DMEM/F-12 (Life Technologies, Grand Island, NY, USA) and RPMI/F-12 (Life Technologies, Grand Island, NY, USA), respectively, at a 5% CO_2 and 37°C atmosphere. Media was supplemented with 100 IU/mL penicillin, 100μg/mL streptomycin (Life Technologies, Grand Island, NY, USA) and 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA). ABT-888 (Selleck Chemicals, Houston, TX, USA) and dacarbazine (Sigma-Aldrich) were stored in aliquots of 10mM in DMSO at -80°C, and freshly thawed before use. Cells were plated at sub-confluency the day prior to treatment, and then incubated in fresh medium containing ABT-888 (0–10μM) for 24 hours, after which dacarbazine was added (0–1000μM) for 2 additional days. DMSO concentrations were normalized across all treatment groups.

Western blotting

Total BON cell lysates following dacarbazine ± ABT-888 treatment were prepared and analyzed by Western blotting as previously described[44]. Each antibody was diluted as follows: 1:2000 for mammalian achaete-scute complex-like1 (ASCL1) (BD Pharmingen, San Diego, CA, USA), 1:3000 for chromogranin A (Zymed Laboratories, San Francisco, CA, USA), 1:10,000 for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Trevigen, Gaithersburg, MD, USA), and 1:1000 for $p21^{Waf1/Cip1}$, cleaved poly (ADP-ribose) polymerase (PARP), phosphorylated ATM, total ATM, and Survivin (Cell Signaling Technology, Beverly, MA, USA). Antibody signals were detected using Supersignal West Femto, Dura, or Pico (Pierce, Rockford, IL, USA) chemiluminescence systems and manufacturers' instructions were adhered to.

Cell viability

BON and H727 cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) rapid colorimetric assay. Both cell lines were seeded in 96-well plates, and treatment groups were plated in sextuplicate. To assess viability following treatment, media was replaced with 25μL of 0.5μg/mL MTT in serum-free media for 3.5 hours at 37°C, followed by addition of 75μL DMSO before measuring optical densities. The remainder of our protocol was followed as previously described[45].

PE Annexin V/7-AAD staining

BON cells treated with dacarbazine (0–600μM) ± ABT-888 (0-10μM) were collected and incubated with PE Annexin V and 7-Aminoactinomycin D (7-AAD) fluorescein solutions (BD Pharmingen, San Jose, CA, USA) according to the manufacturer's protocol. The FACSCalibur™ (BD Biosciences, San Jose, CA, USA) fluorescent-activated cell-sorting (FACS) instrument was used for quantitative fluorescent sorting, and FlowJo v10.0.8 (TreeStar Inc., Ashland, OR, USA) was used for subsequent analysis.

Statistical analyses

Student's t-test was used to compare means between groups and all data are represented as mean ± SEM. CompuSyn® (Paramus, NJ, USA) was used to calculate combination indices (CI) following dacarbazine ± ABT-888 treatment as per the Chou-Talalay method for drug interactions. CI of greater than, equal to, or less than 1 signify antagonistic, additive, or synergistic interactions respectively between two treatments[46].

RESULTS

ABT-888 and dacarbazine synergistically inhibit carcinoid cell proliferation

We set out to determine if BON and H727 cells could be sensitized to dacarbazine with the addition of ABT-888. We observed that both BON and H727 prolifieration were dose-dependently suppressed following dacarbazine doses of up to 1000μM, with IC_{50} values of 218.2μM and 268.6μM, respectively. To determine the correct dose range for concomitant ABT-888 treatment, its monotherapeutic effects were first established using viability assays. ABT-888's IC_{50} in BON and H727 exceeded 50μM, with near 92% of cells still viable at 20μM. We conservatively chose ABT-888 doses of 5μM and 10μM for combination treatment with dacarbazine, as these exerted minimal cytotoxic effect alone, and were within the range necessary, as previously reported, to inhibit its molecular targets and achieve combinatorial benefits[37, 39, 43]. Co-treatment of BON cells with 5μM ABT-888 potentiated dacarbazine-induced cytotoxicity as indicated by CI<1 following 500μM dacarbazine and above. When ABT-888 co-treatment was increased to 10μM, cells appeared further sensitized to dacarbazine, with CIs falling <1 following dacarbazine doses of 400μM and above (Table 1). H727 cells responded similarily, with improved sensitization to dacarbazine at 10μM ABT-888 relative to 5μM. Doses of dacarbazine above 750μM and 400μM interacted synergistically with 5μM and 10μM ABT-888, respectively, signified by CI<1 (Table 2). These curves are represented in Figure 1A-B.

ABT-888 potentiates the effect of dacarbazine on neuroendocrine biomarker expression

To determine the influence of combinatorial ABT-888 and dacarbazine treatment on carcinoid bioactivity we next investigated its effect on expression of neuroendocrine biomarkers of malignancy chromogranin A (CgA), and the basic helix-loop-helix transcription factor achaete-scute complex-like1 (ASCL1), biologically relevant markers of neuroendocrine tumors. CgA is a glycopeptide inherent to neuroendocrine tissue and often used clinically as a biomarker for disease prognosis[47]. ASCL1 is an evolutionarily conserved transcription factor central to neuroendocrine development and highly expressed in

carcinoids[48]. As shown in Figure 1C, ASCL1 and CgA expression responded to dacarbazine monotherapy at doses up to 600μM in BON cells, with CgA only moderately suppressed and ASCL1 reduction more pronounced. This dose range for dacarbazine was chosen since it encompassed both the IC_{50} in BON cells, as well as the window within which combination indices reflected synergy (Table 1–2). Following ABT-888 co-treatment, CgA expression was reduced markedly further, and ASCL1 expression was near entirely diminished (Figure 1C). Collectively, these data indicate that ABT-888 potentiates the anticancer effects of dacarbazine on the neuroendocrine phenotype of carcinoids.

Combination ABT-888 and dacarbazine treatment promotes apoptosis

Through its mechanism of action, ABT-888 is purported to enhance apoptotic induction by impairing the mechanisms that mend dacarbazine-induced DNA damage[37]. To assess the extent of apoptosis in response to ABT-888 and dacarbazine, PE Annexin V/7-AAD was used to probe BON cells following 72 hours of treatment (as described in the Methods section). The percentage of cells in apoptosis (upper right quadrant), preapoptosis (lower right quadrant), necrosis (upper left quadrant) and those still viable (lower left quadrant) were quantified using flow cytometry. Experimental replicates were then averaged and are conveyed in the adjacent bar graph (Figure 2A). ABT-888 alone showed no appreciable increase in apoptosis. However, cells treated with ABT-888 and dacarbazine exhibited marked apoptotic induction relative to dacarbazine alone, almost approaching significance ($p=0.07$). To corroborate this, the degree of PARP cleavage was assessed following combinatorial treatment. Figure 2B shows considerable induction of cleaved PARP expression in BON cells treated with increasing dacarbazine doses in the setting of ABT-888 co-treatment, while dacarbazine alone only produced modest PARP cleavage. Once again, ABT-888 alone had no effect. These data further suggest that ABT-888 sensitizes cells to dacarbazine cytotxocity and apoptosis.

ABT-888 and dacarbazine treatment leads to DNA damage

To further elucidate the mechanism by which ABT-888 and dacarbazine were inducing cell death, we next examined its effect on components of the ataxia telangiectasia mitogen factor (ATM)-mediated DNA-damage response, including ATM and $p21^{Waf1/Cip1}$. Upon DNA damage, ATM is autophosphorylated and activates several downstream checkpoint proteins, ultimately leading to cell cycle arrest[49, 50]. Through its delayed response, ATM phosphorylation also leads to activation of $p21^{Waf1/Cip1}$ and other p53 target genes, ultimately causing cell cycle arrest or apoptosis[51]. We observed that combination treatment increased levels of phospho-ATM and $p21^{Waf1/Cip1}$, suggesting DNA damage from ABT-888 and dacarbazine treatment. Additionally, combination treatment potentiated suppression of the Inhibitor of Apoptosis protein Survivin, a marker of cancer cell malignancy that binds to and inhibits caspase cleavage, blocks G2/M phase progression and thereby promotes cell replication[52]. Together these data indicate that DNA damage occurs secondary to ABT-888 and dacarbazine combination treatment, thus mediating apoptotic induction (Figure 3).

DISCUSSION

Despite recent advances in targeted therapies, there is limited consensus on standardized treatment approaches for metastatic carcinoid tumors[53]. In the setting of distant spread, 5-year survival is only about 38.5% compared to 78.2% for local disease, based on late SEER data[1]. Patients with disseminated bronchopulmonary carcinoids fare very poorly as well with survival approaching only 15% after 5 years, as do those with atypical pulmonary carcinoids with only 25% mortality[54]. Therapeutic management of carcinoids is also made challenging given their long natural histories raising concerns for treatment toxicity. Carcinoids are known to be slow-growing, insidious cancers that follow a subtle yet malignant clinical course. Neuroendocrine in origin, they comprise cells packed with neurosectretory granules containing biogenic and vasoactive peptides including seratonin, histamine, and gastrin, that upon release lead to the debilitating carcinoid syndrome[4]. Other markers of neuroendocrine malignancy include chromogranin A (CgA), and the neuroendocrine-specific transcription factor ASCL1[47, 55]. Cytotoxic therapies have been thought to play a role in treatment of patients with locally advanced or metastatic tumors wherein surgical resection is not curative[7].

In most carcinoid patients, agents like dacarbazine and its sister drug temozolomide have shown little if any benefit compared to patients with other NETs. One particular study showed that only 1 of 14 carcinoid patients achieved objective response to temozolomide and thalidomide therapy[14]. A subsequent study similarily revealed no improvement among carcinoid patients receiving temozolomide and bevacizumab, while 33% of pancreatic NET patients achieved tumor response[15]. Temozolomide-based combination therapy was later associated with an only 2% partial or complete response among carcinoid patients compared to 34% of those with pancreatic NETs[13]. While other studies have associated monotherapy and combinatorial temozolomide regimens with up to 70% efficacy for pancreatic NETs, this approach has generally failed to show efficacy in carcinoids[16, 17]. Studies have attributed the treatment-refractoriness of carcinoids to its discrepant expression of mismatch repair mechanisms that may confer resistance. Archival specimens of carcinoids resistant to temozolomide were less likely to be deficient in the DNA repair enzyme O^6-methylguanine DNA methyltransferase (MGMT) than NETs responsive to treatment[13]. Since temozolomide's and dacarbazine's mechanism relies upon DNA methylation at the O^6-guanine position causing DNA mismatch and subsequent apoptosis, sensitivity to its effects would improve in the setting of impaired MGMT activity as demonstrated in patients with advanced glioblastomas and melanomas[56–58]. The BER pathway has also been acknowledged as a major contributor to temozolomide resistance in other cancer models, but moreover, its disruption in conjuction with temozolomide has been shown to sensitize cells to treatment[18, 19, 22–27]. Pharmacological inhibiton of BER by the PARP inhibitor ABT-888 has been demonstrated to enhance the antitumor effects of dacarbazine and temozolomide, and have advanced to phase I and II clinical trials for pediatric and adult gliomas, hepatocellular carcinoma, and metastatic melanoma with mixed success[33–42]. To improve carcinoid susceptibility to alkylating agent therapy for such candidate patients, we investigated the ability of the PARP inhibitor ABT-888 to enhance the activity of dacarbazine in carcinoids.

Our data show that in gastrointestinal and bronchopulmonary carcinoid cell lines, ABT-888 effectively sensitized cells to dacarbazine cytotoxicity, generating combination indices <1 (Tables 1–2) signifying synergistic interaction, and reduced the expression of neuroendocrine biomarkers of malignancy in BON cells (Figure 1). In addition to potentiating apoptosis (Figure 2), ABT-888 co-treatment profoundly increased levels of phosphorylated ATM and $p21^{Waf1/Cip1}$, indicating induction of the ATM-mediated DNA-damage response pathway[50] (Figure 3). Activation of ATM following DNA damage in response to PARP blockade is central to recruitment of DNA repair proteins, as has been illustrated following pharmacological inhibition of PARP[59]. Other reports have also reported on PARP's participation in the DNA-damage response triggering ATM phosphorylation and recruitment[60, 61]. Relevant the interpretation of our findings, Tanaka et al. demonstrated that phospho-ATM is an accurate indicator of DNA damage following chemotherapy-induced apoptosis[49]. Further aligned with our study, Liu et al. reported that the extent of cytotoxicity following ABT-888 and temozolomide treatment in several cancer lines was proportional to the degree of DNA damage as represented by levels of ATM's immediate target γH2AX[43]. Additionally, as a downstream target of p53 activation following ATM phosphorylation, $p21^{Waf1/Cip1}$ is a key regulator of G2/S checkpoint passage by maintaining G2-phase arrest in the setting of cellular stress[51]. Of note, its expression has been shown to be a direct function of DNA damage given its inextricable link to the ATM-mediated DNA repair process[62]. Previous reports have confirmed that pharmacologic inhibitors of PARP including ABT-888 cause a G2/S phase arrest state during replicative stress, confirmed by an upregulation of $p21^{Waf1/Cip1}$ [63–65]. Combined with the simultaneous suppression of the Inhibitor of Apoptosis gene Survivin alongside PARP cleavage, these results strongly suggest that ABT-888 optimizes dacarbazine-induced cytotoxicity at synergistic doses by inducing DNA damage and subsequent alteration in cell cycle kinetics, suggested by ATM pathway activity, to promote cellular demise.

Common treatment strategies for managing locally advanced or metastatic carcinoids, although not standardized, have employed the use of alkylating agents such as streptozocin-based regimens that are FDA approved for these indications[7]. However, given carcinoids prolonged performance status and indolent growth pattern, the routine use of such treatment approaches often raises toxicity concerns despite their purported therapeutic potential[53]. Even the alternative alkylating agent temozolomide, which has been explored clinically for treating NETs including in phase II trials, has resulted in limited therapeutic benefit in carcinoids specifically, as described in the above discussion[13–17]. Clinical challenges surrounding temozolomide and similar cytotoxic drugs are reflected by a study by Ramirez et al. who although endorse survival benefits from temozolomide and capecitabine therapy in metastatic NET patients, add that adverse reactions forced dose reductions in 24% of patients[66]. Therefore, translating these treatments into effective and rational therapeutic regimens is challenging, particularly due to the long natural histories of carcinoids making formal treatment comparisons difficult to devise. Alternatively, the use of compounds designed to sensitize patients to lower doses of cytotoxic therapies may be of value in this setting. Inhibitors of DNA repair like ABT-888 designed to enhance the therapeutic indices of alkylating agents may offer an option to circumvent long-term toxicity and improve treatment tolerability. Because drug sensitivity has been linked to inherent impairments in

the DNA damage response, it is likely, as our data suggests, that manipulating synthetic lethality through BER-pathway inhibition may improve treatment of carcinoids by targeting malignant cells with dysregulated repair mechanisms that confer resistance while spairing healthy cells[18, 19]. Moreover, we demonstrate that lone ABT-888 treatment has limited toxicity, further supporting its candidacy as a treatment adjunct with favorable toxicity. Initial human trials showed that only a single dose was necessary to achieve adequate plasma concentration necessary for effective PARP inhibition[67]. Since 2007, the National Cancer Institute has evaluated ABT-888 in 88 clinical trials for the treatment of several cancer types including melanoma, gliomas, hepatocellular carcinoma, pulmonary and colorectal cancers. Select trials have combined ABT-888 and temozolomide therapy, and despite offering no clinical suggestion of synergy, have demonstrated excellent tolerability among both pediatric and adult populations[34, 35, 40, 42]. Several studies report modest antitumor activity with some approaching significance, though their general failure to demonstrate favorable drug interaction may be attributed factors like acquired resistance through BER pathway overexpression[41, 42]. Previous *in vitro* reports have revealed that enhanced DNA repair mechanisms and homologous recombination capacity in response to ABT-888 and temozolomide therapy may underlie a learned resistance to this regimen[68]. Hence, the effects of combination therapy may be more profound when these intrinsic resistance mechanisms are inherently or therapeutically disabled. Additionally, given the low number of recruited patients in some of these trials, their results may be considered exploratory rather than confirmatory.

In summary, ABT-888 potentiates dacarbazine-induced cytotoxicity in carcinoid cell lines, while altering the neuroendocrine phenotype. Hence, this therapeutic strategy may be a viable option for circumventing treatment refractoriness while controlling syndrome symptomatology. Given the current clinical characterization and use of ABT-888 and dacarbazine's more tolerable form temozolomide, these findings warrant further investigations into the clinical use of combinatorial treatment for management of locally advanced and metastatic carcinoids.

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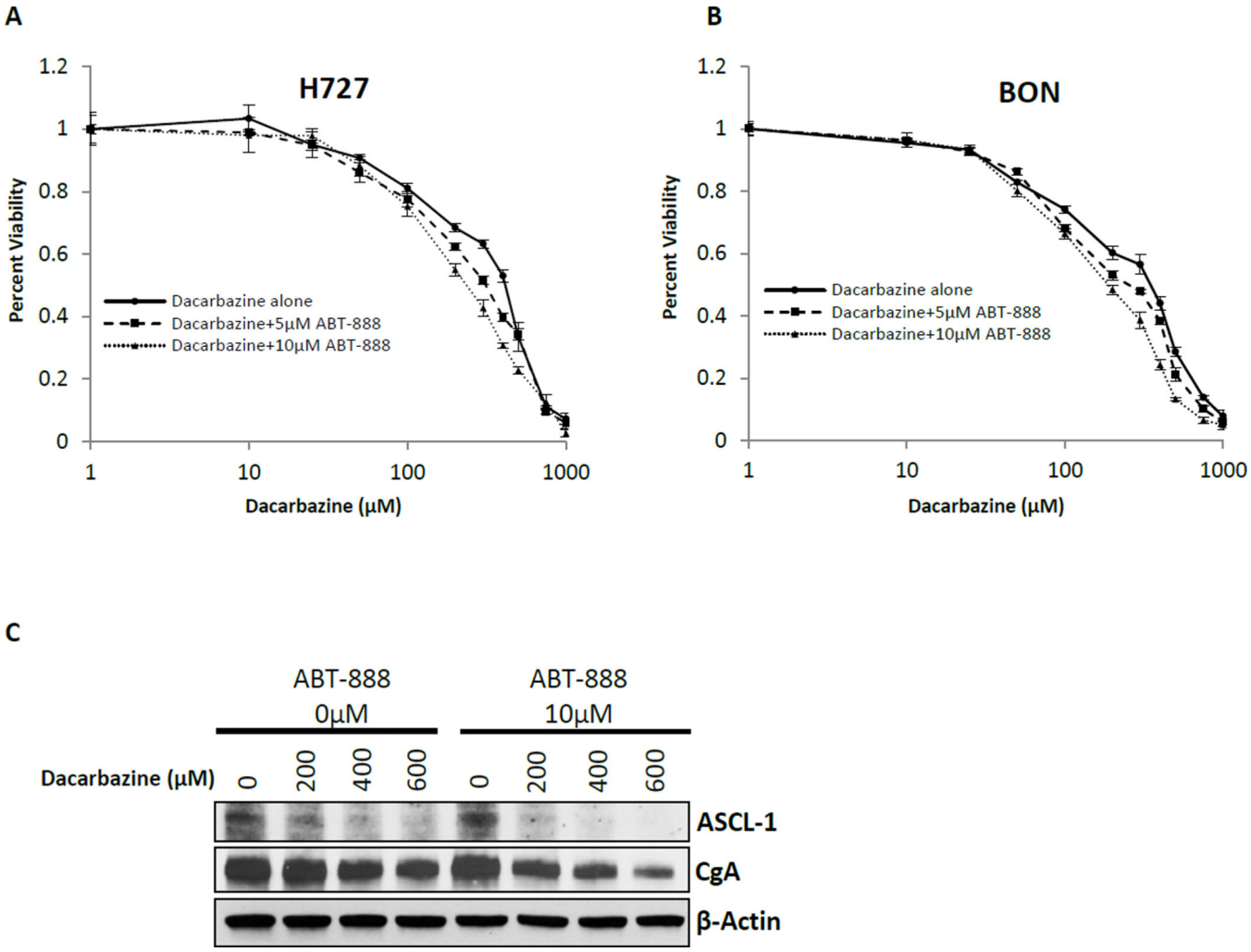

Figure 1. ABT-888 and dacarbazine synergistically inhibit cell growth in BON and H727 lines, while suppressing ASCL1 and CgA

BON GI (A) carcinoid and H727 (B) pulmonary carcinoid cell lines were treated with ABT-888 (0–10μM) for 24 hours, after which dacarbazine was added (0–1000μM) for 2 additional days. A 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay demonstrated dose-dependent reduction following dacarbazine treatment alone in both cell lines. Dacarbazine-induced cytoxicity was potentiated by the addition of 5μM ABT-888. With 10μM ABT-888, both cell lines were further sensitized to dacarbazine treatment. Combination indices indicated synergistic interaction between ABT-888 and dacarbazine in both BON and H727, falling below 1 at higher dacarbazine doses (Table 1–2). Combining ABT-888 (0–10μM) with dacarbazine (0–600μM) treatment enhanced suppression of neuroendocrine biomarkers ASCL1 and CgA in BON cells, relative to dacarbazine's effects alone (C).

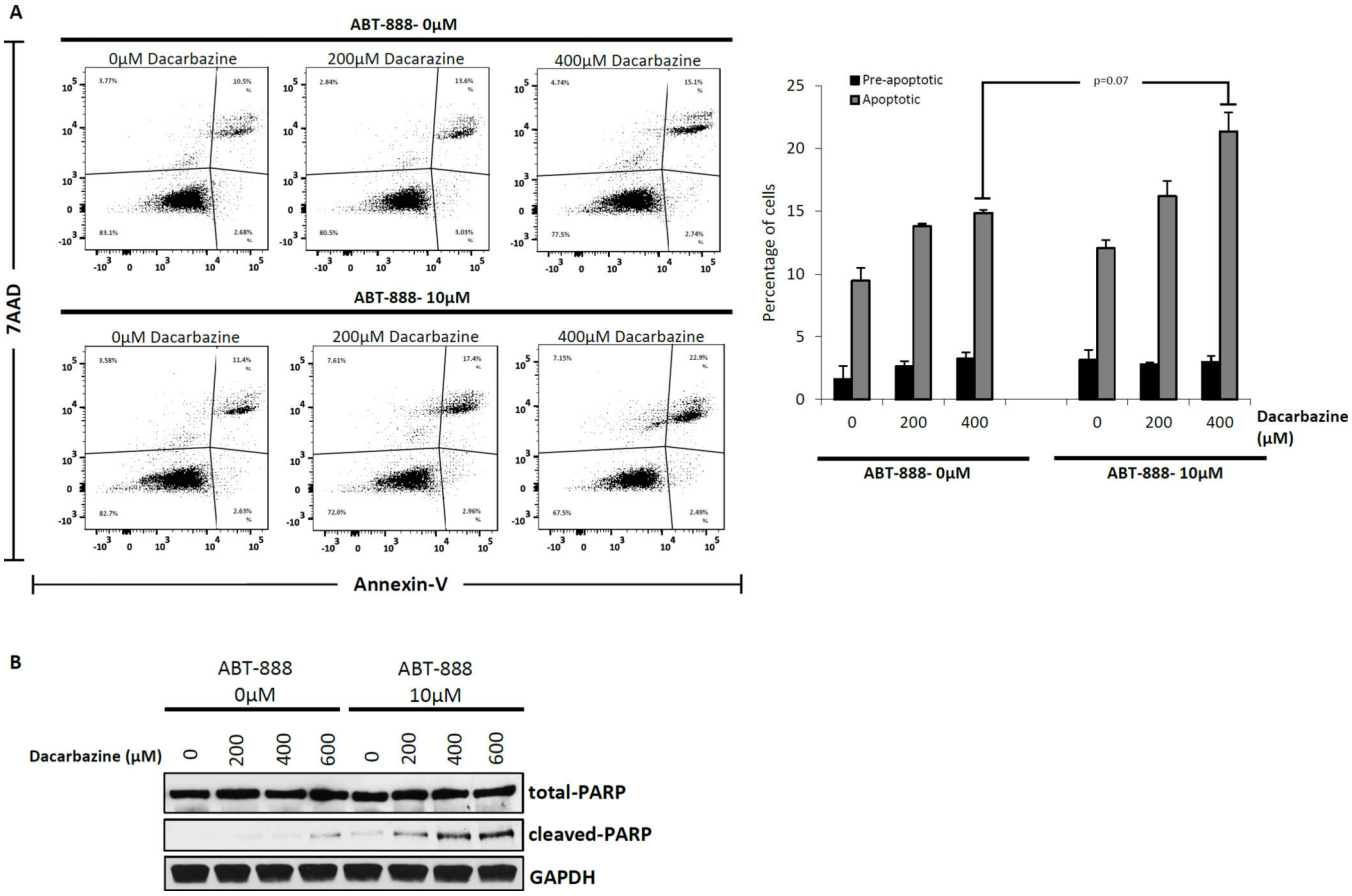

Figure 2. ABT-888 potentiates dacarbazine-induced apoptosis

BON cells treated with a combination of ABT-888 (0–10μM) and dacarbazine (0–400μM) underwent Annexin V/7-AAD staining followed by phosphatidylserine exposure. Using the BD FACSCalibur™ instrument, cells were quantified using fluorescent activated cell sorting, demonstrating an induction of apoptotic populations following 200μM and 400μM of dacarbazine alone. With the addition of 10μM ABT-888 to dacarbazine treament, the percentage of apoptotic cells markedly increased. Within each pane, the upper right quadrant indicates late apoptotic cells (Annexin V-positive/7-AAD-positive), the lower right quadrant represents pre-apoptotic cells, the upper left quadrant represents cells positive for 7-AAD only, and the the lower left quadrant represents viable cells (Annexin V-negative/7-AAD-negative). Data from three experimental replicates were averaged and are represented in the graph on the right (mean ± SEM) (A). Apoptotic induction following the addition of 10μM ABT-888 to dacarbazine (0–600μM) treatment was also indicated by enhanced cleavage of the terminal apoptotic marker PARP (B).

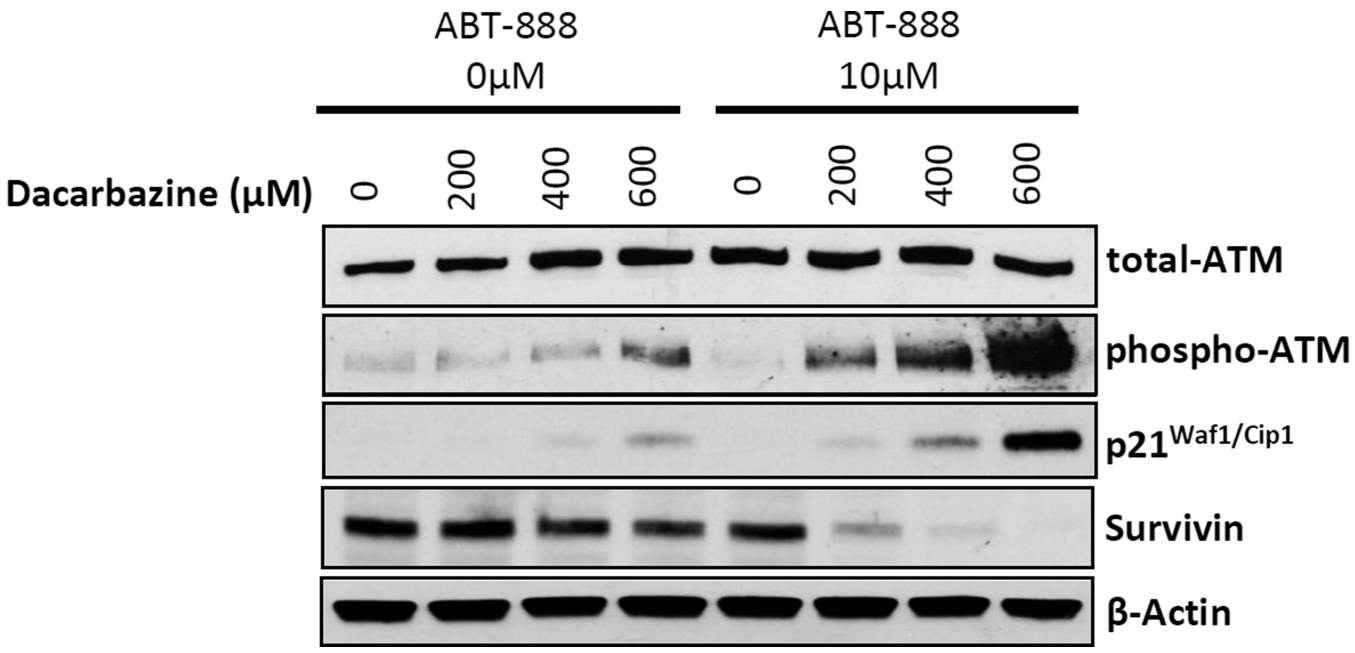

Figure 3. ABT-888 and dacarbazine induce ATM phosphorylation and p21$^{Waf1/Cip1}$ expression

Combined ABT-888 (0–10μM) and dacarbazine (0–600μM) treatment induced phosphorylated-ATM and p21$^{Waf1/Cip1}$, while either agent alone generated minimal expression. These findings indicate activation of the ATM-mediated DNA repair pathway secondary to drug-induced DNA damage. While dacarbazine alone produced no observed effect on expression of the Inhibitor of Apoptosis gene Survivin, the addition of 10μM ABT-888 led to near complete depletion of its expression.

Table 1

Combination indices[1] (CI) following dacarbazine and ABT-888 treatment in BON

	5μM ABT-888	10μM ABT-888
Dacarbazine (μM)	**CI**	
10	1.43	2.08
25	1.60	2.08
50	1.44	1.23
100	1.11	1.12
200	1.12	1.04
300	1.37	1.08
400	1.26	0.75
500	0.74	0.48
750	0.52	0.39
1000	0.42	0.37

[1]Calculated based on the Chou-Talalay method (>1:antagonism, =1:additivity, <1:synergy)

Table 2

Combination indices[1] (CI) following dacarbazine and ABT-888 treatment in H727

	5μM ABT-888	10μM ABT-888
Dacarbazine(μM)	**CI**	
10	2.49	2.59
25	1.32	3.48
50	0.96	1.41
100	1.12	1.19
200	1.22	1.08
300	1.25	1.05
400	1.19	0.96
500	1.25	0.88
750	0.61	0.78
1000	0.54	0.40

[1]Calculated based on the Chou-Talalay method (>1:antagonism, =1:additivity, <1:synergy)