BJA

British Journal of Anaesthesia, 126 (2): 367–376 (2021)

doi: 10.1016/j.bja.2020.10.021
Advance Access Publication Date: 19 November 2020
Clinical Practice

CLINICAL PRACTICE

Intraoperative opioids are associated with improved recurrence-free survival in triple-negative breast cancer

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Abstract

Background: Opioid-induced immunomodulation may be of particular importance in triple-negative breast cancer (TNBC) where an immune response is associated with improved outcome and response to immunotherapy. We evaluated the association between intraoperative opioids and oncological outcomes and explored patterns of opioid receptor expression in TNBC.

Methods: Consecutive patients with stage I–III primary TNBC were identified from a prospectively maintained database. Opioid receptor expression patterns in the tumour microenvironment were analysed using publicly available bulk and single-cell RNA-seq data.

Results: A total of 1143 TNBC cases were retrospectively analysed. In multivariable analysis, higher intraoperative opioid dose was associated with favourable recurrence-free survival, hazard ratio 0.93 (95% confidence interval 0.88–0.99) per 10 oral morphine milligram equivalents increase (P=0.028), but was not significantly associated with overall survival, hazard ratio 0.96 (95% confidence interval 0.89–1.02) per 10 morphine milligram equivalents increase (P=0.2). Bulk RNA-seq analysis of opioid receptors showed that *OPRM1* was nearly non-expressed. Compared with normal breast tissue *OGFR*, *OPRK1*, and *OPRD1* were upregulated, while *TLR4* was downregulated. At a single-cell level, *OPRM1* and *OPRD1* were not detectable; *OPRK1* was expressed mainly on tumour cells, whereas *OGFR* and *TLR4* were more highly expressed on immune cells.

Conclusions: We found a protective effect of intraoperative opioids on recurrence-free survival in TNBC. Opioid receptor expression was consistent with a net protective effect of opioid agonism, with protumour receptors either not expressed or downregulated, and antitumour receptors upregulated. In this era of personalised medicine, efforts to differentiate the effects of opioids across breast cancer subtypes (and ultimately individual patients) should continue.

Keywords: immunomodulation; opioid receptor; opioids; outcome; personalised medicine; recurrence; RNA-seq; triple-negative breast cancer; survival

Received: 15 September 2020; **Accepted:** 15 October 2020

Editor's key points

- Innate immune responses modify many types of cancer; this is likely to be genetically determined.
- Similarly, individual responsiveness to opioids is likely to differ across breast cancer subtypes.
- This study found a protective association between an increased intraoperative opioid dose and improved recurrence-free survival, and this seems to be in part modified by variable opioid receptor gene expression.

While opioids are generally considered to have a negative effect on cancer, definitive clinical evidence to support this association is lacking,[1,2] and counterexamples may be found in recent retrospective studies.[3] At a preclinical level, morphine induces angiogenesis,[4,5] and directly promotes proliferation in renal cancer[6] and hepatocellular carcinoma cell lines.[7] However, this is not the case for all solid tumours.[8] The activation of the endogenous opioid pathway has been shown to suppress proliferation in different types of cancer, including pancreatic, ovarian, and triple-negative breast cancer (TNBC),[9–11] suggesting that opioids may be protective in certain cancers.

Surgery is a key component of breast cancer treatment.[12,13] However, it is associated with a profound neuroendocrine, metabolic, and cytokine response, the magnitude and oncological impact of which may be influenced by the anaesthesia-analgesia technique utilised.[14] In 2006, Exadaktylos and colleagues[15] published the first retrospective study comparing breast cancer outcomes after general anaesthesia with paravertebral block *vs* opioid analgesia and found the former to be associated with improved recurrence-free survival (RFS). However, these results were not confirmed in a recent multicenter RCT.[16]

Breast cancer is a heterogeneous disease. The degree of immune response, which modulates cancer growth, varies between molecular subtypes, being highest in TNBC and human epidermal growth factor receptor 2 (HER2)-positive tumours.[17,18] The magnitude and the type of immune response may be particularly relevant to the opioid-cancer interaction, as opioid-induced immunosuppression may contribute to tumour escape.[19–21]

In this study, we sought to evaluate the association between intraoperative opioids and oncologic outcomes in TNBC. Furthermore, we explored expression of relevant opioid receptors in the tumour microenvironment (TME) in TNBC using publicly available bulk and single-cell RNA-seq data, hypothesising that patterns of receptor expression would reflect observed clinical associations.

Methods

Study population

After institutional review board approval, consecutive patients with primary stage I–III TNBC treated between March 2010 and December 2016 at the Memorial Sloan Kettering Cancer Center were identified from a prospectively maintained database. Tumours with absence of oestrogen receptor (ER) and progesterone receptor staining (<1%) or HER2 staining classified as 0 or 1+ were included. In case of equivocal HER2 status (2+), fluorescence *in situ* hybridisation was performed and considered negative if the HER2-to-probe ratio was <2.0. We excluded patients with synchronous ER-positive contralateral breast cancer or other synchronous cancers, male patients, patients with missing information on adjuvant therapy, anaesthesia or follow up, those with rare histologic subtypes (metaplastic carcinoma, angiosarcoma, malignant phyllodes tumours, salivary gland-like tumours, and neuroendocrine carcinoma), patients with an occult breast primary, and those with no axillary staging (Fig. 1). Locoregional and systemic treatment data, and disease status at last follow-up (no evidence of disease, alive with disease, died of disease, and died of unknown cause) were extracted from medical records. Total intraoperative opioid dose for each opioid type (fentanyl, hydromorphone, and morphine) was extracted from anaesthetic records and converted to oral morphine milligram equivalents (MME), and summed to give the total intraoperative MME dose (for reference, 10 MME is equivalent to i.v. fentanyl 50 μg, a typical bolus dose during general anaesthesia).

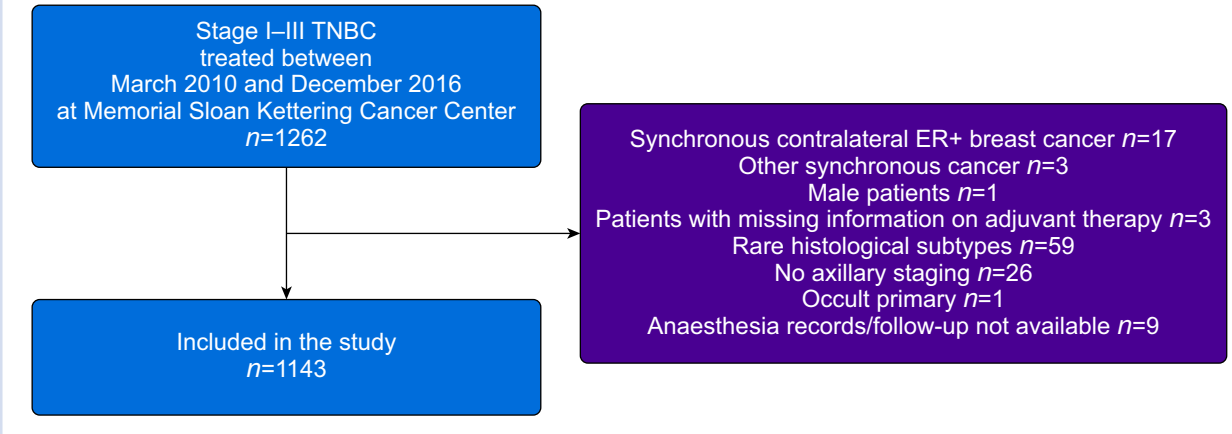

Fig 1. Study flowchart. TNBC, triple-negative breast cancer; ER+, oestrogen receptor-positive.

Table 1 Sociodemographic and clinicopathological characteristics. Data are presented as frequency for categorical variables and median (inter-quartile ranges) for continuous variables.

Characteristics	*n*	Median (IQR) or *n* (%)
Age (yr)	*n*=1143	54 (45–64)
Race		
White		764 (67)
Black or African American		192 (17)
Asian-Far East/Indian		94 (8.2)
Other/unknown		93 (8.1)
BMI (kg m^{-2})		26 (23–31)
Van Walraven comorbidity score		4.0 (4.0–12.0)
ASA physical status		
1/2		592 (52)
3/4		551 (48)
NLR	*n*=1008	2.11 (1.62–2.84)
Intraoperative characteristics		
Intraoperative MME	*n*=1143	30 (20–60)
Regional block		41 (3.6)
Volatile anaesthesia *vs* TIVA		
Volatile anaesthesia		618 (54)
TIVA		525 (46)
Length of surgery (min)		87 (58–158)
Estimated blood loss >500 ml		2 (0.2)
Year of surgery		
2010	*n*=1143	105 (9.2)
2011		156 (14)
2012		152 (13)
2013		191 (17)
2014		145 (13)
2015		198 (17)
2016		196 (17)
Locoregional therapy		
Lumpectomy with WBRT	*n*=1143	505 (44)
Lumpectomy with WBRT and nodal irradiation		116 (10)
Lumpectomy without radiotherapy		40 (3.5)
Mastectomy with PMRT		192 (17)
Mastectomy without PMRT		290 (25)
Pathological features		
Histology	*n*=1143	
Ductal		911 (80)
Apocrine		88 (7.7)
Lobular and mixed		28 (2.4)
Micropapillary		29 (2.5)
Other		87 (7.6)
Tumour grade		
Poorly differentiated		1070 (94)
Moderately differentiated		73 (6.4)
Lymphovascular invasion		344 (30)
Pathological tumour stage		
T1		603 (53)
T2		213 (19)
T3/T4		13 (1.1)
ypT0		117 (10)
ypT1		133 (12)
ypT2		50 (4.4)
ypT3/ypT4		14 (1.2)
Pathological nodal stage		
N0		629 (55)
N1		147 (13)
N2		33 (2.9)
N3		21 (1.8)
ypN0		204 (18)
ypN1		65 (5.7)
ypN2		26 (2.3)
ypN3		18 (1.6)
Systemic therapy		
Adjuvant regimen	*n*=782	
Anthracycline with or without taxane		532 (68)
CMF regimens		178 (23)
Taxane-based with or without platinum		41 (5.2)
Other		31 (4.0)
Neoadjuvant regimen	*n*=313	
ACT		213 (68)
ACT+platinum		66 (21)
Others		34 (11)
Response to neoadjuvant chemotherapy		
No pCR	*n*=313	220 (70)
pCR		93 (30)

BMI, body mass index (kg/m^2); ASA, American Society of Anesthesiologists; MME, oral morphine milligram equivalents; TIVA; total intravenous anaesthesia; CMF; Cyclophosphamide, Methotrexate and 5-Fluorouracil; NAC, neoadjuvant chemotherapy; pCR, pathological complete response; NLR, neutrophil-lymphocyte ratio; ACT Adriamycin, Cyclophosophamide followed by Paclitaxel,WBRT, whole breast radiation therapy, PMRT, post mastectomy radiotherapy.*NLR unknown for 135 patients

Endpoints and statistical analyses

The primary objective of the study was to quantify the association between intraoperative opioid dose and oncological outcomes. The primary endpoint was RFS. Time-to-event was determined from the time of surgical resection to the time of recurrence or death, otherwise censored at the time of last follow-up. The secondary endpoint was overall survival (OS). To estimate the association between intraoperative opioids and oncological outcomes, we used univariable and multivariable Cox proportional hazards regression to calculate hazard ratios (HRs) and 95% confidence intervals (CIs), treating intraoperative MME as a continuous variable. Variables with a *P*-value of <0.1, for either RFS or OS, were included in the multivariable model, while retaining clinically relevant variables. The final multivariable model included surgical procedure (lumpectomy *vs* mastectomy), histology, TNM (tumour–node–metastasis) stage, presence of lymphovascular invasion (LVI), receipt of (neo)adjuvant chemotherapy, response to neoadjuvant therapy, breast and nodal irradiation, neutrophil-lymphocyte ratio,[22] the van Walraven comorbidity score, and anaesthesia technique (presence of regional block and total i.v. *vs* volatile anaesthesia).[23] To assess whether the use of opioids changed over time, we constructed a linear regression model on log-transformed MMEs by year of surgery.

All analyses were two-sided and $P<0.05$ was considered statistically significant. Analyses of the clinical endpoints were conducted using R 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria).

Bulk and single-cell RNA-seq data

To investigate opioid receptor expression in TNBC, we used bulk RNA-seq data from The Cancer Genome Atlas (TCGA) through the National Institutes of Health/National Cancer Institute Genomic Data Commons public database.[24] Tumour sample RNA-seq data were drawn from a cohort of 173 TNBC patients,[25] while another cohort of 113 RNA-seq samples of

normal breast tissue was used for comparison. Data containing raw RNA counts (HTSeq files) without any normalisation were used, and the data were normalised using the standard DESeq2 workflow.[26] This is a significantly more robust approach than the approach used to normalise in the TCGA pipeline (fragments per kilobase million), as DeSeq2 uses a complex statistical framework to better model effect sizes in differential expression experiments.

To assess cellular-level expression of the opioid receptor in specific cell types in the TME, a single-cell RNA-seq data set produced by Karaayvaz and colleagues[27] was used, and 1112 clustered cells from primary TNBC tumours were analysed. Expression data for the specific genes of interest were extracted from the processed data therein (which were therefore already normalised and log2-transformed as described in Step 2 of their methodology, see Supplementary material of Karaayvaz and colleagues[27]).

We extracted expression data for genes of interest from the bulk and single-cell data sets, focusing specifically on receptors known to bind opioids given intraoperatively. These included three canonical opioid receptors (*ORPM1*, *OPRK1*, and *OPRD1*), the endogenous opioid receptor *OGFR*,[28] and Toll-like receptor 4 (*TLR4*).[29] Differential gene expression (whether between tumour and normal tissue for bulk data, or between various cell types for single-cell data) was calculated as the ratio (fold change) of the means of normalised counts for each cluster compared, presented as log2-transformed, with *P*-values corrected for multiple testing (for all genes in the respective data sets) (Benjamini-Hochberg procedure, false-discovery rate). All corrected *P*-values were considered to be significant if $P<0.05$. All statistical analyses were performed using SciPy and statsmodel packages in Python.[30]

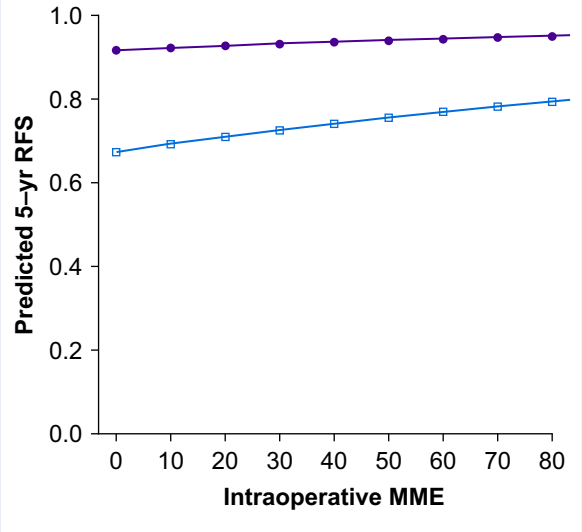

Fig 2. Predicted 5-yr recurrence-free survival by intraoperative opioid dose based on multivariable model for RFS. The purple line is a model patient with T1N0 ductal carcinoma treated with lumpectomy, radiotherapy, and adjuvant chemotherapy, and the blue line is a model patient with T2N1 ductal carcinoma with residual disease after NAC treated with mastectomy and postmastectomy radiotherapy. X-axis is total intraoperative opioid dose converted to oral morphine milligram equivalents. MME, oral morphine milligram equivalents; NAC, neoadjuvant chemotherapy; RFS, recurrence-free survival.

Results

Between March 2010 and December 2016, 1143 patients with stage I–III primary TNBCs treated by the Breast Service, Department of Surgery, Memorial Sloan Kettering Cancer Center met the inclusion criteria (Fig. 1). Clinicopathological features of the entire cohort are shown in Table 1. Median age was 54 yr (inter-quartile range [IQR] 45–64). The majority of tumours were pT1 (53%) and pN0 (55%), and 80% were of ductal histology. Lumpectomy was performed in 661 (58%) cases. Median length of surgery was 63 min (IQR 49–81) in the lumpectomy group and 165 min (IQR 126–206) in the mastectomy group. The median intraoperative MME was 30 (IQR 20–60) and was similar over the study period (*P*-value for trend over time was 0.76).

Recurrence-free survival and overall survival

The 5-yr RFS was 81% (95% CI 79–84%) and 5-yr OS was 86% (95% CI 84–88%). On multivariable analysis, higher intraoperative opioid dose was associated with favourable RFS, HR 0.93 (95% CI 0.88–0.99) per 10 MME increase ($P=0.028$). Fig. 2 shows the predicted 5-yr RFS by given MME for two illustrative patients, one with low-risk patient factors, the other with high-risk features. Other factors associated with worse RFS were lobular or mixed histology (ductal [reference]: HR 1.96 (95% CI 1.03–3.75), $P=0.042$), LVI (no LVI [reference]: HR 1.61 (95% CI 1.17–2.21), $P=0.003$), failure to achieve pathological complete response (no neoadjuvant chemotherapy [reference]: HR 2.34 (95% CI 1.53–3.60), $P<0.001$),T3/T4 stage (T1 [reference]: HR 3.88 (95% CI 1.12–13.4), $P=0.032$), N3 stage (N0 [reference]: HR 3.76, (95% CI 2.02–7.00), $P<0.001$), and lack of breast radiotherapy (RT) after lumpectomy (lumpectomy with RT [reference]: HR 4.47 (95% CI 2.51–7.96), $P<0.001$).

In the multivariable model, intraoperative opioid dose did not significantly affect OS, HR 0.96 (95% CI 0.89–1.02) per 10 MME increase ($P=0.2$). Features associated with OS were N3 disease (N0 [reference]: HR 3.95 (95% CI 1.97–7.89), $P<0.001$), failure to achieve pathological complete response (no neoadjuvant chemotherapy [reference]: 2.42 (95% CI 1.46–4.00), $P<0.001$), lack of breast RT after lumpectomy (lumpectomy with RT [reference]: HR 2.85 (95% CI 1.30–6.26), $P=0.009$), and higher comorbidity score (HR 1.06 (95% CI 1.02–1.11) per 1 point increase, $P=0.002$). The full multivariable models for RFS and OS are shown in Table 2.

Opioid receptor expression

Analysis of bulk RNA-seq expression data from the TCGA-derived TNBC cohort demonstrated expression of both the canonical and non-canonical opioid receptors of interest, though the canonical mu opioid receptor (*OPRM1*) had almost non-existent expression (DESeq2 normalised mean of 0.23, which is three orders of magnitude or more lower than the other opioid receptors; Supplementary Table S1 and Fig. S1). Comparison with normal breast tissue enabled calculation of differential expression of the remaining receptors, revealing that *OGFR*, *OPRK1*, and *OPRD1* were upregulated while *TLR4* was downregulated in the tumour tissue, as compared with normal tissue (Fig. 3 and Supplementary Table S1).

Table 2 Multivariable associations between recurrence-free and overall survival and intraoperative opioids and other relevant clinicopathological factors.

Characteristic	RFS			OS		
	HR	95% CI	P-value	HR	95% CI	P-value
Intraoperative MME (per 10 MME)	0.93	0.88–0.99	0.028	0.96	0.89–1.02	0.2
Regional block	0.83	0.25–2.77	0.8	0.47	0.06–3.57	0.5
TIVA *vs* volatile anaesthesia						
General anaesthesia	—	—		—	—	
TIVA	0.70	0.45–1.08	0.11	0.87	0.52–1.47	0.6
Surgical procedure						
Lumpectomy with breast radiotherapy	—	—		—	—	
Lumpectomy without breast radiotherapy	4.47	2.51–7.96	<0.001	2.85	1.30–6.26	0.009
Mastectomy	1.09	0.66–1.82	0.7	1.14	0.63–2.07	0.7
Postmastectomy radiotherapy						
No	—	—		—	—	
Yes	1.06	0.57–1.99	0.9	0.97	0.47–2.00	>0.9
Nodal radiotherapy						
No	—	—		—	—	
Yes	0.75	0.45–1.26	0.3	0.75	0.41–1.37	0.3
Histology						
Ductal	—	—		—	—	
Apocrine	0.85	0.52–1.41	0.5	0.69	0.36–1.34	0.3
Lobular and mixed	1.96	1.03–3.75	0.042	2.04	0.97–4.29	0.060
Micropapillary	1.09	0.56–2.14	0.8	1.53	0.77–3.05	0.2
Other	0.64	0.35–1.16	0.14	0.61	0.29–1.26	0.2
Lymphovascular invasion						
No	—	—		—	—	
Yes	1.61	1.17–2.21	0.003	1.38	0.95–2.02	0.094
Pathological tumour stage						
ypT0	—	—		—	—	
T1/ypT1	1.38	0.49–3.93	0.5	1.16	0.35–3.89	0.8
T2/ypT2	2.44	0.84–7.07	0.10	2.04	0.59–7.02	0.3
T3/T4 or ypT3/ypT4	3.88	1.12–13.4	0.032	3.87	0.95–15.7	0.058
Pathological nodal stage						
N0	—	—		—	—	
N1/ypN1	1.41	0.93–2.14	0.11	1.32	0.81–2.16	0.3
N2/ypN2	1.82	0.99–3.32	0.053	1.91	0.96–3.79	0.065
N3/ypN3	3.76	2.02–7.00	<0.001	3.95	1.97–7.89	<0.001
Adjuvant therapy						
No chemotherapy	—	—		—	—	
Chemotherapy	0.86	0.58–1.28	0.5	0.73	0.46–1.16	0.2
Response to NAC						
No NAC	—	—		—	—	
No pCR	2.34	1.53–3.60	<0.001	2.42	1.46–4.00	<0.001
pCR	2.03	0.58–7.06	0.3	2.19	0.53–9.04	0.3
NLR	1.04	0.96–1.13	0.4	1.05	0.96–1.15	0.3
van Walraven score	1.03	0.99–1.07	0.10	1.06	1.02–1.11	0.002

CI, confidence interval; HR, hazard ratio; MME, oral morphine milligram equivalents; NAC, neoadjuvant chemotherapy; NLR neutrophil-lymphocyte ratio; OS, overall survival; pCR, pathological complete response (ypT0/ypN0); RFS, recurrence-free survival; RT, radiotherapy; TIVA, total intravenous anaesthesia.

Consistent with this finding, single-cell RNA-seq showed that *OPRM1* was not expressed at a detectable level in any cell type. The same was true for *OPRD1* but not for *OPRK1*, which was found qualitatively to be expressed mainly on tumour cells, although the expression level was not statistically different among different types of cells (Supplementary Fig. S2 and Supplementary Table S2). *OGFR* (Fig. 4) and *TLR4* (Supplementary Fig. S2 and Supplementary Table S2) expression levels were significantly higher in the TME immune cells compared with tumour (epithelial) cells (*OGFR* expression: B-cell *vs* epithelial (tumour) cells: log2 (fold change)=0.2485, *P*=6.59e-15; macrophage *vs* epithelial (tumour) cells: log2 (fold change)= 0.0406, *P*=0.01; T-cell *vs* epithelial (tumour) cells: log2 (fold change)=0.1216, *P*=8.48e-8; *TRL4* expression: macrophage *vs* epithelial (tumour) cells: log2 (fold change)= 0.6283, *P*<1e-24; all *P*-values false-discovery rate corrected; see Supplementary Fig. S3 for differential expression (fold change) for pairwise comparison of all cell types for these receptors).

Discussion

In this large, retrospectively analysed TNBC cohort, we found a protective association of intraoperative opioids with RFS with a 7% decrease in the hazard of recurrence for each 10 MME increase in opioid administration. Previous studies have examined the impact of regional anaesthesia on breast cancer recurrence with inconsistent results.[15,16,31–35] However, none of these studies directly examined the intraoperative

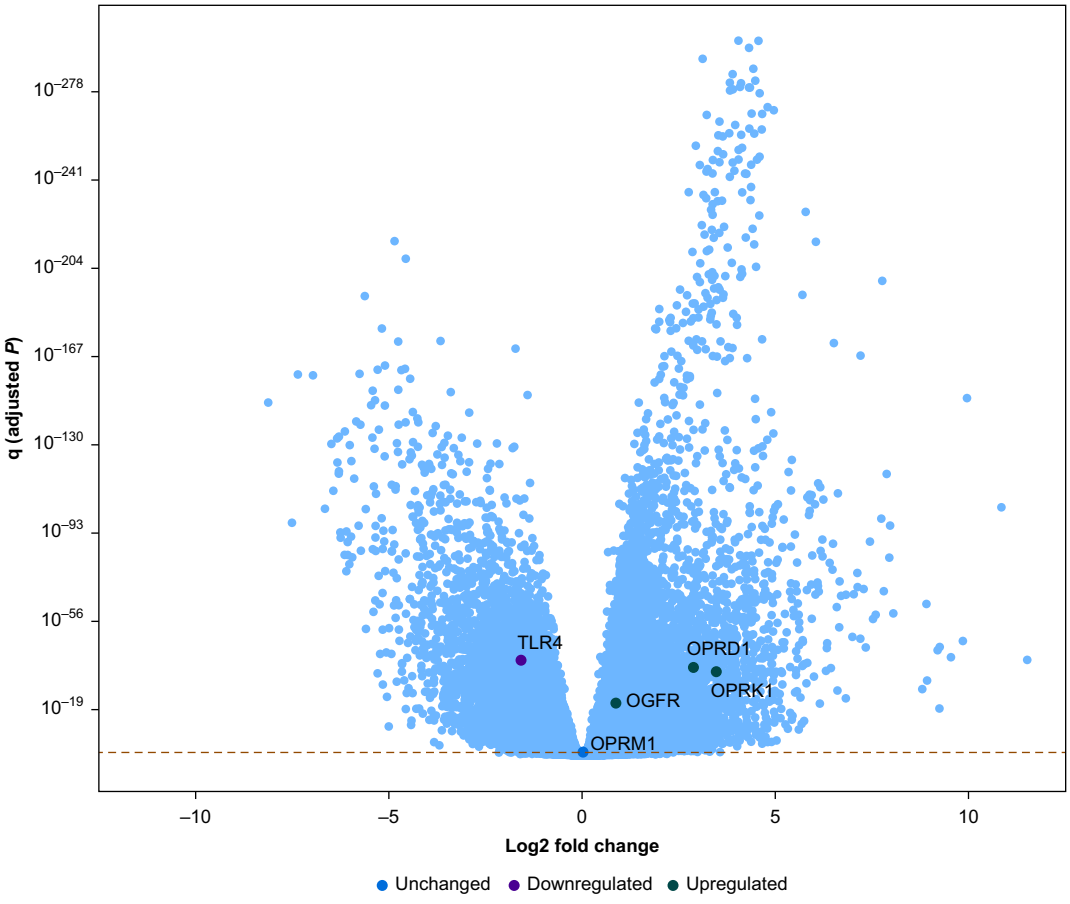

Fig 3. Differential expression of opioid receptor genes in TNBC. Volcano plot showing differential expression of opioid receptor genes in TNBC for tumour *vs* normal breast tissue. log10 q-value (multiple-testing corrected *P*-value) is plotted against log2 (fold change) (tumour *vs* normal) for all differentially expressed genes represented in the TCGA data. Approximately 60 k loci are plotted, including genes and shorter transcribed fragments, such as miRNA. The horizontal dotted line represents q=0.05. Downregulated opioid receptors are labelled in purple, and upregulated receptors are in green. Note that *OPRM1* is expressed at very low, nearly identical levels in both tumour and normal tissue (see Supplementary Table S1). Gene expression data are extracted from TCGA, as described in the Methods section. *OGFR*, opioid growth factor receptor; *OPRD1*, opioid receptor delta 1, *OPRK1*, opioid receptor kappa 1; *OPRM1*, opioid receptor mu 1; TCGA, The Cancer Genome Atlas; *TLR4*, toll-like receptor 4; TNBC, triple negative breast cancer.

opioid dose, but rather used regional blocks as a surrogate. Similar methodology was used in a recent prospective randomised control study by Sessler and colleagues[16] that compared TIVA/regional anaesthesia *vs* a volatile agent/opioid-based anaesthetic. At a median follow-up of 36 months, no difference in breast cancer recurrence (10% in both groups) was observed.[16] Since the design of the trial more than 10 yr ago, our understanding of the heterogeneity of breast cancer has evolved. In TNBC and HER2-positive tumours, lymphocytic infiltration is associated with better prognosis, and is predictive of response to neoadjuvant therapy.[36,37] In the trial of Sessler and colleagues,[16] the majority (78%) of patients had ER-positive disease, the least immunogenic subtype of breast cancer and therefore the least likely to be affected by anaesthetic-analgesic-induced immunomodulation. As such, it is difficult to draw conclusions regarding TNBC specifically, and the inclusion of different breast cancer subtypes in the study may have contributed to the 'negative' result of no association between anaesthetic technique and breast cancer outcome. Further studies in specific breast cancer subtypes examining the effect of opioid dose on breast cancer outcomes are warranted. We note that in our study we found no association between TIVA or the use of regional anaesthesia with either RFS or OS outcomes.

A number of studies over the past decade have found associations between opioid receptor gene expression levels and outcomes in specific cancer types.[38–40] The implication is that at the molecular level, opioid agonism at the receptor has downstream effects that impact the oncological endpoint, and therefore receptor expression level will modulate this relationship. A recent study in lung cancer cells[28] looked beyond single receptor expression levels and considered the possibility that relative expression between multiple opioid receptors may better predict the effect of opioid administration on outcome.[41] In particular, the balance between *OGFR*

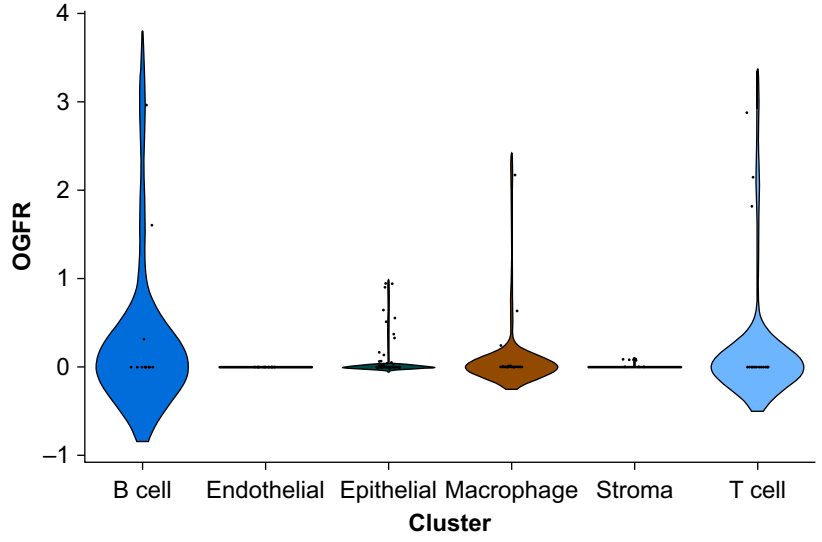

Fig 4. *OGFR* expression in the tumour microenvironment. Violin plots of *OGFR* gene expression distributions by cell type from single-cell RNA-seq data (epithelial cell cluster represents tumour cells). Processed gene expression level data were extracted from Karaayvaz and colleagues[27]; the y-axis represents the expression counts after log2-transformation and normalisation. The normalisation strategy used by Karaayvaz and colleagues[27] allows for negative expression values. *OGFR*, opioid growth factor receptor.

(antitumour) and *OPRM1* (protumour) signalling determined progression of tumour in these cells when exposed to morphine.

In this study we considered not only *OGFR* and *OPRM1*, but also additional receptors both known to bind opioids and to be independently associated with survival outcomes. Previous studies have suggested *OGFR* to be protective in TNBC,[9,41,42] while *TLR4* activation is associated with poor prognosis in TNBC.[43,44] *OPRK1* is tumour-suppressive in a variety of cancer types.[5,7] The role of *ORPD1* is less clear, with antitumour associations shown in TNBC specifically,[45] but also protumour associations in breast cancer generally.[46,47] A variety of evidence links *OPRM1* expression to protumour outcomes in cancer.[28,39,40]

We found in TNBC that differential expression of these receptor genes is consistent with a net protective effect of opioid agonism: protumour receptors are either not expressed (*OPRM1*) or downregulated (*TLR4*), while antitumour receptors (*OGFR*, *OPRK1*, *OPRD1*) are upregulated.

OGFR cell-type specificity (as seen through single-cell RNA-seq) suggests that its protective effect in TNBC is both through direct action in tumour cells (consistent with TNBC cell experiments referenced earlier)[9,42] and possible effects on tumour-infiltrating lymphocytes (TILs). In fact, *OGFR* expression on TILs (T cells, B cells, macrophages) in the TME is significantly higher than in tumour cells. Given the particular importance of TILs in promoting survival in TNBC, this intriguing result suggests that opioids may specifically enhance antitumour TILs action in TNBC, consistent with recent results in other cancer types.[48,49] Taken together, this suggests that the widely espoused view of opioids worsening survival in cancer through their immunosuppressive effects is either too general or too simplistic, and effects are likely to vary by cancer type.

TNBC is an aggressive breast cancer subtype characterised by early relapse and a shorter post-recurrence survival,[50] with limited therapeutic options currently available. Our findings support those of previous studies suggesting that the protective role of *OGFR* observed in this breast cancer subtype may be attributable to both a direct effect on cancer cell inhibition[9,41] and an indirect effect via TILs activity.[48,49] TILs are a known prognostic factor in early TNBC,[36,37] and they are predictive of response to immune checkpoint inhibitors.[51] Given that immunotherapy may become part of the standard treatment of early TNBC in the near future,[52,53] opioid-induced immunomodulation and its potential impact on immunotherapy response[54] is worthy of further investigation.

To our knowledge, this is the first study to directly evaluate the association between intraoperative opioids and RFS and OS outcomes in TNBC. Its strengths include the large sample size and detailed information on intraoperative opioids and clinicopathologic features. Its limitations include its retrospective nature, the lack of detailed data on postoperative opioid consumption (with the caveat that persistent opioid use post-discharge is uncommon at our institution), and the fact that the gene expression data were derived from separate cohorts. As such, we were only able to explore trends and patterns in expression of opioid receptors in TNBC and extrapolate the possible effects in our cohort. We also acknowledge that while the model we are proposing is consistent with the protective effect of opioids seen in our cohort, it is possible that other molecular pathways are relevant to mediating this effect. Finally, we note that we did not differentiate between specific opioid types, but rather converted each to MMEs and considered the combined effect (total intraoperative MME). While this is consistent with human studies in general in order to deal with the heterogeneity of opioids administered in the clinical context, it is possible that different opioids could have different effects on outcomes. To this last point we note that fentanyl accounted for the overwhelming majority of opioids received in our study population (Supplementary Fig. S4).

Conclusions

We found a protective effect of intraoperative opioids on TNBC recurrence. Although opioids are generally considered to have a negative effect on cancer outcomes, our findings show that this may not be the case in TNBC. Analysis of opioid receptor expression using both bulk and single-cell RNA data appears to support the observed protective effect of opioids (suggesting a mechanism that involves both direct effects on tumour cells and promoting TILs activity). As this is a retrospective study (albeit in a large TNBC cohort), associations should be confirmed prospectively. In that context, acquisition of RNA-seq on the actual clinical cohort will enable direct quantitative comparisons at the individual patient level between expression levels of relevant opioid receptors, opioid dose, and outcome, to clarify and better quantify associations suggested by the TCGA and single-cell RNA cohort data. In this era of personalised medicine, efforts to determine whether anaesthetic effects differ across breast cancer subtypes (and ultimately across individual patients) should continue.

Authors' contributions

Study design: GM, KST, PJM, GWF, MM, JSM
Data curation: GM, HVG, PJM, JSM
Data analysis: GM, HVG, MH, JL, KST, PJM, JSM
Writing of original draft: GM, HVG, MH, KST, JSM
Reviewing and editing: GM, GP, KST, JRS, TI, PJM, GWF, MM

Acknowledgements

JSM and HVG acknowledge John Chodera of the Sloan Kettering Institute for his support and insightful discussions. JSM also acknowledges Sahrena London for helpful conversations.

Declarations of interest

MM has received honoraria from Genomic Health. GP is on the scientific advisory board of Merck and Tizona Therapeutics and receives research funding from Takeda Pharmaceuticals. GM was supported by the Ticino Cancer League, the Hanne Liebermann Foundation, the Fondation Ancrage, and the HEMMI-Stiftung.

Funding

Funded in part through NIH/NCI Cancer Center Support Grant P30 CA008748 to Memorial Sloan Kettering Cancer Center.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2020.10.021.

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Handling editor: Paul Myles