Repurposing the selective estrogen receptor modulator *bazedoxifene* to suppress gastrointestinal cancer growth

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**Abstract**

Excessive signaling through gp130, the shared receptor for the interleukin (IL6 family of cytokines, is a common hallmark in solid malignancies and promotes their progression. Here, we established the *in vivo* utility of *bazedoxifene*, a steroid analog clinically approved for the treatment of osteoporosis, to suppress gp130-dependent tumor growth of the gastrointestinal epithelium. *Bazedoxifene* administration reduced gastric tumor burden in *gp130<sup>Y77F</sup>* mice, where tumors arise exclusively through excessive gp130/STAT3 signaling in response to the IL6 family cytokine IL11. Likewise, in mouse models of sporadic colon and intestinal cancers, which arise from oncogenic mutations in the tumor suppressor gene *Apc* and the associated β-catenin/canonical WNT pathway, *bazedoxifene* treatment reduces tumor burden. Consistent with the proposed orthogonal tumor-promoting activity of IL11-dependent gp130/STAT3 signaling, tumors of *bazedoxifene*-treated *Apc*-mutant mice retain excessive nuclear accumulation of β-catenin and aberrant WNT pathway activation. Likewise, *bazedoxifene* treatment of human colon cancer cells harboring mutant *Apc* did not reduce aberrant canonical WNT signaling, but suppressed IL11-dependent STAT3 signaling. Our findings provide compelling proof of concept to support the repurposing of *bazedoxifene* for the treatment of gastrointestinal cancers in which IL11 plays a tumor-promoting role.

**Keywords** colon cancer; gastric cancer; gp130; interleukin-11; interleukin-6

**Subject Categories** Cancer; Digestive System; Pharmacology & Drug Discovery

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provide a rheostat function to link an effective, proliferative wound-healing response to the ensuing inflammatory response of the intestinal epithelium (Ernst et al., 2014). We surmised that the activity of these cytokines becomes rate limiting for the growth of tumors and can be exploited as therapeutic vulnerability.

Due to their solubility and cellular expression, cytokines and their receptors are preferred targets for the development of body-based therapeutics and have become mainstream targets for the treatment of inflammation and other chronic diseases. Accordingly, antibodies directed against IL6, or its cognate ligand-specific IL6Rx co-receptor subunit, are either already in the clinic, or in advanced clinical trials for the treatment of autoimmune conditions and some hematological malignancies. Surprisingly, no such clinically approved antibody reagents have been developed to target IL11 signaling either at the level of the ligand or the IL11Rx receptor subunit.

Previous in silico modeling has revealed that the selective estrogen receptor modulators (SERM) raloxifene and bazedoxifene could interfere with the protein–protein interactions between IL6 and gp130 (Li et al., 2014). These third-generation SERMs are approved in Europe and the United States for the prevention and treatment of postmenopausal osteoporosis without conferring agonistic effects on endometrial, ovarian, and breast tissues (Silverman et al., 2008; Pinkerton et al., 2012; Flannery et al., 2016). Here, we provide the first in vivo evidence that bazedoxifene treatment of mice, which harbor epithelial tumors in the glandular stomach, the small intestine or the colon, with drug doses corresponding to treatment regimens for osteoporosis patients, suppresses tumor growth irrespective of the gender of the host. Akin to our observations with the IL11Rx receptor antagonist IL11-Mutein (Putoczki et al., 2013), we find that bazedoxifene restricts the growth of intestinal tumors by suppressing IL11-mediated signaling rather than by interfering with excessive canonical WNT signaling that arises from bi-allelic inactivation of the Apc tumor suppressor gene. Collectively, our observations suggest that bazedoxifene could be readily repurposed for the treatment of gastric and colon cancers and that bazedoxifene serves as a tool compound for further chemical refinements to increase specificity and affinity of future small molecule IL11 signaling antagonists.

Results

**Bazedoxifene blocks IL11 signaling**

Bazedoxifene is thought to inhibit IL6 signaling by interfering with the formation of the signaling-competent hexameric receptor complex. Bazedoxifene prevents the aggregation of two trimeric receptor complexes, comprised of an IL6 ligand, an IL6Rx co-receptor subunit, and one gp130 subunit, and resulted in suppressed activation of STAT3 (Li et al., 2014). Given the proposed similar hexameric 2:2:2 nature of the IL11:IL11Rx:gp130 complex (Veverka et al., 2012; Putoczki et al., 2014), we investigated whether bazedoxifene could also inhibit IL11-mediated, gp130-dependent STAT3 signaling. We co-expressed human IL11Rx alongside the STAT3-responsive pAPRE-luciferase (luc) reporter construct in HEK293 cells. Treatment with IL11 induced a 15-fold increase in APRE-luc reporter activity, which was antagonized in a dose-dependent manner by bazedoxifene (Fig 1A and Appendix Fig S1A). To ensure this was not a generic effect conferred by antagonistic-acting estrogen analogs, we also tested these cells with tamoxifen (Appendix Fig S1A). Tamoxifen failed to suppress IL11, suggesting a selective effect of bazedoxifene in the inhibition of IL11:IL11Rx:gp130 signaling.

To extend our findings to a more biologically complex response, we exploited the IL3-dependency of the murine BAF/03 pro-B-cell line for their survival and proliferation in vitro (Hilton et al., 1994; Nandurkar et al., 1996). For this, we installed expression constructs encoding gp130 alongside either IL6Rx, IL11Rx, or LIFR in BAF/03 cells. This enabled the subsequent propagation of the corresponding BAF/03 clones with IL6, IL11, or LIF, respectively, in the absence of IL3 (Fig 1B and C). We confirmed that bazedoxifene treatment antagonized IL11-mediated cell proliferation in a concentration-dependent manner (Fig 1B). Corroborating the selective effect that we observed with bazedoxifene on STAT3 transcriptional activity in HEK293T cells, we also found that bazedoxifene but not tamoxifen suppressed IL11-mediated proliferation of BAF/03 cells expressing human IL11Rx (Appendix Fig S1B).

Our observations are consistent with the proposed inhibitory mechanism of bazedoxifene on the hexameric gp130 signaling complex, as bazedoxifene also inhibited IL6-dependent BAF/03 proliferation (Fig 1B). By contrast, bazedoxifene treatment not only failed to antagonize IL3-dependent parental BAF/03 cell proliferation, but also that of the LIFR-expressing clones stimulated with human LIF (Fig 1C) and consistent with LIF forming trimeric LIF:LIFR:gp130 complexes (Gearing et al., 1991; Hammersch et al., 1998). We also excluded that bazedoxifene interfered with gp130 signaling in the absence of ligand or of receptor α-subunits. For this, we exploited a synthetic form of gp130 in which a leucine zipper region of c-jun substitutes for the native extracellular receptor domain and confers ligand-independent homodimerization of the resulting chimeric L-gp130 proteins (Stuhlmann-Laesz et al., 2006). Thus, L-gp130-dependent BAF/03 cell proliferation should be refractory to bazedoxifene inhibition, which we confirmed experimentally (Fig 1C). We surmise from this collective functional data that bazedoxifene disrupts IL11 signaling akin to its proposed action on the signaling-competitive, hexameric IL6 receptor complex.

**In silico modeling of bazedoxifene bound to gp130 Site III residues**

It was been previously predicted that bazedoxifene competes with binding of IL6 in the trimeric IL6:IL6Rx:gp130 complex. It is the interaction between IL6 of one trimer and the gp130 co-receptor of the second trimeric unit that is inhibited thereby preventing the formation of the signaling-competent hexameric complex (Fig 2A and B; Li et al., 2014; Wu et al., 2016). Specifically, structural analysis of the IL6:IL6Rx:gp130 hexameric complex (PDB ID: 1P9M) revealed that IL6 binds to gp130 via residues leucine-57, glutamic acid-59, asparagine-60, leucine-62, tryptophan-157, and leucine-156 in Site III. A 30 Å resolution cryo-electron map of the IL11:IL11Rx:gp130 hexameric complex suggested that its subunits are organized in a similar arrangement to that of the hexameric IL6:IL6Rx:gp130 complex (Neddermann et al., 1996; Matadeen et al., 2007; Putoczki et al., 2014).
Site-directed mutagenesis studies indicated that IL11 interacts with gp130 at Site III via tryptophan-168 and IL6 interacts via tryptophan-157 as amino acids that form part of helix D of these ligands (Barton et al., 1999). We therefore aligned helix D from the crystal structure of IL11 (PDB ID: 4MHL; Putoczki et al., 2014) with that of IL6 to model how IL11 is likely to interact with gp130 at Site III (Fig 2C). When aligned in this manner, tryptophan-157 in IL6 and tryptophan-168 in IL11 indeed interact with gp130 at Site III via tryptophan-168 and IL6 interacts via phenylalanine-36, isoleucine-83, and glutamine-91. In an alternative second binding model, the phenol ring and azepanyl ring of bazedoxifene mimic the interactions between gp130 and tryptophan-157 and leucine-57 residues in IL6 (Appendix Fig S2C and D). The indole ring hydroxyl substituent of bazedoxifene is able to interact through hydrogen bonds specifically with glutamine-78 in gp130, and the indole and phenol rings both form π-π interactions with tyrosine-94. Meanwhile, the leucine-3 residue in gp130 engages through hydrophobic interactions with the indole ring and phenol substituent, as well as with the pendant phenyl ring of bazedoxifene. Finally, the bazedoxifene azepanyl ring extends in a largely hydrophobic region of gp130 comprising cysteine-6, cysteine-32, phenylalanine-36, isoleucine-83, and glutamine-91. In an alternative second binding model, the phenol ring and azepanyl ring of bazedoxifene mimic the interactions between gp130 and tryptophan-157 and leucine-57 in IL6 (Appendix Fig S2E and F). In this binding mode, the bazedoxifene indole ring can form π-π interactions with tyrosine-94 in gp130. Meanwhile, the phenol substituent of bazedoxifene can form a hydrogen bond with asparagine-92 in gp130, and the indole ring hydroxyl substituent could form hydrogen bonds with either the hydroxyl group of tyrosine-94 or the side chain of glutamic acid-12. The leucine-3 residue in gp130 would then form hydrophobic interactions with

Figure 1. Bazedoxifene suppresses IL11-mediated STAT3 signaling activity.

A. Effect of bazedoxifene (BZA) on IL11-induced and STAT3-dependent pAPRE-firefly luciferase reporter activity in HEK293T cells expressing human IL11Rα. Cells were co-transfected with a non-responsive Renilla luciferase plasmid. Results are expressed as relative luciferase units (RLU), that is, firefly luciferase activity normalized against Renilla luciferase activity in each individual culture.

B. Effect of BZA treatment on proliferation of IL11 stimulated BAF/03 murine B-cell lines, as determined by MTS-assay. IL6 stimulation was used as a positive control. Cells were engineered to express human either IL6Rα or IL11Rα, respectively.

C. Effects of BZA treatment, as determined by MTS-assay, on parental BAF/03 cells stimulated with IL3, of LIF receptor (LIFR)-expressing cells stimulated with LIF, or of cells expressing the constitutive active L-gp130 construct.

Data information: Data are mean ± SEM, n = 3 individual cultures, *P < 0.05, **P < 0.01, ***P < 0.001, 2-way repeated-measures ANOVA, Tukey’s multiple comparison test.
the pendant phenyl substituent of bazedoxifene. Importantly, in either of the putative interaction models, bazedoxifene makes multiple interactions with Site III residues of gp130 consistent with the proposed models by Li et al (2014) and Wu et al (2016).

**Bazedoxifene inhibits IL11-dependent STAT3 activation and growth of patient-derived colon cancer organoids**

Given our previous observations that IL11 signaling confers potent activation of STAT3 in gastrointestinal cancer cells (Putoczki et al, 2013), we next examined the effects of bazedoxifene on cytokine-stimulated human gastric and colon cancer cells. We observed that bazedoxifene treatment suppressed IL11-mediated induction of the transcriptionally active, phosphorylated STAT3 (pSTAT3) isoform in a dose-dependent manner (Fig 3A). Reduced pSTAT3 levels were also observed in the estrogen receptor (ER)-negative breast cancer cell line, MDA-MB-231, confirming the ER independence of the bazedoxifene effect on IL11/gp130 signaling (Fig 3A). To ascertain the biological importance of this observation in a pathologically relevant setting, we assessed the effects of bazedoxifene on IL11 signaling in patient-derived samples of colon cancer cells grown as primary cultures. We consistently observed that bazedoxifene treatment decreased pSTAT3 levels in a dose-dependent manner (Fig 3B). These tumor epithelial cells showed higher expression of IL11 mRNA transcript when compared to that of IL6 (Fig 3C). Furthermore, mRNA transcripts for IL6R, IL11R, and SOCS3 were readily detectable, with IL6ST transcripts, encoding gp130 as being the most abundant (Fig 3C). We then exploited patient-derived human colon cancer organoids grown in 3D cultures in the presence of IL11. We observed significantly smaller organoids in the presence of 10 μM bazedoxifene (Fig 3D and E). Collectively, our *in vitro* data in factor-dependent BAF/03 cells, human colon and gastric cancer cell lines, patient-derived colon cancer cells, and organoids provide compelling evidence that bazedoxifene effectively antagonizes IL11-elicted STAT3 signaling and associated cell proliferation *in vitro*.

**Therapeutic bazedoxifene treatment impairs IL11-dependent gastric tumor growth in vivo**

In order to extend our *in vitro* findings to an *in vivo* cancer setting, we utilized the *gp130<sup>7757F</sup>* mouse model of intestinal-type gastric cancer. In this model, gastric adenomas spontaneously and reproducibly develop with 100% penetrance in 4-week-old mice with an absolute genetic dependence on bi-allelic expression of the *Il11ra* and *Stat3* genes, but completely independent of IL6 signaling (Jenkins et al, 2005; Ernst et al, 2008). Importantly, we had previously shown that therapeutic treatment of tumor-bearing, 10- to 13-week-old *gp130<sup>7757F</sup>* mice with either the antagonistic IL11-MuTein peptide (Putoczki et al, 2013), JAK1/2 kinase inhibitors (Stuart et al, 2014), STAT3 antisense oligonucleotides (Ernst et al, 2008), or inducible short hairpin STAT3-RNA (Alorro et al, 2017), reduces tumor burden associated with reduced cell proliferation and increased apoptosis. Thus, we treated 13-week-old *gp130<sup>7757F</sup>* mice with...
Figure 3. Bazedoxifene suppresses IL11-mediated STAT3 signaling activity in human cancer cell lines and patient-derived colon cancer primary cultures and organoids.

A Effects of bazedoxifene (BZA) on IL11-stimulated STAT3 activation (pSTAT3) in gastric (MKN1), colon (LIM2405), and breast (MDA-MB-231) cell lines.

B Effects of bazedoxifene (BZA) on IL11-stimulated STAT3 activation (pSTAT3) in primary cell cultures of isolated colon cancer epithelial cells of three individual colorectal cancer (CRC) patients.

C mRNA expression levels of IL6, IL11, IL6R, IL11R, IL6ST encoding gp130 and SOCS3, a STAT3-regulated gene in epithelial cells derived from six colorectal cancer patients.

D Effects of BZA treatment on IL11-dependent growth of human colon cancer organoids. Organoids were cultured for 6 days in the presence of IL11 (50 ng/ml) and then for a further 7 days in IL11 plus the indicated concentration of BZA. Representative bright-field microscopy images of organoid cultures at 7 days after BZA treatment. Scale bar = 300 μm.

E Relative changes in organoid size after 7 day treatment with vehicle control or BZA as described in (D). Data are mean ± SEM, n = 3 individual cultures, one-way ANOVA, Dunnett’s comparison test.

Source data are available online for this figure.
established gastric tumors for 7 weeks with bazedoxifene at a dose of 3 mg/kg i.p. five times per week (Fig 4A). This dosing was previously used to document inhibitory effects on estrogen-sensitive mouse tissue (Sakr et al., 2014; Flannery et al., 2016). We consistently detected significantly smaller and fewer tumors in the bazedoxifene-treated cohorts compared with the vehicle-treated cohorts (Figs 4B-D and EV1A). Importantly, we observed reduced tumor burden in both, bazedoxifene-treated gp130Y775F male and female mice, suggesting that the tumor suppressive effect was not mediated through bazedoxifene’s SERM activity (Fig 4C and D). We confirmed the latter with our observations that bazedoxifene also reduced tumor burden following depletion of estrogen producing cells in a cohort of female ovariectomized mice (Fig EV2). Consistent with this finding, we also were unable to detect expression of ERα, encoded by the Esr1 gene, in gastric tumors of mice of either gender (Fig 4E). Thus, our observations argue strongly that the anti-tumor activity of bazedoxifene on the gastric epithelium occurs independently of its SERM activity.

We then hypothesized that bazedoxifene treatment of tumor-bearing mice would induce the same signaling “hallmarks” observed upon treatment of gp130Y775F mice with either IL11-Mutein or JAK inhibitors (Stuart et al., 2014). Indeed, we detected reduced levels of the activated (i.e., phosphorylated) isoforms of the signaling proteins STAT3 and Akt (Fig 4F). Akin to our findings with IL11-Mutein therapy (Putoczki et al., 2013; Stuart et al., 2014), tumors from bazedoxifene-treated mice had reduced expression of the pro-survival protein Bcl-xL, of the proliferative marker cyclin D1, and the cancer cell-specific mitosis marker survivin (Fig 4F). These observations correlated with a significant decrease in Ki67-stained tumor epithelium and an increased number of TUNEL-positive, apoptotic cells in tumors from bazedoxifene-treated mice (Fig EV1B). Furthermore, expression of mRNA transcripts for the bona fide STAT3-target genes Socs3, Icam1, and Reg3a, which are both induced by IL11 in gastric tumors (Putoczki et al, 2013), were significantly decreased in tumors from bazedoxifene-treated animals (Fig 4G). Collectively, our data support a mechanism of action whereby bazedoxifene directly impairs intracellular IL11 signaling.

**Bazedoxifene reduces Apc-dependenet colon cancer growth in vivo**

We and others have previously shown that IL11 signaling, and to a lesser extent IL6 trans-signaling, enables and promotes the formation and progression of epithelial tumors that arise in the intestinal epithelium in response to aberrant canonical WNT signaling associated with either loss-of-function mutations in the Apc tumor suppressor gene or gain-of-function mutations in β-catenin (Becker et al., 2004; Ernst et al., 2008; Putoczki et al., 2013). We surmised from these observations that gp130-mediated STAT3 signaling conferred a rate-limiting, permissive signaling for survival and proliferation of intestinal epithelium harboring bona fide mutations in these WNT signaling genes (Phesse et al, 2014).

To explore whether bazedoxifene could also curb Apc-dependent colon cancer development, we exploited Cdx2CreERT2; Apclox mice, which develop tumors within 2 weeks following tamoxifen-depend-ent bi-allelic inactivation of the Apc gene in the epithelium of the colon and cecum (Hinoi et al., 2007; Fig 5A). We treated tumor-bearing Cdx2CreERT2; Apclox mice with bazedoxifene for a 2 week duration, with treatment commencing 2 weeks after tamoxifen administration, and observed a significant reduction in overall tumor burden (Fig 5B–F). Akin to our observation with the gp130Y775F model, bazedoxifene treatment conferred a comparable anti-tumor effect in male and female Cdx2CreERT2; Apclox mice (Fig 5B and E). As the therapeutic effect of bazedoxifene treatment reduced tumor number (Fig 5C) as well as tumor size (Fig 5D), we inferred that bazedoxifene treatment suppressed both tumor initiation (affecting tumor numbers) and progression (affecting tumor size). This result is consistent with our previous findings for a role for STAT3 signaling in models of Apc-dependent colon tumorigene-sis (Phesse et al, 2014). Western blot analysis of colon tumors confirmed strongly reduced levels of pSTAT3 in bazedoxifene-treated mice, coinciding with reduced Bcl-xL, cyclin D1, and survivin expression (Fig 5G). We corroborated these findings by immunohis-tochemical analysis of tumor sections, which revealed reduced pSTAT3 staining in tumor epithelium that coincided with reduced proliferation and increased apoptosis as revealed by staining for Ki67 and TUNEL, respectively (Fig EV3A). Finally, we used qPCR analysis to confirm reduced expression of the bona fide STAT3-target genes Sox3, Icam1, and lgr5 (Fig 5H). To confirm that bazedoxifene treatment reduced the growth of colon cancer cells, we also derived organoids from tumors of Apcmin mice and observed that bazedoxifene treatment reduced IL11-dependent organoid growth (Fig EV3B).

We next ascertained that the striking anti-tumor effect of baze-doxifene treatment also extended to the epithelium of the small intestine, which is also susceptible to the therapeutic benefit conferred by inhibition of the IL11/STAT3 signaling axis at the level of the gp130-associated JAK1/2 kinases (Phesse et al., 2014). For this, we induced intestinal tumors using the stem cell marker locus Lgr5 to confer bi-allelic Apc deletion following tamoxifen treatment of Lgr5CreERT2; Apclox mice. We observed that administration of bazedoxifene for 2 weeks to tumor-bearing Lgr5CreERT2; Apclox mice also significantly reduced overall tumor burden in the small intestine (Fig 6A).

Consistent with our observations in the Cdx2CreERT2; Apclox colon cancer model, immunohistochemical analysis of tumors from baze-doxifene-treated Lgr5CreERT2; Apclox mice revealed a reduction in nuclear pSTAT3 and Ki67 staining that coincided with augmented TUNEL staining (Fig EV4). Tumors from bazedoxifene-treated Cdx2CreERT2; Apclox and Lgr5CreERT2; Apclox mice also showed reduced levels of the STAT3-target gene bmi-1 (Fig 5H and data not shown), encoding a polycomb repressor component that suppresses expression of cell cycle inhibitors, and therefore restricts intestinal tumor growth in Lgr5CreERT2; Apclox; bmi-1+/− mice (Fig 6A; Phesse et al., 2014). Collectively, our data suggest that the anti-tumor mechan-ism of action of bazedoxifene occurs via inhibition of STAT3 signaling and results in suppressed cell survival and proliferation of Apc-mutagenized intestinal epithelium.

**Aberrant WNT signaling is retained in Apc-mutant tumors of bazedoxifene-treated mice**

To rule out that bazedoxifene directly interferes with canonical WNT signaling in tumors of Lgr5CreERT2; Apclox mice, we used immunohistochemistry to detect transcriptionally active surrogates of canonical WNT signaling (nuclear β-catenin) and gp130 signaling...
(pSTAT3) in tumor epithelium from mice in the bazedoxifene- and the vehicle-treated cohorts. While we detected comparable signals for β-catenin, pSTAT3 staining was reduced selectively in tumors collected from mice in the bazedoxifene-treated cohort (Fig EV4).

We functionally confirmed this observation in IL11-stimulated human SW480 colon cancer cells that harbor homozygous impairment mutations in the APC gene and therefore show aberrant WNT signaling. For this, we transfected SW480 cells with either the pTOPFLASH or pAPRE-luc reporter plasmids to specifically record β-catenin- and STAT3-dependent transcriptional activity, respectively. For this, we transfected SW480 cells with either the pTOPFLASH or pAPRE-luc reporter plasmids to specifically record β-catenin- and STAT3-dependent transcriptional activity, respectively. This analysis revealed that bazedoxifene treatment did not suppress aberrant β-catenin-dependent pTOPFLASH activity (Fig 6B), but antagonized IL11-induced pAPRE-luc reporter activity (Fig 6C). Indeed, the effect of bazedoxifene-dependent inhibition of pAPRE-
**Figure 5.** *Bazedoxifene* treatment inhibits growth of endogenous arising colon tumors in *Cdx2CreERT; Apcflx* mice.

A Schematic representation of experimental setup. Colon cancer occurs in adult *Cdx2CreERT; Apcflx* mice 10–14 days after tamoxifen administration (1 mg/day for 3 consecutive days) and associated induction of Cre recombinase activity to confer bi-allelic inactivation of the Apc tumor suppressor gene. *Bazedoxifene* (BZA) treatment was initiated 14 days post-tamoxifen administration and lasted for two consecutive weeks.

B Representative examples of colons (left) and cecum (right) of vehicle- (VEH) and BZA-treated mice as described in (A). Scale bar = 10 mm.

C, D Quantification of total tumor number in the colon of individual mice of the indicated gender (C), and when classified according to the size of individual tumors (D) at the end of 2 weeks of BZA treatment (mean ± SEM, *n* = 12 untreated controls, *n* = 13 BZA-treated group, unpaired Student’s *t* test). Each symbol represents an individual mouse of the indicated gender.

E, F Representative endoscopy images of tumor-bearing mice 2 weeks after vehicle or BZA treatment. Arrowheads indicate tumors (E). Endoscopy score of tumor burden as described in the Materials and Methods section (F) (Unpaired Student’s *t* test).

G Western blot analysis of tumor lysates from (C) for the activated (phosphorylated) isoforms of signaling proteins, as well as for the STAT3 targets survivin, BclXL, and cyclin D1. Each lane corresponds to tumors pooled from an individual mouse.

H, I Changes in mRNA expression of target genes regulated by STAT3 (H) or the canonical WNT/β-catenin pathway (I) in vehicle- and BZA-treated mice. Expression is normalized to mean value of vehicle-treated mice. Data are mean ± SEM, *n* = 5 per group, *P*-values from unpaired Student’s *t* test.

Source data are available online for this figure.
in suppressing the growth of Apc
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Here, we provide the first proof-of-concept study demonstrating SERM-activity independent, anti-tumor effects of bazedoxifene in a comprehensive series of preclinical mouse models for gastrointestinal cancers. We identify interference with IL11 binding to the gp130 receptor signaling subunit as the mechanism by which bazedoxifene suppresses tumor growth in male and females hosts and on cells lacking ERx expression. Akin to our observations that inhibition of gp130 signaling at the level of ligand binding, engagement of the gp130-receptor-associated JAK kinases or ultimately activation of the STAT3 signal transducer suppresses tumor formation in gp130+/+; Lgr5CreERT2; Apcloxlox mice; bazedoxifene treatment replicated these effects. Notably, bazedoxifene also suppressed tumor growth in mutant Apc-driven intestinal

Collectively, our observations derived in several independent, but complementing mouse and human-derived model systems provide orthogonal evidence for the in vivo effects of bazedoxifene in suppressing the growth of Apc-driven cancers, most likely through a process involving the inhibition of IL11 signaling in epithelial tumor cells.

Bazedoxifene treatment does not alter tumor immune responses

While our data thus far argue strongly in favor of bazedoxifene conferring anti-tumor activity directly on epithelial cells via a STAT3-dependent, but SERM-independent mechanism, our next aim was to assess whether the anti-tumor activity of bazedoxifene was conferred in part by systemic, immune cell-dependent mechanisms. For this, we extensively profiled tumor infiltrating immune cells in gp130−/−/CD8+/−/CD8−/−, Ly6Chigh, Ly6Clo monocyte, or CD45+, CD11b+, Ly6Clo granulocytic MDSCs, nor in F4/80+ tumor-associated macrophages between the two cohorts (Appendix Fig S5), despite reports that some SERMs may affect neutrophils and decrease lymphopoiesis (Bernardi et al., 2015; Nordqvist et al., 2017). Collectively, these data suggest that the anti-tumor effect of bazedoxifene is primarily mediated by tumor cell-intrinsic mechanism rather than by altering the host’s anti-tumor immune response.

Discussion

Here, we provide the first proof-of-concept study demonstrating SERM-activity independent, anti-tumor effects of bazedoxifene in a comprehensive series of preclinical mouse models for gastrointestinal cancers. We identify interference with IL11 binding to the gp130 receptor signaling subunit as the mechanism by which bazedoxifene suppresses tumor growth in male and females hosts and on cells lacking ERx expression. Akin to our observations that inhibition of gp130 signaling at the level of ligand binding, engagement of the gp130-receptor-associated JAK kinases or ultimately activation of the STAT3 signal transducer suppresses tumor formation in gp130+/+; Lgr5CreERT2; Apcloxlox and Cdx2CreERT2; Apcloxlox mice; bazedoxifene treatment replicated these effects. Notably, bazedoxifene also suppressed tumor growth in mutant Apc-driven intestinal
tumors and in human colon cancer cell lines without reducing the excessive signaling output of the canonical WNT pathway that constitutes the underlying oncogenically activated driver pathway. Accordingly, bazedoxifene treatment also reduced the growth of patient-derived colon cancer organoids that harbor the APC driver, and possibly compounding additional mutations. However, given that conventional IL11 signaling and IL6 trans-signaling both facilitate the growth of intestinal tumors (Putoczki et al., 2013; Schmidt et al., 2018), our observations do not address the relative contribution by which the bazedoxifene effect is mediated through inhibition of each of these mechanisms. Given the collective contribution of these gp130 cytokines in driving tumorigenesis, bazedoxifene may represent a potent mode for the simultaneous inhibition of conventional IL11 and IL6 trans-signaling in tumors.

Elevated levels of IL6, IL11, their cognate receptor α-subunits, and associated signaling activity, as assessed by the abundance of the activated pSTAT3 isofrom, are commonly observed in colon, gastric, and other solid malignancies and often correlate with increased metastasis and poorer patient outcomes (Kang et al., 2003; Putoczki et al., 2013; Johnstone et al., 2015). In our study, tumor epithelial cells isolated from stage II primary colon cancers, mRNA transcript expression of IL6 and IL11, their cognate receptors, and gp130 were detected although it was the expression levels of gp130 which determined cellular sensitivity to IL6 and IL11 stimulation. This suggests that expression levels of gp130 may be relevant as a biomarker in patient selection for targeted anti-IL6 or anti-IL11 treatments. An anti-tumorigenic effects of bazedoxifene, associated with reduced IL6-dependent pSTAT3 levels, have been demonstrated in xenograft models of rhabdomyosarcoma (Xiao et al., 2017), pancreatic (Wu et al., 2016), liver (Wang et al., 2017), and colon cancer (Li et al., 2018). Our data comprehensively extend these observations and demonstrate significant anti-tumorigenic effects of bazedoxifene in multiple IL11-dependent and immune-competent models of spontaneous gastric, intestinal, and colon cancer.

Bazedoxifene is an indole-based ER ligand with unique structural characteristics not shared with anti-estrogens tamoxifen and raloxifene. Although bazedoxifene binds to ERα with greater affinity than to ERβ, bazedoxifene is a less selective ERα binder than raloxifene from which its structure was derived (Wardell et al., 2013). Upon the identification of raloxifene and bazedoxifene as potential gp130-interacting compounds, Li et al. (2014) proposed a mechanism whereby IL6 inhibition is likely to result from the disruption of hexameric IL6:IL6Rα:gp130 complex formation. Mutagenesis studies demonstrated that tryptophan-157 in IL6 was the most critical residue at the Site III interface (Barton et al., 1999; Boulanger et al., 2003; Veverka et al., 2012). Meanwhile, our crystallographic analysis of IL11, IL6, and the IL6 hexameric signaling complex suggests that the two cytokines interact in a similar manner through Site III with gp130 and show functional conservation between tryptophan-157 in IL6 and tryptophan-168 in IL11 (Putoczki et al., 2014). These insights also predict our findings that bazedoxifene should not affect LIFR signaling as the corresponding signaling-competent trimeric LIF:LIFR:gp130 receptor complex does not rely on Site III-dependent interactions (Huyton et al., 2007). Likewise, the models also foreshadow our experimental observations here that bazedoxifene is unable to interfere with signaling from the chimeric L-gp130 molecule, in which the entire extracellular domain is replaced with a leucine zipper.

Besides the beneficial effect of bazedoxifene on bone mineral density and risk reduction for new vertebral fractures in post-menopausal women (Silverman et al., 2012; Palacios et al., 2015; Peng et al., 2017), bazedoxifene has also been reported to reduce the serum concentrations of total and low-density lipoprotein cholesterol (Stevenson et al., 2015). The well-documented anti-tumor effects of bazedoxifene observed in models for ovarian (Romero et al., 2012) and tamoxifen-resistant breast cancer (Wardell et al., 2013) have been mechanistically attributed to the capacity of bazedoxifene to compete with 17β-estradiol for binding to ERα in these steroid-sensitive tissues. The evidence collectively presented here strongly suggests that the therapeutic effect of bazedoxifene observed on gastrointestinal cancer occurs independent of ERα signaling, as ERα expression in the gastrointestinal epithelium was below detection level. Functionally even more important, bazedoxifene conferred similar therapeutic benefits to male and female mice in all of our in vivo models and also reduced gastric tumor burden in ovariec-tomized female gp130Y757F mice. Likewise, the anti-tumor activity of bazedoxifene in our gastrointestinal cancer models was also not mediated by systemic effects through tumor-associated immune cell subsets even though some SERMs may affect granulopoiesis and lymphopoiesis (Nordqvist et al., 2017).

Treatment of postmenopausal osteoporosis in women with bazedoxifene is approved by the FDA at doses in the range of 20–40 mg daily. Accounting for the pharmacokinetic difference between humans and mice, the FDA-recommended daily intake of 20 mg bazedoxifene for a 60 kg woman corresponds to a dose of approximately 4.1 mg/kg/day in mice (Nair & Jacob, 2016). The latter dose is consistent with the 3 mg/kg dose administered here in some of the previous studies documenting the anti-estrogen activities of bazedoxifene in mice (Sakr et al., 2014; Flannery et al., 2016). Thus, the safety profile of bazedoxifene may confer benefits to a large (aging) population. Besides protecting against bone loss and beneficially affecting cholesterol metabolism (Silverman et al., 2012; Stevenson et al., 2015), here we provide evidence in controlling the growth of adenomatous lesions, where mutations in APC form the initial oncogenic insult in 80% of all metastatic colon cancers. By exploiting the capacity of bazedoxifene to inhibit those inflammatory cytokines that most prominently promote the growth of gastrointestinal tumors, our data provide a compelling argument for the repurposing of bazedoxifene for a potentially novel treatment modality for gastrointestinal cancers.

**Materials and Methods**

**Cell culture and reporter activity assays**

Cell lines were maintained at 37°C in 10% CO2 with appropriate culture media supplemented with 10% (v/v) of fetal calf serum (FCS) and 1% (v/v) of GlutaMAX (Gibco). LIM2405 and MDA-MB231 cell lines in Dulbecco’s modified Eagle’s medium (DMEM)/F-12; HEK293, MKN1, BAF/03IL6R, BAF/03IL11Rα and BAF/03LIFR cell lines in RPMI1640. Transfections were performed with Lipofectamine 2000 according to manufacturer’s instructions (Invitrogen). To transfect HEK293 cells, 150,000 cells were plated in 6-well plates and transfected with 100 ng of pAPRE-luciferase, 100 ng of pcDNA-
hIL11RA, and 100 ng of pRL-TK-Renilla Luciferase expression constructs, with a 3:1 Lipofectamine:DNA ratio. Cells were serum starved 1 day post-transfection and treated with IL11 (10 ng/ml) and bazedoxifene (ranging from 0.003 to 3 μM; Selleck Pharmaceuticals) 2 days post-transfection. Luciferase assays were performed using the dual luciferase reporter kit (Promega) using manufacturer’s instructions. Firefly luciferase readings were normalized to Renilla activity. TOPFLASH and FOPFLASH Reporter assays were performed in SW480 cells using previously published methods (Phesse et al., 2014).

**BAF/03 cytokine-dependent cell proliferation assays**

Transfections in BAF/03 cells were performed with the Amaza Nucleofector (Lonza) with 1 μg plasmid DNA (pcDNA-IL11RA and pcDNA-L-gp130). Parental BAF/03 cells were maintained in 10 ng/ml of IL3, and BAF/03IL11RA, BAF/03IL11RA, and BAF/03IL1fR were maintained in 10 ng/ml of IL3 and 10 ng/ml of IL6, IL11, or LIFR, respectively. Cell proliferation was measured at Day 0 upon treatment initiation and at 24 h post-treatment. Cell proliferation rates were measured on Day 0 upon the initiation of treatment and after 24 h of treatment. Changes in cell proliferation were determined relative to the mean values obtained from cytokine and vehicle treated wells. Data are expressed as fold change. Cell proliferation was determined with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to manufacturer’s instructions with the measurement of absorbance at 490 nm (Thermo Fisher; M6494).

**Structural modeling**

Bazedoxifene was manually docked to gp130 Site III (PDB ID:1P9M) using SYBYL-X 2.1.1 (Certara, L.P. Princeton, NJ, USA) to verify the binding modes previously published. To remove any steric issues that may have arisen during the docking process, the bazedoxifene ligand and gp130 residues within an 8 Å radius of the docked ligand were geometrically optimized within SYBYL-X 2.1.1 using the MMFF94s force field and partial atomic charges, the conjugate gradient convergence method, and 10,000 iterations. To illustrate the structural differences between the two compounds, tamoxifen and bazedoxifene were aligned via their common phenoxypropylamine core. The PyMOL Molecular Graphics System, version 1.8.2.2 (Schrödinger LLC, Cambridge, MA; http://www.pymol.org), was used for all structural visualization, interaction analysis, and figure creation.

**Patient-derived colon cancer primary cell isolation and culture**

Patient-derived colon cancer cells were derived from resection specimens collected from Stage II CRC patients using a previously described protocol (Grillet et al., 2017), under agreement from the Centre Hospitalier Universitaire de Nimes Human Ethics Committee (#2011-A01141-40). Informed consent was obtained from all subjects and experiments performed in accordance with the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. Briefly, tumor samples were incubated for 5 min in HBSS + 0.4% bleach, washed five times in HBSS, minced into 2-mm³ fragments, and dissociated using liberase H (0.26 U/ml, Roche) diluted in Accumax (Sigma-Aldrich) for 2 h at 37°C. Thereafter, isolated cells were filtered through a 40-μm mesh and single-cell suspensions were plated in DMEM, supplemented with FBS, glutamine, 100 μg/ml gentamicin, and 40 μg/ml ciprofloxacin.

**Human cancer organoid culture and treatment**

Human cancer cultures were established and maintained as described previously with modifications (Jung et al., 2011). For assay setup, the passaged organoids were harvested and broken down into small fragments mechanically using a 26-G needle. The fragments were mixed with Matrigel (BD Biosciences #356231) and plated mechanically using an Epmotion Automated workstation 5073c (Eppendorf) into 384-well plates (Corning #3985). Approximately 200 fragments per well were embedded in 8 μl Matrigel and seeded in 384-well plates. The Matrigel was allowed to polymerize at 37°C for 60 min after which 80 μl of growing media was dispensed into each well using the Epmotion 5073c. On Day 6 of the culture, the Epmotion Automated workstation 5073c (Eppendorf) was used to replenish media with treatments. The experiment used in the study was terminated on Day 13 (Day 7 after treatment). The cultures were monitored by microscopy throughout the experiment where bright-field image stacks were acquired of each used well on most days.

**Human cancer organoid image acquisition, processing, and scoring**

The detailed image acquisition and analysis procedures for acquiring images in 96-well plates have been described previously (Hirokawa et al., 2014; Tan et al., 2015). In this experiment, 384-well plates (Corning #3985) were used. The microscopy was conducted using a Nikon Eclipse Ti-U microscope with a 4× objective lens and motorized stage (Prior Scientific, H117). Bright-field image stacks of each well were captured on a Nikon DS-R2i camera (Nikon Inc, MQA17000) using the NIS-Elements software (Nikon, Basic Research version 4.40). The depth images of each FOV (well) were then stacked using the EDF algorithm in Fiji. The processed images were analyzed semi-automatically using a customized Fiji-script where objects identified were bordered a region of interest (ROI) and these ROIs were tabulated and analyzed. The resulting ROIs were manually curated, and the objects sizes and mean intensities acquired.

**Animal experiments**

All animal experiments were conducted in accordance with ethics protocols approved by Austin Health and La Trobe University Animal Ethics Committees. Transgenic C57BL/6 gp130Y757F, Lgr5CreERT2, Apcflox and Cdx2CreERT2, Apcflox mice were bred and maintained under specific pathogen-free housing conditions. Mouse housing conditions included a constant temperature of 24°C with a 12-h light/dark cycle, 5 mice per cage with food and water ad libitum as described previously (Tebbutt et al., 2002; Hinoi et al., 2007; Phesse et al., 2014). Experiments were performed with mice of both genders, 9–10 weeks of age in all experiments except for those with gp130Y757F mice, which were used at 13 weeks of age. To induce
Cre-mediated exon deletion, a single-daily intra-peritoneal (i.p.) injection of tamoxifen was administered over three consecutive days in the Lgr5CreERT2, Apcflox and Cdx2CreERT2, Apcflox mice. Bazedoxifene (3 mg/kg, Selleckchem) dissolved in 10% v/v dimethyl sulfoxide (DMSO) and 90% v/v sunflower oil daily was i.p. administered to animals for 5 days of the week, for a period of 7 consecutive weeks in gp130V577F, and 2 weeks in Lgr5CreERT2, Apcflox and Cdx2CreERT2, Apcflox mice. Vehicle-treated control cohorts were administered 100 μl of 10% v/v DMSO and 90% v/v sunflower oil daily for the same duration. All experiments were performed in age-matched mice. Tumor onset and progression in the distal colon, in Cdx2CreERT2, Apcflox mice, were monitored by endoscopy as previously described (Putoczki et al, 2013). Mice were euthanized by CO2, and tissue was collected for formalin fixation for immunohistochemistry and snap-frozen for Western blot and qPCR analyses.

Ovariectomy

Female gp130V577F mice were anesthetized using 4% isoflurane, and a small (1 cm) transversal (vertical) incision was made in the skin in the center of the lower back and dissected from the underlying fascia to reach the abdominal wall. A small (5 mm) incision was made in the abdominal wall and the adipose tissue surrounding the ovary to expose the ovary and connected uterine horn. The vessel between the ovary and uterine horn was ligated, and the ovary was dissected out. The uterine horn was then replaced into the abdominal cavity, and the procedure was then repeated for the other side. The skin wound was then closed and secured with a sterile surgical clip. The mice were placed in a heated cage and closely monitored for 2 h.

Gene expression analysis

Quantitative reverse-transcriptase PCR was performed on RNA isolated from cell lines, tumours, and whole stomach, small intestine, and colon lysates. RNA extraction was performed using the ReliaPrep RNA Cell Mini-Prep System (Promega) according to the manufacturer’s instructions. First-strand cDNA synthesis using 1.0 μg total RNA was performed using SuperScript™ III First Strand Kit (Life Technologies) primed by random hexamer primers. mRNA was quantified using primer sequences (Appendix Table S1) using the SensiMix SYBR (Bioline) on the 7500 Fast Real Time PCR System (Applied Biosystems). Relative expression was calculated using the comparative CT method (2^−ΔΔCT) method after normalization to Hprt as the housekeeping control gene (Coulson et al, 2017). Comparative real-time PCR results were performed in triplicates for all samples.

Western blot analysis

Protein lysates containing a total of 30 μg of protein, 25% NuPAGE LDS sample buffer, and 10% NuPAGE reducing agent were heat-denatured and separated on precast 4–12% NuPAGE Bis-Tris mini gels (Thermo Fisher Scientific). The SeeBlue Plus2 Protein Standard ladder was used to determine protein band sizes (Thermo Fisher Scientific). Proteins were transferred to PVDF or nitrocellulose membranes using iBlot™ Gel Transfer Stacks for 7 min with 23V using the iBlot Gel Transfer Device (Thermo Fisher Scientific). The PVDF or nitrocellulose membranes with transferred proteins were then blocked with Odyssey blocking buffer (TBS; Li-COR) for 1 h at room temperature. Membranes were incubated overnight at 4°C with primary antibodies diluted in solution of Odyssey blocking buffer (TBS) and TBST (1:1). After washes in TBST, secondary antibody incubations were performed at room temperature for 1 h (Appendix Table S2). Following washing in TBST, the membranes were visualized using the Licor Odyssey Imaging system.

Immunohistochemistry

Immunohistochemistry was performed using antibodies directed against pSTAT3, Ki67, and β-catenin as previously published (Putoczki et al, 2013; Phesse et al, 2014; Coulson et al, 2017). 4-μm-thick sections from paraffin blocks were rehydrated, antigen retrieval performed with citrate buffer (0.1%) by microwaving for 15 min. Sections were hydrogen peroxidase–quenched for 20 min (H2O2, 3%) and blocked for 30 min in TBST supplemented with 10% goat serum and incubated overnight at 4°C with primary antibody (Appendix Table S2). Sections were incubated with biotinylated secondary antibody (1:1,000) for 1 h at room temperature and subsequently incubated with Vectastain Elite ABC HRP Kit (Vector Laboratories). Chromogenic staining was performed using the DAB peroxidase kit, and the sections were counterstained using Mayer’s hematoxylin (H-3401, Vector Laboratories). Apoptosis was quantified by the in situ apoptosis detection kit, ApopTAG (Millipore) according to the manufacturer’s instructions.

Stained sections were imaged with an Aperio Slide Scanner (Leica Biosystems). Whole-tumor sections were selected and immunostaining for β-cadherin, Ki67, pSTAT3, and cleaved caspase 3 were quantified using Aperio ImageScope Positive Pixel Count Algorithm, written specifically for detection of nuclear, cytoplasmic, or membrane immunostains. The algorithm measurements include signal intensity (graded score of 1–3 with increasing signal intensity) and calculation of the fraction of pixels with a positive-color measurement. Immunostaining was measured as a percentage of the total tumor area including epithelial and stromal cells. Data are presented as mean ± SEM, n = 5 animals per treatment group. For pSTAT3 immunostaining, data are presented as percentage positive cells stained for signal intensity > 3. Data are presented as percentage positive pixels of the total number of pixels analyzed.

Flow cytometry

Colons were harvested from animals, and cells were dissociated in digestion buffer (2.5% FCS, 1 mg/ml collagenase III, 0.4 U/ml dispase, and 2 U/ml DNase in HBSS) for 30 min at 37°C. The cells were incubated in FACS buffer (2.5% of FCS in PBS) with the relevant antibodies for 30 min on ice. The following fluorochrome-conjugated antibodies were used for flow cytometry analysis: EpCAM (clone G8.8) #17579180, CD4 (clone GK1.5), F4/80 (clone BM8), Ly6c (clone HK1.4) all from e-Biosciences; CD8 (clone 53-6.7); B220 (clone RA3-6B2), Ly6g (clone 1A8) all from BD Biosciences; PD1 from Invitrogen #46998582; CD45.2 (clone GK1.5), F4/80 (clone BM8), EpCAM (clone M5/114.16) from the WEHI monoclonal antibody laboratory. TCRβ (Biolegend #109208; Appendix Table S3). Dead cells were excluded by using SytoxBlue (Invitrogen). The data were analyzed by using FlowJo software version 10.3 (FlowJo, LLC 2006-2017). Gating
The paper explained

Problem
Colon and stomach cancers collectively are among the most common malignancies and arise in the epithelial lining of the gastrointestinal tract. Specifically, gp130 signaling becomes rate limiting for the growth of gastrointestinal tumors arising from oncogenic driver mutations in Apc. In the present study, we investigate whether pharmacological targeting of gp130 with the selective estrogen receptor modulator bazedoxifene, clinically approved for the treatment of osteoporosis, is an effective treatment in preclinical models of gastric and colon cancers.

Results
Our in silico modeling suggests that the inhibitory activity of bazedoxifene is due to its ability to mimic interactions between the Site III interface of gp130 receptor and tryptophan-157 in the IL6, or tryptophan-168 in the IL11 cytokines. Bazedoxifene suppresses gastric tumor growth in gp130+/−/− mice irrespective of their gender, in which tumors arise through excessive IL11-dependent STAT3 signaling. Strikingly, in sporadic colon cancer models based on aberrant activation of β-catenin/canonical WNT pathway, bazedoxifene treatment also reduces tumor burden in mice following conditional ablation of the Apc suppressor gene using the Ly56CreERT2 or Cdx2CreERT2-driver alleles. Consistent with our observation that nuclear accumulation of β-catenin remains unaffected in colonic tumors of bazedoxifene-treated mice, bazedoxifene treatment of human SW480 colon cancer cells harboring mutant APC did not affect canonical WNT signaling, but suppressed IL11-dependent STAT3 signaling.

Impact
Our data suggest that gp130-dependent Stat3 activation is an important and pharmacologically targetable therapeutic opportunity in gastrointestinal cancers.

Statistical analysis
Data were analyzed using GraphPad PRISM software for statistical analysis with Students’ t test. Normal distribution was calculated by using the Kolmogorov–Smirnov test. For multiple analysis, statistical significance was calculated using ANOVA, followed by Tukey or Dunnett’s post hoc test for multiple comparisons where appropriate. A P-value < 0.05 was considered significant. Animals were randomized into treatment arms to remove subjective bias; tumor burden was assessed by an investigator with no prior knowledge of treatment details.

Expanded View for this article is available online.

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Author contributions
A.L.C and M.E designed research; PT, JH, ARP, CVT, KT, NJH, DB, SA-S, and A.L.C performed research; TLN, ACP, MB, AMS, MDWG, DB, FH, MWP, TLP, ME, and A.L.C analyzed data; A.L.C, TLN, and ME wrote the paper. All authors approved the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest.

For more information
Gp130/IL6ST gene and protein links
(i) https://www.ncbi.nlm.nih.gov/gene/3572
(ii) https://www.omim.org/entry/600694

IL11 and IL6 gene and protein links
(i) https://www.ncbi.nlm.nih.gov/gene/3572
(ii) https://www.omim.org/entry/147681
(iii) https://www.ncbi.nlm.nih.gov/gene/3569
(iv) https://www.omim.org/entry/147620

Author Website links
(i) https://www.onjcancercentre.org/research/research/our-research-programs/cancer-therapeutics-development-group
(ii) https://www.onjcancercentre.org/profile/ashwini-chand
(iii) https://www.onjcancercentre.org/research/research/our-research-programs/cancer-inflammation-laboratory
(iv) https://www.onjcancercentre.org/profile/prof-matthias-ernst

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