Influence of opioids on immune function in patients with cancer pain: from bench to bedside

Running title: Opioids and immune function in patients with cancer pain

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Abstract

In patients with cancer, opioids are principally used for the management of acute surgical and chronic cancer-related pain. However, opioids have many non-analgesic effects, including direct and indirect effects on cancer cells and on anti-tumour immunity (natural killer (NK) cells, macrophages and T-cells). Direct effects on immune cells are manifested via opioid and non-opioid Toll-like receptors, whereas indirect effects are manifested *via* the sympathetic nervous system and hypothalamic-pituitary-adrenal axis. Opioids can also decrease/alter immune cell infiltration into the tumour microenvironment. Animal models have shown that this is not a class effect, in that morphine and fentanyl suppress NK cell cytotoxicity; buprenorphine does not affect NK cell cytotoxicity, whereas tramadol increases NK cell cytotoxicity, reducing metastasis. In healthy individuals, morphine suppresses and fentanyl enhances NK cell cytotoxicity. In patients undergoing surgery, fentanyl decreased and tramadol increased NK cell cytotoxicity; clinical outcomes were not determined. Meta-analyses of opioid-sparing surgical studies report an association between improved recurrence-free and/or overall survival with regional/neuraxial anaesthesia compared with systemic opioids. In patients receiving opioids for non-surgical cancer-related pain, morphine has variable effects on immunity; clinical outcomes were not assessed. Although there is a potential association between systemic opioid administration and shorter survival in cancer patients with a prognosis of months to years, studies have not been designed to primarily assess survival, as a consequence of which causality cannot be apportioned. Pain is immunosuppressive, so analgesia is important. Opioids for cancer-related pain will continue to be recommended until definitive data on the effects of opioids on clinical outcomes in specific patient groups becomes available.

Keywords: opioids, immunity, cancer, pain, natural killer (NK) cells, survival, sur gery

Abbreviations

GAB1: GRB2 Associated Binding Protein 1

HPA: hypothalamic pituitary adrenal

IVME: intravenous morphine-equivalent dose

MOP: mu opioid peptide

NK cells: natural killer cells

NPY: neuropeptide Y

PAG: periaqueductal gray

PCR: polymerase chain reaction

PI3 kinase: Phosphoinositide 3-kinase

SNS: sympathetic nervous system

STAT3: signal transducer and activator of transcription-3

TLR-4: Toll-like receptor-4

Plain Language Summary

Some opioids influence the functioning of the immune system, and this has been shown to affect cancer growth and progression in animal models. Although some opioids affect different aspects of immune function in humans, their effect on the cancer or the patient remains unknown. However, some of the surgical studies to date report an association between improved clinical outcomes with lower doses of systemic opioids. Pain suppresses immunity and immunity is important for protecting against cancer. Despite the uncertainty, adequate control of cancer-related pain is important and opioids will therefore continue to be recommended for its management.

Opioids and the immune system

Opioids are a diverse range of drugs that act on opioids receptors. In patients with cancer, opioids are principally used for the management of cancer-related pain in the long-term, and cancer surgery pain in the shorter term. As with all drugs, opioids can elicit a range of undesired consequences (Boland *et al.*, 2013), including the functioning of the immune system (Grace *et al.*, 2015; Ramaswamy *et al.*, 2017; Wigmore *et al.*, 2016).

The immune system has a crucial role in controlling and potentially eradicating cancer cells in individuals with, or developing, cancer. Many immune cells are involved in anti-tumour immunity including natural killer (NK) cells, T cells, mast cells, dendritic cells and macrophages, as well as soluble immune mediators such as cytokines and chemokines (Table 1) (Boland *et al.*, 2014b; Liang *et al.*, 2016; Nguyen *et al.*, 2014). NK cell activity is a critical endpoint in the immunotoxicological evaluation of pharmaceuticals (Agency, 2000) and a consensus statement for the use of opioids for alleviating chronic pain in elderly people proposes that the effects of opioids on immune function should be considered (Pergolizzi *et al.*, 2008).

Given that patients with cancer are also at risk of infection, it is important that the anti-infection arm of the immune system is maintained at an effective level. However, there is evidence that opioids could increase the risk of infection in patients with cancer (Shao *et al.*, 2017; Suzuki *et al.*, 2013), as a retrospective study has shown that patients treated with morphine developed more infections than those treated with oxycodone (Suzuki *et al.*, 2013). Furthermore, the risk of infection has been shown to increase by 2% for each 10 mg rise in the oral morphine equivalent daily dose; with no difference between opioids (Shao *et al.*, 2017).

Opioids can influence the establishment and progression of cancer in many ways, including indirect effects on cancer growth via angiogenesis and host immunity (Afsharimani *et al.*, 2011; Gach *et al.*, 2011; Gupta *et al.*, 2002; Koodie *et al.*, 2014). Opioids also have the potential to directly influence the growth of cancer cells, as cancer tissue overexpresses *mu* opioid peptide receptors (MOP) (Lennon *et al.*, 2012; Mathew *et al.*, 2011; Nguyen *et al.*, 2014; Singleton *et al.*, 2014). Overexpression of the MOP receptor on cancer cells in murine models has been shown to increase the growth of lung cancer (Lennon *et al.*, 2014), and decreasing MOP receptor activity (using MOP receptor small hairpin RNA mice or the opioid receptor antagonist methylnaltrexone) has been shown to reduce the growth of lung cancer

(Mathew *et al.*, 2011). Furthermore, MOP receptor overexpression has been associated with the development of metastasis in patients with prostate, oesophageal and lung cancer (Singleton *et al.*, 2014; Zhang *et al.*, 2015; Zylla *et al.*, 2013). Morphine influences the proliferation and survival of cancer cells via direct effects on tumour cell DNA cleavage, mitogen-activated protein kinase, Src, Gab-1, PI3 kinase, Akt and STAT3 signalling pathways (Lennon *et al.*, 2014; Lennon *et al.*, 2012; Mathew *et al.*, 2011).

The remainder of this review will concentrate on the cancer-related immune effects of opioids. Drug/molecular target nomenclature conforms to British Journal of Pharmacology's Concise Guide to Pharmacology (Alexander *et al.*, 2015).

Mechanisms of immune effects of opioids

Numerous mechanisms underlying the influence of opioids on immune cells have been described (Figure 1). *In vivo*, opioids can modulate immune function by direct effects on immune cells, and via indirect effects which involve the central nervous system and its release of immune mediators (Al-Hashimi *et al.*, 2013; Borner *et al.*, 2008; Campana *et al.*, 2010).

For opioids to have direct effects on immune cells, immune cells must express opioid receptors, or opioids must be able to have effects via non-opioid receptors present on immune cells (e.g. Toll-like receptor-4) (Xie *et al.*, 2017). Although morphine can directly interact with MOP receptors on immune cells *in vitro* (Borner *et al.*, 2008), the presence of functional opioid receptors on immune cells, as determined using radioligand antibody binding studies, Western blot and polymerase chain reaction (PCR) analysis continues to be disputed (Al-Hashimi *et al.*, 2016; Al-Hashimi *et al.*, 2013; Borner *et al.*, 2009; Campana *et al.*, 2010; Glattard *et al.*, 2010; Kraus, 2009; Langsdorf *et al.*, 2011; Williams *et al.*, 2007). One possibility for the discrepancy is that MOP receptor expression depends upon immune cell activation, in that the expression of MOP receptors in T cells is non-constitutive (Borner *et al.*, 2008). In activated human T cells, MOP receptor mRNA is increased to levels about 1% of those in neurons and can produce functional MOP receptors in T cells (Borner *et al.*, 2007). Many extracellular signals, including the cytokines IL-1, IL-4, IL-6, TNF and IFN-γ, control MOP receptor gene transcription and cell surface opioid receptor expression in immune cells (Kraus, 2009; Langsdorf *et al.*, 2011). Toll-like receptor-4 is involved in innate

immune system activation. *In vitro* and *in silico* techniques have shown that many commonly used opioids can have direct effects on innate immunity via activation of the Toll-like receptor-4 (Franchi *et al.*, 2012; Grace *et al.*, 2015; Hutchinson *et al.*, 2010; Khabbazi *et al.*, 2016).

There are two principal pathways via which the central immunosuppressive effects of morphine can be mediated. These effects have been primarily identified on the basis of animal model-derived data and might therefore differ between species (Al-Hashimi *et al.*, 2013; Fecho *et al.*, 1996). Acute morphine administration acts on the peri-aqueductal gray and sympathetic nervous system (which innervates lymphoid organs) to release immunosuppressive biological amines that can suppress NK cell cytotoxicity (Fecho *et al.*, 1996; Gomez-Flores *et al.*, 2000; Hernandez *et al.*, 1993; Irwin *et al.*, 1988; Weber *et al.*, 1989). An additional arm to the sympathetic nervous system pathway is the action of acute morphine/diamorphine via D1 receptors in the nucleus accumbens shell. This increases the release of neuropeptide Y from the sympathetic nervous system, which in turn acts on peripheral Y1 receptors to inhibit splenic NK cell cytotoxicity (Saurer *et al.*, 2009; Saurer *et al.*, 2006). Prolonged use of opioids increases activity in the hypothalamic-pituitary-adrenal axis, thereby increasing the release of immunosuppressive glucocorticoids, and decreasing T cell function and NK cell cytotoxicity. As pain/stress activates this axis, opioids used as analgesics might be protective (Borman *et al.*, 2009; Hernandez *et al.*, 1993; Mellon *et al.*, 1998; Zhang *et al.*, 2011).

As well as having direct and indirect effects on immune cell activation, MOP receptor activation can inhibit NK cell migration. Fewer NK cells are in the tumours of wild-type compared to MOP receptor knockout mice (Boehncke *et al.*, 2011). Morphine has been shown to reduce leukocyte migration in a murine lung tumour model (Koodie *et al.*, 2014), and NK cell infiltration into breast cancer tissue is decreased in women who have received more perioperative systemic opioids (Desmond *et al.*, 2015). For effective anti-tumour immunity, immune cells need to be in the tumour microenvironment, and a lack of immune cell infiltration could be detrimental to the establishment of protective anti-tumour immunity. However, morphine has been shown to be protective in a murine Lewis lung carcinoma model by reducing immune cell infiltration and angiogenesis (Koodie *et al.*, 2014). Morphine can also elicit anti-tumour effects that are mediated via modulation of paracrine communication between cancer cells and non-malignant cells in the tumour microenvironment (Afsharimani *et al.*, 2014), and can prevent the pro-angiogenic interaction

between macrophages and breast cancer cells (Khabbazi *et al.*, 2016). Furthermore, morphine modulates cells in the tumour microenvironment via many mechanisms, some of which increase and others decrease tumour aggressiveness (Khabbazi *et al.*, 2015).

However, it should be noted that not all immune cells are beneficial to the anti-tumour response. Immunosuppressive 'regulatory' T cell populations can suppress the immune response (Takeuchi *et al.*, 2016), and meta-analyses have reported increased regulatory T cell infiltration into breast cancers is associated with a poorer survival (Shou *et al.*, 2016; Wang *et al.*, 2016). Incubating blood from patients with gastric or breast cancer with morphine, sufentanil or fentanyl *in vitro* increases the number of regulatory T cells (Gong *et al.*, 2014; Hou *et al.*, 2016). Furthermore, although peri-operative treatment of women undergoing breast cancer resection with sufentanil or fentanyl equally decreased regulatory T cell numbers at day 1, numbers were increased on day 7 (Gong *et al.*, 2014). Given the capacity of regulatory T cells to inhibit anti-tumour immunity, these effects could therefore be detrimental (Takeuchi *et al.*, 2016).

Animal studies: Anti-tumour immunity and tumour growth

Many studies have described how opioids can affect immunity, most of which have been based on the acute administration of morphine. However, the duration of opioid administration (as well as the opioid itself) are likely to differential effects on immune function (Liang *et al.*, 2016; Xie *et al.*, 2017).

NK cells are key participants in effective tumour immunosurveillance. Suppression of NK cell cytotoxicity correlates with increased tumour growth and metastasis in animal models (Franchi *et al.*, 2007; Gaspani *et al.*, 2002; Shavit *et al.*, 2004). The literature generally reports that morphine (Franchi *et al.*, 2007; Yeager *et al.*, 1995) and fentanyl (Franchi *et al.*, 2007; Martucci *et al.*, 2004; Shavit *et al.*, 2004) decrease NK cell cytotoxicity, that buprenorphine does not affect NK cell cytotoxicity (Franchi *et al.*, 2007; Martucci *et al.*, 2004) and that tramadol enhances it (Gaspani *et al.*, 2002; Sacerdote *et al.*, 2000) (Table 2). However, effects on NK cell cytotoxicity is likely to be dose-dependent, as low-dose morphine has been shown to stimulate cytotoxicity, and high-dose morphine to induce an initial increase in cytotoxicity followed by a suppression of cytotoxicity in pigs (Borman *et al.*, 2009).

The differential effects of opioids on immune function and the development of tolerance is illustrated by a series of murine experiments by Martucci *et al* (Martucci *et al.*, 2004). In these studies, single dose fentanyl only decreased lymphocyte proliferation, whereas chronic fentanyl reduced lymphocyte proliferation and splenic NK cell cytotoxicity, IL-2 and IFN-γ production at 24 hours. NK cell cytotoxicity recovered by day 3, whereas lymphocyte proliferation, IL-2 and IFN-γ production only recovered by day 7. After the 7-day infusion, the administration of twice the dose of fentanyl for 1 or 3 days to these mice did not affect any immune parameter, thereby suggesting that once developed, increasing the dose did not overcome immunological non-responsiveness (Martucci *et al.*, 2004). In the same study, buprenorphine had no effect on any of the measured parameters at any time point (Martucci *et al.*, 2004).

The effect of the timing of morphine administration on cancer progression has been studied in a murine model of breast adenocarcinoma. If given before the development of cancer, morphine had no effect on cancer growth, whereas morphine increased the progression of established cancer and decreased survival via interactions with tumour μ-opioid receptors (Nguyen *et al.*, 2014).

Several surgery-based studies have assessed how different opioids, and the timing of opioid administration, affect cancer progression. In a series of rodent experiments, Franchi and co-workers showed that morphine and fentanyl decreased NK cell cytotoxicity and increased MADB106 cell-derived lung metastases, whereas buprenorphine did not (Franchi *et al.*, 2007). Surgery alone decreased NK cell cytotoxicity and increased MADB106 lung metastases. In the surgical model, perioperative buprenorphine reversed the suppressive effects of surgery on NK cell cytotoxicity and MADB106 lung metastases, whereas morphine and fentanyl did not (Franchi *et al.*, 2007). Decreased NK cell cytotoxicity correlated with high corticosterone levels, thereby suggesting the involvement of the hypothalamic-pituitary-adrenal axis (Franchi *et al.*, 2007). Fentanyl has been shown to suppress NK cell cytotoxicity in rats, and this suppression correlates to increased tumour load when fentanyl is administered close to the time of MADB106 tumour cell inoculation, but not when fentanyl was administered 6 hours before tumour inoculation (Shavit *et al.*, 2004). Thus, the acute administration of morphine and fentanyl around the time of tumour resection in animals could be detrimental to immune function and cancer outcomes.

In another series of rodent experiments, Gaspani *et al* demonstrated that tramadol increased, and morphine decreased NK cell cytotoxicity in non-operated rats (Gaspani *et al.*, 2002). Surgery reduced NK cell cytotoxicity, and this reduction in cytotoxicity correlated with increased numbers of MADB106 cell-derived lung metastases. Morphine did not influence surgery-induced NK cell cytotoxicity suppression, whereas tramadol prevented this and reduced lung metastasis (Gaspani *et al.*, 2002). The (+) enantiomer of tramadol inhibits serotonin uptake and is immunostimulatory, and this effect is inhibited by the serotoninergic antagonist metergoline (Sacerdote *et al.*, 1999). It has also been shown that an increased serotoninergic tone stimulates NK cell cytotoxicity (Mossner *et al.*, 1998). Thus, the effect of tramadol on NK cell cytotoxicity following surgery might be the summation of stimulation of NK activity, which is also present in non-operated rats, due to serotonin re-uptake inhibition, and the reduction of surgical pain which is itself immunosuppressive (Gaspani *et al.*, 2002; Page, 2003; Page *et al.*, 2001). For potentially immunosuppressive opioids, there could be a balance between the immunosuppressive effect of the opioid and the reduction of putative immunosuppression of pain (

Figure) (Page, 2005).

A recent study using mouse models of breast cancer has shown that perioperative morphine did not affect tumour growth, peripheral blood or tumour-infiltrating immune cells (although NK cells were not assessed), even when surgery was performed (Doornebal *et al.*, 2015). The difference between this and the other presented studies, could be that the tumours in this study are grown *in vivo* and transplanted from mouse to mouse without being cultured on plastic, the orthotopic rather than intravenous inoculation of cancer cells, female mice being used (different species and genders could respond differently) and the 14-day dosing schedule of morphine which was initiated once mammary tumours had developed, or 1 day after mastectomy (Afsharimani *et al.*, 2015; Doornebal *et al.*, 2015).

Healthy volunteer studies

Several studies have assessed the effect of opioids in healthy volunteers (Table 2). These have the advantage of being able to evaluate the effect of opioids on both direct and indirect opioid-immune pathways in humans, as opposed to *in vitro* studies which can only assess direct pathways, albeit in a very controlled way (Boland *et al.*, 2014a). However, it should be noted that healthy volunteer study results cannot necessarily be extrapolated to patients with cancer, as the inflammatory and immune cell activation context are different.

In a volunteer study, morphine was shown to decrease NK cell cytotoxicity; with higher doses it took over 24 hr for the NK cell cytotoxicity to normalise after the morphine was stopped (Yeager *et al.*, 1995). In two volunteer studies, acute administration of intravenous fentanyl increased NK cell cytotoxicity, an effect which resulted from an increase in the proportion of NK cells in the peripheral blood, rather than an increase in the cytotoxicity of individual NK cells (Yeager *et al.*, 2002; Jacobs *et al.*, 1999). Although these studies suggest that morphine suppresses NK cell cytotoxicity in healthy subjects, with a more prolonged suppression at a higher dose, and that fentanyl increases NK cell cytotoxicity, we cannot extrapolate healthy volunteer studies to the surgical/clinical setting.

Human cancer surgical studies

There is pre-clinical evidence that the immune response at the time of surgical removal of cancer is critical, that some opioids can be detrimental to the immune response and that this

can promote cancer progression (Byrne *et al.*, 2016; Gaspani *et al.*, 2002; Shavit *et al.*, 2004). As *in vitro* conditions of most studies do not reproduce the biology of cancer *in vivo*, and that there are differences between opioids (Borner *et al.*, 2013), immune response and clinical outcomes to different opioids need to be studied in humans undergoing surgical resection of cancer. During surgery, opioids are just one of many factors that might affect cancer progression/metastasis (Byrne *et al.*, 2016). Opioids also influence cancer growth in animal models via numerous mechanisms, with the potential influence on anti-tumour immunity being just one of these (Afsharimani *et al.*, 2011; Gach *et al.*, 2011; Jaura *et al.*, 2014).

In patients undergoing surgery, surgical stress variably reduces NK cell cytotoxicity, and different opioids (and the dose used) have different effects on NK cell cytotoxicity. In 30 patients undergoing surgery for uterine carcinoma to whom were administered fentanyl, anaesthetic medications and either morphine or tramadol, NK cell cytotoxicity was not affected by the surgery or by morphine (trend for inhibition only). However, tramadol increased NK cell cytotoxicity at 2 hours post-treatment, despite similar analgesia (Sacerdote *et al.*, 2000). In a randomised controlled trial of 40 patients undergoing surgery for malignant or benign conditions, post-operative NK cell cytotoxicity was reduced, with high-dose fentanyl delaying the post-operative recovery of NK cell cytotoxicity (Beilin *et al.*, 1996). The long-term impact or overall outcome of different opioids and doses were not determined.

The influence of morphine with or without flurbiprofen (the flurbiprofen group consumed less morphine) on immune status has been evaluated in 60 patients undergoing surgery for gastric cancer. In the morphine treated patients, T and NK cell numbers decreased at 2 hours after incision, and NK cell numbers had not returned to baseline at 5 days after surgery. In the flurbiprofen group, NK cell numbers at 2 hours and T and NK cell numbers at 1 day were higher, despite similar levels of analgesia (Shen *et al.*, 2014). In a randomised controlled trial of 25 patients undergoing neck surgery, fentanyl suppressed NK cell cytotoxicity more than flurbiprofen on day 1, but not day 2 postoperatively (Narahara *et al.*, 2013). The difference could either be a beneficial effect of the flurbiprofen or a negative effect of the opioid (Boland *et al.*, 2016a; Hooijmans *et al.*, 2015).

Although NK cell cytotoxicity correlates with tumour growth and metastasis in animal models, cancer outcomes have not been assessed in human cancer surgery settings (Table 2). Furthermore, the magnitude of change in NK cell cytotoxicity which is needed to produce a

clinically-relevant effect is unknown. It is therefore difficult to definitively attribute a decrease in NK cell cytotoxicity to poorer outcomes in patients with cancer.

Systemic opioid sparing surgical studies

Many studies have assessed the effects of regional analgesia vs. systemic opioids on clinical outcomes. In general, these compare clinical outcomes between pain control with regional/neuraxial analgesia and systemic opioids in patients having cancer surgery.

Meta-analyses for the effect of neuraxial blockade on cancer surgery have generated mixed findings from the heterogeneous and mostly retrospective studies that were included. One concluded that there was no advantage for overall or progression-free survival (Cakmakkaya *et al.*, 2014), whereas another suggested there might be a benefit for neuraxial blockade for prostate cancer surgery, but not for colonic cancer (Pei *et al.*, 2014). The most recent and largest meta-analysis (incorporating findings from 21 studies) reported an association between improved recurrence-free and overall survival with neuraxial anaesthesia (compared to general anaesthetic alone), especially in patients having colorectal cancer surgery (Weng *et al.*, 2016).

Many reviews have considered the various perioperative factors which might influence cancer-related outcomes. They suggest that although pre-clinical studies indicate a benefit of regional anaesthesia and stress response reduction in cancer formation, there is no clear association between regional/neuraxial anaesthesia for cancer surgery and tumour recurrence and cancer-related survival benefit from the heterogeneous and mostly retrospective studies. However, there might be an association with improved overall survival (Sun *et al.*, 2015; Vogelaar *et al.*, 2016). This needs confirmation and causality explored in prospective studies.

A randomised controlled trial of 503 patients undergoing abdominal surgery for cancer resection compared the effect of general anaesthesia with either epidural analgesia or postoperative systemic opioids (median 3-day morphine dose: 0mg epidural group; 107mg opioid group) showed no difference in cancer recurrence and mortality at 2-3 years between the two groups (Myles *et al.*, 2011). In a prospective cohort study of 34,188 cancer survivors who had resections for early stage breast cancer, opioid prescriptions were not associated with breast cancer recurrence (Cronin-Fenton *et al.*, 2015). This echoes previous studies indicating that opioids do not increase *de novo* cancer risk.

As surgical stress response and increased glucocorticoids can cause immune suppression, some of the benefits from regional anaesthesia may result from better analgesia (reducing pain-associated immune suppression), along with attenuation of the surgical stress response, decreases in levels of endogenous opioids and the lower doses of systemic opioids (Al-Hashimi *et al.*, 2013; Byrne *et al.*, 2016; Juneja, 2014).

Data on the influence of opioid-sparing techniques for the surgical resection of cancer are mixed, and are primarily derived from retrospective studies. Although the most recent meta-analysis reported an association between improved recurrence-free and overall survival with neuraxial anaesthesia (Weng *et al.*, 2016), more randomised controlled trials are needed in different cancers to elucidate causation, some of which are underway (Buggy *et al.*, 2015; Byrne *et al.*, 2016; Connolly *et al.*, 2016). In the meantime, there is consensus that there is currently insufficient evidence to change perioperative practice until the results of definitive randomised controlled trials are available (Buggy *et al.*, 2015; Byrne *et al.*, 2016; Connolly *et al.*, 2016).

Effects of opioids on anti-tumour immunity and survival in patients with cancer

Many patients who are not undergoing surgery receive long-term opioids for cancer-related pain, which is a different scenario in terms of the immune effects of opioids and their clinical consequences.

Effects of opioids on immunological parameters that are relevant to anti-tumour immune potential in patients with cancer have been reviewed previously (Boland *et al.*, 2014b). Five human studies which assessed the immune effects of morphine in patients with cancer showed variable effects on immunologic end points. Given that none of these studies measured the clinical effects, it is not possible to know the clinical significance of these (Boland *et al.*, 2014b).

Opioids, via immune (and non-immune) effects can influence cancer progression in animal studies and other patient groups. The effect of opioids on prognosis in patients with cancer not undergoing surgery has been systematically reviewed (Boland *et al.*, 2015). In the 13

studies of patients in the last days/weeks of life, there were mixed effects of opioids on survival. These studies were short term with poor methodology, sometimes only including patients with a very limited life expectancy such as from hospice admission, or only measured opioids that were taken in the last day(s) of life (Boland *et al.*, 2015). The best quality end of life study, a secondary data analysis, showed that an intravenous morphine-equivalent dose (IVME) above 20 mg/day was associated with a shorter survival compared with ≤17 mg/ day (the lowest dose group); mean survival: 27 days for patients on ≤17 mg/day IVME vs 12 days for patients on 20-25 mg/day IVME (Portenoy *et al.*, 2006). Studies in patients with a longer prognosis (months to years) tended to be larger and of better quality. Six out of seven studies described an association between strong systemic opioid use or increasing dose and shorter survival. However, these studies did not have survival as a primary, appropriately powered, endpoint. Furthermore, they were limited, in that they included variable populations, starting points for opioid use, durations of opioid administration and from when survival was measured. The main confounding factor is that the control groups were not directly matched (i.e. not patients who had refractory severe symptoms, but did not choose opioids), and that greater analgesic requirements and shorter survival is likely to be mediated by painful progressive cancer. As a consequence, these studies cannot show causality, only associations (Boland *et al.*, 2015).

Pharmacogenetic factors are also important. In 2039 women with breast cancer, the A118G MOP receptor polymorphism, which confers a reduced receptor response to opioids, was associated with increased cancer-related survival in invasive breast cancer (Bortsov *et al.*, 2012). Although, opioid consumption was not recorded, patients with the A118G polymorphism need a higher morphine dose for analgesia. It is thus likely that the A118G patients received more opioids and had increased cancer–related survival (Bortsov *et al.*, 2012). Furthermore, Chinese people with the A118G MOP receptor polymorphism had a lower incidence of oesophageal cancer (Wang *et al.*, 2013). However, this might be cancer-type dependent, as North-eastern Polish patients with the A118G allele are more likely to develop breast cancer (Cieslinska *et al.*, 2015).

Future studies

The variable findings from *in vitro*, animal or healthy volunteers cannot be extrapolated to clinical settings, between the different clinical settings or between opioids (Juneja, 2014;

Ramaswamy *et al.*, 2017). Therefore, appropriate prospective studies in each of the clinical settings in which are used are required. These need to focus on long-term clinical studies and be undertaken in patients needing long-term continuous and intermittent opioids for non-surgical cancer-related pain, in specific cancer types (Xie *et al.*, 2017). They should also elucidate mechanisms by analysing the tumours for mitogenic activity, immune cell status in circulation and tumours, pain, symptoms and survival-related outcomes. *In vitro*, animal and healthy volunteer studies should principally be used to explore mechanisms, as the environments can be carefully controlled. These should use clinically relevant conditions, including opioid concentrations, in the experimental design (Boland *et al.*, 2016b). Furthermore, subset analysis of NK cells is needed (as $CD56^{bright}$ and $CD56^{dull}$ NK cells exhibit different functionality) (Tabellini *et al.*, 2014), and pharmacogenomics, such as the A118G MOP receptor polymorphism, should be taken into account.

Patients taking opioids for cancer-related pain could be on opioids for a long time, thus tolerance could develop. Given that they might also be on opioids intermittently, it is important to know if regular or intermittent opioids are more immune protective and how this influences clinical outcomes. As pain is immunosuppressive, the effects of pain in patients and how opioids influence immunity and clinical outcomes in these populations need further study. Appropriately designed and powered studies assessing clinical outcomes of opioid use in patients with cancer are required to inform the optimal use of opioids in these patient groups.

Conclusion

Evidence from preclinical, healthy volunteer, clinical and surgical models suggests that different opioids variably influence protective anti-tumour immunity. There are discrepancies in the results of these studies which might be partly explained by differing methodologies, species used, opioid used and the dose and duration of administration. However, in general the literature reports that morphine and fentanyl decrease NK cell cytotoxicity *in vivo* (although short-term *in vivo* exposure to fentanyl in healthy humans increases NK cell numbers and cytotoxicity), buprenorphine does not affect NK cell cytotoxicity and tramadol enhances it. This change in NK cell cytotoxicity correlates with tumour growth and metastasis in rodent models. Opioid dose and duration of administration can influence outcome.

Although clinical evidence is sparse, data suggest that perioperative opioid sparing may lead to better long-term outcomes. However, high-quality perioperative and chronic cancer-related pain studies are needed. Given that current data from patients with cancer are inconclusive, definitive recommendations about how adequate analgesia is best achieved cannot be made and opioids for cancer-related pain will continue to be recommended.

Author contributions

JWB drafted the article. Both authors intellectually contributed to the article and approved the final version to be published.

Conflicts of Interest statement

The authors do not have any conflicts of interest.

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Figures and figure legends

Figure 1: Peripheral and central mechanisms of opioid-induced immune suppression. Opioids can have direct effects on immune cells which express appropriate receptors such as mu opioid peptide (MOP) receptors and Toll-like receptor-4 (TLR-4). They can also have immunosuppressive effects via central mechanisms. Acute opioid administration enhances activity in the periaqueductal gray (PAG) matter which activates the sympathetic nervous system (SNS). The SNS innervates lymphoid organs, such as the spleen, and this activation induces the release of biological amines which suppress splenic lymphocyte proliferation and NK cell cytotoxicity (Fecho *et al.*, 1996; Irwin *et al.*, 1988). Second, prolonged use of opioids increases hypothalamic pituitary adrenal (HPA) axis activity and glucocorticoid production, which decrease NK cell cytotoxicity (Fecho *et al.*, 1996; Mellon *et al.*, 1998). Morphine can also act via D1 dopamine receptors in the nucleus accumbens shell, increasing the release of neuropeptide Y (NPY) and reducing splenic NK cell cytotoxicity in rodent models (Saurer *et al.*, 2006). Reproduced from (Boland *et al.*, 2014b) with permission by British Journal of Cancer; http://www.nature.com/bjc/journal/v111/n5/full/bjc2014384a.html.

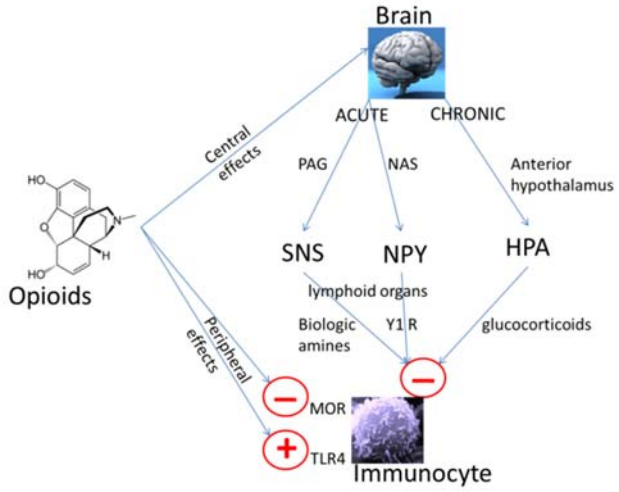
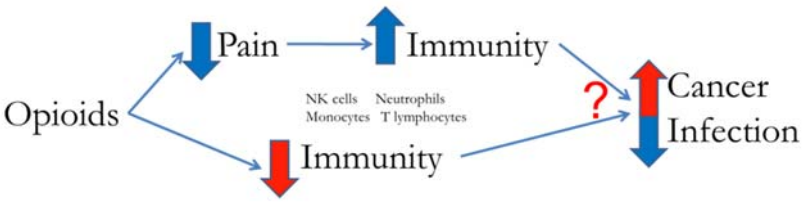


Figure 2: Opioids, pain, immunity and cancer.
Pain is immunosuppressive and may worsen outcomes in rodent models of cancer (Page, 2003; Page *et al.*, 2001). By reducing pain, opioids might have a beneficial effect on immune function and cancer (Gaspani *et al.*, 2002; Page, 2005). However, opioids which suppress immune function may decrease anti-tumour immunity and promote the development of cancer; the balance of these effects is critical (Shavit *et al.*, 2004).



Tables

Table 1: Role and function of the main immune cells.

Cell	Role	Function	Arm
Dendritic cell	Antigen presentation	Presentation of antigenic peptides in the context of MHC class I and II molecules and the delivery of essential co-stimulatory molecules	Innate
Natural killer cell	Anti-tumour Anti-viral	Release of cytotoxic molecules (granzymes, perforin)	Innate
Neutrophil	Anti-bacterial / fungal	Phagocytosis and oxidative burst	Innate
Monocyte-Macrophage lineage	Anti-bacterial / fungal	Phagocytosis and oxidative burst	Innate
$CD4^+$ T cell	Immune coordination / regulation	Regulating the activity of other immune cells	Adaptive
Regulatory T cell ($CD4^+$, $CD25^+$, $FOXP3^+$, $CD127^{low}$, plus other markers)	Immune regulation/ suppression	Immune system modulation, maintaining tolerance to self-antigens, preventing autoimmune disease, potential barrier to the development of protective anti-tumour immunity	Adaptive
$CD8^+$ T cell	Cytotoxicity	Eradication of virally-infected cells and cancer cells. Induction of apoptosis by i) release of cytotoxins (perforin, granulysin, granzymes) ii) direct cell-cell contact	Adaptive
B cell	Antibody production	Antibody production	Adaptive

Adapted with permission by British Journal of Cancer from (Boland et al., 2014b); http://www.nature.com/bjc/journal/v111/n5/full/bjc2014384a.html.

Table 2: Summary of the effects of opioids on NK cell cytotoxicity and cancer outcomes in the different models

	Animal		Healthy volunteer	Human cancer surgery	
Opioid	**NK cell cytotoxicity**	**Cancer**	**NK cell cytotoxicity**	**NK cell cytotoxicity**	**Cancer**
Morphine	Decrease/no effect on suppressive effects of surgery	Increase	Decrease	No effect/ delayed post-operative recovery	Not assessed
Fentanyl	Decrease/no effect on suppressive effects of surgery	Increase	Increase	Delayed post-operative recovery	Not assessed
Buprenorphine	No effect/reversed the suppressive effects of surgery	No effect	Not assessed	No effect/ reversed the suppressive effects of surgery	Not assessed
Tramadol	Increase/reversed the suppressive effects of surgery	Decrease	Not assessed	Increase/ reversed the suppressive effects of surgery	Not assessed