Original Article

Perioperative COX2 and β-Adrenergic Blockade Improves Biomarkers of Tumor Metastasis, Immunity, and Inflammation in Colorectal Cancer: A Randomized Controlled Trial

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BACKGROUND: Preclinical studies have implicated excess release of catecholamines and prostaglandins in the mediation of prometastatic processes during surgical treatment of cancer. In this study, we tested the combined perioperative blockade of these pathways in patients with colorectal cancer (CRC). **METHODS:** In a randomized, double-blind, placebo-controlled biomarker trial involving 34 patients, the β-blocker propranolol and the COX2-inhibitor etodolac were administered for 20 perioperative days, starting 5 days before surgery. Excised tumors were subjected to whole genome messenger RNA profiling and transcriptional control pathway analyses. **RESULTS:** Drugs were well-tolerated, with minor complications in both the treatment group and the placebo group. Treatment resulted in a significant improvement ($P < .05$) of tumor molecular markers of malignant and metastatic potential, including 1) reduced epithelial-to-mesenchymal transition, 2) reduced tumor infiltrating $CD14^+$ monocytes and $CD19^+$ B cells, and 3) increased tumor infiltrating $CD56^+$ natural killer cells. Transcriptional activity analyses indicated a favorable drug impact on 12 of 19 a priori hypothesized CRC-related transcription factors, including the GATA, STAT, and EGR families as well as the CREB family that mediates the gene regulatory impact of β-adrenergic– and prostaglandin-signaling. Alterations observed in these transcriptional activities were previously associated with improved long-term clinical outcomes. Three-year recurrence rates were assessed for long-term safety analyses. An intent-to-treat analysis revealed that recurrence rates were 12.5% (2/16) in the treatment group and 33.3% (6/18) in the placebo group ($P = .239$), and in protocol-compliant patients, recurrence rates were 0% (0/11) in the treatment group and 29.4% (5/17) in the placebo group ($P = .054$). **CONCLUSIONS:** The favorable biomarker impacts and clinical outcomes provide a rationale for future randomized placebo-controlled trials in larger samples to assess the effects of perioperative propranolol/etodolac treatment on oncological clinical outcomes. ***Cancer* 2020;126:3991-4001**. © *2020 American Cancer Society*.

KEYWORDS: catecholamines, clinical trial, colorectal cancer, EMT, inflammation, metastases, perioperative period, prostaglandins, stress, transcription factors.

INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignancy worldwide, accounting for approximately 900,000 deaths annually.[1] Resection of the primary tumor is the central curative approach, but the 5-year mortality rate is approximately 30% to 35%, with a majority of deaths attributable to metastatic disease.[2] Although surgery is a cornerstone in cancer treatment, extensive preclinical research has shown that it can also promote the formation of new metastases and the growth or outbreak of preexisting micrometastases.[3-5] The biological mechanisms underlying the prometastatic effects of surgery are numerous,[3-5] and many are triggered or accelerated by paracrine and/or neuroendocrine stress-inflammatory responses to surgery.[6]

Specifically, catecholamines and prostaglandins have been implicated repeatedly in promoting cancer metastasis.[4,5] These factors are released during the perioperative period due to 1) stress and anxiety experienced by patients,[7] which also trigger proinflammatory processes[8]; 2) surgical procedures,[9] including anesthesia, tissue damage, hypothermia, and nociception; and 3) prevalent prostaglandin secretion by the malignant tissue.[10] Thus, catecholamines and prostaglandins are simultaneously elevated during the perioperative period, both locally and systemically.[4,5] Mechanisms throughout which catecholamines and prostaglandins contribute to the prometastatic effects of surgery include 1) direct impact on tumor cells, promoting their growth, invasion capacity, resistance to cell death,

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secretion of proangiogenic factors,[3-5,11] and epithelial-to-mesenchymal transition (EMT)[12], and 2) suppression of antimetastatic immunity, including reduced cytotoxic T lymphocyte and natural killer (NK) activities and disrupted Th1/Th2 cytokine balance.[3] Consistent with these preclinical results, primary tumor expression of COX2 is a negative prognostic index in patients with CRC, and CRC metastases exhibit higher levels of COX2 than the primary tumor.[13] Thus, the perioperative blockade of catecholamine and prostaglandin signaling may prove beneficial through pleiotropic mechanisms.

Indeed, preclinical in vitro and in vivo studies have shown that inhibition of β-adrenergic signaling and/or prostaglandin synthesis can reduce the immunosuppressive and prometastatic effects of stress and surgery in several tumor lines and models,[4,5] including liver metastases of colon cancer.[14] Specifically, the perioperative use of the β-blocker propranolol and the COX2 inhibitor etodolac were each shown to reduce postoperative metastases and/or mortality rates in several animal models.[14-17] Importantly, the combination of these 2 drugs was most effective and is often the only effective approach,[14,16,17] as both catecholamines and prostaglandins are upregulated perioperatively, and each alone can promote metastasis by activating the cAMP-PKA signal transduction pathway in tumor cells and their microenvironment as well as in immunocytes. Therefore, given the limited patient number in the current trial, only the combination of propranolol and etodolac was studied.

Here, we report the results from the first randomized, placebo-controlled biomarker clinical trial testing the combined perioperative use of propranolol and etodolac in patients with CRC. The primary objective of the trial was to study the drug impact on tumor biomarkers associated with long-term cancer outcomes. Specifically, excised primary tumors were subjected to messenger RNA (mRNA) profiling and transcriptional control pathway analyses based on a priori hypotheses to study prometastatic and inflammatory indicators, including EMT, cancer-promoting transcription factors (TFs), and tumor-infiltrating leukocytes (TILs). The second objective was to ascertain the safety of the treatment by clinically assessing short-term complication rates and 3-year recurrence rates.

MATERIALS AND METHODS

Patients

Thirty-four patients with a median age of 58 years (range, 30-77 years) who had been diagnosed with CRC without known metastatic disease were recruited at the Sheba Medical Center, Ramat-Gan, Israel. Exclusion criteria included 1) any contraindications for the study drugs, 2) chronic use of any β-blocker or COX inhibitor, and 3) chronic autoimmune disease (a complete list of inclusion/exclusion criteria is provided in Supporting Section S1). The protocol (ClinicalTrials.gov identifier NCT00888797) was approved by the institutional review board of Sheba Medical Center, and written informed consent was obtained from all patients.

Study Design and Drug Treatment

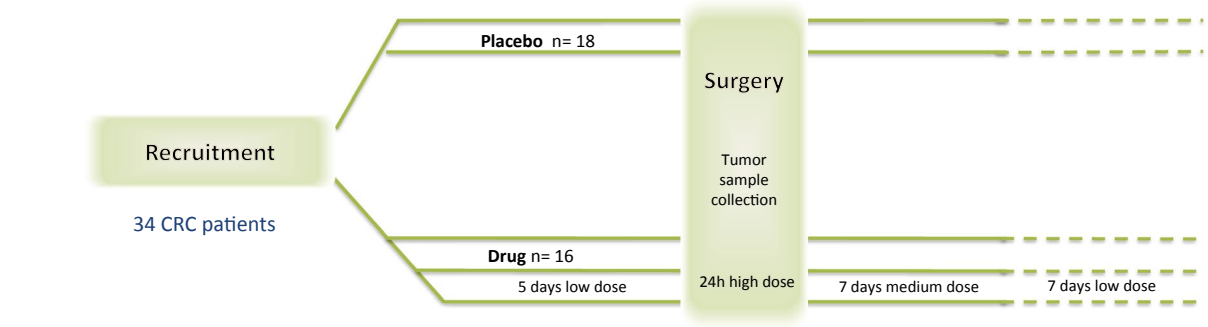
We conducted a randomized, double-blind, placebo-controlled biomarker trial. Drug or placebo was administered for 20 consecutive days, starting 5 days before the tumor resection. Oral etodolac (400 mg twice a day) was administered throughout the treatment period. Propranolol was administered orally in titration using extended release formulations at 20 mg twice a day during the 5 days preceding surgery; 80 mg twice on the day of surgery; 40 mg twice a day after the day of surgery for 7 postoperative days; and 20 mg twice a day for the last 7 days. Identical schedule and capsules were used for placebo and medication. Drug intake and compliance were monitored by a clinical research coordinator, based on returned pill packs and patients reports. Protocol compliance was defined as consuming >60% of treatment pills and >75% (from each medication) during days −1 and 0 and days 1 and 2 postoperatively. An intent-to-treat analysis included 2 patients in whom metastases were detected after recruitment and patients who were drug incompliant. Random assignment of patients was conducted by the pharmacy in randomization blocks of 10 (1:1 ratio). The study drugs were dispensed by the investigational drug services pharmacy at the hospital.

Endpoints and Assessments

Clinical outcomes, including safety parameters and disease recurrence, were prospectively recorded on case report forms. Excised tumor tissues were fixed in 4% formaldehyde and stored as formalin-fixed, paraffin-embedded blocks. Five 5-μm sections were taken for gene expression profiling conducted at the UCLA Social/Neuroscience Genomics Core Laboratory.

Gene Expression Profiling and Bioinformatic Analysis

Detailed methods have been described previously[18] and are provided in Supporting Section S5. Briefly, mRNA



Drug treatment:

Etodolac	*No treatment*	400mg/b.i.d	400mg/b.i.d	400mg/b.i.d	400mg/b.i.d
Propranolol	*No treatment*	20mg/b.i.d	**80mg/b.i.d**	40mg/b.i.d	20mg/b.i.d

FIGURE 1. Schematic presentation of the design and time schedule of the study. A randomized, double-blind, placebo-controlled biomarker trial was conducted in patients with colorectal cancer who were administered placebo or propranolol and etodolac for 20 consecutive days, starting 5 days before surgery. Propranolol doses increased during the day of surgery and decreased each consecutive week postoperatively. Tumor tissue was collected during surgery. b.i.d., twice a day; CRC, colorectal cancer.

was extracted from formalin-fixed, paraffin-embedded tumor sections and subjected to genome-wide transcriptional profiling with quantile normalization. Of the 28 protocol-compliant patients, 3 patients in the placebo group and 2 patients in the drug treatment group who had rectal cancer showed complete response to neoadjuvant therapy, and 3 tumors were not found in the tumor bank. Thus, gene expression analyses were conducted in the remaining 20 protocol-compliant patients, 9 patients who received drug treatment, and 11 patients who received placebo. Due to the limited number of tissue samples available for analysis, we did not attempt to adjust for any demographic or cancer-related patient characteristics in the primary reported analyses, but verified that their introduction in secondary analyses did not alter the outcomes. A priori hypotheses regarding EMT polarization and tumor-infiltrating leukocyte transcriptomes were tested using transcript origin analyses. A priori hypotheses regarding activity of CRC-relevant transcription control pathways were tested using TELiS bioinformatic analysis of TF binding motifs in the promoters of all genes showing ≥1.25-fold differential expression, using TRANSFAC position-specific weight matrices as described previously.[19] Specifically, this analysis was performed for 19 hypothesized TFs: GATA1, GATA2, STAT1, STAT3, EGR2, EGR3, c-MYB, deltaEF1/ZEB1, ETS1, NFY-C, PAX2, AP4, NF-κB, AP-1, HSF1, IRF1, ISRE, GRE, and the CREB family of TFs that mediate gene regulatory responses to cAMP/PKA signaling (including β-adrenergic and prostaglandin receptor systems). A full discussion of the clinical significance of the hypothesized TFs is provided in Supporting Section S5.

Statistical Analyses

All analyses were 2-sided ($\alpha < .05$), and were conducted only for a priori hypothesized group differences in progression-related transcriptome profiles of malignant tissue (EMT; tumor associated-monocyte, B cell, and NK cell transcripts; and cancer-promoting TFs). Survival analyses were conducted for 3-year DFS for intent-to-treat and for protocol-compliant patients. A full description of the statistical and power analyses is provided in Supporting Section S4.

RESULTS

Demographics and Drug Compliance

Thirty-four patients were randomly assigned to receive combined propranolol/etodolac perioperative treatment or placebo. The study design is provided in Figure 1, and the CONSORT diagram is provided in Figure 2. No stratification by tumor site, stage, or grade was made given 1) the relatively small sample size and 2) the fact that staging was not known until randomization was completed. Because these characteristics are associated with different metastatic spread patterns,[20] we tested and verified that the 2 groups did not differ in these or in any other cancer-related characteristics (Table 1) and conducted ancillary analyses incorporating these covariates to verify their lack

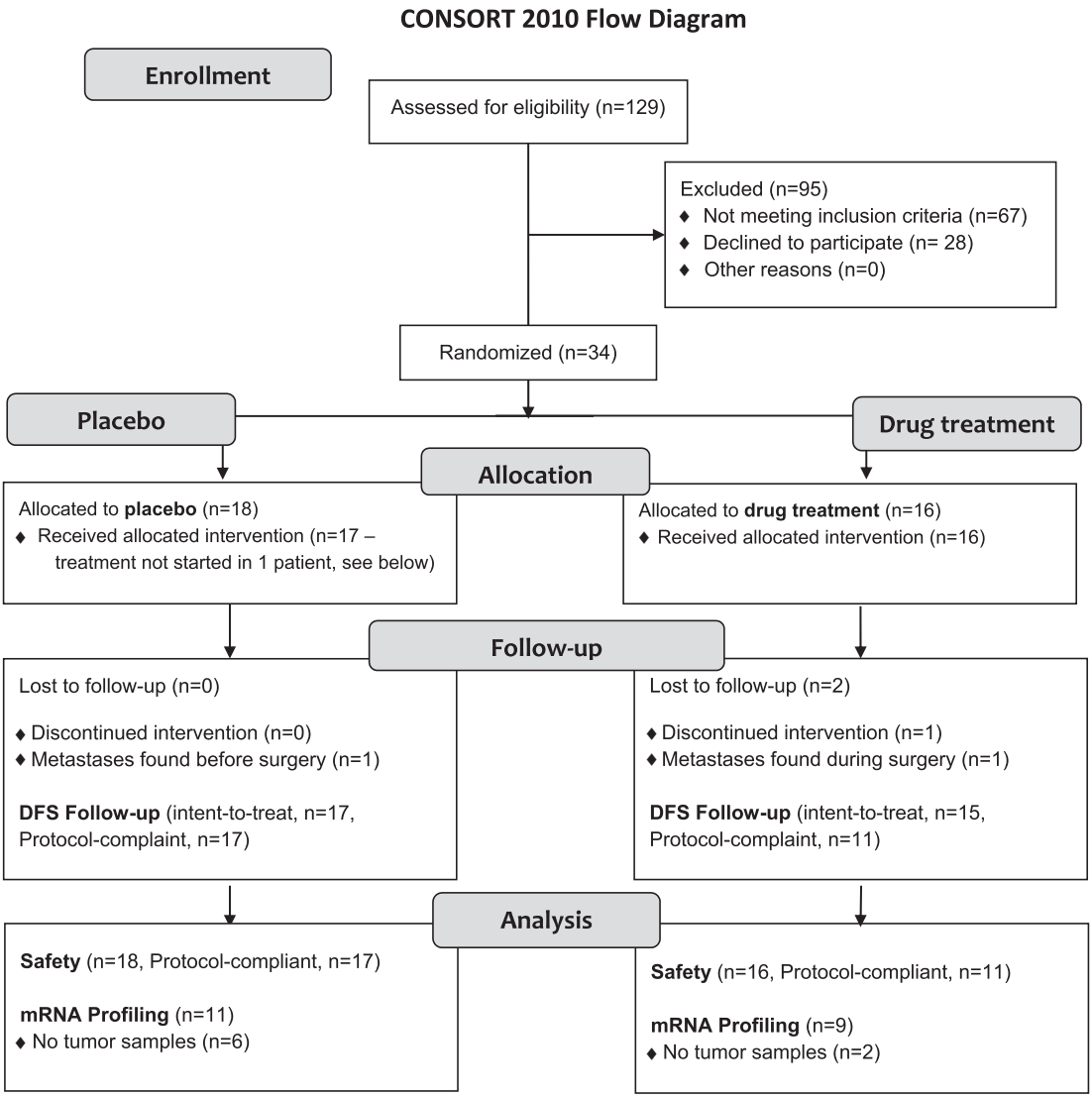


FIGURE 2. CONSORT diagram of clinical trial enrollment, treatment, and analyses. Intent-to-treat includes patients that were randomly assigned to treatment, but not those with known metastases before or during surgery. DFS, disease-free survival; mRNA, messenger RNA.

of impact on group differences. Regarding demographic characteristics, the 2 groups differ in sex, with more men randomly assigned to drug treatment and more women randomly assigned to placebo ($P = .042$ intent-to-treat analysis, $P = .06$ protocol-compliant analysis). However, women have improved survival compared with men[21,22] and lower probability of developing invasive cancer.[2] Thus, this sex difference works against our hypothesis that the drug treatment will improve clinical outcomes.

In intent-to-treat analysis, drug compliance differs between groups (91.56% placebo vs 74.8% drug; $P = .047$). Excluding 1 patient whose treatment was ceased preoperatively by the principal investigator, compliance was similar between groups (91.56% vs 92.5%). In protocol-compliant patients, overall compliance was 93.3% with similar group compliance (Table 1).

Safety Outcomes

Both intent-to-treat and protocol-compliant analyses indicated that drug-related adverse event rates and postoperative adverse event rates (up to 30 days after surgery) were equivalent between the 2 groups (Table 2 and Supporting Section S2). Importantly, no

TABLE 1. Baseline Demographic and Clinical Characteristics

	Intent-to-Treat (n = 34)		Protocol-Compliant (n = 28)		mRNA Profiling (n = 20)		
	Placebo (n = 18)	Drugs (n = 16)	Placebo (n = 17)	Drugs (n = 11)	Placebo (n = 11)	Drugs (n = 9)	P^a
Age at surgery, mean (range)	54.8(39-73)	57.6 (30-77)	55.7 (40-73)	57.4 (30-77)	56.2 (40-73)	59.4 (40-77)	NS
BMI, mean (range)	26.6 (18.7-36.6)	25.1 (15.6-33.7)	27 (21.8-36.6)	25.6 (15.6-33.7)	27.7 (22.2-36.6)	26.9 (23.1-33.7)	NS
Weight, kg, mean (range)	72.8 (50-100)	71 (50-95)	73.9 (50-100)	73 (55-95)	75.8 (50-100)	76.1 (65-95)	NS
Sex							
Men	**6**	**11**	6	8	4	6	.042
Women	**12**	**5**	**11**	**3**	7	3	.06
Smoking status							
Yes	1	1	1	1	0	1	NS
No	16	15	15	10	11	8	NS
NA	1	0	1	0	0	0	NS
Cancer stage							
T0	1	3	1	3	0	1	NS
T1	0	2	0	2	0	1	NS
T2	3	2	3	2	2	2	NS
T3	13	8	12	4	8	5	NS
NA	1	1	1	0	1	0	NS
Lymph node involvement							
N0	13	11	12	8	7	6	NS
N1	3	4	3	3	3	3	NS
N2	1	0	1	0	0	0	NS
NA	1	1	1	0	1	0	NS
Tumor site							
Left colon	5	3	4	1	4	1	NS
Right colon	3	2	3	1	1	1	NS
Rectum	10	11	10	9	6	7	NS
Any time point metastasis							
None	12	12	12	11	8	8	NS
Postoperative	5	1	**5**	**0**	3	1	.063
Preoperative	1	1	0	0	0	0	NS
Preoperative NACRT							
Yes	8	7	8	6	4	4	NS
No	2	4	2	4	1	3	NS
NA	8	5	7	1	6	2	NS
Postoperative NACRT							
Yes	12	7	**12**	**4**	9	4	.0905
No	2	4	2	4	1	3	NS
NA	4	5	3	3	1	2	NS
Drug compliance							
Average intake	91.56%	74.8%	96.76%	89.67%	96.21%[b]	92.6%[b]	.047
Noncompliance rate	1/18[c]	5/16[c]	0/17	0/11	0/11	1/9[d]	NS

Abbreviations: BMI, body mass index; mRNA, messenger RNA; NA, not available; NACRT, neoadjuvant chemo-radiation therapy; NS, nonsignificant.
[a]Significant and marginally- significant values apply to data in boldface type.
[b]Preoperative.
[c]Complete information about treatment compliance (per patient) is provided in Supporting Section S2.
[d]One patient's treatment was ceased by the principal investigator postoperatively due to breathing difficulties (pulmonary edema) and low blood pressure. For mRNA analyses purposes, this patient was considered drug-compliant (100% of drug dosages administered at treatment stage 1 and 75% of drug dosages administered on day −1 and day of surgery).

severe surgical complications were observed in patients in the treatment group versus 1 event in a patient in the placebo group. Additional details and a discussion of safety outcomes and considerations are provided in Supporting Section S3 and in the supplementary data of Shaashua et al.[18]

Tumor Gene Expression and Bioinformatics Analyses

Genome-wide transcriptional profiling of tumor tissues identified 277 genes showing ≥1.25 fold up-regulation in tumors from patients in the treatment group versus patients in the placebo group, and 294 genes were equivalently downregulated.

TABLE 2. Safety Data

	Intent-to-Treat (n = 34)		Protocol-Compliant (n = 28)		mRNA Profiling (n = 20)		
	Placebo (n = 18)	Drugs (n = 16)	Placebo (n = 17)	Drugs (n = 11)	Placebo (n = 11)	Drugs (n = 9)	P^a
No. of severe surgical complications	1/18	0/16	1/17	0/11	1/11	0/9	NS
Potential drug-related AEs during intervention phase[b]							
Type of AE							
None	17	13	16	8	10	7	NS
Mild	0	0	0	0	0	0	NS
Moderate	1	2	1	2	1	1	NS
Severe	0	1	0	1	0	1	NS
No. of AEs per patient							
0	17	13	16	8	10	7	NS
1	0	2	0	2	0	1	NS
2	1	1	1	1	1	1	NS
Postoperative complications[c]							
No. of AEs per patient							
0	13	10	12	6	8	5	NS
1	4	2	4	2	2	1	NS
2	1	2	1	1	1	1	NS
3	0	1	0	1	0	1	NS
4	0	0	0	0	0	0	NS
5	0	1	0	1	0	1	NS

Abbreviations: AE, adverse event; mRNA, messenger RNA; NS, nonsignificant.
All events are nonserious adverse events. Complete information about specific adverse events per patient is provided in Supporting Section S2.
[a]All nonsignificant values are $P > .15$.
[b]Five days before surgery to 14 days after surgery (20 days total).
[c]Up to 30 days after surgery.

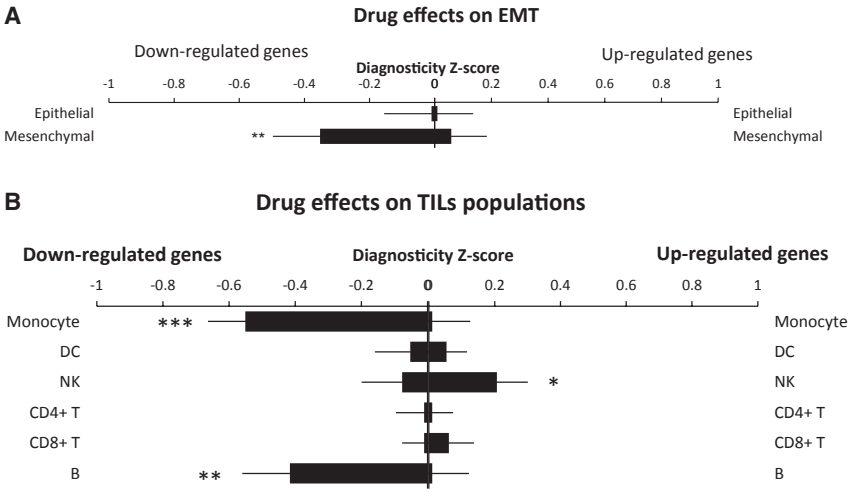


FIGURE 3. Effect of drug treatment on primary tumor transcriptome indicators of EMT, tumor-infiltrating leukocytes, and prometastatic or colorectal cancer prognostic relevant transcription factors (TFs). (A) Effects of drug treatment on EMT gene expression of genes showing ≥1.25-fold change in tumors from patients receiving drug treatment versus placebo. (B) Effects of drug treatment on expression of genes derived from monocytes, dendritic cells (DC), natural killer (NK) cells, $CD4^+$ and $CD8^+$ T cells, and B cells. (C) Effect of drug treatment on TF binding motifs associated with CRC progression and survival, and inflammatory TFs, in the promoters of all genes showing ≥1.25-fold differential expression from patients receiving drug treatment versus placebo. ^The potential positive (✓), negative (✗), or inconclusive (?) prognostic value is based on existing literature (see Supporting Section S5). Data are presented as the mean ± SEM. ¥Group differences (marginally significant $P < .1$). *$P < .05$. **$P < .01$. ***$P < .001$. CRC, colorectal cancer; EMT, epithelial-to-mesenchymal transition; IFN, interferon.

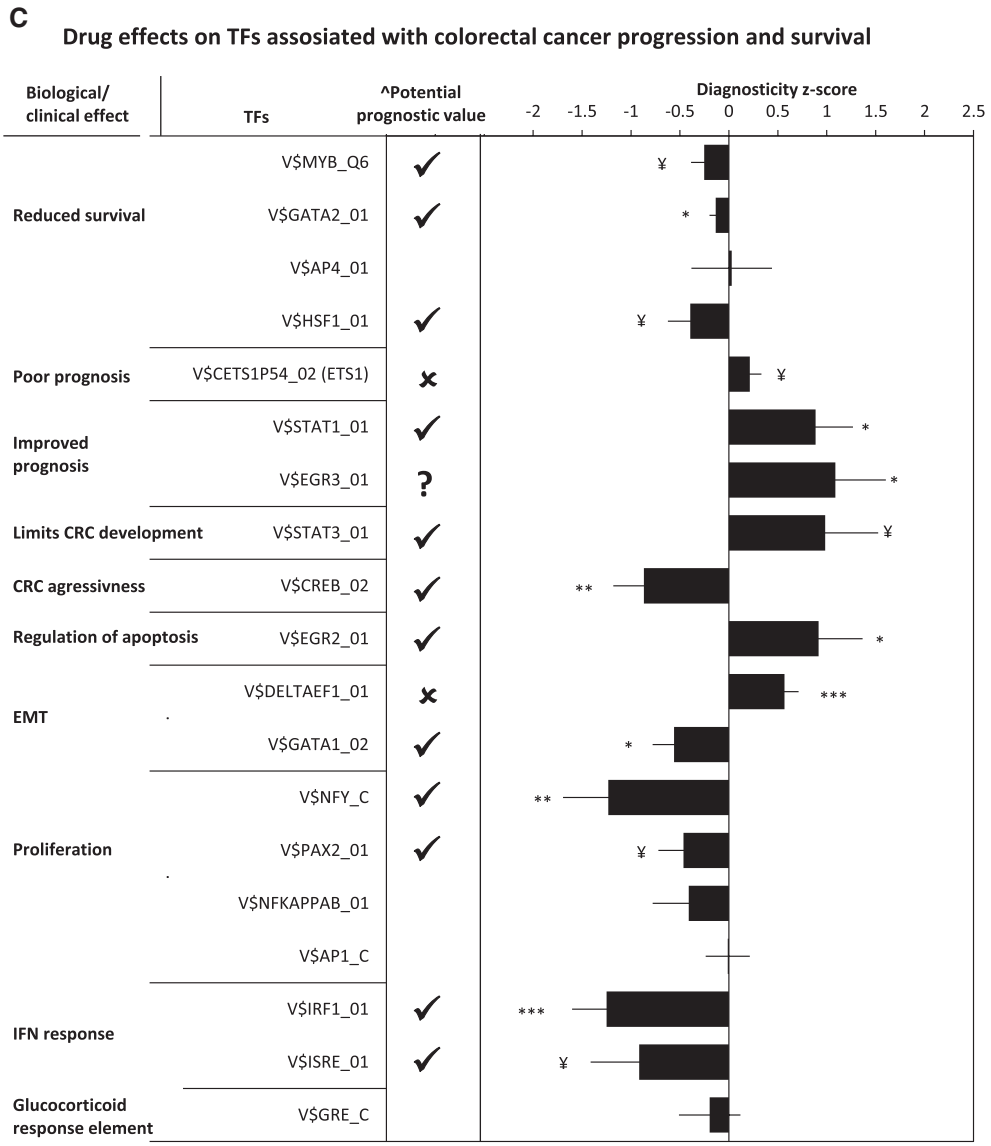


FIGURE 3. *Continued.*

A priori–specified bioinformatics analyses, using epithelial and mesenchymal reference cell transcriptomes as comparison points, showed that genes down-regulated by drug treatment were characteristic of mesenchymal polarization ($P = .008$), whereas genes up-regulated in association with drug treatment showed no significant polarization toward either epithelial or mesenchymal phenotypes (Fig. 3A). Transcriptome analyses of TIL populations (Fig. 3B) indicated that genes upregulated in response to drug treatment were derived from $CD56^+$ NK cells ($P = .0144$), whereas down-regulated genes were derived from $CD14^+$ monocytes ($P < .0001$) and $CD19^+$ B cells ($P = .002$).

Promoter-based bioinformatic analyses of TF regulation of differentially expressed genes was conducted, testing for 19 specific TFs that have previously been linked to prometastatic processes, inflammation, and tissue invasion (Fig. 3C). A full description of each of the 19 TFs analyzed, the drug impact, and the clinical predictive values are provided in Fig 3C and Supporting Section S5.

Our results revealed 1) a reduction in CREB ($P = .0062$), c-MYB ($P = .0619$), GATA1 ($P = .0113$), GATA2 ($P = .0321$), NFY-C ($P = .0085$), IRF1 ($P = .0005$), ISRE ($P = .0663$), HSF1 ($P = .0874$), and PAX2 ($P = .0722$) and 2) an increase in STAT1 ($P = .0218$), STAT3 ($P = .0702$), EGR2 ($P = .0427$),

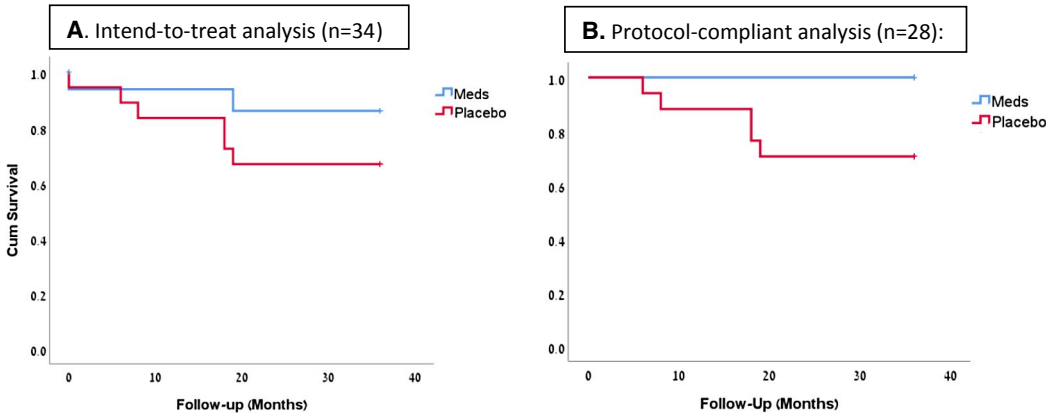


FIGURE 4. Kaplan-Meier survival analysis and log-rank test significance of a 3-year follow-up of any type recurrence disease-free survival based on (A) intent-to-treat analysis of patients (n = 34), in which recurrence was indicated in 2 of 16 patients receiving drug treatment versus 6 of 18 patients receiving placebo (log-rank test, P = .239). (B) Protocol-compliant patients with no presurgical metastases (n = 28), in which recurrence was indicated in 0 of 11 patients receiving drug treatment versus 5 of 17 patients receiving placebo (log-rank test, P = .054).

EGR3 (P = .0366), deltaEF1 (P = .0001), and ETS1 (P = .0722). Overall, among the 19 TFs tested, drug treatment altered 12 toward positive clinical impact, had no significant or undetermined effects on 5, and altered 2 in a potentially unfavorable direction (Fig 3C). Beyond these a priori hypotheses, the incidental findings for 13 additional TF binding motifs are detailed in Supporting Section S5.

Clinical Outcomes

This study was not designed or powered to assess drug effects on disease recurrence and survival, though we did collect data on 3-year recurrence rate to assess long-term safety. The results revealed a favorable trend toward reduced CRC recurrence in treated patients. Specifically, in protocol-compliant patients, metastases occurred in 0 of 11 patients who received drug treatment versus 5 of 17 patients who received placebo (P = .063). Kaplan-Meier survival analysis (log-rank test for recurrence at 3-year follow-up) showed a marginally significant (P = .054) reduction in recurrence (Fig. 4B). In intent-to-treat patients, the same analysis indicated a nonsignificant trend toward reduced recurrence in patients who received drug treatment (2/16) versus patients who received placebo (6/18) (P = .239; Fig. 4A).

DISCUSSION

Approximately one third of patients with CRC with no detectable metastases who undergo surgery with curative intent will develop metastatic disease within 3 years of surgery.[23] Recent studies have provided evidence that CRC may metastasize even before detection of the primary tumor.[24,25] The short perioperative period is now believed to be critical in determining the fate of this minimal residual disease that can progress, remain dormant, or regress. Mechanisms that are involved affect tumor proliferation and apoptosis, angiogenesis, and immunity[4] and often potentiate each other, creating a self-perpetuating "snowball effect" of accelerated malignant growth and/or escape from dormancy, leading to eventual disease recurrence.[26] The common biological perioperative drivers of these effects are catecholamines and prostaglandins. The intensified impact of the short perioperative period, together with the key role played by catecholamines and prostaglandins in promoting minimal residual disease,[3-5] provide a strong biological rationale for the simultaneous inhibition of catecholamines and prostaglandins as conducted in the present study. Additionally, similar recent clinical trials by us and by other investigators have provided evidence for the efficacy of perioperative blockade of catecholamines and/or prostaglandins,[18,27-29] as is also detailed below.

The treatment significantly reduced colorectal tumor EMT polarization, as indicated by reduced mesenchymal phenotype. These findings are consistent with in vivo and in vitro preclinical studies indicating that β-adrenergic blockade and COX2 inhibition can each inhibit EMT in models of human cancers, including

CRC.[30,31] This reduction also parallels our findings of reduced EMT polarization in patients with breast cancer who were randomly assigned to the same drug regimen,[18] and a similar reduction was recently reported by a different research group in patients with breast cancer who were treated with propranolol.[28] Alterations in intracellular processes that affect TFs, mRNA levels, and EMT are known to occur within hours to a few days after extracellular signals, as in a study that showed alterations in EMT markers as soon as 3 hours following EGF treatment.[32] Thus, we ascribe the reduction in EMT polarization to processes occurring during the 5 days of drug treatment before tumor excision. EMT is critically involved in CRC progression and was previously shown to promote tumor cell migration, invasion, self-sufficiency of growth signals, and resistance to apoptosis.[33] EMT of colorectal tumors is associated with COX2 overexpression,[34] local and distant recurrence,[35] positive lymph node status, and reduced 5-year survival rates.[36] Thus, the present reduction of EMT by the drug treatment is a positive indicator for long-term cancer outcomes.

Drug treatment increased tumor-infiltrating NK cells. Because most colorectal tumors display diminished major histocompatibility complex class I expression and are thus potential targets for NK cell–mediated killing,[37] tumor-infiltrating NK cells are expected to be a positive prognostic index and were indeed associated with improved DFS and overall survival (OS) in patients with CRC.[38] Unfortunately, the NK cell population in CRC is usually scarce.[39] To the best of our knowledge, the current study is the first to find an increase in tumor infiltration of NK cells in patients with CRC, which may have favorable clinical ramifications.

Monocyte recruitment by tumors was shown in several animal models to be enhanced by β-adrenergic signaling and to promote cancer progression.[40] Herein, our treatment reduced monocyte infiltration. In human CRC, infiltrating monocytes commonly transform into tumor-associated M2 macrophages (TAMs)[41] and support tumor progression, metastasis, and chemoresistance.[42,43] Finally, drug treatment reduced tumor-associated B cells, which constitute a significant proportion of TILs in colorectal tumors.[44] Although the role of B cells in human CRC is not well characterized, B cell–deficient mice exhibit spontaneous regression of CRC.[45] Overall, the changes in TIL composition induced by drug treatment suggest favorable effects.

We studied the impact of drug treatment on 19 TFs with known CRC prognostic value or expected drug effects based on a priori hypotheses. These included the CREB family of TFs that mediate the effects of β-adrenergic and prostaglandin receptors on gene expression, which confirmed the molecular activity of the 2-drug intervention regimen employed herein.

Among the 19 TFs tested, drug treatment altered 12 toward positive clinical impact. These TFs regulate tumor COX2 expression and inflammatory status, as well as tumor EMT, proliferation, invasiveness, and proangiogenic signaling. Most of these TFs are also associated with risk for recurrence, metastatic disease, or survival in patients with CRC. The treatment had no significant or undetermined effects on 5 TFs, and altered 2 TFs in a potentially unfavorable direction. Overall, these outcomes suggest a predominately favorable impact of the perioperative propranolol/etodolac protocol on tumor gene regulatory pathways in CRC.

The present study documents a favorable safety profile of drug treatment, as indicated by equivalent short-term complications and adverse event rates between groups. This safety profile is consistent with previous findings in patients with breast cancer who were treated with a shorter regimen[18,27] as well as various recent perioperative regimens of each drug alone.[5,46] Our study was not designed or powered to assess drug effects on disease recurrence and survival, but we did collect data on 3-year recurrence rate to assess long-term safety. The results revealed a favorable trend toward reduced CRC recurrence in treated patients. These findings suggest no reported long-term adverse effects, and the trends toward favorable clinical outcomes are consistent with the positive molecular biomarker results reported above; this finding was expected given that those biomarkers were selected based on known predictive value to DFS and OS in patients with CRC.

Our study has some limitations. First, the generalizability of the results is limited given that 1) the study was conducted in a single medical center and 2) approximately 50% of the patients were ineligible to participate due to exclusion criteria. Second, the study followed a between-patient design, but within-subject repeated measures may have yielded a clearer understanding of the mechanisms underlying the drugs' effects. A pretreatment biopsy of the excised tumor or repeated liquid biopsies[47] studying circulating cell-free tumor DNA[48] and/or exosomes along treatment period are feasible and may have provided additional information. Most importantly, it is critical for future studies to test the long-term impact of the perioperative propranolol/etodolac protocol in a clinical trial powered to assess DFS and OS.

In conclusion, this clinical trial is the first to investigate the combined perioperative inhibition of β-adrenoceptors and COX2 in patients with CRC. The outcomes indicate a favorable impact on molecular biomarkers associated with tumor growth and metastatic disease. We recently observed similar favorable effects of a similar drug treatment regimen on biomarkers in patients with breast cancer,[18,27] which strengthens the relevance and generalizability of the current findings. Overall, our findings indicate that the perioperative propranolol/etodolac protocol is empirically safe, easy to administer, and inexpensive and has overall favorable molecular impacts on tumor tissues. These findings also provide a strong rationale for future clinical trials in larger samples to assess the impact of this protocol on clinical endpoints such as disease recurrence and survival.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Rita Haldar: study conceptualization; data curation; formal analysis; funding acquisition; project administration; writing–original draft; writing–review and editing. **Itay Ricon-Becker:** study conceptualization; data curation; formal analysis; funding acquisition; project administration; writing–original draft; writing–review and editing. **Arielle Radin:** data curation; writing–review and editing. **Mordechai Gutman:** methodology; study supervision; writing–review and editing. **Steve W. Cole:** formal analysis; funding acquisition; resources; software; study supervision; writing–original draft; writing–review and editing. **Oded Zmora:** study conceptualization; funding acquisition; methodology; resources; study supervision; writing–original draft; writing–review and editing. **Shamgar Ben-Eliyahu:** study conceptualization; formal analysis; funding acquisition; methodology; resources; study supervision; writing–original draft; writing–review and editing.

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