REVIEW ARTICLE

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Impact of CCR5-inhibitors on cancer treatment – a systematic review of preclinical evidence

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Abstract

Introduction and Objective. In view of the role of the CCR5 chemokine receptor in tumour development and progression, researchers have investigated the effects of its inhibition in different types of cancer. Although promising results have been reported, the efficacy, safety, and methodological quality of the studies need to be analyzed before their application in clinical practice. The aim of this review is to evaluate the approaches used to inhibit CCR5 and assess its effects on cancer development. Additionally, the methodological quality of preclinical animal studies aree evaluated.

Review Methods. A systematic review was performed according to PRISMA guidelines retrieving, and analyzing 19 original studies. To analyze the risk of bias and quality of the preclinical studies, the SYRCLE tool (Systematic Review Centre for Laboratory Animal Experimentation) was used.

Brief description of the state of knowledge. Despite the wide methodological variability found in the reviewed studies, some common characteristics were observed. Most experiments (73.68%; n=14) used immunosuppressed mice in their induction models, and the response to CCR5 inhibition was primarily assessed by measuring tumour size. Maraviroc (MVC) was the most frequently used CCR5 inhibitor (73.68%; n=14).

Summary. The results provided significant evidence that CCR5 inhibition is a promising target for cancer treatment. However, by mapping the risk of bias across all investigated studies, this review provides objective support for guiding future research with more rigorous methodologies, ensuring clear evidence of the impact of CCR5 inhibition on cancer development and progression.

Key words

CCR5, cancer, systematic review, chemokine receptor, malignant neoplasms

INTRODUCTION

Several studies have reported the role of chemokines and their receptors in various stages of tumour development and progression [1–3]. In particular, the chemokine receptor CCR5 has emerged as an important focus of study due to its involvement in multiple aspects of tumour growth and progression [4–7].

The CCR5 chemokine receptor belongs to the superfamily of receptors with seven G protein-coupled transmembrane domains that bind a variety of cytokines, including CCL3 (MIP1α), CCL3L1, CCL4 (MP-1β), CCL5 (RANTES), CCL8 (MCP2), CCL11 (Eotaxin), CCL13 (MCP-4) and CCL16 (HCC-4) [8, 9]. The activation of CCR5 modulates the physiological functions of various immune (T lymphocytes, macrophages, eosinophils, myeloid suppressor cells, microglia and dendritic cells) and stromal (fibroblasts, endothelial and adipose cells) cells [10]. CCR5 is also highly expressed in different types of cancer [6,11–14].

Different studies have demonstrated not only the indirect effects of CCR5, such as promoting the migration of various cell types to the tumour micro-environment, but also its direct effects when expressed by cancer cells [4–7, 11–14]. Accordingly, high CCR5 expression has been associated with poor prognosis in patients with breast cancer, Hodgkin lymphoma, pancreatic cancer, and oral cancer [5, 11, 13–14]. This potential role in carcinogenesis has been explored in several studies investigating the impact of CCR5 inhibitors on cancer development [4–7, 11, 14]. Although promising results have been reported across various types of cancer, the efficacy and safety of these treatments – as well as the quality of the studies supporting them – need to be thoroughly evaluated before their application in clinical practice.

Considering the role of chemokine receptors in the pathogenesis and progression of tumour lesions, and that currently available evidence is fragmented, the present study uses a systematic review framework to investigate the impact

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of CCR5 inhibitors on cancer treatment in preclinical animal models. In addition to mapping cancer types and CCR5 inhibitors, the study investigates dosimetry characteristics and the effects of these inhibitors on histopathological, biochemical, and immunological outcomes, as well as tumour progression and survival rates. The methodological quality of all reviewed studies was evaluated, pointing out the main limitations/sources of bias in the accumulated evidence that must be overcome in further investigations.

MATERIALS AND METHOD

Guiding question and definitions. The guiding question was structured considering the PICO (= Problem, I= Intervention, C = Comparison and O = outcome) strategy [15]. Thus, the following guiding question was adopted in the review: Could animals with cancer and treated with CCR5 inhibitors exhibit improved histopathological, biochemical, and immunological outcomes, as well as reduced tumour progression and mortality rates, compared to untreated animals? To answer this question, a structured methodological protocol was defined which was registered in the PROSPERO (International Prospective Register of Systematic Reviews) database (Register No. CRD42023368156).

Search strategy and research algorithm. To retrieve research records, four different electronic databases were used, which consisted of two levels of search: a direct search in electronic databases indirect screening of reference lists of all studies identified in the direct search [16]. The Pubmed/Medline, Scopus, Web of Sciences and EMBASE databases were used in the primary search. A strategy based on specific search algorithms was developed for each database. Chronological limits were not adopted [17]. The complete search strategy and the results found are described in Table S1.

Prisma workflow and records screening. In this review, only animal model studies that investigated the potential effect of CCR5 blockade on tumour development and progression were included. Initially, all search records retrieved in electronic databases were loaded into the Mendeley Reference Management Program (Mendeley, London, UK), which was used to remove duplicates by comparing indexing metadata (e.g., titles, authors, year, volume, edition, publication journal, and doi) of all databases. The complete PRISMA workflow obtained from ther search strategy is presented in Figure 1.

Eligibility criteria and inter-rater agreement. Eligible taken into consideration and inclded comprised preclinical studies, investigations based on specific inhibition of the CCR5 chemokine receptor, cancer studies, investigations of the direct and/or indirect effect of CCR5 inhibition on cancer development. Studies were considered irrelevant and excluded if they exclusively investigated *in vitro* or human systems, were secondary research (e.g. literature reviews, editorials, letters, notes and conference abstracts), grey literature (studies not formally published or peer-reviewed), the absence of an untreated control group, studies with combined treatments where was not possible to isolate the effect of CCR5 receptor inhibition, and studies based on knockdown and/or silencing of the CCR5 gene. All exclusion criteria were equally applied in the primary and secondary search strategies.

Data extraction. Data extraction from pre-clinical *in vivo* studies was categorized as follows: publication characteristics: authors, year of publication, and country in which the study was conducted; experimental model – animal species, lineage, gender and age; specific treatment – CCR5 specific inhibition/blocking drug, concentration, frequency, time and rout of treatment; disease model – only cancer; and reproductive outcomes – inhibition of tumour growth and/or cell proliferation, promotion of cell apoptosis, metastasis inhibition, metastasis remission, tumour size decrease, inhibition of antineoplastic treatment resistance and blockage of angiogenesis.

Risk of bias. The SYRCLE's risk of bias tool was used to assess potential sources of bias in animal studies. This tool is based on the Cochrane Risk of Bias (ROB) tool and originally adjusted for specific aspects of bias that have a relevant impact on animal intervention studies [18]. The overall and individual result obtained from the SYRCLE's strategy was graphically expressed using the Review Manager (RevMan) software, version 5.3 (Fig. 2).

RESULTS

Characteristics of animal models and included studies. From 412 records identified in all electronic databases, 19 relevant studies were recovered in full-text and reviewed, with 14 studies identified in the primary search and 5 from the secondary screening (Fig. 1).

There was a predominance of studies (84%; n= 16) that used mice as an animal model, while only 3 (16%) studies used rats. The main lineage used in these studies was athymic nude mice (T-cell-deficient) (32%; n=6 mice and 11%; n=2 rats). Moreover, 5 (26%) studies reported severe combined immuno-deficiency (T/B/NK cell deficiency and macrophage tolerance for human cells) NOD/SCID mice, 1 (5%) study reported C57 mice, 3 (16%) studies reported Balb/c mice, 1 (5%) study reported using FVB mice, and 1 (5%) study used WAG rats (Tab. 1).

Characteristics of tumour models. Breast cancer (n= 6; 32%) was the most common cancer type in studies, followed by colorectal and prostate cancers (16%; n=3 studies each), and gastric cancers (11%; n=2 studies). Hodgkin's lymphoma, pancreatic, liver, kidney and lung cancer were reported in 1 (5%) study each (Tab. 1). Most studies used the injection of cancer cells by different routes, including subcutaneous (42%; n= 8), intravenous (26%; n= 5), intraperitoneal and intracardiac (5%; n= 1 each). One study (5%) did not specify the route through which the tumour cells were injected. The remaining studies used tumour tissue orthotopic implantation (n=4; 21%) and cancer induction by choline-deficient diet supplemented with ethionine (CDE) (n=1; 5%) (Tab. 1).

In most studies (63%; n=12), the treatment was started immediately after the confirmation of tumour presence. In one specific study, trials were also performed starting treatment 10 days after and 5 days before cancer cell injection. Although the treatment has started after cancer cell injections, the interval between tumour induction and initiation of treatment was quite variable in 6 studies (32%) (Tab. 2). In addition, only 1 study (5%) started treatment 5 days before tumour cell injection (Tab. 2).

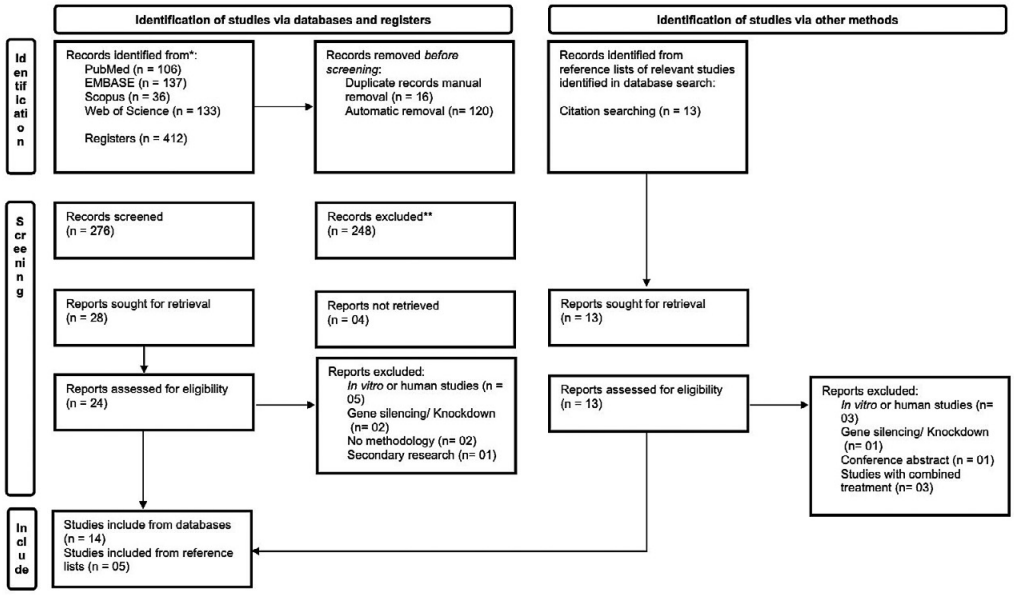

Figure 1. PRISMA flow diagram describing the stages of selection of eligible studies

Table 1. Characteristics of tumor induction models in animals

Author	Specie/Lineage	Age	Sex	Induction model/cell line	Type of cancer
ZHANG et al., 2012	Nude mice	(-)	Male	SC injection of M12 prostate cancer cell line	Prostate cancer
VELASCO-VELÁZQUEZ et al., 2012	NOD/SCID mice	8 weeks	Female	Tail vein injection of bioluminescent human breast adenocarcinoma MB-MDA-231 cells	Breast cancer
MENCARELLI et al., 2013	NOD/SCID mice	8 weeks	Male	IP injection of gastric cancer human cell MKN45 SC injection of gastric cancer human cell lines MKN74 or MKN45	Gastric cancer
OCHOA-CALLEJERO et al., 2013	C57BL/6 mice	5 weeks	Male	Hepatocellular carcinoma model induced by choline-deficient diet supplemented with ethionine in the drinking water	Hepatocellular carcinoma
ARNATT et al., 2013	Nude mice	(-)	(-)	SC injection of M12 prostate cancer cell line	Prostate cancer
SICOLI et al., 2014	FVB mice	12 weeks	Male	SC injection of bioluminescent prostate epithelial cells transformed with the v-Src oncogene at one dorsal flank or into the left ventricle of the heart	Prostate cancer
HALVORSEN et al., 2016	Balb/c mice	8-12 weeks	Female	Orthotopic injection of mammary carcinoma 4T1, 4T07 and 67NR cells into the fourth mammary fat pad.	Lung cancer
TANABE et al., 2016	Balb/c Balb/c-nude	7 weeks	Female Male	Orthotopic injection of murine colon 26 cells Orthotopic injection of human colon KM12C cells	Colorectal cancer
WANG et al., 2017	Nude mice	6 weeks	Male	SC coinjection of human gastric cancer AGS cells and human leukemic monocyte lymphoma U937 cells	Gastric cancer
JIAO et al., 2018	NOD/SCID mice	8 weeks	Female	IC injection of Luc2-expressing breast cancer SUM-159 cells	Breast cancer
NISHIKAWA et al., 2019	KSN/slc nude mice	8-11 weeks	Female	SC coinjection of human colorectal cancer HCT116 cells transfected with CCR5 or empty vector plus human BM-derived MSC cells into the flanks	Colorectal cancer
NIE et al., 2019	NOD/SCID mice	6 weeks	Female	Implantation of patient-derived malignant breast specimens into the mammary fat pads	Breast cancer
CASAGRANDE et al., 2019	Nude micea NSG miceb	4 weeks	Femalea Maleb	Injection of classic Hodgkin lymphoma L-540 cells into the flanka Injection of classic Hodgkin lymphoma L-428 cells into the flankb	Hodgkin lymphoma
PERVAIZ et al., 2019	Nude rats	6-8 weeks	Male	Saphenous artery injection of bioluminescent human breast cancer MDA-MB-231 cells	Breast cancer
HUANG et al., 2020	Nude rats	5-8 weeks	Female	Mesenteric vein injection of bioluminescent human pancreatic Suit2-007 cells	Pancreatic cancer
ZAZO et al., 2020	SCID/beige mice	6 weeks	Female	SC injection of breast cancer BT-474.rT cells into the right flank	Breast cancer
ZHOU et al., 2020	BALB/c mice	4-6 weeks	Female	SC injection of murine renal adenocarcinoma RENCA cells Orthotopic injection of murine renal adenocarcinoma RENCA cells	Renal carcinoma
PERVAIZ et al., 2021	WAG/Rij rats	6-8 weeks	Male	Hepatic portal vein injection of bioluminescent rat colon adenocarcinoma CC531 cells	Colorectal cancer
JIAO et al., 2021	Nude mice	8 weeks	Female	Tail vein injection of bioluminescent human breast adenocarcinoma MB-MDA-231 cells	Breast cancer

(-) Data not reported
(MSC) Mesenchymal stem cells. (BM) Bone marrow. (NOD/SCID) Nonobese diabetic/severe combined immunodeficient. (NSG) NOD/SCID gamma chain deficiente. (FVB) Friend leukemia virus B. (SC) subcutaneous; (IV) Intravenous; (IP) Intraperitoneal; (IC) Intracardiac

Table 2. General characteristics of CCR5 blocking/inhibition interventions performed in the studies

Author	CCR5 inhibitor	Control group	Treated group	Route of administration	Treatment period
ZHANG et al., 2012	Anibamine; Compound 38	Saline solution	0.3 mg/kg of the anibamine or compound 38	IV (lateral tail vein)	1x every 4 days/ 16 days
VELASCO-VELÁZQUEZ et al., 2012	MVC	5% DMSO in acidified water	MVC 8 mg/kg	OG	Test 1: 2x day/ 5 weeks Test 2: 28 days starting from day 10 after the injection of cells Test 3: 5 days before the injection of cells
MENCARELLI et al., 2013	MVC	Not specified	Test 1: MVC 10 mg/kg Test 2: MVC 50 mg/kg	Test 1: IP Test 2: O (?)	Test 1: 7 days starting day 3 after tumor induction Test 2: 12 hours for 20 days starting from day 10 after the injection of cells
OCHOA-CALLEJERO et al., 2013	MVC	Tap water	300 mg/L of MVC in drinking water	O	17 weeks
ARNATT et al., 2013	Compound 18	Saline solution	Compound 18 at a dose of 0.3 mg/kg	IV	1x every four days/ 16 days
SICOLI et al., 2014	MVC	5% DMSO in acidified water	MVC 8 mg/kg	O (?)	12 hours for 5 days before the injection of cells
HALVORSEN et al., 2016	MVC	5% DMSO in acidified water	MVC 31 mg/kg	OG	1x day for 14 days starting from day 10 after the injection of cells
TANABE et al., 2016	MVC	Not specified	MVC 30 mg/kg	O (?)	Test 1: Every 2 days for 15 days starting from day 2 after the injection of cells Test 2: Every 2 days for 28 days starting from day 2 after the injection of cells
WANG et al., 2017	MVC	Not specified	MVC 10 mg/kg	II	Twice weekly for 24 days
JIAO et al., 2018	MVC	5% DMSO in acidified water	MVC 08 mg/kg	OG	2x day/6 weeks
NISHIKAWA et al., 2019	MVC	100 µl PBS with 5% DMSO	MVC 30 mg/kg	IP	1x day for 14 days starting from day 7 after the injection of cells
NIE et al., 2019	MVC	IgG	Test 1: MVC 10 mg/kg Test 2: MVC 03 mg/kg Test 3: MVC 10 mg/kg	IP	Test 1: 2x day/ 2 days Test 2: 1x day/ 40 days(B) Test 3: 1x day/ 40 days(C)
CASAGRANDE et al., 2019	MVC	PBS	MVC 10 mg/kg	IP	Test 1: Every day for 12 days (L-540 cells) Test 2: Every other day for 38 days (L-428 cells)
PERVAIZ et al., 2019	MVC	Not specified	MVC 25 mg/kg	IP	Test 1: 1x day for 4 weeks starting from day 2 after the injection of cells Test 2: 1x day for 3 weeks starting from day 7 after the injection of cells
HUANG et al., 2020	MVC	Autoclaved ddH2O and 100 µl KolliphorR EL	MVC 15mg/kg	IP	1x day/ 21 days
ZAZO et al., 2020	MVC	Human IgG1κ	MVC 10 mg/kg	IP	Every other day for 3 weeks
ZHOU et al., 2020	Anti-CCR5 antibody	Isotype control antibody at a dose of 100 µg	Anti-CCR5 antibody systemically at a dose of 100 µg	Systemical	2x weekly
PERVAIZ et al., 2021	MVC	KolliphorR EL (cremophor EL) as emulsifier (100 µl/ rat) and double distilled autoclaved water was prepared (500 µl/rat)	MVC at 25 mg/kg	IP	1x day for 3 weeks starting from day 2 after the injection of cells
JIAO et al., 2021	Leronlimab; MVC	Not specified	Test 1: leronlimab 2 mg/mouse Test 2: MVC 8 mg/kg Test 3: leronlimab 2 mg/mouse	IP	Test 1: 2x weekly/ 8 weeks Test 2: 2x weekly/ 8 weeks Test 3: 2x weekly/ 30 weeks.

(?) Incomplete information
(MVC) Maraviroc; (IgG) Immunoglobulin G; (ddH2O) double-destilled water; (DMSO) Dimethyl sulfoxide; (PBS) Phosphate-bufferid saline
(SC) subcutaneous; (IV) Intravenous; (IP) Intraperitoneal; (IC) Intracardiac; (II) Intratumoral injection; (O) Oral; (OG) Oral gavage

Characteristics of treatment protocols. Table 2 depicts the main characteristics of treatments protocol in each study. To assess the effects of CCR5 inhibition, most studies (79%; n=15) used MVC. Only 1 (5%) study performed independent trials to evaluate not only MVC, but also Leronlimab effects on CCR5 inhibition in cancer. The treatment was also performed with Compound 18 and anti-CCR5 antibody in 1 (5%) study each. Independent assays with Anibamine and Compound 38 was reported in only 1 (5%) study (Tab. 2). The dose and frequency of treatment were quite heterogeneous. The main route for drug administration was intraperitoneal (42%; n= 8), followed by oral gavage (32%; n= 06) and intravenous route (11%; n=2). Only 1 study (5%) administered the treatment both intraperitoneally and orally. Intra-tumoural injection and systemic routes were reported in 1 (5%) study each (Tab. 2). The vehicle used in the control group was not specified in 5 (26%) studies. In the remaining studies, dimethyl sulfoxide (DMSO) (26%; n=5), saline solution (11%; n=2), water (16%;

Table 3. Macro and microstructural outcomes of the studies included in this systematic review

Author	CCR5-inhibitor	Outcomes
		Macrostructural findings
ZHANG et al., 2012	Anibamine Compound 38	Tumor volume/mm³ (Anibamine) NT: 268.86 ± 45.90 T: 174.87 ± 21.85* (Compound 38) NT: 268.86 ± 45.90 T: 96.17 ± 19.67*
VELASCO-VELÁZQUEZ et al., 2012	MVC	Mice with metastatic tumors (%) NT: 84.69 T: 50.81* Tumor area (μm²) x104 NT: 8.96 ± 1.41 T: 3.43 ± 1.14* Tumor growth - Lung metastasis (x108 p/s/cm²/sr) NT: 9.82 ± 4.87 T: 0.90 ± 0.00 Growth of established metastasis (x109 p/s/cm²/sr) NT: 0.68 ± 0.07 T: 0.74 ± 0.05
MENCARELLI et al., 2013	MVC	Intraperitoneal injection: Peritoneal nodules (MKN45) (Nº) NT: 23.0 ± 2.8 T: 7.2 ± 1.4* Mesenteric nodules (MNK45) (Nº) NT: 13.7 ± 2.4 T: 2.4 ± 0.7* Total volume/mm³ (Peritoneal and mesenteric nodules (MNK45) (Nº) NT: 832.0 ± 59.0 T: 336.0 ± 62.0* Body weight loss (% vs day 0) NT: 7.91 ± 0.83 T: 7.97 ± 1.13
MENCARELLI et al., 2013	MVC	Subcutaneous injection (xenograft model): Volume of Nodule /mm³ (MKN45) NT: 582.36 ± 75.0 T: 366.20 ± 48.52* Volume of Nodule /mm³ (MKN74) NT: 835.71 ± 128.57 T: 442.85 ± 35.71*
OCHOA-CALLEJERO et al., 2013	MVC	After 17 weeks treatment: Survival (%) NT: 33.78 T: 75.34* Body weight (g) NT: 19.72 ± 1.48 T: 24.93 ± 0.74* Liver relative body weight NT: 0.067 ± 0.001 T: 0.063 ± 0.000* Spleen relative body weight NT: 0.011 ± 0.001 T: 0.006 ± 0.001* Number macroscopic tumors NT: 65.82 ± 9.11 T: 18.22 ± 8.10* Tumor max. diameter (mm) NT: 16.50 ± 3.77 T: 2.98 ± 0.78*
ARNATT et al., 2013	Compound 18	Tumor volume/mm³ NT: 269.60 ± 87.91 T: 95.23 ± 41.02*
SICOLI et al., 2014	MVC	Total of body metastasis tumor burden (x109 p/s/cm²/sr) NT: 3.85 ± 1.40 T: 1.65 ± 0.79 Tibia metastasis (x108 p/sec/cm²/sr) NT: 3.93 ± 1.61 T: 1.36 ± 0.49* Brain metastasis (x108 p/sec/cm²/sr) NT: 3.53 ± 1.21 T: 1.63 ± 0.76*
HALVORSEN et al., 2016	MVC	Tumor volume/mm³ NT: 1234.72 ± 77.77 T: 1098.61 ± 68.05
TANABE et al., 2016	MVC	Tumor volume/mm³ (15 days) NT: 154.17 T: 76.04* Tumor volume/mm³ (28 days) NT: 213.15 T: 100.65*
WANG et al., 2017	MVC	Tumor volume/mm³ NT: 3033.81 ± 212.56 T: 1391.30 ± 502.41*
JIAO et al., 2018	MVC	Tumor volume/lung (×107 p/sec/cm2/sr) NT: 10.05 ± 4.4 T: 3.01 ± 1.16
NISHIKAWA et al., 2019	MVC	Tumor volume/mm³ (HCT116-EV + MSCs) NT: 335.29 ± 79.41 T: 220.58 ± 79.41 (HCT116-CCR5 + MSCs) NT: 1051.09 ± 183.94 T: 481.75 ± 157.66
NIE et al., 2019	MVC	Tumor volume/mm3 (at dose 3 mg/kg) NT: 107098.70 ± 22399.72 T: 57399.28 ± 11899.85* (at dose 10 mg/kg) NT: 107098.70 ± 22399.72 T: 14699.82 ± 0.00*
CASAGRANDE et al., 2019	MVC	Median survival (%) (L540 cells) NT: 13 days T: 15.5 days Tumor volume/mm3 (L-540 cells) NT: 880 ± 88 T: 435 ± 75* Tumor volume/mm3 (L-428 cells) NT: 966.82 ± 108.05 T: 369.71 ± 85.30* BODY WEIGHT (g) (L-540 cells) NT: 19.52 ± 1.09 T: 20.98 ± 0.72 (L-428 cells) NT: 24.82 ± 2.15 T: 24.60 ± 1.94

Author	CCR5-inhibitor	Outcomes
		Macrostructural findings
PERVAIZ et al., 2019	MVC	Tumor growth (x109p/s/cm²/sr) (Treatment from 2nd day) NT: 6.71 ± 0.46 T: 2.89 ± 0.44 (Treatment from 7th day) NT: 6.71 ± 0.46 T: 6.53 ± 0.72 Liver weight (g) NT: 13.75 ± 3.03 T: 9.13 ± 1.92
HUANG et al., 2020	MVC	
ZAZO et al., 2020	MVC	Tumor volume/mm² NT: 164.44 ± 11.85 T: 125.18 ± 8.88
ZHOU et al., 2020	Anti-CCR5 antibody	Subcutaneous injection: Tumor volume/mm³ (112 days) NT: 1794.64 ± 196.42 T: 455.35 ± 232.14* Tumor volume/mm³ (140 days) NT: 1782.60 ± 228.26 T: 1728.26 ± 195.65
PERVAIZ et al., 2021	MVC	Tumor growth (x1010 p/s/cm²/sr) NT: 8.92 ± 0.28 T: 0.92 ± 0.00* Liver weight (gm) NT: 38.54 ± 3.35 T: 13.40 ± 2.51*
JIAO et al., 2021	Test 1: leronlimab Test 2: MVC	Tumor size - Lung metastasis (x 109 p/s/cm2/sr) (Leronlimab) NT: 860 × 106 T: 3.7 × 106 (Maraviroc) NT: 860 × 106 T: 0.4x × 106 Treatment start at 7 weeks: Survival (%) - (Leronlimab) NT: 0.00 T: 28.6
VELASCO-VELÁZQUEZ et al., 2012	MVC	Lung colonization (Cells per field) NT: 9.05 ± 1.03 T: 5.16 ± 0.87
OCHOA-CALLEJERO et al., 2013	MVC	Number microscopic tumors NT: 4.62 ± 1.00 T: 0.70 ± 0.33* Number apoptosis (per 10 fields - 40x) NT: 7.26 ± 0.80 T: 3.54 ± 0.86* Proliferation index % (40x) NT: 44.90 ± 3.39 T: 25.66 ± 4.15* Fibrotic area % (liver) NT: 7.26 ± 0.21 T: 4.89 ± 0.21*
HALVORSEN et al., 2016	MVC	Tumor cells in the lungs (No) x107 NT: 8.00 ± 0.46 x107 T: 5.89 ± 0.51 x107*

(MVC) Maraviroc; (NT) Not treated; (T) Treated; (BM) Bone marrow
Statistical difference: *P≤0.05

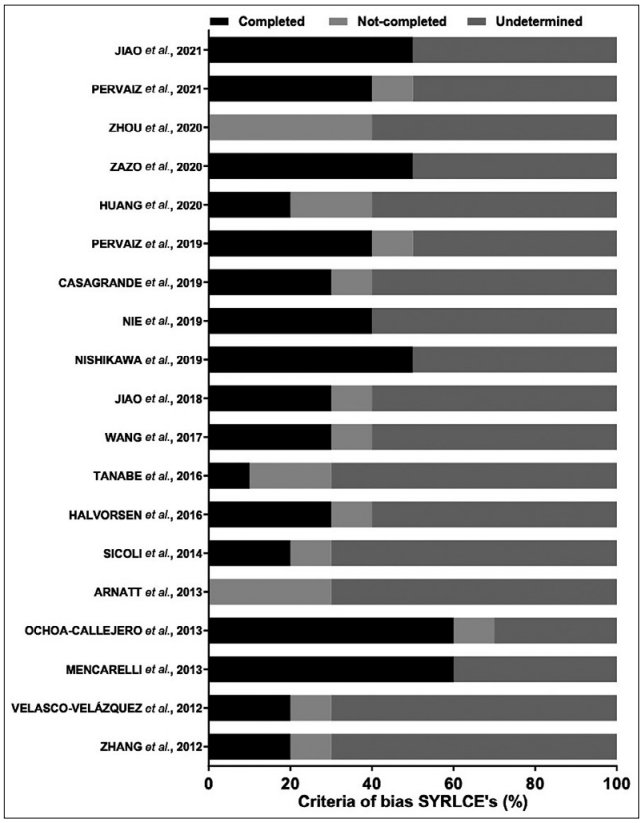

Figure 2. Analysis of the risk of bias in each study included in the systematic review

n=3), isotype control antibody (n=3), PBS (5%; n=1) were used in the control group (Tab. 2).

Effect of ccr5 inhibition on cancer outcomes. A wide variety of outcomes associated with CCR5 inhibition were observed; however, the main outcome reported was reduction in tumour size. The reduction of primary tumours was related to different mechanisms, including reduction of the migration of fibroblasts, macrophages, and mesenchymal stem cells [11,19–21]; reduced cancer cell proliferation [4,7,22]; suppression of DNA Methyltransferase 1 [23]; cancer cell necrosis [24]; and cellular apoptosis, as well as decreased proliferation in a CDE diet-induced model (Tab. 3) [25]. Among studies that analyzed the reduction in size of metastatic tumours, 1 study clarified its reduction by intratumoural necrosis and decrease of cancer cell proliferation [26], while another study reported that MVC decreased CCL8-mediated migration of CCR5+ regulatory T cells. [27] (Tab. 3).

Among other effects, there are reports on increased animal survival [11,25,28], maintenance of body weight [11,25,26], remission of metastases [29], reduction of metastatic tumour burden [30], and reduction in the number of macroscopic tumours [6,25] (Tab. 3).

The microstructural outcomes were related to the reduction of metastatic cells [6,27], decrease in the number of microscopic tumours, and percentage of hepatic fibrotic area (Tab. 3) [25].

Among immunological findings, a reduction in the rate of leukocyte destruction was observed, as well as the reduction

Table 4. Biochemical and immunological outcomes of the studies included in this systematic review

Author	CCR5-inhibitor	Biochemical and immunological findings
OCHOA-CALLEJERO et al., 2013	MVC	Liver damage (After 16 weeks treatment) Transaminases ALT (IU/L): NT: 878.61 ± 68.36 T: 508.67 ± 46.24* AP (IU/L): NT: 976.74 ± 122.09 T: 488.37 ± 78.48* Bilirubin (mg/dl): NT: 1.63 ± 0.34 T: 0.54 ± 0.21* Chemokines: CCL2 (pg/ml) NT:205.29 ± 26.49 T: 94.37 ± 16.55* CCL3 (pg/ml) NT: 4.03 ± 0.22 T: 4.18 ± 0.64 CCL4 (pg/ml) NT: 26.07 ± 3.03 T: 16.96 ± 3.29 CCL5 (pg/ml) NT: 19.02 ± 0.78 T: 15.09 ± 1.25 CCL11 (pg/ml) NT: 14.87 ± 3.16 T: 7.75 ± 0.63* CCXCL10 (pg/ml) NT: 42.91 ± 5.56 T: 31.78 ± 5.16
HALVORSEN et al., 2016	MVC	Treg cells as a % of CD4+ cells in the lungs Leucocytes distribution: (CD4+ CD25+ Foxp3+) NT: 11.69 ± 1.01 T: 8.65 ± 0.55* (CCR5+ CD4+ CD25+ Foxp3+) NT: 6.07 ± 0.36 T: 4.23 ± 0.27*
TANABE et al., 2016	MVC	Relative Expression of target (treatment 15 days) Protein: bFGF NT: 1.01 ± 0.17 T: 0.76 ± 0.15* CTGF NT: 1.04 ± 0.32 T: 0.89 ± 0.27 EGF NT: 1.01 ± 0.13 T: 0.54 ± 0.22* EGFR NT: 1.06 ± 0.38 T: 0.47 ± 0.09* EREG NT: 1.04 ± 0.36 T: 1.68 ± 0.37* HB-EGF NT: 1.01 ± 0.18 T: 1.33 ± 0.21 HGF NT: 1.03 ± 0.31 T: 1.65 ± 0.37 PDGF NT: 1.00 ± 0.09 T: 0.88 ± 0.20
TANABE et al., 2016	MVC	Relative Expression of target (treatment 15 days) Protein: VEGF NT: 1.01 ± 0.18 T: 1.24 ± 0.15 (treatment 15 days) Protein: Ly6G (counts/field) NT: 365.21 ± 125.21 T: 349.56 ± 182.60 F4/80 (%area/field) NT: 10.10 ± 2.55 T: 8.52 ± 2.92 CD31 (%area/field) NT: 8.24 ± 1.35 T: 7.51 ± 1.56 αSMA (%area/field) NT: 6.05 ± 2.19 T: 0.87 ± 0.17* Type I collagen (%area/field) NT: 8.34 ± 1.77 T: 3.23 ± 0.62* CD11b+Gr-1+cells (%) NT: 21.09 T: 26.56 CD25+ Foxp3+ cells (%) NT: 1.22 T: 1.15 mRNA expression (Treatment 28 days) Protein: EGF NT: 1.00 ± 0.26 T: 0.25 ± 0.04*
		(Treatment 28 days) Protein: Type I collagen (%area/field) NT: 14.24 ± 2.34 T: 10.33 ± 1.25* αSMA (%area/field) NT: 27.82 ± 10.08 T: 13.91 ± 2.08* CD31 (%area/field) NT: 1.88 ± 0.30 T: 1.94 ± 0.97
CASAGRANDE et al., 2019	MVC	Protein: CD68+ (AU) (L-540 cells) NT: 99.62 ± 8.30 T: 7.54 ± 0.00* (L-428 cells) NT: 99.62 ± 8.30 T: 24.90 ± 0.75*
ZHOU et al., 2020	Anti-CCR5 antibody	Orthotopic injection - Cell number (per 105 cells) Leucocytes distribution: (Treg) NT: 1204.91 ± 409.83 T: 663.93 ± 245.90 (Treg CCR5+) NT: 786.88 ± 303.27 T: 139.34 ± 73.77 (Treg CCR5-) NT: 295.08 ± 196.72 T: 475.40 ± 221.31
ZHOU et al., 2020	Anti-CCR5 antibody	Cell number (per 105 cells) Leucocytes distribution: (CD8+ T) NT: 2687.50 ± 500.00 T: 3718.75 ± 593.75 (CCR5+ CD8+ T) NT: 812.50 ± 156.25 T: 718.75 ± 156.25 (CCR5- CD8+ T) NT: 1906.25 ± 375.00 T: 2781.25 ± 687.50 Leucocytes distribution: (Macrophage) NT: 5437.50 ± 531.25 T: 4937.50 ± 906.25 (CCR5+ M) NT: 1843.75 ± 687.50 T: 1312.50 ± 437.50 (CCR5- M) NT: 3656.25 ± 500.00 T: 3718.75 ± 718.75 (CD4+ T) NT: 4218.75 ± 1125.00 T: 4000.00 ± 1125.00 (CCR5+ CD4+ T) NT: 1468.75 ± 593.75 T: 1156.25 ± 218.75 Leucocytes distribution: (Macrophage) NT: 5437.50 ± 531.25 T: 4937.50 ± 906.25 (CCR5+ M) NT: 1843.75 ± 687.50 T: 1312.50 ± 437.50 (CCR5- M) NT: 3656.25 ± 500.00 T: 3718.75 ± 718.75 (CD4+ T) NT: 4218.75 ± 1125.00 T: 4000.00 ± 1125.00 (CCR5+ CD4+ T) NT: 1468.75 ± 593.75 T: 1156.25 ± 218.75 (CCR5- CD4+ T) NT: 2625.00 ± 593.75 T: 2781.25 ± 906.25 Cell number (per 105 cells) Leucocytes distribution: (CD4+T) NT: 5230.76 ± 1923.07 T: 3769.23 ± 1307.69 (CD8+T) NT: 2461.53 ± 384.61 T: 3615.38 ± 692.30* (DC) NT: 1076.92 ± 461.53 T: 3076.92 ± 1076.92* Cytokine: (IFNγ+) NT: 3000.00 ± 1000.00 T: 5307.69 ± 1461.53* Protein: (GZMB+) NT: 846.15 ± 307.69 T: 1769.23 ± 461.53* (PRF1+) NT: 230.76 ± 0.00 T: 846.15 ± 384.61* (MHCII+) NT: 3615.38 ± 615.3 T: 4846.15 ± 1769.23

Author	CCR5-inhibitor	Biochemical and immunological findings
ZHOU et al., 2020	Anti-CCR5 antibody	Protein: (CD80+) NT: 1076.92 ± 615.38 T: 3384.61 ± 769.23* Protein: (CD86+) NT: 153.84 ± 0.00 T: 1076.92 ± 461.53* (PDL1+) NT: 18307.69 ± 4076.92 T: 6000.00 ± 2461.53* (PD1+) NT: 4384.61 ± 2000.00 T: 3076.92 ± 1000 (CTLA4+) NT: 923.07 ± 307.69 T: 153.84 ± 76.92* Cell number (per 105 cells) Leucocytes distribution: (CD4+T cell) NT: 5529.95 ± 2488.47 T: 4147.46 ± 1244.24 (CD8+T) NT: 3870.96 ± 1658.98 T: 2764.97 ± 414.74 (DC) NT: 3456.22 ± 967.74 T: 3870.96 ± 829.49
		Cytokine: (IFNγ+) NT: 4009.21 ± 1658.98 T: 6774.19 ± 2211.98 Protein: (GZMB+) NT: 967.74 ± 276.49 T: 2073.73 ± 414.74* (PRF1+) NT: 829.49 ± 138.24 T: 1244.24 ± 414.74 (MHCII+) NT: 4976.95 ± 1244.24 T: 5806.45 ± 1658.98 (CD80+) NT: 2626.72 ± 552.99 T: 3179.72 ± 1105.99 (CD86+) NT: 1244.24 ± 414.74 T: 1244.24 ± 414.74 (PDL1+) NT: 19907.83 ± 4423.96 T: 22949.31 ± 3870.96 (PD1+) NT: 5253.45 ± 1105.99 T: 5115.20 ± 1520.73 (CTLA4+) NT: 552.99 ± 276.49 T: 414.74 ± 414.74

(MVC) Maraviroc; (AU) Arbitrary units; (Treg) Regulatory T cells; (ALT) Alanine aminotransferase; (AP) Alkaline phosphatase; (CCL2) CC motif chemokine ligand 2; (CCL3) CC motif chemokine ligand 3; (CCL4) CC motif chemokine ligand 4; (CCL5) CC motif chemokine ligand 5; (CCL11) CC motif chemokine ligand 11; (CXCL10) CXC motif chemokine ligand 10; (CCR5) CC chemokine receptor type 5; (bFGF) Basic fibroblast growth factor; (CTGF) Connective tissue growth factor; (EGF) Epidermal growth factor; (EGFR) Epidermal growth factor receptor; (EREG) Epiregulin; (HB-EGF) Heparin binding epidermal growth factor-like growth factor; (HGF) Hepatocyte growth factor; (PDGF) Platelet derived growth factor; (VEGF) Vascular endothelial growth factor; (Ly6G) Lymphocyte antigen 6 complex locus G; (αSMA) Alpha smooth muscle actin; (M) Macrophage; (DC) Dendritic cells; (IFNγ) Interferon gamma; (GZMB) Granzyme B; (PRF1) Perforin 1; (MHC II) Major histocompatibility complex class II; (PDL1) Programmed cell death ligand 1; (PD1) Programmed cell death; (CTLA4) Cytotoxic T-lymphocyte associated protein 4.
Statistical difference: *P≤0.05

Table S1. Complete search strategy with search filters and number of studies recovered in databases PubMed-Medline, Embase, Scopus, and Web of Science

PubMed-MEDLINE – Search filters	Records
#1 Disease: (neoplasm[MeSH Terms] OR neoplasms[MeSH Terms] OR cancer[Title/Abstract] OR "cancerous lesions"[Title/Abstract] OR "tumor lesions"[Title/Abstract] OR "malignant lesions"[Title/Abstract])	4.270.020
#2 Intervention (CCR5 inhibitor): ("CCR5 Receptor Antagonists"[MeSH Terms] OR CCR5[Title/Abstract] OR "CCR5 Antagonism"[Title/Abstract] OR "CCR5 inhibitors"[Title/Abstract])	9.658
#3 Type of study: (preclinical [Title/Abstract] OR "in vivo"[Title/Abstract])	1.127.887
#4 Combined search (#1 AND #2 AND #3)	106
*Database search was concluded in August 11, 2022 at 17:03 p.m.	

Embase – Search filters	Records
#1 Disease: (neoplasm:de,ab,ti OR cancer:de,ab,ti OR "cancerous lesions":de,ab,ti OR "tumor lesions":de,ab,ti OR "malignant lesions":de,ab,ti)	4.506.488
#2 Intervention (CCR5 inhibitor): ("CCR5 Receptor Antagonists":de,ab,ti OR CCR5:de,ab,ti OR "CCR5 Antagonism":de,ab,ti OR "CCR5 inhibitors":de,ab,ti)	18.161
#3 Type of study: (preclinical:de,ab,ti OR "in vivo":de,ab,ti)	1.508.851
#4 Combined search: #1 AND #2 AND #3	304
#5 Search limit: NOT ([MEDLINE]/lim)	137
*Database search was concluded in August 11, 2022 at 17:15 p.m.	

SCOPUS – Search filters	Records
#1 Disease: (TITLE-ABS-KEY(neoplasm) OR TITLE-ABS-KEY(cancer) OR TITLE-ABS-KEY("cancerous lesions") OR TITLE-ABS-KEY("tumor lesions") OR TITLE-ABS-KEY("malignant lesions"))	4.605.773
#2 Intervention (CCR5 inhibitors): (TITLE-ABS-KEY("CCR5 Receptor Antagonists") OR TITLE-ABS-KEY(CCR5) OR TITLE-ABS-KEY("CCR5 Antagonism") OR TITLE-ABS-KEY ("CCR5 inhibitors"))	15.516
#3 Type of study: (TITLE-ABS-KEY(preclinical) OR TITLE-ABS-KEY("in vivo"))	1.397.129
#4 Combined search: #1 AND #2 AND #3	230
#5 Search limit (Sources): AND NOT INDEX (Medline)	36
*Database search was concluded in August 11, 2022 at 17:21 p.m.	

Web of Science – Search filters	Records
#1 Disease: (TS=neoplasm OR TS=cancer OR TS="cancerous lesions" OR TS="tumor lesions" OR TS="malignant lesions")	3.027.560
#2 Intervention (exercise): (TS="CCR5 Receptor Antagonists" OR TS=CCR5 OR TS="CCR5 Antagonism" OR TS="CCR5 inhibitors")	11.519
#3 Type of study: (TS=preclinical OR TS="*in vivo*")	1.292.265
#3 Combined search: #1 AND #2 AND #3	133
*Database search was concluded in August 11, 2022 at 17:30 p.m.	

Table S2. Risk of bias in all original preclinical studies according to the Syrcle's quality index

Studies	1. Was the allocation sequence adequately generated and applied?	2. Were the groups similar at baseline or were they adjusted for confounders in the analysis?	3. Was the allocation to the different groups adequately concealed during?	4. Were the animals randomly housed during the experiment?	5. Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?	6. Were animals selected at random for outcome assessment?	7. Was the outcome assessor blinded?	8. Were incomplete outcome data adequately addressed?	9. Are reports of the study free of selective outcome reporting?	10. Was the study apparently free of other problems that could result in high risk of bias?	11. Individual score (%)
ZHANG et al., 2012	?	?	?	?	?	?	?	Yes	No	Yes	20
VELASCO-VELÁZQUEZ et al., 2012	?	No	?	?	?	?	?	?	Yes	Yes	20
MENCARELLI et al., 2013	Yes	Yes	Yes	?	?	?	?	Yes	Yes	Yes	60
OCHOA-CALLEJERO et al., 2013	Yes	No	Yes	Yes	?	?	?	Yes	Yes	Yes	60
ARNATT et al., 2013	?	?	?	?	?	?	?	No	No	No	0
SICOLI et al., 2014	?	?	?	?	?	?	?	No	Yes	Yes	20
HALVORSEN et al., 2016	?	No	?	?	?	?	?	Yes	Yes	Yes	30
TANABE et al., 2016	?	No	?	?	?	?	?	?	No	Yes	10
WANG et al., 2017	?	No	?	?	?	?	?	Yes	Yes	Yes	30
JIAO et al., 2018	?	No	?	?	?	?	?	Yes	Yes	Yes	30
NISHIKAWA et al., 2019	Yes	Yes	?	?	?	?	?	Yes	Yes	Yes	50
NIE et al., 2019	?	Yes	?	?	?	?	?	Yes	Yes	Yes	40
CASAGRANDE et al., 2019	?	Yes	?	?	?	?	?	Yes	Yes	No	30
PERVAIZ et al., 2019	Yes	No	?	?	?	?	?	Yes	Yes	Yes	40
HUANG et al., 2020	?	No	?	?	?	?	?	Yes	No	Yes	20
ZAZO et al., 2020	Yes	Yes	?	?	?	?	?	Yes	Yes	Yes	50
ZHOU et al., 2020	?	No	?	?	?	?	?	No	No	No	0
PERVAIZ et al., 2021	Yes	No	?	?	?	?	?	Yes	Yes	Yes	40
JIAO et al., 2021	Yes	Yes	?	?	?	?	?	Yes	Yes	Yes	50
Total score / Yes (n)	7	6	2	1	0	0	0	14	14	17	
Total score (%)	36.84	31.57	10.52	5.26	0	0	0	73.68	73.68	89.47	

From: HOOIJMANS, Carlijn R. et al. SYRCLE's risk of bias tool for animal studies. BMC Medical Research Methodology, [s. l.], v. 14, n. 1, p. 43, 2014. Disponível em: BMC Medical Research Methodology.
(Yes) indicates low risk of bias; (No) indicates high risk of bias; and (?) indicates an unclear risk of bias.

of growth factors and cytokines in primary tumours (Tab. 4) [11,19,24,25,27]. Furthermore, 1 study reported decreased levels of liver damage markers, including transaminases, alkaline phosphatase and bilirubin (Tab. 4) [25].

Risk of bias by the SYRCLE tool. No study met all methodological quality criteria, indicating potential risks of bias in different domains. Aspects of methodological quality, such as investigator blindness of treatment groups, blindness of animals randomly selected for evaluation, and evaluator blindness during data collection, were underestimated in all studies (Tab. S2). In addition, animal allocation sequencing, baseline animal characteristics, allocation concealment, and random housing of animals, were not well described in most of the reviewed studies. On the other hand, incomplete result data (74%; n=14), selective results (74%; n=14), other potential sources of bias (e.g., pharmacological treatment route of administration and *in vivo* cancer induction model) (89%; n=17), were the best evaluated domains (Tab. S2). This vast methodological variability founded in the studies, impaired a meta-analysis of the results.

DISCUSSION

In view of the high incidence and mortality rates associated with various cancers worldwide, and the many studies demonstrating that CCR5 inhibitors are potentially relevant for more efficient anticancer therapies [4,7,24,28,30], this systematic review was designed to evaluate the effects of CCR5 inhibition on cancer, and to verify the quality of the preclinical animal studies with CCR5 inhibitors.

Although most studies have shown wide methodological variability, there was a predominance of studies that used immunosuppressed mice as the animal model. The use of immunocompromised mice xenografted with human cancer cells is a valuable model for the investigation of anticancer drug efficacy by simulating the physiology of cancer patients

[31–33]. The advantages also related to these models include the facts that the tumour is derived exclusively from *Homo sapiens*, the easy reproducibility of obtaining and monitoring the growth of a homogeneous tumour mass in almost any part of the body, the rapid tumour development, and the possibility to study a specific type of cancer [31–33]. Therefore, the predominance of the use of nude or NOD/SCID mice in most studies retrieved for the current study was a good choice.

There was a predominance of studies that used injection of cancer cells to establish tumours. This preclinical model induced by cancer cell transplantation, especially into immunosuppressed mice, is a valuable approach for clinical predictability of anticancer drugs in humans [31]. Although there is no perfect model, tumour grafts retain histopathologic and genomic characteristics similar to those of primary human tumours, mimicking at least some features of human cancer [34]. Nonetheless, once cancer cell lines were transplanted by different routes and in a non-native microenvironment, they might give different responses, and even lose their ability to metastasize [35], consequently increasing the chance of failure to reproduce the results in clinical trials.

CCR5, a G protein-coupled chemokine receptor, plays a crucial role in regulating cell migration and the inflammatory response [3,8]. In cancer, its over-expression has been associated with tumour progression by promoting a microenvironment that facilitates immune evasion, invasion, and metastasis [5–6,11,14]. CCR5 activation can stimulate extracellular matrix remodelling and angiogenesis, thereby facilitating the dissemination of tumour cells [6,12,14]. Given its biological relevance, blockade of this receptor has been investigated as a therapeutic strategy, particularly in tumours with high CCR5 expression.

It was found that most studies prioritized the analysis of a single CCR5 inhibitor, with MVC being the most commonly used. The ability of this drug to selectively inhibit CCR5, in addition to its excellent safety profile and clinical efficacy, has stimulated investigation into its effects on cancers with high expression of this receptor [36]. Furthermore, the predominance of studies using MVC is also justified by the reduced cost and time required for its repurposing, compared to other inhibitors that have not yet been used in clinical practice.

The dosage schedules and methods of medication administration were quite heterogeneous, hindering the comparison and reproducibility of results. The use of different administration methods can affect both the absorption and metabolism of the drug [31]. Another concern relates to the 2 studies that reported oral drug treatment without specifying whether the delivery was via gavage or drinking water, limiting comparison of dose-dependent responses due to the undefined amount of medication ingested at *ad libitum* in the exposure model [26,30]. Additionally, the robustness of 5 studies was also compromised due to the lack of control-related information, including dose and/or vehicle used.

Response to CCR5 inhibition was primarily assessed by measurement of the tumour size. Although most studies reported a reduction in tumour size, the mechanisms underlining this effect was quite variable or unclear. In fact, the linear measurements of tumour size form the basis for assessing treatment response in many clinical trials of anticancer therapeutics. However, due to certain technical limitations restricting volumetric evaluation, a combined effort should have been made not only to qualify the tumour volume, but also to clarify its underlying mechanisms [37].

Considering a critical interpretation of the evidence, most studies presented potential risks of bias in the different domains evaluated. In general, the methodological quality of the articles was questionable due to the absence of detailed information about the allocation and randomization of the animals, baseline characteristics, blindness of the assessments of the results of interventions received by the animals, and evaluation of the results. Unfortunately, despite methodological advances and the availability of more sensitive and specific analytical tools, bias-related elements continue to be replicated.

It is important to emphasize that these bias elements do not indicate flaws in the experimental protocols, they only indicate limitations in the research reporting. Thus, a clear there is a clear need for standardization of preclinical cancer models with the focus on bias-reducing methods, not only to improve the quality of the reports, but also to reduce possible speculations about false-positive results. Hence, new, well-designed preclinical studies supported by evidence of their ability to predict clinical success or failure of CCR5 inhibition in cancer, are needed.

CONCLUSIONS

The results obtained provide significant evidence that CCR5 inhibition represents an important option for cancer treatment. However, by mapping the risk of bias across all the studies investigated, the review offers objective support for delineating future studies with greater methodological rigor, providing clear evidence regarding the impact of CCR5 inhibition on cancer development and progression.

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