Systematic Review & Meta-Analysis

Role of midazolam on cancer progression/survival - An updated systematic review

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ABSTRACT

Background and Aims: Cancer is a leading cause of mortality worldwide. Despite advancements in cancer management, cancer progression remains a challenge, requiring the development of novel therapies. Midazolam is a commonly used adjunct to anaesthesia care for various surgeries, including cancer. Recently, there has been a growing interest in exploring the potential role of midazolam as an anticancer agent; however, the exact mechanism of this linkage is yet to be investigated thoroughly. **Methods:** Based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline, this systematic review presented aggregated evidence (till November 2022) of the effects of midazolam on cancer progression and survival. All primary research article types where midazolam was administered *in vivo* or *in vitro* on subjects with cancers were included. No restrictions were applied on routes of administration or the type of cancer under investigation. Narrative synthesis depicted qualitative findings, whereas frequencies and percentages presented numerical data. **Results:** Of 1720 citations, 19 studies were included in this review. All articles were preclinical studies conducted either *in vitro* (58%, 11/19) or both *in vivo* and *in vitro* (42%, 8/19). The most studied cancer was lung carcinoma (21%, 4/19). There are two main findings in this review. First, midazolam delays cancer progression (89%, 17/19). Second, midazolam reduces cancer cell survival (63%, 12/19). The two major mechanisms of these properties can be explained via inducing apoptosis (63%, 12/19) and inhibiting cancer cell proliferation (53%, 10/19). In addition, midazolam demonstrated antimetastatic properties via inhibition of cancer invasion (21%, 4/19), migration (26%, 5/19), or epithelial-mesenchymal transition (5%, 1/19). These anticancer properties of midazolam were demonstrated through different pathways when midazolam was used alone or in combination with traditional cancer chemotherapeutic agents. **Conclusion:** This systematic review highlights that midazolam has the potential to impede cancer progression and decrease cancer cell survival. Extrapolation of these results into human cancer necessitates further investigation.

Keywords: Cancer, cancer progression, cell line, metastasis, midazolam, neoplasms, surgery, surgical procedures, survival

INTRODUCTION

Cancer remains a major public health concern worldwide despite significant advances in treatments. In 2020, 19.3 million new cancer cases and 10 million cancer deaths were recorded worldwide.[1] Benzodiazepines have been investigated for their effects on cancer progression, among which midazolam has shown interesting results. Midazolam is commonly used peri-operatively as an anxiolytic, sedative, hypnotic, and adjunct to anaesthesia care in cancer surgery.[2] Midazolam is characterised by its fused benzene/diazepine rings, which bind to central and peripheral benzodiazepine receptors. Peripheral benzodiazepine receptors have been examined

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in various pathologic and physiologic processes, including cell proliferation and apoptosis.[3] These roles and previous investigations suggest a possible role for midazolam in cancer therapy and delayed cancer progression.

However, the current literature is conflicting, and midazolam's role in cancer progression and cell survival is not fully understood. Some anti-tumour immunity studies using mice models found no change in T-lymphocyte levels or cancer progression.[4] A review by Jiao *et al*.[5] examined the biochemical properties of midazolam on cancer and collated studies with cancer cell lineages, mainly lymphoma cells and Leydig tumour cells, and highlighted some of the key mechanisms of probable anticancer properties of midazolam. However, the role of midazolam on well-defined cancer outcome(s) was not studied systematically. In addition, it has been over five years that no other systematic synthesis of evidence in this area was conducted.

This updated systematic review aims to amalgamate current research on midazolam's effects on cancer cell progression and survival. The results of this review also incorporate suggested mechanisms of such effects.

METHODS

This systematic review followed all Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[6] and was registered with International Prospective Register of Systematic Reviews (PROSPERO) vide registration number CRD42022340081.

Study selection

We included all primary research article types, including randomised controlled trials and prospective, retrospective, and cross-sectional survey studies available in English. The detailed inclusion strategy is defined along the 'PICO (Patient/Population, intervention, comparison and outcomes)' criteria:

Population (type of participants/subjects): Either human or animal, with no restriction on age, gender, or type of cancer or *in vitro* experimental models.

Intervention (type of intervention): Midazolam for any purpose, including premedication, anti-anxiety, anti-seizure, anaesthetic induction, sedation, and other peri-operative use through any route.

Comparison: Comparators such as no midazolam, placebo, and other medications were included.

Studies were excluded if they were case series, case reports, correspondences, letters, or abstracts or were not written in English.

Outcome(s)

The primary outcome is cancer progression (or if it continues progressing) after using midazolam. The effect on progression was assessed and assigned a letter grade according to a predetermined scale [P – Progression observed; N - No change observed in cancer progression; A - Anti-progression (inhibit cancer progression); M - Mixed results; I - Inconclusive results].

The secondary outcome was survival if the study was conducted *in vivo* and cell viability if the study was conducted *in vitro*. Clinical cancer studies commonly utilise standard clinical definitions (overall survival, progression-free survival, recurrence rate, etc.) for cancer progression and survival. All studies in the pilot search were found to be experimental (*in vitro* or *in vivo*); therefore, the modified progression and survival definitions were interchangeably used for cancer cell proliferation and survival for this review.

Information source

With the help of an information specialist (RC), a systematic search was performed in Ovid Medline Epub Ahead of Print, in-process and other non-indexed citations, Ovid Medline (R) Daily, Embase, Cochrane central registry of controlled trials, Cumulative Index to Nursing and Allied Health Literature (CINAHL), and Web of Science to retrieve all potentially relevant studies. The search was conducted from inception until November 7, 2022, by using predefined search criteria. Keywords such as midazolam, cell lines, neoplasms, cancer, surgery, surgical procedures, and operative were used [Supplementary Table 1a and b].

Data extraction

All studies identified via the search strategy were uploaded to Covidence.org and deduplicated. Two authors (AS and AR) independently screened the studies for titles and abstracts. Full text for titles that passed the initial screening was retrieved and screened for eligibility (AS and AR). Any disagreement in the initial or full-text eligibility review was resolved by the senior corresponding author (TC). Studies that did not meet all the inclusion criteria during full-text screening were excluded, and the reasons for exclusion were identified. Documentation of retrieved/selected studies

adhered to the PRISMA guidelines [Supplementary Table 2].[6] Data were abstracted by the two authors (AS and AR) independently, and a standardised Microsoft Excel form for data collection and management was used. Extracted information included study identifications such as authors and country, study design, level of evidence, type of cancer, subject, intervention medication, control agent (if any), doses, assays used in the studies, and pathways for different effects of midazolam.

Quality/bias and level of evidence

A bias assessment using the Newcastle–Ottawa scale (NOS)[7] was planned for the included articles.

Data summary and synthesis

Due to the inherent characteristics of the data derived from the included animal studies and *in vitro* models, a narrative synthesis approach was adopted to summarise qualitative findings. Frequencies and percentages were utilised for the description of numerical data.

RESULTS

A total of 1720 articles were screened from various databases and cross-reference searches. Out of these, 19 articles were included in the final analysis. Of the 16 studies excluded after full-text review, the most common cause of exclusion was the unavailability of full text [number of articles (n)=4], conference abstract (n = 4), and cancer progression not being a measured outcome (n = 3). The PRISMA flow diagram provides a detailed account of the search locations and exclusion reasons [Figure 1].

Study characteristics

Eleven of the 19 articles were conducted *in vitro*, and the other eight were combinatory of both *in vitro* and *in vivo* studies. As per the evidence levels outlined by Turk in his introduction to evidence-based medicine article,[8] all included studies corresponded to level-V evidence. This is considered the least valid form of evidence. Results from level-V evidence can be used to inform and design more rigorous studies such as cohort studies or randomised control trials; however, determining treatment efficacy with level-V evidence is challenging.[8] The type of cancer investigated varied greatly, with multiple occurrences only for neuroblastoma (n = 2), hypopharyngeal squamous cell carcinoma (n = 2), mouse Leydig tumour (n = 2), and hepatocellular carcinoma (n = 2). Varying concentrations of midazolam, up to 1000 μM *in vitro* and 50 mg/kg *in vivo*, were used throughout the studies [Table 1].

Notably, all the included articles exclusively pertained to experimental *in-vivo* and *in-vitro* studies. Consequently, there was an absence of cohort or case-control studies, rendering the application of the NOS bias assessment inapplicable in this context.

Primary outcome

Approximately 89% of the studies (n = 17) concluded that midazolam-treated groups delayed or stopped cancer progression [Table 2]. The remaining studies concluded no change in cancer progression (n = 1)[4] and mixed results (n = 1).[15] The definition of 'delayed cancer progression' varies amongst studies. Most *in-vitro* studies used biochemical markers such as caspase-3 activity, deoxyribonucleic acid (DNA) fragmentation, reactive oxygen species (ROS) generation, 3,4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) assay and 5-bromo-2-deoxyuridine (BrdU) assay to measure changes in proliferation or viability. A few *in-vitro* studies also used microscopy techniques to observe morphological changes. *In vivo*, mice model studies used physical tumour weight and volume measurements. Midazolam decreased the tumour volume in one *in-vivo* study.[18] Midazolam was administered subcutaneously to adult male Bagg Albino (BALB/c) immunodeficient nude mice with A549 tumours at a dosage of 2.5 mg/kg per day for five days. Although there were no differences in animal weight observed, midazolam had a significant effect in reducing tumour size. It significantly reduced the tumour burden compared to the control group, with a 1.7-fold reduction ($P < 0.05$).[18] Midazolam delayed or inhibited tumour growth compared to controls in six studies.[12,20-22,24,25] In human leukaemia (K562) and colon cancer (HT29) cell lines, midazolam significantly suppressed the proliferation of K562 cells and HT29 at 100 and 200 μM, respectively. Growth-inhibitory concentrations, the concentration (mM) of midazolam that achieved 50% growth inhibition of cells - presented as % of control of midazolam, were 171.5 and 148.5 μM for K562 and HT29 cells, respectively.[12]

Four studies noted that they utilised high concentrations of midazolam, which may not be feasible in clinical applications.[10,11,14,16,25] For example, the mean [standard deviation (SD)] of 50% cytotoxic concentration of midazolam in human oral squamous cell carcinoma (OSCC) cell line HSC-2 was

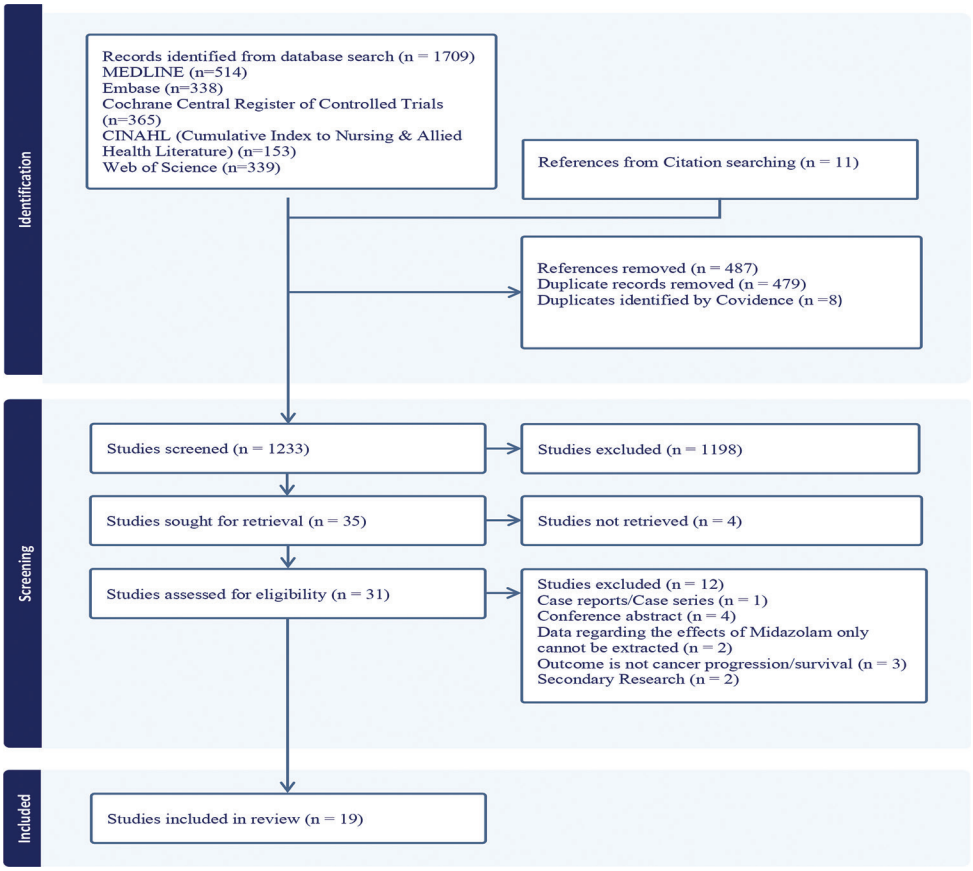

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection. n: Number of studies

119 (0.8) μM and 232 (59.0) μM in HSC-3;[10] in FaDu human hypopharyngeal squamous cell carcinoma, cells were incubated with concentrations of up to 100 μM midazolam for 24 and 48 h.[11] Again, a high concentration of 100 μM of midazolam was applied in human malignant glioma cells, greatly exceeding the clinical range.[16]

Secondary outcome

Approximately 63% of the studies (n = 12) showed decreased cancer cell viability upon treatment with midazolam. Of the other seven studies, five did not measure the effect of midazolam on cancer cell viability. Ohno *et al*. demonstrated that midazolam decreased the viability of both cancer and normal human cells.[10] In FaDu human hypopharyngeal squamous cell carcinoma, cells were incubated with 0, 6.25, 12.5, 25, 50, and 100 μM midazolam for 24 and 48 h. After 24 h of midazolam treatment, cell viability decreased at 50 and 100 μM. By 48 h, the cell viability decreased at 6.25 μM and 100 μM, a 47.1% reduction.[11] Mishra *et al*. found that different concentrations impacted the duration needed to induce apoptosis. High concentrations (300 μM) induced late apoptosis, whereas low concentrations (100 μM) induced early apoptosis.[12] The last remaining study found mixed results in terms of cell viability. This study found midazolam to have a protective effect on neuroblastoma cells at concentrations of <79.4 μM but reduced cell viability at higher concentrations.[15]

DISCUSSION

This systematic review indicated the potential inhibitory effects of midazolam on cancer progression and cell survival through several signalling pathways. In this review, midazolam was found to delay cancer progression by inhibiting cell proliferation and induction of apoptosis. In addition, midazolam exerted an anti-metastatic effect by hindering cell invasion and migration in various cancer types. Our review demonstrated that midazolam may alter the efficacy of traditional anticancer agents such as chemotherapy and immunotherapy. The exact mechanisms involved remain unclear as all the studies were short-term *in-vitro* or *in-vivo* models.

The studies identified many mechanisms for this delay/halt of proliferation. The most common mechanism was the mitochondrial intrinsic pathway[9,12,18,19] and induced apoptosis.[4,20,22,24] Other mechanisms include

Table 1: Study characteristics of included articles summarising interventional and control groups along with author conclusions regarding the effect of midazolam on various cancer lines

Author	*Study Design*	Model Type of Cancer	Subject	Intervention Medication
Kushida *et al.*, 2007[4]	*In Vitro* + *In Vivo*	Thymoma	Murine thymoma cells (EL4) (H-2b) (105) Male C57BL/6 mice* Murine	Propofol
Stevens *et al.*, 2011[9]	*In Vitro*	T-lymphoma, Neuroblastoma,	Human Jurkat T-lymphoma	Midazolam
			Human neuroblastoma cell lines	Midazolam
				Flumazenil on pretreated Midazolam cells (100, 200 μM)
			Rat primary neurons	Midazolam on pretreated cells with Q-VD (10 μM).
Ohno *et al.*, 2012[10]	*In Vitro*	Oral Squamous Cell Carcinoma and Glioblastoma.	Human OSCC (HSC-2, HSC-3, HSC-4, NA, Ca9-22) Human Glioblastoma (T98G, U87MG)	Midazolam
Dou *et al.*, 2013[11]	*In Vitro*	Hypopharyngeal Squamous Cell Carcinoma	FaDu Human Hypopharyngeal Squamous Cell Carcinoma (ATCC HTB-43)	Midazolam
Mishra *et al.*, 2013[12]	*In Vitro* + *In Vivo*	Leukaemia & Colon Cancer	Human Leukaemia (K562) Human Colon Cancer (HT29) Female 5-week-old BALB/c-nu mice*	Midazolam
Dou *et al.*, 2014[13]	*In Vitro*	Hypopharyngeal squamous carcinoma cells	Human head and neck hypopharyngeal squamous carcinoma cells (FaDu)	Midazolam[1]
				Midazolam[2]
So *et al.*, 2014[14]	*In Vitro*	Mouse Leydig Tumour	Mouse Leydig Tumour (MA-10)	Midazolam
Braun *et al.*, 2015[15]	*In Vitro*	Human Neuroblastoma	Human Neuroblastoma (SHEP)	Midazolam
Chen *et al.*, 2016[16]	*In Vitro*	Human Malignant Glioma	Malignant glioblastoma T98-MG cells	Midazolam
So *et al.*, 2016[17]	*In Vitro*	Mouse Leydig Tumour	Mouse Leydig Tumour (MA-10)	Midazolam
Wang *et al.*, 2018[18]	*In Vitro* + *In Vivo*	Lung Carcinoma & Neuroglioma	Human Lung Carcinoma (A549) Human Neuroglioma (H4) Adult male BALB/C immunodeficient nude mice*	Midazolam
Jiao *et al.*, 2018[19]	*In Vitro*	Human NSCLC	A 549 NSCLC	Midazolam
				Midazolam, miR-520d-5p mimic transfection, miR-520d-5p mimic transfection in+Midazolam
				Midazolam, siSTAT3 alone, siSTAT3in+Midazolam
Qi *et al.*, 2020[20]	*In Vitro* + *In Vivo*	Hepatocellular carcinoma	HepG2 Human hepatocellular carcinoma cells Nude mice aged 6 weeks *	Midazolam
				Midazolam, miR-124-3p inhibitor, miR-124-3p mimics, Midazolam + miR-124-3p mimics, Midazolam + miR-124-3p inhibitor
Seo *et al.*, 2020[21]	*In Vitro* + *In Vivo*	Melanoma	Animal (Male C57BL/6 mice) Melanoma B16F10, HPMVECs	Midazolam[4]
				Midazolam[5]
				Midazolam + VEGF, NAC + VEGF, cystamine + VEGF6
Sun *et al.*, 2021[22]	*In Vitro* + *In Vivo*	NSCLC	Six-week-old male BALB/c nude mice CR-NSCLC cell line (A549/DDP and H1299/DDP)	Midazolam Cisplatin Midazolam + Cisplatin
Lu *et al.*, 2021[23]	*In Vitro*	Lung alveolar adenocarcinoma Breast cancer (luminal, oestrogen positive)	Human A549 lung alveolar adenocarcinoma Breast cancer cell line MCF-7	TGFβ TGF β + Midazolam
Oshima *et al.*, 2022[24]	*In Vitro* + *In Vivo*	Pancreatic ductal adenocarcinoma	PDAC transgenic 6-week-old mice Murine PDAAC cell lines	Midazolam
				Midazolam + receptor antagonist (PK1119)
Kang *et al.*, 2022[25]	*In Vitro* + *In Vivo*	HCC	Human HCC-LM3 Human Hep-3B HCC Mouse Hepa1-6 HCC Male C57BL/6 mice (5-6 weeks old) *	Midazolam
Shen *et al.*, 2022[26]	*In Vitro*	HCC	HCC cell strain (Hep3B) HCC cell strain (SK-HEP-1) Human Hepatocyte line (THLE-2)	Midazolam

Contd...

Table 1: Contd...

Author	Intervention		Control Group	Conclusion
	Dose	Duration		
Kushida *et al.*, 2007[4]	IP 50 mg/kg OD	3 days and 3 weeks before and after inoculation with tumour cells, respectively	Midazolam (25 mg/kg) Saline or intralipid (5 mL/kg)	Midazolam does not affect cytotoxic T- lymphocyte activity against tumours in mice spleen.
Stevens *et al.*, 2011[9]	(50, 70, 100, and 150 μM)	24 h	Medium (negative control), Staurosporine 0.125 μM (positive control)	Midazolam induces apoptosis in the human lymphoma and neuroblastoma cell lines in a concentration-dependent manner.
	(100, 200, 300, and 400 μM)	24 h	Medium (negative control), Staurosporine 0.125 μM (positive control)	
	(0, 20, and 200 μM),	24 h	Flumazenil on pretreated cells with a control medium	
	(0, 5, 7.5, 10, 25, 50, 75, 100 μM)	48 h	Midazolam without pre-treatment with Q-VD.	
Ohno *et al.*, 2012[10]	(15.7, 31.25, ., 500, 1000 μM)	48 h	Midazolam (in non-cancer cells)	Midazolam was cytotoxic to OSCC cell lines but only at concentrations much greater than clinically accepted. Midazolam was also cytotoxic to some skin and oral cells.
Dou *et al.*, 2013[11]	(0, 25, 50, 100 μM)	24 h, 48 h	No Midazolam (0 μM)	Midazolam exhibits anti-proliferative effects in human hypopharyngeal squamous cell carcinoma, but concentrations are too high to apply to cancer therapy.
Mishra *et al.*, 2013[12]	(0.83 mg/kg OD - *in vivo*) (0, 10, 30, 100, 200 μM - *in vitro*)	12 days	Saline	Midazolam inhibited leukaemia and colon cancer progression
Dou *et al.*, 2014[13]	(0, 6.25, 12.5, 25, 50, and 100 μM)	24 h, 48 h	Solvent	Midazolam inhibits the proliferation of human head and neck squamous carcinoma cells by downregulating p300.
	(0, 25, 50, and 100 μM)	48 h	Tubulin	
So *et al.*, 2014[14]	(6, 30, 150 μM)	24 h	Midazolam (0 μM)	Midazolam causes cell cycle arrest and induces apoptosis in Leydig mice tumour cells.
Braun *et al.*, 2015[15]	(1, 2, 4, ., 512 μM)	24 h	Temozolomide Midazolam (0 μM)	At low concentrations (<79.4 μM), Midazolam had a protective effect on the neuroblastoma cells, but at doses higher than 79.4 μM, it greatly reduced cell viability.
Chen *et al.*, 2016[16]	(0, 25, 50, and 100 μM)	24 h, 48 h	Solvent Control	Midazolam decreased cell proliferation in malignant glioma by inhibiting TRPM7 currents and calcium influx, resulting in G 0 -G 1 -phase cell-cycle arrest.
So *et al.*, 2016[17]	(30, 150 μM)	24 h	Midazolam (0 μM)	Midazolam induces apoptosis of Leydig mice tumour cells.
Wang *et al.*, 2018[18]	(2.5 mg/kg OD)	5 days	Naive Control Vehicle Control	Midazolam inhibited the growth of lung tumours in xenograft mice and inhibited proliferation in lung carcinoma and neuroglioma cells.
Jiao *et al.*, 2018[19]	(10, 20, 40, 80 μg/mL)	24 h, 48 h	Midazolam (0 μg/mL)	Midazolam suppressed cancer cell growth by suppressing STAT3 and inducing apoptosis in NSCLC.
	(10 μg/mL)[3]	48 h	Midazolam (0 μg/mL)	
	(10 μg/mL)[3]	48 h	Midazolam (0 μg/mL)	
Qi *et al.*, 2020[20]	(10, 20, 40 μg/mL)	24 h, 48 h	Midazolam (0 μg/mL)	Midazolam inhibits cell proliferation and promotes apoptosis of human hepatocellular carcinoma cells by elevation of micro-RNA-124-3p and suppressing the PIM-1 gene.
	(10 μg/mL)[3]	25 days	Midazolam (0 μg/mL)	

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Table 1: Contd...

Author	Intervention		Control Group	Conclusion
	Dose	Duration		
Seo *et al*., 2020[21]	(0, 10 mg/kg)	1,3,5 days	Midazolam (0, 10 mg/kg) -treated normal mice,	Midazolam inhibits VEGF-induced trans-endothelial migration of melanoma cells but does not affect cancer cell metastasis and proliferation.
	(0, 10 mg/kg)	Q3 day (6 times total)	Midazolam (0, 10 mg/kg) -treated normal mice	
	20 μmol/L3	90 mins	VEGF (0,10 ng/mL)	
Sun *et al*., 2021[22]	(10 μg/mL) (50 μg/mL) (10 μg/mL+50 μg/mL)	0, 6, 12, 24, 48 h	Midazolam (0 μg/mL) + cisplatin (0, 50 μg/mL)	Midazolam demonstrated tumour-suppressing effects by increasing cisplatin sensitivity in CR-NSCLC cells.
Lu *et al*., 2021[23]	(10 ng/mL) (10 ng/mL) +(5,10, 20 μg/mL).	12, 24, 48 h	Midazolam (10 μg/mL) negative control Midazolam (0 μg/mL). + TGFβ (0 ng/mL)	Midazolam inhibits cell proliferation, migration, invasion, and the epithelial-mesenchymal transition process in lung and breast cancers.
Oshima *et al*., 2022[24]	(30 mg/kg/day) (30 mg/kg/day + 3 mg/kg/day)	HE	Water	Midazolam suppresses pancreatic ductal adenocarcinoma progression via inhibiting cell proliferation, cancer-associated fibroblasts, and local infiltration of tumour-associated inflammatory cells.
Kang *et al*., 2022[25]	(50 μM, 75 μM, 100 μM, 150 μM, 200 μM)/ (1 mg/kg QOD)	21 days	Midazolam (0 μM) Saline	Midazolam inhibited the growth of hepatocellular carcinoma in both in vitro and in vivo models in human and mouse cell lines.
Shen *et al*., 2022[26]	(0-10 μg/mL)	48 h	Midazolam (0 μg/mL)	Midazolam inhibits hepatocellular carcinoma metastasis, especially when used in conjunction with miR-217.

HCC - Hepatocellular Carcinoma, OSCC - Oral Squamous Cell Carcinoma, OD - Once Daily, QOD - Every Other Day, Q3 day - Every 3 Days, HE - Until Humane End Point, Non-small cell lung cancer (NSCLC), CR-NSLC- Cisplatin-resistant NSCLC, TGFβ- Ttransforming growth factor-beta, CTL- Cytotoxic T lymphocytes, HPMEs- Human pulmonary microvascular endothelial cells, VEGF - Vascular endothelial growth factor. 1: to measure FaDu cell growth, 2: to measure the expression of p300, 3: Midazolam dose, 4: for hyperglycaemia-induced vascular leakage assessment in diabetic mice, 5: for hyperglycaemia-induced cancer metastasis assessment in diabetic mice, 6: To assess the midazolam effect on vascular leakage in HPMVECs, IP- Intraperitoneal. *Cells implanted into the animal model

the p53 pathway,[17] necrosis, transient receptor potential melastatin (TRPM)7 inhibition,[11,16] S-phase cell cycle arrest,[12] etc. One study[15] did not recognise specific mechanisms by which midazolam reduced the cytotoxicity of temozolomide in neuroblastoma cells. Kushida *et al*. measured T-lymphocyte activity against spleen tumour cells, and the midazolam group fared similarly to the saline control.[4] Ohno *et al*. found midazolam cytotoxic to oral squamous cell carcinoma and regular oral cells but not cytostatic. It is important to note that these results were observed when using high concentrations of midazolam, reaching 1000 μM, which exceeded clinical standards.[10] Interestingly, Seo *et al*. found lower doses of midazolam (6, 30, and 150 μM) more effective in inducing apoptosis in mouse Leydig tumour cells.[14]

In this review, the effects of midazolam were mainly demonstrated *in vitro*. Limited studies demonstrated midazolam's inhibitory role on cancer progression and survival *in vivo*.[12,18-22,24,25] This discrepancy between the number of *in-vivo* and *in-vitro* studies might be attributable to the potentially toxic effects of high doses of midazolam on animal tissues. In evaluating drug effects on cancer, *in-vitro* studies are usually prioritised as an initial step before *in-vivo* studies as they provide a controlled environment.[27] *In-vivo* experiments often require more resources and longer time frames and are subject to ethical regulation to prevent the overuse of animals.[28]

In this review, midazolam inhibited cancer cell progression in various cancer cell lines; human non-small cell lung cancer (NSCLC)[18,19,22,23] and hepatocellular carcinoma[20,25,26] were the most studied cancer types. Midazolam also suppressed the progression of human T-cell lymphoma and neuroblastoma,[9] oral squamous cell carcinoma and glioblastoma,[10] hypopharyngeal squamous cell carcinoma,[11,13] leukaemia and colon cancer,[12] mouse Leydig tumour,[14,17] neuroglioma,[18] melanoma,[21] pancreatic ductal adenocarcinoma,[24] breast cancer (oestrogen positive),[23] and malignant glioma.[16] Midazolam appeared to inhibit cancer cell proliferation in hypopharyngeal squamous cell carcinoma, leukaemia, colon cancer, neuroglioma, NSCLC, breast, pancreatic, glioblastoma, and hepatocellular carcinoma. Midazolam also exerted anti-metastatic properties by inhibiting cancer cell

Table 2: Summary of primary and secondary outcomes of the included studies and the described pathway of effect

Author	Assays	Primary Outcome (Cancer Progression)	Secondary Outcome (Effect on Cell Survival)	Mechanism (Pathway)
Kushida *et al.*, 2007[4]	CTL flow cytometry	N*	NA	Cellular Immune Response
Stevens *et al.*, 2011[9]	XTT	A	D	Mitochondrial pathway
Ohno *et al.*, 2012[10]	MTT, Caspase Activation, Autophagy, Electron Microscopy, UV Irradiation	A	D	Necrosis
Dou *et al.*, 2013[11]	MTT, BrdU, Lactate dehydrogenase release	A	NA	TRPM7 Inhibition
Mishra *et al.*, 2013[12]	WST, MTT, Mitochondria Membrane Potential, DCFH-DA	A	D	Mitochondrial Intrinsic Pathway S Phase Cell Cycle Arrest No×2-dependent ROS Suppression
Dou *et al.*, 2014[13]	MTT and BrdU incorporation RT-PCR and Western blotting	A	NA	Cyclin-dependent kinase/ retinoblastoma pathway
So *et al.*, 2014[14]	Flow Cytometry	A	D	t-Bit, JNK, and p38 Pathways p-Akt Downregulation, caspase cascade
Braun *et al.*, 2015[15]	XTT, BrdU	M	M	N/A
Chen *et al.*, 2016[16]	(BrdU) cell proliferation, MTT, caspase-3 activity, lactate dehydrogenase release, immunofluorescence	A	N	Inhibited the TRPM7 expression
So *et al.*, 2016[17]	Micro BCA Protein Assay, MTT, annexin V/PI double staining	A	D	p53 Pathway (with the involvement of autophagy) ER stress
Wang *et al.*, 2018[18]	MTT, CCK8, Wound healing	A	D	Mitochondrial Intrinsic Pathway, PBR pathway, Cell Migration
Jiao *et al.*, 2018[19]	MTT, LIVE/DEAD, flow cytometry, and Western blot	A	D	Mitochondria Intrinsic Apoptosis Pathway, miR-520d-5p, STAT3 pathway
Qi *et al.*, 2020[20]	Transwell, EdU and colony formation, flow cytometry, Dual-luciferase reporter gene, Western blot	A	D	Apoptosis, Cell Proliferation, Migration, Invasion
Seo *et al.*, 2020[21]	In vitro permeability, MTT, Wound healing, trans-endothelial migration	A	NA	Vascular Endothelial Growth Factor Induced Intracellular Events, Lung Metastasis
Sun *et al.*, 2021[22]	MTT assay, trypan blue staining, Dual-luciferase reporter gene system, flow cytometry,	A	D	Apoptosis, proliferation, microRNA
Lu *et al.*, 2021[23]	MTT, (EdU) cell proliferation, wound healing and Transwell migration and invasion, and western blot	A	NA	TGF Pathway, Cell Migration, Invasion, Epithelial-mesenchymal Transition, Peripheral Benzodiazepine Receptors
Oshima *et al.*, 2022[24]	Proliferation	A	D	Proliferation via Cyclins and Cyclin-dependent Kinases, Apoptosis
Kang *et al.*, 2022[25]	CCK8, EdU, Transwell, wound healing	A	D	NF-κB pathway
Shen *et al.*, 2022[26]	MTT, Transwell	A	D	miR-217 Upregulation

P-Cancer continues progressing, N-No change in cancer progression, A-Anti-progression(inhibits/delays cancer progression), M-Mixed results(both P and A), I-Inconclusive results. Secondary Outcome Abbreviations: D - Decreased cell viability, I - Increased cell viability, N - No effect on cell viability, M - Mixed results on cell viability (both increase and decrease), N/A - Not Available, *Indirect interpretation of the study results, EdU - 5-ethyl-20-deoxyuridine, BrdU- 5-Bromo-2-deoxyuridine, CCK8- Cell counting kit-8, CTL- Cytotoxic T lymphocytes, MTT- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, WST-Water-soluble tetrazolium, XTT-2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-carboxanilide-2H-tetrazolium

migration,[18,20,23,25,26] invasion,[18,20,23,26] and epithelial–mesenchymal transition.[23] Midazolam also decreased lung metastasis of melanoma cells by demonstrating an inhibitory effect on the vascular endothelial cells, with no direct effect on the migration or the proliferation of the melanoma cells.[21]

Our secondary outcome in this systematic review was cancer cell survival upon midazolam treatment, with a high proportion of the research studies reporting decreased cell survival. Midazolam has been found to modulate apoptosis in cancer cells by activating several signalling pathways.[29] Apoptosis is a programmed cell

death mechanism that eliminates damaged or abnormal cells and maintains tissue homeostasis.[30] A complex network of signalling pathways regulates the process of apoptosis, and dysregulation of these pathways can contribute to various diseases, including cancer and neurodegeneration.[30] Generally, there are extrinsic and intrinsic pathways of apoptosis. The former activates downstream death-inducing signalling complex (DISC) and caspase-8 in response to death receptor activation. Alternatively, mitochondrial outer membrane permeability is the key to the intrinsic pathway to activate caspase-9 through cytochrome-c and Bcl-2 proteins.[31]

Another mechanism may involve tumourigenesis in human cancers associated with the abnormal expression of microribonucleic acids (miRNAs).[32] In addition, necrosis was one of the mechanisms postulated by Ohno *et al*. as to how midazolam decreased cancer cell survival in this review. Their experiments revealed high rates of cytotoxicity without inducing significant apoptosis and induced vacuoles and mitochondrial swelling along with plasma membrane injury.[10]

Numerous studies suggested either one of both apoptotic pathways. In human lymphoma and neuroblastoma cell lines, with increasing concentrations at 50, 70, 100, and 150 μM and 100, 200, 300, and 400 μM, respectively, midazolam toxicity switched from caspase-dependent apoptosis (mitochondrial pathway) to necrosis, and these effects appeared to be unrelated to gamma amino butyric acid-A (GABAA) receptor or the peripheral benzodiazepine receptor.[9] Midazolam showed pro-apoptotic effects by the activation of the intrinsic pathway of apoptosis in human leukaemia and colon cancer, neuroglioma, and lung carcinoma cell lines. This may be through suppressing reactive oxygen species production.[12,18] Midazolam activated extrinsic and intrinsic caspase cascades to induce apoptosis in MA-10 mouse Leydig tumour cells.[17] Midazolam induced apoptosis in NSCLC by upregulating miRNAs (miR-520d-5p) and suppressing signal transducer and activator of transcription (STAT) 3 activity.[19] Similarly, midazolam accelerated apoptosis of hepatocellular carcinoma (HCC) cells by elevating miRNA levels (miR-124-3p) and suppressing the PIM-1 gene.[20] Shen *et al*. supported this and demonstrated that midazolam suppressed HCC by elevating miR-217 levels. Midazolam induced apoptosis in cisplatin-resistant CR-NSCLC by upregulating miR-194-5p but downregulated HOOK3 in the CR-NSCLC cells.[22] Another study demonstrated the downregulation of cyclins/CDKs and cell cycle arrest and induction of apoptotic and non-apoptotic cell death by midazolam in mouse models of pancreatic ductal adenocarcinoma.[24] In oral squamous cell carcinoma cell lines, midazolam demonstrated cytotoxicity by inducing necrosis rather than apoptosis.[10]

The effect of midazolam on cell survival and the type of cell death induced was reported to vary by concentration. Midazolam induced apoptosis in the human lymphoma and neuroblastoma cell lines in a concentration-dependent manner; at higher doses, the cell death mechanism changed from apoptosis to necrosis.[9] Similar results were reported by Braun *et al*., who found that at low concentrations (<79.4 μM), midazolam had a protective effect on neuroblastoma cells. Still, it greatly reduced cell viability at doses higher than 79.4 μM. They concluded that midazolam demonstrated a biphasic dose-response relationship in human neuroblastoma cells, where pretreatment with midazolam reduced the anticancer effect of the chemotherapeutic agent temozolomide in neuroblastoma cells, where subsequent coincubation with midazolam amplified the toxicity of temozolomide on HCC cells.[15] This cytotoxicity of midazolam extended to affect the normal cells as well. Midazolam demonstrated cytotoxicity by activating apoptosis in primary rat neuron cells[9] and necrosis in normal human oral cells.[10]

Another proposed mechanism of the demonstrated inhibitory effects of midazolam on cancer progression may be related to the tumour microenvironment, which has been identified as a complex network of cells, including cancer, immune and stromal cells, cytokines, and extracellular matrix components.[33] Cytotoxic T lymphocytes (CTL) constitute a major anti-tumour component in the tumour microenvironment. CTL suppresses tumour growth by inducing apoptosis, necrosis, and interferonγ-dependent cell-cycle arrest.[34] Kang *et al*. examined the effects of midazolam on HCC microenvironment. By experimenting on both HCC cell lines and a C57BL/6 mouse model, Kang *et al*. demonstrated that midazolam downregulated the expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB pathway), increased concentration of cytokines interferon-gamma and tumour necrosis factor α (TNF α), and lowered the concentration of CD8+ T-cell fatigue markers.[25] This finding contradicts an earlier study by Kushida *et al*., who postulated that

the microenvironment surrounding immune cells substantially influences the nature of developing immunity against cancer. Therefore, cytotoxic T lymphocyte-mediated destruction is essential in developing anti-tumour immunity. In that study, midazolam was found to not affect CTL activity *in vitro* in mouse spleen cells.[4]

In addition, midazolam seems to enhance the sensitivity of some cancer cells to traditional chemotherapy and immunotherapy. Some studies have shown that midazolam can also enhance the sensitivity of some cancer cells to chemotherapy and immunotherapy. For example, Kang *et al*. demonstrated that midazolam potentiated the efficacy of anti-PD-1 on HCC. Midazolam inhibited proliferation, invasion, and migration by moderating the nuclear factor kappa B (NF-κB) pathway and reducing the exhaustion of CD8+ T cells.[25] Sun *et al*. found that midazolam enhanced the sensitivity of non-small cell lung cancer to cisplatin, a traditional chemotherapy drug, in lung cancer cells. The study revealed that midazolam reduced cisplatin resistance in CR-NSCLC by regulating the miR-194-5p/HOOK3 axis, promoting cell apoptosis, and suppressing cell proliferation.[22]

The study has the following limitations. First, limiting the included studies to those published in English might have led to a lack of capture of essential research findings published in other languages, limiting this review's comprehensiveness. Second, all included studies in this review are experimental (in vitro/ in vivo), which has implications for their relevance in the clinical context. Although the data from our review suggests that tumour cell progression may be inhibited by midazolam, many factors, including the cell line or the animal model, variation of the experimental protocols, and the absence of various confounding factors in the clinical settings, might have affected the outcome of these laboratory studies. The clinical standard definition(s) of progression and survival could not be applied for this review and may limit the valid comparison and conclusion. Among the included studies, the route of midazolam delivery (e.g. intraperitoneal and subcutaneous) was variable, given that some studies were *in vitro* and others were *in vivo*. Thus, it is not easy to properly generalise midazolam's results and actual effect. Long-term use of midazolam at a high dose could also cause side effects, including its potential neuronal cytotoxicity. As a result, additional clinical research is needed to determine the appropriate dosage and assess the efficacy and safety of midazolam in clinical settings, either alone or as an adjunct to conventional chemotherapeutic agents. Moreover, the absence of meta-analysis limits the ability to draw statistically significant conclusions and identify the source of heterogeneity or explore the origin of bias, which could impact the reliability and validity of the results. Therefore, these results should be extrapolated with caution in clinical settings. It is still uncertain how relevant and applicable these effects of midazolam are in clinical settings, especially to patients of cancer who are undergoing surgery.

CONCLUSION

The *in-vivo* and *in-vitro* studies pointed out that using midazolam may be associated with anticancer modulations, including delaying cancer progression and decreasing cancer cell survival. Future studies should investigate the mechanisms underlying the observed effects of midazolam on tumour growth and survival, as well as the potential interactions with other cancer treatments. In addition, clinical trials should be conducted to evaluate the impact of midazolam on cancer outcomes in patients undergoing cancer surgery or other related procedures.

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Conflicts of interest
There are no conflicts of interest.

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APPENDIX

Supplementary Files:

1) Supplementary Table 1a: Summary of databases search results

2) Supplementary Table 1b: CINAHL search strategy

3) Supplementary Table 2: PRISMA 2020 check list

Supplementary Table 1a: Summary of databases search results

Database	Number of articles
MEDLINE	514
Embase	338
Web of Science	339
Cochrane Library	365
CINAHL	153
Subtotal	1709
Duplicates	−479
Total	1230

November 07, 2022

Database: OVID Medline Epub Ahead of Print, In-Process and Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present

Search Strategy:

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1. midazolam.mp. or Midazolam/(15956)
2. (versed or buccolam or dalam or doricum or hypnovel or midazo or midazol or nayzilam or ozalin).mp. [mp = title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (1315)
3. 1 or 2 (17192)
4. exp Neoplasms/(3753851)
5. exp Cell Line, Tumor/(569936)
6. (cancer or tumour* or tumor* or malign* or carcinom*).mp. [mp = title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (4024915)
7. or/4-6 (5158354)
8. 3 and 7 (1154)
9. exp Surgical Procedures, Operative/(3473342)
10. exp Preoperative Care/(72581)
11. exp Perioperative Care/(157929)
12. exp Postoperative Period/(61381)
13. (surg* or preoperat* or pre-operat* or perioperat* or peri-operat* or postoperat* or post-operat* or operat*).mp. [mp = title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (4411395)

14. or/9-13 (5719311)
15. 8 and 14 (514)

Embase (old one)

Database: Embase <1974 to 2022 November 07>

Search Strategy:

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1. midazolam.mp. or midazolam/(58509)
2. (versed or buccolam or dalam or doricum or hypnovel or midazo or midazol or nayzilam or ozalin).mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (2824)
3. 1 or 2 (60161)
4. exp neoplasm/(5235111)
5. exp tumor cell line/(425759)
6. (cancer or tumour* or tumor* or malign* or carcinom*).mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (6098849)
7. or/4-6 (6853163)
8. 3 and 7 (8239)
9. exp surgery/(5515579)
10. (surg* or preoperat* or pre-operat* or perioperat* or peri-operat* or postoperat* or post-operat* or operat*).mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (5846592)

11 9 or 10 (7488049)

12. 8 and 11 (5615)

Embase (new one)

Database: Embase < 1974 to 2022 November 07>

Search Strategy:

---

1. exp neoplasm/(5235111)
2. exp tumor cell line/(425759)
3. (cancer or tumour* or tumor* or malign* or carcinom*).mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (6098849)
4. or/1-3 (6853163)
5. exp surgery/(5515579)
6. (surg* or preoperat* or pre-operat* or perioperat* or peri-operat* or postoperat* or post-operat* or operat*).mp. [mp = title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (5846592)
7. 5 or 6 (7488049)

8. 4 and 7 (2237862)
9. *midazolam/(10499)
10. (midazolam or versed or buccolam or dalam or doricum or hypnovel or midazo or midazol or nayzilam or ozalin).ti. (7372)
11. 9 or 10 (10987)
12. 8 and 11 (338)

Web of Science

5. #1 AND #2 AND #3 339
4. #1 AND #2 1,393
3. TS=(surg* or preoperat* or pre-operat* or perioperat* or peri-operat* or postoperat* or post-operat* or operat*) 5,289,746
2. neoplasm* or cancer or tumour* or tumor* or malign* or carcinom* (Topic) 4,485,314
1. midazolam or versed or buccolam or dalam or doricum or hypnovel or midazo or midazol or nayzilam or ozalin (Topic) 35,320

Cochrane Library

Search Name:

Date Run: 08/11/2022 22:03:21

Comment:

ID Search Hits

#1 MeSH descriptor: [midazolam] explode all trees 3250
#2 midazolam or versed or buccolam or dalam or doricum or hypnovel or midazo or midazol or nayzilam or ozalin 13507
#3 #1 or #2 13507
#4 MeSH descriptor: [Neoplasms] explode all trees 90265
#5 MeSH descriptor: [Cell Line, Tumor] explode all trees 390
#6 cancer or tumour* or tumor* or malign* or carcinom* 255736
#7 #4 or #5 or #6 268799
#8 #3 and #7 2057
#9 MeSH descriptor: [Surgical Procedures, Operative] explode all trees 130308
#10 MeSH descriptor: [Preoperative Care] explode all trees 6160
#11 MeSH descriptor: [Perioperative Care] explode all trees 12825
#12 MeSH descriptor: [Postoperative Period] explode all trees 6305
#13 surg* or preoperat* or pre-operat* or perioperat* or peri-operat* or postoperat* or post-operat* or operat* 382829
#14 #9 or #10 or #11 or #12 or #13 417367
#15 #8 and #14 1531
#16 #15 in Trials 365

CINAHL

Supplementary Table 1b: CINAHL search strategy				
#	Query	Limiters/Expanders	Last Run Via	Results
S14	S8 AND S13	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	153
S13	S9 OR S10 OR S11 OR S12	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	1,503,383
S12	TX surg* or preoperat* or pre-operat* or perioperat* or peri-operat* or postoperat* or post-operat* or operat*	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	1,289,726
S11	(MH 'Post-operative Care') OR (MH 'Post-operative Period')	Search modes – Boolean/ Phrase	Interface – EBSCOhost Research Databases Search Screen – Advanced Search Database – CINAHL	36,085
S10	(MH 'Pre-operative Care') OR (MH 'Pre-operative Period')	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	25,444
S9	(MH 'Surgery, Operative+')	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	746,827
S8	S3 AND S7	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	459
S7	S4 OR S5 OR S6	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	987,732
S6	TX cancer or tumour* or tumor* or malign* or carcinom*	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	849,544
S5	(MH 'Cell Line, Tumor')	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	19,586
S4	(MH 'Neoplasms+')	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	642,377
S3	S1 OR S2	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	5,042
S2	TX versed or buccolam or dalam or doricum or hypnovel or midazo or midazol or nayzilam or ozalin	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	757
S1	(MH 'Midazolam') OR 'midazolam'	Search modes - Boolean/ Phrase	Interface - EBSCOhost Research Databases Search Screen - Advanced Search Database - CINAHL	4,308

Supplementary Table 2: PRISMA 2020 check list			
Section and topic	**Item #**	**Checklist item**	**Location where item is reported**
TITLE			
Title	1	Identify the report as a systematic review.	PROSPERO
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Reviewed
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Proposal
Objectives	4	Provide an explicit statement of the objective (s) or question (s) the review addresses.	Proposal
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Proposal
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Proposal
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Summary of Search Strategies
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Proposal/ PROSPERO
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Proposal/ PROSPERO
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Tables 1 & 2
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Manuscript
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool (s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	N/A
Effect measures	12	Specify for each outcome the effect measure (s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	N/A
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Proposal
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Proposal
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Proposal/ PROSPERO
	13d	Describe any methods used to synthesize results and provide a rationale for the choice (s). If meta-analysis was performed, describe the model (s), method (s) to identify the presence and extent of statistical heterogeneity, and software package (s) used.	Proposal
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Proposal
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Proposal

Contd...

Supplementary Table 2: Contd...			
Section and topic	**Item #**	**Checklist item**	**Location where item is reported**
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Proposal
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Proposal
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	PRISMA Flow Diagram
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	PRISMA Flow Diagram
Study characteristics	17	Cite each included study and present its characteristics.	Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	N/A
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Results
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Results
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Results
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Results
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Results
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Discussion
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Discussion
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Discussion
	23b	Discuss any limitations of the evidence included in the review.	Discussion
	23c	Discuss any limitations of the review processes used.	Discussion
	23d	Discuss implications of the results for practice, policy, and future research.	Discussion
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Abstract/ Methods
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Methods
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	PROSPERO
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	N/A
Competing interests	26	Declare any competing interests of review authors.	N/A
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Manuscript

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