**Detailed Methods**

**Study design and procedure:** Patients were recruited in three Medical Centers (Sheba, Rabin, and Kaplan) located in the greater Tel Aviv metropolitan area, Israel. **Random assignment of patients to placebo or treatment was conducted by the pharmacy that prepared the medication kits. Each two consequent kit numbers (e.g., 1, 2 or 105, 106) had a placebo kit and a drug kit in a random order determined by a coin-flip.**

**Verification of drug consumption:** Patients reported to Clinical Trial Associates the number of doses taken, and returned the empty vials after use. While in the hospital, medication was provided by the nurse who also verified their consumption.

To see the full trial protocol please address the corresponding author

**Power analysis and patients numbers:** Based on our previous studies with similar indices ([1](#_ENREF_1),[2](#_ENREF_2)) power calculations ( error = 0.2, 2-sided, = 0.05, expected diagnosticity z score of 0.2, and SD of 0.2), indicated n=8/group for primary hypotheses, and n=12/group for secondary hypotheses ( error = 0.2, 2-sided, = 0.05, estimated effect size of 30% difference between groups, SD = 0.37). **Based on these estimates, sample size was planned for n=16 per group, assuming attrition and other potential obstacles. Recruitment terminated once n=18 per group was achieved.**

**Preparation of blood samples:** Blood samples were maintained at room temperature after collection while being transferred to our laboratory for processing. Assays were performed exactly 2 hr after blood collection. One tube (10ml without preservative/anticoagulant) was used to harvest serum (20 min centrifugation at 930g), and the second (10ml with 300 Unit preservative-free heparin) for immediate assessment of (i) induced cytokine production, and (ii) FACS analyses.

**Flow cytometry procedure and analyses:** A standard whole blood flow cytometry procedure was used to assess the number of leukocyte sub-populations and the expression levels of different activation markers.([3](#_ENREF_3)) Lymphocytes, granulocytes, and monocytes were identified based on forward and side scatters. NK cells were identified as CD3- (APC-eFluor-conjugated anti-human-CD3, eBioscience) lymphocytes which express CD16+ (PerCP-eFluor-conjugated anti-human-CD16, eBioscience) and/or CD56+ (APC-conjugated anti-human-CD56, eBioscience). Expression level of CD11a (FITC-conjugated anti-human-CD11a, eBioscience) on NK cells was assessed. Monocytes were identified based on forward and side scatters and as CD14++ (PE-conjugated anti-human-CD14, eBioscience), and CD16 (PerCP-eFluor-conjugated anti-human-CD16, eBioscience) expression levels were categorized as bright, dim, or negative. Numbers of leukocytes in each subpopulation was evaluated based on a known number of microbeads added to each sample ([3](#_ENREF_3)).

**Induced cytokine production:** 500 ml whole blood was diluted 1:1 in complete RPMI-1640 media (supplemented with 10% fetal calf serum, 50 mg/ml of gentamicin, 2 mM of L-glutamine, 0.1 mM of nonessential amino-acids, and 1 mM of sodium pyruvate), containing 5g of lipopolysaccharide (LPS) and 5g of Polyhydroxyalkanoates (PHA), and was incubated for 21 hrs in 100% humidity 37 °C before supernatant was harvested.

**ELISA assessment of soluble factors in serum and supernatant:** The following kits were used based on manufacturer instruction. Cortisol, high sensitive IL-6, and CRP (R&D systems; Minneapolis, MN, USA); high sensitive IL-10 (eBioscience San Diego, CA, USA); and IL-12 and IFN (Peprotech, Rocky Hill, NJ, USA). All samples of each patient were assayed in duplicates within the same plate, and the intra assay coefficient of variance (CV%) was 1-4%.

**Gene Expression Profiling and Bioinformatic Analysis:** Total RNA was extracted from five 5m FFPE sections, tested for suitable mass (Nanodrop ND1000; Thermo Scientific, Rockford, IL) and subjected to genome-wide transcriptional profiling using Illumina Human HT-12 v4 Expression BeadChips (Illumina Inc., San Diego, CA) in the University of California Los Angeles Neuroscience Genomics Core Laboratory, as previously described ([4](#_ENREF_4),[5](#_ENREF_5)). Expression data were quantile normalized and log2-transformed for standard linear model analyses assessing the average difference in expression of each gene transcript between the two groups (drug treatment vs. placebo) while controlling for tumor stage. These point estimates of differential expression for each gene served as input into second stage gene set-based bioinformatics analyses testing specific a priori hypotheses regarding mesenchymal vs. epithelial polarization of the tumor transcriptome, prevalence of transcriptome signatures of major leukocyte subsets (CD4+ and CD8+ T cells, B cells, NK cells, monocytes, and plasmacytoid dendritic cells), and activity of specific transcription factors previously implicated in breast cancer progression and metastasis (i.e., pro-inflammatory factors NF-B/cRel and AP-1, STAT family mediators of cytokine signaling, GATA factors, the oxidative stress response factor NRF-2, neuroendocrine-response factors CREB and GR, and myeloid lineage activation factors EGR1-EGR4/NGFIC). A priori hypotheses regarding EMT polarization and tumor-associated leukocyte transcriptomes were tested using Transcript Origin Analyses ([6](#_ENREF_6)) to relate the genes found to be differentially expressed by ≥ 1.25-fold difference in average expression between groups (drug treatment vs. placebo) in this study to previously published reference transcriptome profiles derived from mesenchymal- vs. epithelial-polarized breast cancer cells (GSE13915) ([7](#_ENREF_7)) or isolated leukocyte subsets (CD4+ and CD8+ T cells, B cells, NK cells, monocytes, and plasmacytoid dendritic cells; GSE1133) ([8](#_ENREF_8)). A priori hypotheses regarding activity of breast-cancer relevant transcription control pathways were tested using TELiS bioinformatic analysis of transcription factor binding motifs (TFBMs) in the promoters of genes found to be differentially expressed in these analyses ([9](#_ENREF_9)), using TRANSFAC position-specific weight matrices for inflammation-related pathways (NF-B/cRel, AP-1), GATA family factors GATA1-GATA3, cytokine response factors STAT1 and STAT3, the oxidative stress response factor NRF-2, neuroendocrine response factors CREB and glucocorticoid receptor (GR), and EGR family transcription factors EGR1-EGR4/NGFIC ([10](#_ENREF_10)), as previously described ([4](#_ENREF_4),[5](#_ENREF_5)). Statistical testing of bioinformatics results was based on standard errors derived from bootstrap resampling of linear model residual vectors over all genes assayed (which accounts for any potential correlation across genes) ([11](#_ENREF_11)).

**The use of propranolol and etodolac**

Propranolol and etodolac were chosen based on our animal studies that compared various beta-blockers and COX-2 inhibitors ([12](#_ENREF_12)). To ease the translation of our studies to patients, we have chosen drugs that are commonly used in humans for other indications, and that are known for their safety profile. Given the efficacy and promising results that these drugs had in the preclinical studies, we continued to use them in the current study**.**

**The rationale for the duration of the treatment and its initiation 5 days prior to surgery**

The timing and dosing schedule was selected to achieve optimal -adrenergic and COX-2 inhibition at the time of surgery and for several days afterward, while maintaining a clinically feasible treatment duration and providing for standard ramp-up in dosing of propranolol. Biological stress responses start prior to surgery, so the treatment was initiated 5 days before surgery. The “window” between diagnosis and surgery is often shorter than 2 weeks, so a longer initiation period would not be routinely feasible. Initiating treatment prior to surgery also enabled the patient to adjust to propranolol through exposure to a low initial dose before initiating the higher dose on the day of surgery. After surgery, we chose to terminate the treatment as soon as possible after the physical trauma of the surgery subsides, to minimize potential adverse effects of the drugs that may develop with prolonged exposure. Future studies will be required to determine if using a more prolonged post-operative treatment period offers any substantial benefit in terms of long-term outcomes (e.g., disease-free or overall survival).

**Safety concerns**

Some concerns have been raised regarding the perioperative use of β-blockers and COX inhibitors (as detailed below), but not regarding non-selective β-blockers or semi-selective COX-2 inhibitors, such as propranolol and etodolac used herein. Importantly, several lines of evidence suggest the safety and potential beneficial long-term outcomes of these drugs when used in the context of cancer and the perioperative period.

Previously, the perioperative use of β-blockers was recommended for patients at risk of post-operative cardiac events by the European Society of Cardiologists (ESC), American College of Cardiology (ACC), and the American Heart Association (AHA) ([13](#_ENREF_13)). Heavily influencing a reassessment of this recommendation was the 8,351-patient POISE trial published in 2008 ([14](#_ENREF_14)). This study demonstrated both beneficial and adverse effects of 100 mg of extended release metoprolol, a selective 1 antagonist, when initiated on the day of surgery and continued for 30 days postoperatively. Although metoprolol was associated with a reduction in postoperative myocardial infarction (176 [4.2%] vs 239 [5.7%] patients; HR = 0.73, 0.60-0.89; p=0.0017), it was also associated with a higher postoperative mortality rate (129 [3.1%] vs 97 [2.3%] patients; HR = 1.33, 1.03-1.74; p=0.0317) due to hypotension and stroke (41 [1.0%] vs 19 [0.5%] patients; HR = 2.17, 1.26-3.74; p=0.005). Important characteristics of this study were moderate to high dosing, and lack of dose escalation with commencement of pharmacotherapy on the day of surgery – features noted by the investigators to contribute to the morbidity from perioperative 1-blockade. Furthermore, a large cohort study (n=44,092) that followed POISE concluded on 2013 that the observation of increased stroke is specific to metoprolol, in contrast to other 1 blockers ([15](#_ENREF_15)).

Because the POISE trial recruited patients at elevated risk of postoperative cardiac events, the ACC and the AHA recommend evaluating perioperative β-blocker administration on an individual (per-patient) basis ([13](#_ENREF_13)). Furthermore, on 2014 the ACC/AHA and ESC recommend initiating pre-operative therapy sufficiently before surgery to assess safety and tolerability, and stress the risk of initiating β-blocker therapy on the day of surgery ([16](#_ENREF_16),[17](#_ENREF_17)). ESC recommend that β-blockers will be administered no less than 2 days before surgery and continued post-surgery ([16](#_ENREF_16)), as indeed implemented in our protocol. In the current study, we also excluded patients with elevated risk of postoperative cardiac events.

In regard of COX-2 inhibitors, concerns have been raised about an association between perioperative COX-2 inhibition and risks for cardiovascular events and bone healing. However, recent (2016) protocols of ERAS (Enhanced Recovery After Surgery) Society conclude that current evidence does not justify the avoidance of NSAIDs during the short perioperative period in patients with low cardiovascular risk ([18](#_ENREF_18)). A number of studies examining perioperative use of COX-2 inhibitors have not found an increase in renal, cardiac, or thrombotic complications ([19-22](#_ENREF_19)). Multiple population studies have shown that COX-2 inhibitors can be given to patients for prolonged durations in the non-surgical setting without increase in thrombotic complications ([23](#_ENREF_23)). However, in those patients at elevated risk of heart failure, myocardial infarction, or stroke (which we excluded from the current study), NSAIDs should be administered at a low dose and for a short duration ([24](#_ENREF_24)).

Disturbances to tissue healing is clearly a concern in the perioperative context, but recent animal studies have reported no adverse effects of either propranolol, etodolac, or both on the healing rate of colon anastomosis, skin, and muscle (most relevant to cancer surgeries), and actually have reported some beneficial effects ([25](#_ENREF_25),[26](#_ENREF_26)).

Overall, the low risk of using each of these drugs alone in patients without contraindication seems manageable. Although their adverse effect profiles do not overlap, the risks for their combined used has been assessed thus far only in a limited number of patients, and concur with a short- and long-term safety of their combined use ([27](#_ENREF_27),[28](#_ENREF_28)). Additionally, in the current study and in a parallel study in operated colorectal cancer patients ([29](#_ENREF_29)), we observed no perioperative treatment-related serious or moderate adverse events. Perhaps most important, the chronic use of COX inhibitors and of -adrenergic blockers have been associated with improved cancer outcomes in several types of cancer and medical circumstances, in very large numbers of patients ([30-32](#_ENREF_30)). Thus, hypothetical long-term risks of the combined use of propranolol and etodolac appear unlikely, and should be weighed against the positive outcomes reported by translational, epidemiological, and clinical studies, as well as those observed in the current biomarker trial.

**References**

1. Greenfeld K, Avraham R, Benish M, Goldfarb Y, Rosenne E, Shapira Y*, et al.* Immune suppression while awaiting surgery and following it: dissociations between plasma cytokine levels, their induced production, and NK cell cytotoxicity. Brain Behav Immun **2007**;21(4):503-13.

2. Cole SW, Hawkley LC, Arevalo JMG, Cacioppo JT. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. Proc Natl Acad Sci U S A **2011**;108(7):3080-5.

3. Matzner P, Hazut O, Naim R, Shaashua L, Sorski L, Levi B*, et al.* Resilience of the immune system in healthy young students to 30-hour sleep deprivation with psychological stress. Neuroimmunomodulation **2013**;20(4):194-204 doi 10.1159/000348698

000348698 [pii].

4. Cole SW, Arevalo JM, Takahashi R, Sloan EK, Lutgendorf SK, Sood AK*, et al.* Computational identification of gene-social environment interaction at the human IL6 locus. Proc Natl Acad Sci U S A **2010**;107(12):5681-6 doi 10.1073/pnas.0911515107

0911515107 [pii].

5. Cole SW, Hawkley LC, Arevalo JM, Sung CY, Rose RM, Cacioppo JT. Social regulation of gene expression in human leukocytes. Genome Biol **2007**;8(9):R189 doi gb-2007-8-9-r189 [pii]

10.1186/gb-2007-8-9-r189.

6. Cole SW, Hawkley LC, Arevalo JM, Cacioppo JT. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. Proc Natl Acad Sci U S A **2011**;108(7):3080-5 doi 10.1073/pnas.1014218108

1014218108 [pii].

7. Choi YL, Bocanegra M, Kwon MJ, Shin YK, Nam SJ, Yang JH*, et al.* LYN is a mediator of epithelial-mesenchymal transition and a target of dasatinib in breast cancer. Cancer Res **2010**;70(6):2296-306 doi 10.1158/0008-5472.CAN-09-3141

0008-5472.CAN-09-3141 [pii].

8. Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D*, et al.* A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci U S A **2004**;101(16):6062-7 doi 10.1073/pnas.0400782101

0400782101 [pii].

9. Cole SW, Yan W, Galic Z, Arevalo J, Zack JA. Expression-based monitoring of transcription factor activity: the TELiS database. Bioinformatics **2005**;21(6):803-10 doi 10.1093/bioinformatics/bti038

bti038 [pii].

10. Wingender E, Dietze P, Karas H, Knuppel R. TRANSFAC: a database on transcription factors and their DNA binding sites. Nucleic Acids Res **1996**;24(1):238-41 doi 5s1013 [pii].

11. Efron B, Tibshirani RJ. An introduction to the bootstrap. CRC press; 1994.

12. Benish M, Bartal I, Goldfarb Y, Levi B, Avraham R, Raz A*, et al.* Perioperative use of beta-blockers and COX-2 inhibitors may improve immune competence and reduce the risk of tumor metastasis. Ann Surg Oncol **2008**;15(7):2042-52.

13. Priebe HJ. The controversy of peri-operative ss-blockade: what should I do? Eur J Vasc Endovasc Surg **2014**;47(2):119-23 doi 10.1016/j.ejvs.2013.11.005

S1078-5884(13)00700-4 [pii].

14. Devereaux PJ, Yang H, Yusuf S, Guyatt G, Leslie K, Villar JC*, et al.* Effects of extended-release metoprolol succinate in patients undergoing non-cardiac surgery (POISE trial): a randomised controlled trial. Lancet **2008**;371(9627):1839-47 doi 10.1016/S0140-6736(08)60601-7

S0140-6736(08)60601-7 [pii].

15. Ashes C, Judelman S, Wijeysundera DN, Tait G, Mazer CD, Hare GM*, et al.* Selective beta1-antagonism with bisoprolol is associated with fewer postoperative strokes than atenolol or metoprolol: a single-center cohort study of 44,092 consecutive patients. Anesthesiology **2013**;119(4):777-87 doi 10.1097/ALN.0b013e3182a17f12.

16. Kristensen SD, Knuuti J. New ESC/ESA Guidelines on non-cardiac surgery: cardiovascular assessment and management. Eur Heart J **2014**;35(35):2344-5 doi 10.1093/eurheartj/ehu285

ehu285 [pii].

17. Fleisher LA, Fleischmann KE, Auerbach AD, Barnason SA, Beckman JA, Bozkurt B*, et al.* 2014 ACC/AHA guideline on perioperative cardiovascular evaluation and management of patients undergoing noncardiac surgery: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. J Am Coll Cardiol **2014**;64(22):e77-137 doi 10.1016/j.jacc.2014.07.944

S0735-1097(14)05536-3 [pii].

18. Feldheiser A, Aziz O, Baldini G, Cox BP, Fearon KC, Feldman LS*, et al.* Enhanced Recovery After Surgery (ERAS) for gastrointestinal surgery, part 2: consensus statement for anaesthesia practice. Acta Anaesthesiol Scand **2016**;60(3):289-334 doi 10.1111/aas.12651.

19. Wattchow DA, De Fontgalland D, Bampton PA, Leach PL, McLaughlin K, Costa M. Clinical trial: the impact of cyclooxygenase inhibitors on gastrointestinal recovery after major surgery - a randomized double blind controlled trial of celecoxib or diclofenac vs. placebo. Aliment Pharmacol Ther **2009**;30(10):987-98 doi 10.1111/j.1365-2036.2009.04126.x

APT4126 [pii].

20. Cheung R, Krishnaswami S, Kowalski K. Analgesic efficacy of celecoxib in postoperative oral surgery pain: a single-dose, two-center, randomized, double-blind, active- and placebo-controlled study. Clin Ther **2007**;29 Suppl:2498-510 doi 10.1016/j.clinthera.2007.12.008

S0149-2918(07)00376-1 [pii].

21. Huang MT, Chen ZX, Wei B, Zhang B, Wang CH, Huang MH*, et al.* Preoperative growth inhibition of human gastric adenocarcinoma treated with a combination of celecoxib and octreotide. Acta Pharmacol Sin **2007**;28(11):1842-50 doi 10.1111/j.1745-7254.2007.00652.x.

22. Lee YS, Kim H, Brahim JS, Rowan J, Lee G, Dionne RA. Acetaminophen selectively suppresses peripheral prostaglandin E2 release and increases COX-2 gene expression in a clinical model of acute inflammation. Pain **2007**;129(3):279-86 doi S0304-3959(06)00559-8 [pii]

10.1016/j.pain.2006.10.020.

23. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ **2006**;332(7553):1302-8 doi 332/7553/1302 [pii]

10.1136/bmj.332.7553.1302.

24. Food U, Administration D. FDA Drug Safety Communication: FDA strengthens warning that non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs) can cause heart attacks or strokes. Accessed August **2015**;25.

25. Benjamin B, Hazut O, Shaashua L, Benish M, Zmora N, Barshack I*, et al.* Effect of beta blocker combined with COX-2 inhibitor on colonic anastomosis in rats. Int J Colorectal Dis **2010**;25(12):1459-64 doi 10.1007/s00384-010-0992-8.

26. Hazut O, Shaashua L, Benish M, Levi B, Sorski L, Benjamin B*, et al.* The effect of beta-adrenergic blockade and COX-2 inhibition on healing of colon, muscle, and skin in rats undergoing colonic anastomosis. Int J Clin Pharmacol Ther **2011**;49(9):545-54 doi 8913 [pii].

27. Effect of coadministered beta blocker and COX-2 inhibitor to patients with pancreatic cancer prior to receiving albumin-bound (Nab) paclitaxel. Bhattacharyya GS, Babu KG, Bondarde SA, Biswas G, Ranade A, Parikh PM*, et al.*2015.

28. Effect of coadministration of propranolol and etodolac (VT-122) plus sorafenib for patients with advanced hepatocellular carcinoma (HCC). de la Torre AN, Castaneda I, Hezel AF, Bascomb NF, Bhattacharyya GS, Abou-Alfa GK2015.

29. Zmora O, Shaashua L, Gutman M, Ben-Eliyahu S. The perioperative use of a beta-adrenergic blocker and a COX-2 inhibitor in colorectal cancer patients for the prevention of cancer recurrence: A preliminary study assessing feasibility and safety. 2016; Brighton, UK. BBI.

30. Horowitz M, Neeman E, Sharon E, Ben-Eliyahu S. Exploiting the critical perioperative period to improve long-term cancer outcomes. Nat Rev Clin Oncol **2015**;12(4):213-26 doi 10.1038/nrclinonc.2014.224

nrclinonc.2014.224 [pii].

31. Cole SW, Sood AK. Molecular pathways: beta-adrenergic signaling in cancer. Clin Cancer Res **2012**;18(5):1201-6 doi 10.1158/1078-0432.CCR-11-0641

1078-0432.CCR-11-0641 [pii].

32. Ricon I, Hiller J, Ben-Eliyahu S. The combined blockade of b-adrenoceptor and COX-2 during the perioperative period to improve long-term cancer outcomes. International Anesthesiology Clinics‏, **2016**.