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Review

Systematic Reviews and Quality Appraisal of *In Vitro* Cancer Studies: Investigation of Current Practice

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Abstract. *Background/Aim: To assess the quantity and quality of systematic reviews of in vitro cancer studies. Materials and Methods: PubMed, MEDLINE, Embase, Web of Knowledge and PROSPERO databases were searched. Articles described as systematic reviews of in vitro studies, focused on or relevant to cancer and published in English were selected and appraised using an adapted version of AMSTAR 2 'critical domains'. Results: From 4,021 records, 41 reviews described as systematic and cancer-related were identified. Publication dates indicate increasing frequency of systematic review conduct. Mean number of databases searched was three (range=1-8). Thirty-six reviews (88%) reported search methods, 35 (85%) specified inclusion criteria, 26 (63%) reported study selection methods, and 21 (51%) used reporting guidelines. Only 13 reviews (32%) involved formal quality assessment. Conclusion: Detailed investigation of reviews of cancer-relevant in vitro studies indicates need for further development and use of robust search strategies, appropriate quality assessment tools, and researchers with relevant skills.*

Systematic methods for collating research have been used to answer specific questions since James Lind published *A Treatise on the Scurvy* in 1753 (1). The rise of clinical epidemiology and evidence based medicine have encouraged and necessitated the application of research results into clinical practice, with Archie Cochrane's seminal text *Effectiveness and Efficiency* (1972) having made a significant impact (2). To ensure practice based on 'best evidence', research was to be identified, selected and assessed using systematic methods that were transparent, objective and reproducible. The first set of systematic reviews focused on pregnancy and childbirth but the scope quickly expanded to include a wide range of health interventions. In most cases, systematic reviews collate, analyse and synthesise (either narratively or statistically, as in meta-analysis) the results of randomised controlled trials (RCTs) (3). The results of a series of RCTs that individually may not have provided conclusive results on whether particular interventions were effective could, when combined, achieve clear answers to such questions.

Alongside the increasing scope of systematic reviews (and meta-analyses), there has been a considerable development in techniques and tools to support their conduct. Thus, reporting tools such as PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (4) were developed and are now widely used, while training programmes, checklists and criteria exist to support health professionals in reading and appraising research (5). Together with these initiatives, developments have taken place in statistical techniques to enable increasingly sophisticated approaches to combining the results of different trials (6).

While far less extensive than the developments in clinical/human research, similar initiatives have been established in other fields of research. In animal research, the CAMARADES initiative (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) has facilitated international, collaborative approaches to collating data and a supportive framework for groups involved in such work (7). Reporting guidelines for *in vivo* studies have also been published (8) in toxicology, and the Evidence Based Toxicology Collaboration is currently undertaking a number of projects including advocating use of systematic methods in conducting chemical risk assessments (9).

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Table I. *Search strategies for each database.*

Database	Search terms and limits
MEDLINE	(TI systematic review) AND (MH *In vitro* Techniques OR TI *In vitro*)
PubMed	((("Neoplasms"[Mesh] or cancer or tumor or tumour)) AND ((("*In vitro* Techniques"[Mesh]) OR "*in vitro*"[Title/Abstract]) AND systematic[sb])
Embase	((systematic review).m_titl.) AND *in vitro* study/
Web of Knowledge (Databases: WOS, BIOSIS, KJD, RSCI, SciELO).	TITLE: (*in vitro*) AND TITLE: (systematic review) AND TOPIC: (cancer)
PROSPERO	Vitro (limited to Health Area: cancer)

KJD: Korean Journal Database; MeSH/MH: medical subject heading; RSCI: Russian Science Citation Index; sb: subset; SciELO: Scientific Electronic Library Online; TI: title; WOS: World of Science.

It has previously been claimed that adherence to reporting guidelines in systematic reviews of *in vitro* studies is generally poor (10). Reporting assessed against the PRISMA tool resulted in a mean overall quality score of reported items of 68%. The authors scored the papers on whether or not specific items in the PRISMA checklist were reported but not on precisely what methods were used (*i.e.*, they assessed whether databases and quality criteria were reported but not which criteria were used or which databases were searched). While criteria for assessment of the quality of studies is crucial for unbiased, reliable reviews of the research literature, assessment of relevance of the technique or method employed is also a key element. Increasing numbers of systematic reviews of *in vitro* studies over the previous 10 years were noted but reporting did not appear to improve over this period.

While these findings are important to the field, only systematic reviews published up to 2016 were included. In addition, a wide range of research areas was covered by the included systematic reviews, with at least 50% relating to dental or orthodontic research. There exists a need for detailed investigation of contemporary use of the systematic review approach in the cancer field due to specific challenges in representing cancer in *in vitro* models and in conducting human relevant *in vitro* cancer research. The current investigation addresses this gap. It is also crucial that systematic reviews are conducted effectively and appropriately to the field to reveal unnecessary replication and/or poor research in the field so that optimal use is made of future resources.

This review uses systematic methods to analyse the prevalence, focus, and methods used in publications presented as systematic reviews of *in vitro* studies in cancer or of particular relevance to the cancer field.

Materials and Methods

The current investigation involved a systematic analysis of current practice in reporting, evidence identification, and quality assessment in systematic reviews of *in vitro* studies relating to cancer.

Search and screening methods. Searches were carried out for systematic reviews involving *in vitro* methods in the field of cancer or with relevance to cancer. Databases searched included PubMed, MEDLINE, Embase, Web of Knowledge, and the PROSPERO International Prospective Register of Systematic Reviews. Searches were conducted between April and July 2018 and updated in December 2019. PubMed, MEDLINE and Embase were searched again in April 2021. Search terms are shown in Table I. Search results were downloaded into Excel and Abstrackr. Resulting titles and abstracts were screened for relevant articles with reference to inclusion/exclusion criteria. Screening of all records was conducted by at least two of three reviewers (KP, MB, and GP) working independently, with disagreements resolved through discussion or by consultation with a third reviewer. All study titles were screened for potential relevance to cancer research and potentially relevant articles checked for specific reference to cancer by searching the full-text. The purpose of this additional screen was to identify reviews of generic relevance to *in vitro* cancer research that were not explicit in title/abstract descriptions or indexing.

Inclusion/exclusion criteria. Articles were included that met all of the following criteria:

- Title/abstract/article indicating a systematic review
- Included studies using *in vitro* research methods and/or an article described as a systematic review of *in vitro* studies
- Focus on cancer or relevant to cancer research (and including a discussion on relevance)
- Published in English

Articles that were not described by the authors as a systematic review, included other research methods (*e.g.*, review of *in vivo* plus *in vitro* research), had no obvious or direct relevance to the cancer field or were published in languages other than English were excluded.

Data extraction and appraisal. Once relevant studies were identified, information was extracted on to a template. This included: area of focus, aim, whether the PRISMA framework was used, other guidelines or quality assessment criteria used, whether these frameworks were adapted or amended for assessment of *in vitro* studies, databases searched, strategy and search terms reported, inclusion criteria, screening and selection process, data extraction process and method of synthesis. Data was extracted by one author (MB or KP), and checked for accuracy by a second author (KP or GP).

Appraisal of each systematic review was carried out using a set of criteria originally developed for clinical studies. Based on

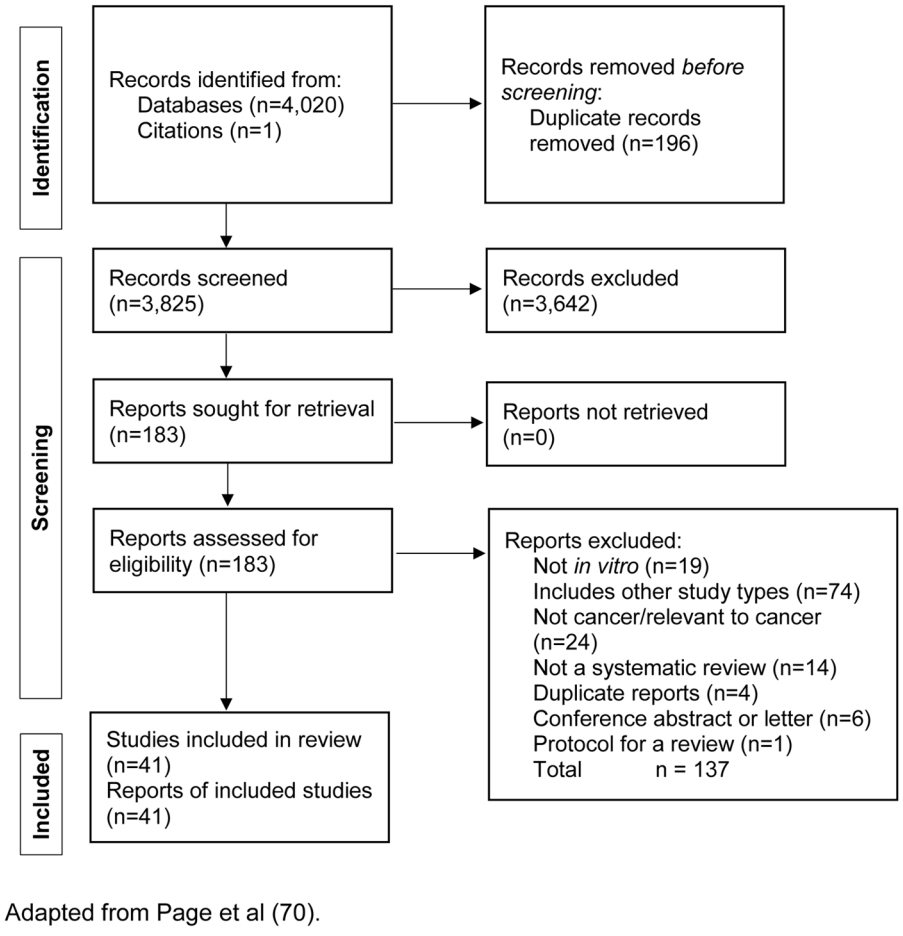

Figure 1. *PRISMA flow diagram for identification and screening of studies for review. Adapted from Page et al. (70).*

AMSTAR (A MeaSurement Tool to Assess systematic Reviews) 2 'critical domains' (11), the following were assessed:

- adequacy of the literature search;
- justification for excluding individual studies by assessing if inclusion criteria were reported;
- risk of bias from individual studies being included in the review;
- effect on the interpretation of the results (by checking whether the quality of included studies had been assessed and if the result and impact of this assessment was referred to in the abstract);

We also recorded whether screening, selection and data extraction were appropriately done (including whether at least two researchers had been involved in each stage). Pre-registration of a protocol prior to commencing the review was not assessed, as there is currently no facility to register systematic reviews other than those involving human or animal studies (12). Publication bias was also not possible to assess.

Results

A total of 4,021 records were retrieved by the searches; after removal of duplicates (records in more than one database that related to the same paper), 3,825 remained, which were screened with reference to the inclusion/exclusion criteria. One hundred and eighty-three records were selected as possibly relevant. Two researchers (two of MB, KP and GP) independently assessed the full text of the papers. Of these, 41 were finally included (13-53). Figure 1 shows the screening process. Table II provides a summary of the basic characteristics. The assessment of the main methods and reporting of the reviews is presented in Table III.

Publication dates and journal of publication. The publication dates indicated an increasing number of systematic reviews of *in vitro* studies in recent years with 21 published since 2019, 12 between 2016 and 2018, 4 in the previous 3-year period and only 4 prior to 2013. Reviews were published in the following types of journals: cancer-specific (10 reviews); pharmacology/toxicology/pharmaceutics related (4 reviews); general journals with broad scientific and medical scope (5 reviews); with the remainder published in a wide range of specialist journals.

Table II. *Focus and aim of included systematic reviews.*

Authors	Area of focus	Aim (page on which aim is stated)
Asweto *et al*. 2017 (13)	Generic	'(To) summarize(s) the apoptosis signal pathways and some ligands involved in SiNPs induced apoptosis, and provides the current evidence of SiNPs induced apoptosis mechanisms' (p. 192)
Barabadi *et al*. 2019a (14)	Colorectal	'To evaluate the anticancer potential of biologically synthesized AuNPs against colorectal cancer cells' (p. 652)
Barabadi *et al*. 2019b (15)	Lung	'To evaluate the anticancer potential of biologically synthesized AuNPs against lung cancer' (p. 323)
Barabadi *et al*. 2020 (16)	Hepatic	'To evaluate the anticancer activity of biogenic AuNPs against hepatic cancer cells' (p. 5) (and the underlying molecular mechanisms)
Batista-Napotnik *et al*. 2016 (17)	Generic	'To review published results in a systematic and comprehensive way... statistical analysis of published results to determine whether nsEP of different durations affect cells differently' (p. 2)
Brown *et al*. 2021 (18)	Breast	'To summarize the direction and magnitude of effect sizes reported in the existing body of literature that explore the role of exercise on modulating breast cancer progression *in vitro* only' (p. 254)
Bus *et al*. 2012 (19)	Oesophageal	'To review the literature on cell lines and incubation conditions for studying BE development' (p. 149)
Cardoso-Pavan *et al*. 2015 (20)	Head and neck	'To verify the *in vitro* anti-tumour effects of statins on head and neck squamous cell carcinoma' (p2) Note: both *in vitro* and *in vivo* studies were included
Chew *et al*. 2020 (21)	Thyroid	'To summarize how thyroid *in vitro* culture models have evolved and highlight how *in vitro* models have been fundamental to thyroid cancer research' (p. 1)
da Silva *et al*. 2020 (22)	Generic	'To conduct a systematic review regarding the use of PBM in tumoral cells, addressing the different types of lasers and parameters used' (p. 524)
de Campos *et al*. 2018 (23)	Cervical	'To identify validated reference genes currently used to normalize RT-qPCR data in cervical cancer cell lines' (p. 139)
Deng *et al*. 2016 (24)	Breast	'To evaluate the diagnostic accuracy of Raman spectroscopy system in the detection of malignant breast lesions' (p. 1) Note: described as review of *in vitro* but *ex vivo* and *in vivo* studies included
Doktorovova *et al*. 2014 (25)	Generic	'To compare the published results' (p. 2) (of *in vitro* studies on lipid nanoparticle carriers)
Gianfredi *et al*. 2017a (26)	Breast	'To review the mechanisms whereby the epigenetic modifications induced by bioactive dietary compounds will impact cancer cell survival' (p. 975)
Gianfredi *et al*. 2017b (27)	Breast	'To evaluate the effect of SFN and EGCG on breast cancer (BC) cells cultured *in vitro*' (p. 126)
Gizzo *et al*. 2015 (28)	Endometrial	'To evaluate *in vitro* mechanisms of actions of RAL on normal (premenopausal human endometrium) and neoplastic endometrial-derived cell lines (Ishikawa cell line) to explain *in vivo* endometrial effects of RAL' (p. 498)
Graybill *et al*. 2017 (29)	Various	'To provide a comprehensive analysis of published studies which details all cancerous tissues tested for orexin receptor expression and the effects of orexin stimulation of these receptors…' (p. 2)
Gupta *et al*. 2019 (30)	Generic	'To present a systematic compilation of the *in vitro* cytotoxic and anticancer properties of various N-substituted isatins and illustrates their mechanism of action to overcome MDR by acting as microtubule-destabilizing agents.'(p. 1)
Hattori *et al*. 2019 (31)	Sarcoma	'To understand the present status of sarcoma cell lines and identify their current challenges, we systematically reviewed reports on sarcoma cell lines' (p. 1)
Kirkegaard *et al*. 2017 (32)	Colorectal	'To identify studies describing *in vitro* models used to investigate cancer cell growth/proliferation, cell migration, cell invasion and cell death of serum taken pre- and postoperatively from patients undergoing colorectal tumor resection' (p. 1)
Laaksonen *et al*. 2010 (33)	Oral	'To review current knowledge on the effects on EMD on oral tissues, particularly with respect to carcinoma' (p. 2)
Ling *et al*. 2021 (34)	Generic	'To reveal whether TiO2-NPs cause genotoxicity *in vitro*' (p. 2057)
Malinowski *et al*. 2020 (35)	Generic	'in this systematic review recent evidence of metformin influence on miRNAs and CSCs regulation in solid tumors will be discussed and summarized' (p. 2)
Manfroi *et al*. 2020 (36)	Thyroid	'Evaluate data on the expression, location, and activity of GPER1, as well as mechanisms mediating its effects in the thyroid' (p. 2)
Mesas *et al*. 2021 (37)	Colon	'To analyze the antitumor activity of the bioactive components present in extracts from Solanaceae and Cucurbitaceae families using different in *in vitro* models of colon cancer' (p. 1)
Mondadori *et al*. 2020 (38)	Generic	'To provide an exhaustive summary of the advanced microfluidic 3D models that have been designed to study the extravasation of cancer and immune cells…' (p. 1)
Muller (Mueller) 2009 (39)	Generic	'reevaluation of the ability of various genetic toxicology *in vitro* tests to predict rodent carcinogenicity and proposed changes to international guidelines governing their use' (p. 131)

Table II. *Continued*

Table II. *Continued*

Authors	Area of focus	Aim (page on which aim is stated)
Orange *et al*. 2020 (40)	Generic	'To systematically review and meta-analyze the effects of acute exercise-conditioned serum on cancer cell growth *in vitro*' (p. 2)
Peltanova *et al*. 2019 (41)	Head and neck	'focus on describing these major subpopulations of (immune) cells and other factors influencing the TME and will discuss their function in the development of cancer, in particular HNSCC' (p. 1)
Prashanth *et al*. 2021 (42)	Brain	'To determine whether *in vitro* models can replace *in vivo* models to assess the brain permeability of novel drugs for brain cancer' (p. 1)
Raj *et al*. 2021 (43)	Generic	'To assess the effect of human MSC derived conditioned media (CM) on human cancer cell lines' (p. 1)
Raju *et al*. 2017 (44)	Generic	'To review original research articles using cancer cell lines as a tool to understand carcinogenesis and to identify the genes involved in tumour development' (p. 2329)
Raphaelli *et al*. 2020 (45)	Skin	'To investigate the protective role of fruit phytochemicals against melanoma skin cancer from *in vitro* studies.' (p. 1009)
Rego *et al*. 2017 (46)	Head and neck	'To summarize the available literature on the *in vitro* anti-tumour effects of metformin on HNSCC' (p. 554)
Rotelli *et al*. 2015 (47)	Colorectal	'To evaluate the *in vitro* effects (of the Mediterranean diet) on colorectal cancer cell lines' (p. 145)
Salamanna *et al*. 2016 (48)	Generic	'To summarise the current status of advanced and alternative 3D *in vitro* bone metastases models' (p. 44803)
Saravanan *et al*. 2020 (49)	Breast	'To evaluate the effectiveness of biogenic AuNPs for the treatment of breast cancer and their anticancer molecular mechanisms through *in vitro* studies' (p. 3577)
Stordal *et al*. 2009 (50)	Generic	'To determine which genetic modifications in cell lines have created the inverse cisplatin/paclitaxel resistance phenotype' (p. 355)
Tripodi *et al*. 2021 (51)	Skin	'This review systematically examines the current evidence describing the effects of PBM on dermal fibroblasts *in vitro*'. .. 'to collate …, to summate the effects..., to analyze the strengths and weaknesses of the existing literature' (p. 2)
Zhurakivska *et al*. 2018 (52)	Generic	'To summarize the data available in the scientific literature concerning the *in vitro* activity of FWGE on malignant cells' (p. 1)
Ziółkowska-Suchanek 2021 (53)	Lung	'To summarize the data in the field of NSCLC tumor hypoxia, including biology, biomarkers, *in vitro* and *in vivo* studies and hypoxia imaging and detection' (p. 2) Note: title indicates *in vitro* only

AuNP: Gold nanoparticles; BE: Barrett's esophagus; CSC: cancer stem cell; EMD: Emdogain; FWGE: fermented wheat germ extract; HNSCC: head and neck squamous cell carcinoma; MDR: multidrug-resistant; MSC: mesenchymal stem cell; NSCLC: non-small cell lung cancer; nsEP: nanosecond electric pulses; PBM: photobiomodulation; RAL: raloxifene; SiNPs: silica nanoparticles; TiO2-NPs: titanium dioxide nanoparticles; TME: tumor microenvironment; p.: page number.

Areas of focus. Table II shows the areas of focus for included reviews. Most frequently, the reviews focused on multiple cancers or had generic relevance (16 reviews). Where the type of cancer was specified, breast (5 reviews) (18, 24, 26, 27, 49) and colorectal (4 reviews) (14, 32, 37, 47) were most frequent, followed by head and neck (3 reviews) (20, 41, 46), lung (2 reviews) (15, 53), skin (2 reviews) (45, 51) and thyroid cancers (2 reviews) (21, 36), with single reviews on remaining cancers. Based on the stated aims, a range of aims were described including analysing, summarising, investigating, identifying, categorising, evaluating, verifying and comparing research on the specific topic.

Use of reporting guidelines. Twenty of the reviews claimed to be reported according to PRISMA guidelines and a further nine used the PRISMA flowchart (or similar) to show the process of selecting studies for inclusion. The remaining 12 did not mention PRISMA although one mentioned the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (STROBE) (65). Of the 21 reviews published since 2019, 16 referred to PRISMA while 5 did not.

Databases searched. A range of databases was used to identify relevant studies to include in the systematic reviews. Four reviews did not report databases searched (30, 39, 41, 53). The three databases most frequently utilised were PubMed and/or MEDLINE (n=37, 90%), followed by Embase (n=18, 44%) and Web of Science/Web of Knowledge (n=16, 39%). Other databases reported to have been used in more than one review also included Scopus (13), Science Direct (8), Proquest (4), EBSCO (3), Google Scholar (3), CINAHL (2) and LILACS (Latin American and Caribbean Health Sciences) database (2). Several reported resources are publishers' repositories rather than true bibliographic databases (that is, they are platforms

Table III. *Analysis of the review methods and reporting.*

Authors	Reported as per PRISMA	Guidance or criteria used to assess quality	Databases searched	Reporting of search (any specific *in vitro* search terms)	Inclusion criteria	Screening and selection process reporting	Data extraction process reporting	Synthesis of results	Reference to quality in conclusions
Asweto *et al.* 2017 (13)	No*	Guidelines for assessing *in vitro* studies (54)	WoS, PubMed, Embase, CNKI	Strategy and terms reported	Reported	People, process, numbers of articles	People, process	Narrative plus tables	No
Barabadi *et al.* 2019a (14)	Yes	Not reported	Cochrane, WoS, ProQuest, PubMed, Embase, Scopus, Science Direct	Terms only reported (cell line*)	Reported	Process, numbers of articles	Numbers of people, process, data	Narrative plus tables	NA
Barabadi *et al.* 2019b (15)	No*	Not reported	Cochrane, WoS, PubMed, Scopus, Science Direct, ProQuest, Embase	Terms only reported (cell line*)	Reported	Process, numbers of articles	Data	Narrative plus tables	NA
Barabadi *et al.* 2020 (16)	No*	Not reported	PubMed, Scopus, Embase, WoS, Science Direct, ProQuest, Cochrane	Terms only reported (cell line*)	Reported	Numbers of people, process, numbers of articles	Numbers of people, process, data	Narrative plus tables	NA
Batista-Napotnik *et al.* 2016 (17)	No*	Risk of bias based on Cochrane criteria (55) (Note: 2008 version cited)	Science Direct, IEEE Xplore, SpringerLink, WoS, HighWire Press, Compendex, IngentaConnect, PubMed	Strategy and terms reported	Reported	Process, numbers of articles	People, process, data	Narrative, tables and meta-analysis	No (but high risk of bias studies excluded from statistical analysis)
Brown *et al.* 2021 (18)	Yes	NTP OHAT tool (56) and criteria from (57)	MEDLINE, PubMed, EBSCO, Cochrane, WoS, BioMed Central	Terms only reported (*in vitro*)	Reported	People, process, numbers of articles	Numbers of people, process, data.	Narrative, tables and meta-analysis	No
Bus *et al.* 2012 (19)	No	Not reported	PubMed, Cochrane library, Embase	Terms only reported (cell line, culture)	Reported	Numbers of articles only	Not reported	Narrative plus tables	NA
Cardoso-Pavan *et al.* 2015 (20)	Yes	GRADE (58) (adapted for *in vitro*)	Cochrane, MEDLINE, Embase, LILACS, PubMed	Strategy and terms reported.	Reported	People, process, numbers of articles	People, process, data	Narrative and tables	No
Chew *et al.* 2020 (21)	No*	Not reported	Embase, MEDLINE and PubMed, Google scholar	Strategy and terms reported (*in vitro*)	Reported	Numbers of articles only	Not reported	Narrative and tables	NA
da Silva *et al.* 2020 (22)	Yes	Not reported	PubMed/ MEDLINE, Embase, Cochrane Library	Strategy and terms reported.	Reported	Numbers of people, process, numbers of articles	Numbers of people, process	Narrative and tables	NA
de Campos *et al.* 2018 (23)	No	Not reported	PubMed	Strategy and terms reported.	Reported	Numbers of articles only	Not reported	Narrative and tables	NA
Deng *et al.* 2016 (24)	Yes	Risk of bias using (QUADAS-2) (59)	PubMed, Embase	Strategy and terms reported.	Reported	Process, numbers of articles	Numbers of people, data	Narrative, tables and meta-analysis	No

Table III. *Continued*

Table III. *Continued*

Authors	Reported as per PRISMA	Guidance or criteria used to assess quality	Databases searched	Reporting of search (any specific *in vitro* search terms)	Inclusion criteria	Screening and selection process reporting	Data extraction process reporting	Synthesis of results	Reference to quality in conclusions
Doktorovova *et al*. 2014 (25)	No	Not reported	PubMed, ISI Web of Knowledge	Strategy and terms reported.	Not reported	Not reported	Reported data	Narrative and tables	NA
Gianfredi *et al*. 2017a (26)	Yes	Not reported (mentions quality but used as exclusion criteria)	PubMed, Scopus	Strategy and terms reported.	Reported	People, process, numbers of articles	Not reported	Narrative	NA
Gianfredi *et al*. 2017b (27)	Yes	Not reported (except for: tested *in vitro* at least in triplicate; clear description of study design and findings)	PubMed/ MEDLINE, Scopus	Strategy and terms reported.	Reported	People, process, numbers of articles	People, process, data	Narrative, tables and meta-analysis	No
Gizzo *et al*. 2015 (28)	No	Not reported (except for generic statement on reporting and data)	MEDLINE, Embase ScienceDirect, Cochrane Library	Terms only reported (*in vitro* activity)	Partially reported	Numbers of articles only	Not reported	Narrative and tables	No
Graybill *et al*. 2017 (29)	No*	Not reported (except: assessed for potential bias based on funding or author affiliations)	PubMed, EBSCO	Strategy and terms reported.	Reported	Process, numbers of articles	Not reported	Narrative and tables	No
Gupta *et al*. 2019 (30)	No	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Narrative (tables do not refer to studies)	NA
Hattori *et al*. 2019 (31)	No	Not reported	Cellosaurus (cell line database); PubMed	Reported for Cellosaurus only	Reported for cell lines only	Numbers of articles only	For cell lines only	Narrative (tables do not refer to studies)	NA
Kirkegaard *et al*. 2017 (32)	Yes	Newcastle-Ottawa quality assessment (60) (adapted)	PubMed, Embase	Strategy and terms reported (cell line)	Reported	People, process, numbers of articles	Not reported except for quality assessment	Narrative and tables	No
Laaksonen *et al*. 2010 (33)	No	Not reported	PubMed, Embase	Strategy and terms reported.	Reported	Numbers of articles only	Not reported	Narrative (tables do not refer to studies)	NA
Ling *et al*. 2021 (34)	No*	ToxRTool checklist (61)	PubMed, WoS, Embase, CNKI, Wan Fang, VIP, CBM	Strategy and terms reported	Reported	Process, numbers of articles	People, process, data	Narrative, tables and meta-analysis	No
Malinowski *et al*. 2020 (35)	Yes	Risk of bias and reporting using criteria from (62)	PubMed, MEDLINE	Strategy and terms reported	Reported	People, process, numbers of articles	People, process, data	Narrative and tables	Yes (states studies had low risk of bias)
Manfroi *et al*. 2020 (36)	Yes	States that quality not rated because of differences in study designs	PubMed, Scielo, Cochrane Library	Strategy and terms reported.	Reported	Numbers of people, process, numbers of articles	Not reported	Narrative and tables	NA

Table III. *Continued*

Table III. *Continued*

Authors	Reported as per PRISMA	Guidance or criteria used to assess quality	Databases searched	Reporting of search (any specific *in vitro* search terms)	Inclusion criteria	Screening and selection process reporting	Data extraction process reporting	Synthesis of results	Reference to quality in conclusions
Mesas *et al*. 2021 (37)	Yes	2-part questionnaire: 1) filter questions for *in vitro* 2) quality of the study	PubMed, SCOPUS, WoS, Cochrane Library	Strategy and terms reported.	Reported	People, process, numbers of articles	People, process, data	Narrative and tables	No
Mondadori *et al*. 2020 (38)	No*	Quality and risk of bias assessed by adapting methods from (62) and (63)	PUBMED, Embase	Strategy and terms reported (microfluidic* microscale/ micro-scale* organ-on-a-chip/ organ-on-chip)	Reported	People, process, numbers of articles	People, process, data	Narrative and tables	No
Muller 2009 (39)	No	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Narrative	NA (but mentions ECVAM)
Orange *et al*. 2020 (40)	Yes	Risk of bias using modified RTI tool (64)	PubMed, WoS, Scopus, SportDiscus, CINAHL	Strategy and terms (cell line*, *in vitro*)	Reported	People, process, numbers of articles	People, process, data	Narrative, tables and meta-analysis	Yes
Peltanova *et al*. 2019 (41)	No	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Narrative and tables	NA
Prashanth *et al*. 2021 (42)	Yes	Not reported	PubMed, Embase, MEDLINE, Scopus	Strategy and terms reported (*in vitro* mode*)	Reported	Numbers of articles only	Not reported	Narrative and tables	NA
Raj *et al*. 2021 (43)	Yes	Customized risk of bias tool was formulated	PubMed, SCOPUS, WoS	Strategy and terms reported (*in vitro*)	Reported	People, process, numbers of articles	People, data	Narrative and tables	No
Raju *et al*. 2017 (44)	No (but refers to STROBE (65)	Not reported except for 'evaluation of the methodologies used and assessed the validation of the study results' (p2330)	PubMed, Google Scholar, EBSCO, Science Direct	Terms only reported (cell lines)	Reported	People, process, number of articles	Some data	Narrative and tables	No
Raphaelli *et al*. 2020 (45)	Yes	Not reported	PubMed/ MEDLINE, Bireme, WoS, ScienceDirect.	Strategy and terms reported	Reported	Numbers of people, process, numbers of articles	Data	Narrative and tables	NA
Rego *et al*. 2017 (46)	Yes	GRADE (58)	Cochrane Library, Embase, LILACS, MEDLINE, PubMed	Strategy and terms reported.	Reported	People, process, numbers of articles	People, process, data	Narrative and tables	No
Rotelli *et al*. 2015 (47)	No*	Not reported	MEDLINE-PubMed, Scirus, Google	Strategy and terms reported (*in vitro* studies in human colorectal carcinoma cells)	Reported	Numbers of articles only	Not reported	Narrative (table does not refer to studies)	NA
Salamanna *et al*. 2016 (48)	Yes	Not reported	PubMed, Scopus, Web of Knowledge	Strategy and terms reported**	Reported	Numbers of articles only	Not reported	Narrative and tables	NA

Table III. *Continued*

Table III. *Continued*

Authors	Reported as per PRISMA	Guidance or criteria used to assess quality	Databases searched	Reporting of search (any specific *in vitro* search terms)	Inclusion criteria	Screening and selection process reporting	Data extraction process reporting	Synthesis of results	Reference to quality in conclusions
Saravanan *et al*. 2020 (49)	Yes	Not reported	Cochrane, Scopus, PubMed, WoS, Science Direct, ProQuest, Embase	Terms only reported (cell line*)	Reported	Numbers of people, process, numbers of articles	Numbers of people, process, data	Narrative and tables	NA
Stordal *et al*. 2009 (50)	No	Not reported	MEDLINE	Terms only reported (cell line)	Reported	Not reported	Not reported	Narrative and tables	NA
Tripodi *et al*. 2021 (51)	Yes	States that risk of bias not carried out	PubMed, Embase, CINAHL, SCOPUS, WoS	Strategy and terms reported	Reported	Numbers of people, process, numbers of articles	Data	Narrative and tables	NA
Zhurakivska *et al*. 2018 (52)	Yes	Not reported	PubMed, Scopus, WoS	Strategy and terms reported.	Reported	Numbers of people, process, numbers of articles	People, data	Narrative and tables	NA
Ziółkowska-Suchanek 2021 (53)	No	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Narrative and tables	NA

*Except for flow chart based on PRISMA; **(three dimensional OR 3D OR 3d) AND (culture OR coculture OR model OR system OR *in vitro* culture OR *in vitro* co-culture OR *in vitro* model OR *in vitro* system); CNKI: China National Knowledge Infrastructure; ECVAM: European Centre for Validation of Alternative Methods; GRADE: Grading of Recommendations, Assessment, Development and Evaluations; NA: not applicable; NTP OHAT: National Toxicology Program's OHAT Risk of Bias Rating Tool for Human and Animal Studies; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; QUADAS-2: revised tool for the Quality Assessment of Diagnostic Accuracy Studies; RTI: Research Triangle Institute (RTI) Item Bank for cross-sectional studies; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology; WoS: Web of Science.

for accessing the collections of publications of specific publishers rather than the structured and indexed records from a wide range of publications found in bibliographic databases). The Cochrane Library/CENTRAL was used in 12 reviews, even though this database focuses primarily on systematic reviews of human clinical trials. The mean number of databases searched for all studies was three (range=1-8). Only two reviews involved a search of databases in a language other than English (Chinese) (13, 34) and two covered the Latin American and Caribbean literature (20, 46).

Search strategies. Thirty-six reviews (88%) provided some information on how they had searched for relevant studies, while the remaining five provided no information on this. Of the 36 providing some information, nine provided only a list of search terms with no details on how these were combined as a strategy while both search terms and strategy were reported in the remaining 27 reviews. The strategies ranged from a simple approach in which terms representing the two main concepts were combined, to highly sophisticated strategies making full use of indexing terms, Boolean logic and other search techniques. Few strategies incorporated search terms that were specific for *in vitro* studies. Of those used, the terms *in vitro* (including *in vitro*, *in vitro* mode, *in vitro* model, *in vitro* activity) and 'cell line(s)' were the most frequently applied. Relevant indexing terms used by the National Library of Medicine to index MEDLINE records, such as In Vitro Techniques, Culture Techniques or more specific terms such as Cell Culture Techniques were not used.

Assessment of methods used to produce review. The majority of reviews (35, 85%) reported the inclusion criteria used in selecting studies, with only six not reporting this information. One review only reported cell lines included (31). In 26 reviews, at least minimal information was provided on the process of screening search results while, in 13 reviews, the numbers of records retrieved by the searches and the reviewers carrying out the screening of results were both reported. In a further 9, only the numbers of records were reported and, in the remaining six, information on the screening and selection process was not presented. Thirteen reviews reported the process of data extraction and appraisal including the

personnel involved and the data items extracted. Five reported two of the three items (people, process and data). Six reported only the data items extracted while one just reported cell lines. The remaining 16 reviews provided no details.

Quality assessment criteria. Only 13 reviews (32%) incorporated a formal, structured assessment of the quality of the studies included in the review. Twenty-one reviews made no reference to quality assessment and two stated specifically that quality was not assessed (36, 51). One review reported using quality criteria which were used as exclusion criteria (26) while one or more generic criteria was used to assess studies for inclusion in three reviews (27-29); for example, statements such as "assessed for potential bias based on funding or author affiliations". Two reviews (35, 38) used criteria from previously published systematic reviews and one review (13) drew on criteria compiled from the results of a previously published scoping review on quality of toxicologically relevant studies (54). In two reviews, a customised risk of bias or quality tool was developed for use (37, 43).

Five reviews (17, 24, 34, 44, 46) indicated that the following tools were used without any reported adaptations by review authors: Cochrane risk of bias (55); Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (59); ToxRTool (61); STROBE (65); Grading of Recommendations, Assessment, Development and Evaluations (GRADE) (58) respectively. Authors of three reviews (20, 32, 39) adapted or modified validated criteria for use with *in vitro* studies. These included GRADE (adapted for *in vitro*) (58); Newcastle-Ottawa quality assessment (adapted) (60), modified Research Triangle Institute (RTI) Item Bank for cross-sectional studies (64). In one case, the criteria were based on a combination of two sets of criteria: the National Toxicology Program's Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool for Human and Animal Studies (56) and criteria from a published paper suggesting standards for *in vitro* research (57).

Existing quality assurance criteria were adapted by authors with the intention of increasing their relevance to *in vitro* methods. Adaptation to the GRADE framework was described thus: 'we have classified *in vitro* studies using comparable baselines, comparing them to the RCT studies published by Xiao *et al*. in 2013 using the GRADE method' (20). The authors noted the lack of standardised available criteria for assessment of *in vitro* studies. Adaptation of the Newcastle-Ottawa scale included adding an assessment of *in vitro* data collection methods and if cancer cells were used as the *in vitro* model system (32).

Methods for synthesis of the results. A narrative or descriptive approach was used in all reviews to present the overall results. Tables summarising the included studies (or selected characteristics of these) supplemented this in 35 reviews (85%). Only six attempted a quantitative synthesis (meta-analysis).

Discussion

The aim of this investigation was to assess the quantity and quality of published reviews of *in vitro* studies described as systematic, and to investigate the quality assessment methods employed by review authors. We identified a relatively small number of completed systematic reviews relevant to cancer that focused on *in vitro* methods. This was consistent with expectations based on initial scoping searches.

The aims of the systematic reviews were very varied and, in some cases, it appeared that the main aim was simply to scope the field of research. This is not the main value of the systematic review approach, which has been designed to facilitate an objective, structured assessment of the research to answer a pre-specified question. The techniques that comprise a systematic review, however, such as comprehensive searching for research and clear presentation of the strengths and weaknesses of each study, may well be valuable in helping to collate research, reduce duplication and clearly demonstrate advances (and limitations) in techniques. Nevertheless, for systematic reviews of *in vitro* research to be effective, searching for relevant research must be comprehensive so that there is no bias in the set of studies included or omission of key studies.

The core AMSTAR 2 criteria include 'Adequacy of the literature search' but, while many reviews reported a search strategy, few were optimal or made use of the search facilities available. This suggests that more use could be made of specialist information staff and/or training for researchers in the field. While search strategies (use of AND/OR operators to combine terms, for example) were reported in 36 reviews (88%), most reported generic searches without describing strategies for each database and, therefore, it is not possible to assess if and how changes to the strategies may have affected the results. Given that the average number of databases searched in included reviews was three (range=1-8), this reporting standard was relevant to the majority of cases here considered.

While the majority of researchers carrying out reviews searched either PubMed or MEDLINE, there was limited use of other databases. This includes those with a specific focus on pre-clinical or basic biological research which may be relevant to reviews of studies using *in vitro* methods. At present, much reliance is placed on one source (the MEDLINE database either in this format or via the PubMed interface). Other databases may improve searches: in the clinical field, this is certainly true (66) but may also be the case for *in vitro* research. For example, Embase covers nearly 3,000 journals that are not covered by MEDLINE

(and has been particularly focused on pharmacology and related literature). Further research may be necessary to quantify the effect that exclusion of this and other databases may have on evidence retrieval for *in vitro* reviews. Similarly, few review teams made use of specific index terms as search terms even though these have the potential to improve the efficiency of searching. This step is crucial for ensuring the reviews offer a truly systematic overview of available evidence, and minimise related risks (*e.g.*, unnecessary replication, failure to identify key findings relevant to future study designs, selection bias in papers included in meta-analyses).

It is also recommended that systematic reviews justify including and excluding individual studies by reporting inclusion criteria. The majority (35) did report the inclusion criteria. The number of records retrieved and screened were also reported by many of the reviews and this increases transparency of the searching and selection processes, *i.e.*, how effectively the review team identified the relevant studies. Fewer reviews (18) reported how the studies were treated; little information was provided on what data was extracted from each study and how this was done. The lack of relevant data may be due to limitations in reporting in the original studies. One systematic analysis specifically focused on the challenges of conducting systematic reviews of *in vitro* studies found that insufficient reporting of essential cell culture parameters prevented cross-study comparisons (67).

The most noticeable difference between these reviews and the key indicators of an effective systematic review was in quality assessment of included studies. A previous analysis of systematic reviews of *in vitro* research in a wide range of areas found that only 29.2% (19/65) reported data on risk of bias (quality) for each study (10). In the current analysis, less than half of the reviews carried out any assessment of quality and less than a third included a formal, structured assessment. The lack of widely accepted quality criteria was also very apparent as a wide range of different tools and techniques were used. In some cases, authors produced their own checklist while in others, criteria were adapted from existing checklists in the clinical field.

Criteria have previously been proposed that specifically focused on the *in vitro* methods comprising items including clear delineation of cell viability, cell type, culture medium and constituents (67). Other authors have found it necessary to add their own guiding examples to generic criteria, *e.g.*, for the criterion 'Clear description of research setting', Rahman *et al*. added the following examples: 'Culture set-up, name and type of insert membrane, temperature' (68). Only a minority of reviews used published criteria for assessment of quality in included studies and 13 different sets of criteria were employed. Of particular note were the three reviews in which authors adapted quality assessment criteria developed for use in evaluation of clinical research. The justification given in these cases was that such criteria were not appropriate to *in vitro* studies in their original form. This indicates an area for further investigation with regard to the applicability of more general quality criteria within this field. Lastly, even when quality was assessed, there was minimal reference to this when presenting conclusions. This means that it is not possible to assess the effect on the interpretation of the results.

Limitations in reporting may be linked to the comparatively low use of established reporting criteria for systematic review in the included studies, as poor reporting of systematic reviews of *in vitro* studies has previously been identified (10). While the numbers of such publications had increased over a 10-year period, reporting quality was not significantly improved over the same period. This current analysis found that while there was increasing reference to the PRISMA reporting guidelines over recent years, the potential for significant improvement in reporting was also revealed.

Finally, several reviews were described as systematic even though they exhibited none of the characteristics of such a review. For example, no methods section was present nor were indications of how studies were identified, selected or assessed. It is important that the description systematic review is restricted to those reviews that are conducted according to generally agreed methods.

Recommendations from this review are the following: 1. The description 'systematic review' is restricted to those reviews that include methods for identifying, selecting, and assessing included studies; 2. Searches for studies are carried out on more than one database and make use of well-constructed search strategies; 3. Methods for screening, data extraction, and quality assessment are reported (and preferably involve more than one reviewer/researcher); 4. Criteria for the assessment of *in vitro* research are adopted; 5. Results of the above stages are reported appropriately; 6. Conclusions refer to quality of the included studies.

Further to this, reporting guidelines that are better suited to the *in vitro* field are required. These could take the form of a PRISMA extension, which has been used widely in other fields to adapt these general guidelines for specific contexts (*e.g.*, PRISMA extension for Ecology and Evolutionary Biology), that includes criteria such as: Describe the type of replication unit (*e.g.* individuals, broods, study sites) (69).

Limitations in this review relate to the challenges in identifying studies using *in vitro* methods and in determining relevance to cancer. In order to focus on systematic reviews of *in vitro* studies, we excluded a large number of reviews that discussed *in vitro* studies alongside other study types and this resulted in a small number of reviews for this analysis. To extend the range of reviews assessed, we included those that made specific reference to relevance to cancer. However, this required examination of the full text of each article and further potentially relevant reviews may have been omitted. Lack of

standards for systematic reviews of *in vitro* studies also impacted on assessment of methods, therefore, criteria were adapted from a tool widely used for clinical studies. Nevertheless, the results indicate that there is potential value in the systematic review approach to address a range of pertinent questions: this has already led to international efforts to develop appropriate techniques in the field of animal research and development of the approach now needs to receive a similar level of attention in the cancer *in vitro* field.

This review article indicates that concerns regarding consistency in application of reporting and quality assurance criteria in primary *in vitro* studies are also relevant to reviews of those studies relating to research in cancer. The present study makes several contributions. Firstly, it is, to our knowledge, the first study to provide a review of current practice and reporting standards in systematic reviews of cancer-related *in vitro* studies. It provided further support for findings of an initial exploratory search, that reporting standards for evidence retrieval and quality assessment are highly variable and that lack of common quality assessment criteria for assessment across studies is a limitation for reviewers. It also demonstrates that there is an interest in the use of the systematic review method in the field and that efforts to develop and adapt current approaches to the needs of the *in vitro* field would be of value. Further development of the systematic review approach is now required to optimise its use and value in the *in vitro* field and overcome the current limitations. This will require development and use of robust search strategies, appropriate quality assessment tools and researchers with the relevant skills.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

Karen Pilkington conceived the study and designed the searches. All Authors were involved in the screening and selection process, and data extraction and analysis. Mike Bracher drafted the paper and all authors revised the paper and contributed to the final manuscript.

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