Constitutively activated STAT3 and STAT5 are expressed in a wide variety of human malignancies including solid and hematopoietic cancers and often correlate with a poor prognosis and resistance to multiple therapies. Given the well established role of STAT3 in tumorigenesis, inhibition of Janus-activated kinase 2 (JAK2) activity might represent an attractive therapeutic approach. Using a mouse model of colitis-induced colorectal cancer, we show that a novel, orally active, selective JAK2 inhibitor, CEP-33779, induced regression of established colorectal tumors, reduced angiogenesis, and reduced proliferation of tumor cells. Histopathologic analysis confirmed reduced incidence of histologic-grade neoplasia by CEP-33779. Tumor regression correlated with inhibition of STAT3 and NF-κB (RelA/p65) activation in a CEP-33779 dose-dependent manner. In addition, the expression of proinflammatory, tumor-promoting cytokines interleukin (IL)-6 and IL-1β was strongly reduced upon JAK2 inhibition. The ability of CEP-33779 to suppress growth of colorectal tumors by inhibiting the IL-6/JAK2/STAT3 signaling suggests a potential therapeutic utility of JAK2 inhibitors in multiple tumors types, particularly those with a strong inflammatory component.

Introduction

There is a growing recognition that inflammation plays a critical role in tumor initiation and progression (1, 2). Elevated levels of inflammatory cytokines like interleukin (IL)-6, IL-11, and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been shown in a wide spectrum of human and experimental tumors, in plasma of cancer patients, and often correlated with a more malignant and refractory disease (3). Tumor-associated inflammation contributes to tumor growth and progression through multiple mechanisms including increased proliferation and antiapoptotic signaling in tumor cells, promotion of angiogenesis, tumor immune evasion, and metastasis (1, 2). Most inflammatory signals promote tumorigenesis by activating NF-κB and Janus-activated kinase (JAK)/STAT signaling pathways, both in tumor and stroma cells. Constitutively activated STAT3 and STAT5 are expressed in a wide variety of human malignancies including solid and hematopoietic cancers and often correlate with a poor prognosis and resistance to therapies (4, 5). Among the common cancers with a strong inflammatory component are gastrointestinal tumors including colorectal carcinomas.

Colorectal cancer is one of the most fatal malignancies worldwide and develops spontaneously or as a long-term complication of chronic bowel inflammation such as in Crohn’s disease or ulcerative colitis (6). The risk of colorectal cancer development is determined by genetic predisposition combined with environmental influences such as bacterial infections that disrupt the mucosal barrier of the gastrointestinal tract leading to aberrant inflammation (7). The risk of colorectal cancer development in patients with inflammatory bowel disease (IBD) such as ulcerative colitis or Crohn’s disease is much higher than in the general population (8) and in ulcerative colitis patients is directly related to the length and extent of bowel inflammation (9, 10). The mechanism of this induced carcinogenesis has been linked to free radicals released during chronic colonic inflammation that damage epithelial DNA and/or alter cell proliferation or survival promoting oncogenesis (11). Patients with colorectal cancer have often elevated levels of IL-6 in the serum (12), and constitutively activated STAT3, which is expressed in the majority of colorectal tumors (13, 14) is associated with a significantly higher mortality. Constitutive STAT3 activation in colorectal cancer-derived cell lines promoted proliferation and invasiveness of tumor cells in culture (15) and growth of tumor xenografts (13) in an IL-6–dependent manner (16).
The critical role of inflammation in colorectal cancer was confirmed and further elaborated in azoxymethane/dextran sodium sulfate (AOM/DSS)-induced colitis-associated cancer models (17). These models showed that immune cells infiltrate preneoplastic lesions and that tumors secrete multiple cytokines that promote a localized inflammatory response and mediate proliferation and survival of premalignant intestinal epithelial cells (IEC), resulting in tumorigenic transformation (18). IL-6 was shown to be a critical cytokine promoting tumor initiation and progression (19, 20) in accordance to clinical data that showed a correlation between circulating IL-6 and clinical activity of IBD (21). Elevated expression of IL-6 and other inflammatory cytokines were driven by activated NF-kB in myeloid cells, whereas activation of NF-kB in IECs promoted survival of premalignant cells (18). The cytoprotective and protumorigenic activity of IL-6 in models of colitis-associated cancer was shown to be mediated by STAT3 as its genetic ablation in IECs inhibited tumor induction and growth (17, 19, 20). Importantly, the phenotype of mice deficient in STAT3 and IL-6 knockout mice were remarkably similar in the AOM/DSS model indicating a critical role of the IL-6/JAK2/STAT3 signaling pathway in tumorigenesis of colitis-associated cancer. Conversely, exogenous administration of IL-6 or genetic hyperactivation of STAT3 resulted in accelerated tumor growth and increased tumor burden (20) further establishing the significance of IL-6 and STAT3 activation in colitis-associated cancer. These data confirmed previous reports that showed that IEC-specific inactivation of SOCS3, an endogenous inhibitor of JAK2/STAT3 signaling, led to increased STAT3 activation and higher tumor burden of colitis-associated cancer (22). Similarly, gp130 mutations that prevent binding of SOCS3 resulted in hyperactivation of STAT3 and growth of spontaneous gastric tumors (23, 24). Thus, preponderance of clinical and preclinical evidence shows that the IL-6/JAK2/STAT3 signaling plays a critical role in development and progression of colitis-associated cancer and provides a rationale for pharmacologic targeting of this pathway. In this context, the antitumor efficacy of CEP-33779, a novel, highly selective JAK2 inhibitor, was evaluated in an AOM/DSS model of colitis-associated cancer. We report here that administration of CEP-33779 in a therapeutic mode resulted in a reduction in colorectal tumor mass, decreased levels of proinflammatory cytokines, and inhibition of STAT3 and NF-kB activation. In addition, histologic reductions in cancer grades were observed, and a negative impact on the tumor microenvironment including reduced tumor cell proliferation and angiogenesis was observed upon JAK2 inhibition. This report shows for the first time that an orally active, highly selective, JAK2 kinase inhibitor can reverse clinical grade disease in a mouse model of colitis-induced colorectal cancer supporting the rationale for targeting the JAK2/STAT pathway in colorectal cancer with a small-molecule JAK2 inhibitor.

Materials and Methods

Animals and facilities

The in-life portions of these experiments were carried out at Cephalon, Inc. Six- to 8-week-old female Balb/cJ (The Jackson Laboratory, catalog# 000651) mice were maintained on a 24-hour light/dark cycle, with food and water available ad libitum. All experimental animal procedures were approved by and in accordance with the regulations of the Institutional Animal Care and Use Committee of Cephalon, Inc. Body mass for each animal was determined by a Mettler Toledo scale every 1 to 2 weeks.

Compound

CEP-33779 was prepared in a manner analogous to the 5-step method described elsewhere (25). Full biochemical and molecular characterization of CEP-33779 was described elsewhere (26). The structure of CEP-33779 is shown in Fig. 1A and referenced elsewhere (25). Compound, CEP-33779, was provided orally in suspension form, dissolved in dimethyl sulfoxide (1% final concentration; Sigma) and further reconstituted in PEG400 (Emerald BioSystems) to a desired concentration for oral administration.

Colitis-induced colorectal cancer model and study design

The colitis-induced colorectal cancer or colitis-associated cancer model (27–29) as it will be referred to in Fig. 1B was optimized for AOM concentrations ranging from 5 to 15 mg/kg with lower doses resulting in smaller tumor burden and higher concentrations leading to increased mortality (data not shown). The concentration and length of exposure for oral DSS was also optimized from 2.5% to 5% wt/vol of DSS with lower doses generating fewer and smaller polyps and higher doses leading to increased mortality due to severe IBD and dehydration (data not shown). Mice were allowed gel food near the end of the study: separate experiments confirmed that gel food did not interfere with drug effectiveness or extent of disease (data not shown). An abbreviated colitis-induced colorectal cancer model was developed based on published reports (27–29). Briefly, mice were allowed to acclimate for 1 week before being injected with 12 mg/kg of AOM (Sigma) at a total volume of 100 μL, i.p., once, 7 days before the first DSS treatment. Mice were provided DSS water at a concentration of 3% wt/vol (3 g/100 mL dH2O; MP Biomedicals; molecular weight = 36,000 – 50,000). Two cycles of 7 days DSS and 7 days regular water/rest were followed by 2 abbreviated cycles consisting of 4 days DSS and 3 days of regular water/rest. This abbreviated protocol resulted in a decreased mouse mortality without changes in tumor size or frequency (data not shown). At the end of the last DSS-water cycle, mice were provided water for 3 days and the extent of tumor formation was determined in 1 to 2 mice. In most cases, 100% of the mice had significant tumor burden. After confirmation of tumor burden, mice were randomized, grouped, and

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initial measurements were recorded including body mass, fecal scores, fecal observations, and fecal occult blood testing then treated with CEP-33779 or controls or vehicle as indicated in Fig. 1B.

Colon processing and mass
All animals were sacrificed at the end of the study (day 66), and the large bowels were flushed with saline and excised. Bowel length was measured from the base of the cecum to the anal verge then were cut open longitudinally along the main axis then washed with saline. The large bowel was macroscopically inspected, imaged (see Fig. 2B), and cut into 4 equal sizes for different analyses [pharmacokinetic/pharmacodynamic (PK/PD), cytokines, histology scoring, frozen sections for immunofluorescent chemistry]. Colon size/mass was determined with intact colon (Fig. 2A).

Luminex analysis of colon tissue cytokines
One of the 4 pieces of colon was frozen in dry ice, cooled isopentene and stored at −80°C until ready for digestion and analysis using previously published methods (30). Colon pieces were cut to 5 × 5 mm segments and kept on dry ice in round bottom polypropylene tubes (BD Falcon; no. 352059). Protein concentration was adjusted to 3 mg/mL with a bicinchoninic acid (BCA) protein assay (Pierce; no. 83228). A minimum of 25 µL of this extraction was used in a mouse cytokine 10-plex bead cytokine kit (Invitrogen; no. LMC0001) according to manufacturer’s recommendations.

Figure 1. Compound and experimental design: treatment of colitis-induced colorectal cancer using a novel, orally active, JAK2 kinase inhibitor, CEP-33779. A, chemical structure of CEP-33779, a 1,2,4-triazolo[1,5a]pyridine derivative. B, mice were treated as described in Materials and Methods and as outlined in the listed groups. High (55 mg/kg), medium (30 mg/kg), and low (10 mg/kg) twice daily, oral, doses of CEP-33779 were provided to mice that had developed colitis-induced colorectal cancer initially induced by the carcinogen AOM and propagated via chronic inflammation induced by DSS-water. Mice were also treated with standard of care agent, Irinotecan at 7 mg/kg, i.p., once daily for the first 5 days on study. The study included disease and nondisease vehicle-treated mice. Mice were treated for a total of 3 weeks before being sacrificed for end of study analyses that included PK analyses for compounds levels in the spleen and plasma, tumor PD for pSTAT3 and ppNF-κB levels, tumor cytokines, colon length, mass and histopathology. CAC, colitis-associated cancer; CRC, colorectal cancer; po, orally; bid, twice a day dosing.
room temperature for 1.5 hours. Samples were stained with secondary Ab following the same procedure as used for primary staining. DAPI (Invitrogen) staining was carried out according to manufacturer’s instructions. Intracellular staining for Ki-67 was done in PBS/0.1% Tween 20/1% bovine serum albumin solution. Slides were cover slipped with mounting solution (Biomed) with antifading agents, set for 24 hours and kept at 4°C until imaged using Metamorph Imaging Software and a BX61 series Olympus fluorescent microscope. Images were merged with Metamorph Imaging Software, and quantitation was done by thresholding the marker signal, thresholding the DAPI signal for nucleated cells, normalizing the marker values against the DAPI values for that corresponding image section as to prevent the counting of false positives. All images are a merged series of 3 different channels captured for the same exposure time. Antibodies used for staining included antimouse CD31/PECAM-1-PE (eBioscience), rabbit antimouse Ki-67 (Abcam), goat antirabbit-FITC (Abcam), DAPI (Invitrogen).

**Histology**

For histologic analyses, the colon from each animal was removed and fixed in 10% buffered formalin for 48 hours on an orbital rocker at 25°C, then washed overnight with running dH2O and stored in 70% ethanol at 4°C until ready to be processed. Colon stains used for histologic analyses included hematoxylin and eosin (H&E), congo red for amyloid protein, and trichrome stains (Wistar Institute). Only H&E-stained sections are shown in this report; however, all stained sections were evaluated in a blinded fashion by an independent board certified veterinary medical pathologist (GetAPath Consulting LLC, New Bolton, PA) for scoring purposes. Multiple stains were used to determine score values. The method used for colorectal histopathologic assessment and grading was based on several published reports (31–33).

**Western blotting and NF-κB analysis**

Tumor-carrying colon areas were excised 3 hours after the last dosing, and tumor extracts were prepared in a Triton-based lysis buffer (Cell Signaling Technologies) supplemented with protease inhibitors (Roche Applied Science). Protein concentration was determined by BCA protein quantitation kit (Pierce), and equal amounts of extracts were resolved on SDS-PAGE gels (BioRad) and transferred to nitrocellulose filters. Protein expression was analyzed with specific antibodies according to the manufacture’s recommendation. All antibodies were from Cell Signaling Technologies: phosphoSTAT3, STAT3, phosphor-p65, and p65. For PK analysis, plasma and tumor levels of CEP-33779 were determined 3 hours after the last dosing as described elsewhere (26).

**Data and statistical analysis**

All Luminex assays were analyzed by linear regression curves to determine concentration of analyte following data acquisition. Mann–Whitney nonparametric and 1- or 2-way ANOVA were used as statistical tests where noted in figure legends depending on the experiment and tested hypothesis. P < 0.05 was considered significant. Statistical software used was Graph Pad Prism (version 5.01, 2007), calculations were conducted on Microsoft Office Excel (Professional, 2003).

**Results**

**CEP-33779-mediated JAK2 inhibition reduces overall tumor burden**

To assess potential therapeutic utility of JAK2 inhibitors in colorectal cancer, we evaluated the antitumor efficacy of CEP-33779 in the AOM/DSS model of colitis-associated
cancer. The initial model optimization studies were based on several published reports and led to the model shown in Fig. 1B (data not shown, see Materials and Methods; refs. 34–37). The shortened, 4-day DSS cycles allowed for the mice to recover, mitigating morbidity, while still generating the same number of polyps and similar overall tumor burden with near 100% penetrance. After a tumor induction phase had been completed, mice were grouped and treated with increasing doses (10 mg/kg, 30 mg/kg, and 55 mg/kg) of CEP-33779 as shown in Fig. 1B. At the end of the study (day 66), the large bowels were excised and tumor burden was evaluated. Twice a day oral dosing of CEP-33779 resulted in a marked reduction of distal colon mass in all CEP-33779–treated groups (10, 30, and 55 mg/kg) versus the vehicle group with the highest decrease of 47% observed for the 55 mg/kg group (Fig. 2A; *, P < 0.05). In contrast, treatment with irinotecan (7 mg/kg) resulted in a modest reduction of colon mass (Fig. 2A–B). The reduction in colon mass reflected a pronounced decrease in tumor burden for all CEP-33779–treated groups. In contrast to the vehicle group in which multiple, well vascularized tumor nodules were observed, CEP-33779 administration resulted in an almost complete shrinkage of tumors in most animals; few remaining tumor nodules were small, poorly vascularized, and had a necrotic appearance (Fig. 2B). Irinotecan-treated animals did not exhibit any significant reduction in tumor burden, in accordance with the colon mass data. Thus, CEP-33779, applied therapeutically, showed a pronounced antitumor efficacy in the AOM/DSS model of colitis-associated cancer. Biochemical analyses of tumor-bearing colons showed that CEP-33779 at 10, 30, and 55 mg/kg strongly inhibited STAT3 activation (Fig. 3) compared with the vehicle group with levels of activation similar to or below those observed in naive, nondiseased animals. The strong PD activity of CEP-33779 was consistent with its antitumor efficacy at all tested doses. Together, these results show that inhibition of the

Figure 3. Pharmacokinetics and pharmacodynamics of CEP-33779-treated colorectal tumors and levels of tumor NF-κB. Mice were treated according to the experimental design shown in Fig. 1. Distal colon/bowel was segmented into 4 equal parts. One part was used for pSTAT3 PK/PD and another for tumor-phosphorylated p65 (pp65)/NF-κB. A, Western blot radiograms from blotted colon extracts from nondiseased (naïve), diseased (CAC), vehicle-treated, and CEP-33779-treated, 10, 30, 55 mg/kg, mice for activated STAT3 (pSTAT3) and total STAT3 (Tot-STAT3). B, ratio of phosphorylated STAT3 over total STAT3 normalized to vehicle control (100%) from the 3-hour extracts of treated colitis-associated cancer mouse colons. C, tumor/colon PK (CEP-33779 compound levels) 3 hours postdosing. D, tumor/colon-activated NF-κB levels over that of total NF-κB (p65) as normalized to a percentage of vehicle control (100%) from the 3-hour extracts of treated colitis-associated cancer mouse colons. A, Western blot analyzed using Gel Pro Software. CAC, colitis-associated cancer.
JAK2/STAT3 signaling in colitis-associated cancer provides an attractive therapeutic approach.

**JAK2 inhibition decreases NF-κB activation modulating the proinflammatory tumor microenvironment**

Previous studies in colitis-associated cancer models showed a critical role of NF-κB in tumor initiation and development as its inactivation suppressed proliferation of IECs during colitis-associated cancer induction (18). NF-κB mediated its protumorigenic activity by promoting expression of inflammatory cytokines, mostly IL-6, in tumor and immune cells (19, 20). Given the marked antitumor efficacy of CEP-33779 in the colitis-associated cancer model, it was important to determine whether inhibition of the JAK2/STAT3 signaling suppressed NF-κB activation and tumor levels of IL-6 and other cytokines implicated in tumorogenesis. Administration of CEP-33779 resulted in a marked, dose-dependent inhibition of p65 (RelA) phosphorylation in colorectal tumors (Fig. 2D) and significant downregulation of IL-6 and IL-1β tumor levels as compared with vehicle-treated animals (Fig. 4). Of note is the very robust effect of CEP-33779 at all tested doses on IL-6 expression, which was suppressed down to the level observed in nondiseased animals. Inhibition of expression of IL-6 and IL-1β was associated with decreased tumor burden. In contrast, irinotecan, which showed no efficacy in this model also failed to down-regulate expression of proinflammatory cytokines (Fig. 4). Together, these results showed that inhibition of the JAK2/STAT3 signaling in the colitis-associated cancer model suppressed multiple mediators critical for tumor initiation and progression.

**JAK2 inhibition impacts neoplasia by restricting tumor cell proliferation and angiogenesis**

Independent pathologist scored trichrome, congo red, and H&E-stained sections using literature supported scoring systems were used to measure the magnitude of inflammation and stage of neoplasia in sectioned colorectal tumors at the end of the study (see Materials and Methods for score definitions). Dysplasia/neoplasia was defined as mucosa that exhibited an epithelial maturation toward the upper portion of the crypt, with recognizable goblet and absorptive cells having basally located nuclei with elongated and crowded nuclei confined to the lower portion of the crypt. Representative H&E-stained sections show the extent of inflammation and hyperproliferation in the vehicle-treated animals that is absent in the nondiseased animals and highly reduced in the CEP-33779 (55 mg/kg) treated group (Fig. 5A). Pathologist scores show that the 2 highest oral doses of CEP-33779, 30 and 55 mg/kg, significantly reduced the dysplasia/neoplasia total score by 22% to 35% below that of the vehicle group (Fig. 5B; *, P < 0.05; **, P < 0.01).

Frozen sections of tumor were cut and stained for microvascular density (MVD) with the endothelial cell marker CD31/PECAM-1 (38). Nuclei/DNA was stained blue using conventional staining techniques with DAPI dye, and intracellular staining of proliferation marker Ki-67 was used to mark proliferating tumor cells (39). Myeloid-derived suppressor cells (MDSC) were also counted with the colocalization of CD11b/Gr-1 and CD49d, the latter, was recently identified as a reliable surface marker for MDSCs (40). There was no change in the numbers of MDSCs between CEP-33779–treated and vehicle control groups (data not shown), however both MVD (i.e., CD31/PECAM-1) and Ki-67+ (i.e., proliferating, G1-2/S) tumor cells were significantly impacted by CEP-33779 administration (Fig. 6B; *, P < 0.05). Representative immunofluorescent images from treated mice showed strongly decreased Ki-67 staining in CEP-33779–treated tumors (Fig. 6A). Importantly, in contrast to tumors from the vehicle group, which displayed uniform and organized cellular structure, the CEP-33779 treated tumors seemed disorganized and exhibited multiple acellular areas suggesting a drug-mediated tumor cell death. In addition, decreased MVD was observed in the CEP-33779–treated tumors (Fig. 6A). Software-counted events from multiple mice per group were quantified and are summarized on Fig. 6B. Inhibition of JAK2 by CEP-33779 resulted in a significant decrease in CD31/PECAM-1–stained vessels as normalized to total DAPI staining in 55 and 30 mg/kg groups (Fig. 6B, left; *, P < 0.05). Reduction in MVD likely
contributed to inhibition of proliferation of tumor cells as indicated by a decreased Ki-67 expression as normalized to total DAPI staining for the 55 mg/kg group (Fig. 6B, right; *P < 0.05). These results provide further support for the clinical evaluation of a JAK2 inhibitor for the treatment of colorectal cancer.

Discussion
Constitutively activated STAT3 and STAT5 are expressed in a wide variety of human malignancies including solid and hematopoietic cancers and often correlate with a poor prognosis and resistance to therapies (4, 5, 41). Importantly, both tumor and infiltrating immune cells can express activated STATs. In most cancers, constitutive activation of STAT3 and STAT5 is mediated by high levels of cytokines and growth factors present in the tumor microenvironment. These cytokines that include IL-6, IL-11, IL-23, LIF, GM-CSF, and others use receptor-associated JAK2 kinase to activate STAT3 and STAT5. Although STAT3 can be activated by other kinases including SRC, EGFR, and VEGFR, published reports (42) and our results (Dobrzanski, unpublished data) showed that in tumor cells JAK2 is the major inducer of STAT3 phosphorylation. Given a well established role of STAT3 in tumorigenesis, inhibition of JAK2 activity might represent an attractive therapeutic approach. Here, we report that CEP-33779, a novel, orally active, selective JAK2 inhibitor strongly suppressed growth of established tumors in an AOM/DSS–induced model of colorectal cancer. It should be noted however, that standard of care anti-inflammatory agents for IBD (i.e., 5-ASA and sulfasalazine, 100 mg/kg, 3x week, orally) were also tested in this model early during the optimization and validation phase and these drugs were able to reduce serum IL-1β, increase body mass and colon length similar to CEP-33779, however, no head to head comparisons were completed with these agents and CEP-33779 in the same study (data not shown).

Administration of CEP-33779 resulted in significant inhibition of tumor growth, including regression of many tumors (Fig. 2), that correlated with inhibition of STAT3 activation (Fig. 3). Downregulation of NF-κB activity by a JAK2 inhibitor might be a strong contributor to its antitumor efficacy in the colitis-associated cancer model. The reversible acetylation of RelA/p65 regulates the duration of its nuclear activation (43), and STAT3 has been shown to directly interact with RelA in the nucleus and to recruit p300 acetylase that in turn acetylates RelA, extending its transcriptional activity in an IκB-kinase-independent manner (44). Indeed, STAT3-driven cell autonomous nuclear retention of NF-κB represents an important mechanism of NF-κB activation in cancer (45). Inhibition of STAT3 activation by a JAK2...
inhibitor would reduce the nuclear pool of STAT3 resulting in a suppressed NF-κB activation. This mechanism would explain CEP-33779–mediated downregulation of NF-κB activity in the colitis-associated cancer model. In addition, inhibitory effects of JAK2 suppression on recruitment and activation of immune cells that express high levels of activated NF-κB-like myeloid cells and T cells (18) would provide a cell nonautonomous mechanism of CEP-33779–mediated repression of NF-κB activity.

The simultaneous inhibition of JAK2/STAT3 and NF-κB signaling pathways is likely to have synergistic effects on expression of many critical drivers of tumorigenesis and therefore could be an important factor mediating a strong antitumor efficacy of CEP-33779. Among genes regulated by STAT3 and NF-κB, either synergistically or individually, are many coding for antiapoptotic proteins like Bcl-2, Bcl-xL, Mcl-1, surviving and for mediators of cell proliferation like cyclin-D and cyclin-B. Indeed, inactivation of STAT3 in epithelial cells suppressed tumor formation in a mouse model of colitis-associated cancer, in part, by attenuating expression of Bcl-xL and survivin (19, 20). Downregulation of expression of multiple antiapoptotic factors resulting in tumor cell death provides a likely mechanism for CEP-33779–mediated tumor regression (Fig. 2) and vast necrotic areas observed in treated tumors (Fig. 6). Inhibition of expression of cyclins would explain a decreased proliferative index as shown by effects of CEP-33779 on Ki-67 expression (Fig. 6). In addition, suppression of tumor angiogenesis by CEP-33779 (Fig. 6) would further contribute to tumor regression.

JAK2/STAT and NF-κB pathways regulate expression of a wide spectrum of cytokines and chemokines in the tumor microenvironment, which control activation of immune cells as well as growth and survival of premalignant and malignant epithelial cells (45). These cytokines include IL-1β, IL-6, IL-11, IL-23, TNFα, and others. IL-6 has been shown to play a critical role in a
development and progression of colitis-associated cancer (19, 20), and we showed that its levels in tumors were dramatically reduced by CEP-33779 in our model. Combinatorial effects of STAT3 and NF-κB inactivation could explain such a robust inhibition of IL-6 expression. In addition, inhibition of JAK2/STAT3 signaling could lead to a decreased recruitment of myeloid cells, which are a significant source of IL-6 in the colitis-associated cancer model (18). Thus, CEP-33779 not only inhibited IL-6 initiated signaling but could also suppress IL-6 expression within treated tumors. Given a well documented role of the IL-6/JAK2/STAT3 signaling in tumorigenesis of many cancers (17, 46, 47), the ability of CEP-33779 to suppress this pathway suggests a potential therapeutic utility of JAK2 inhibitors in multiple tumors, particularly those with a strong inflammatory component. There is growing evidence that the JAK/STAT pathway mediates multidirectional interactions between tumor and stromal cells that are critical for tumor growth and progression and this report confirms the ability of JAK2 inhibitors to interfere with those interactions and provides a rationale for their clinical application in oncology.

Disclosure of Potential Conflicts of Interest
All authors on this manuscript are employees of Cephalon, Inc.

References

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