UNIVERSIDADE FEDERAL DE ALFENAS

JOÃO LUCAS CORRÊA DE ANDRADE

POTENCIAL PROGNÓSTICO E TERAPÊUTICO DO RECEPTOR DE QUIMIOCINAS CCR5 NA CARCINOGÊNESE

Alfenas/MG

2023

JOÃO LUCAS CORRÊA DE ANDRADE

POTENCIAL PROGNÓSTICO E TERAPÊUTICO DO RECEPTOR DE QUIMIOCINAS CCR5 NA CARCINOGÊNESE

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas pela Universidade Federal de Alfenas. Área de concentração: Biologia Celular, Molecular e Estrutural das doenças agudas e crônicas.

Orientadora: Profª. Drª. Carine Ervolino de Oliveira

Alfenas/MG

2023

Sistema de Bibliotecas da Universidade Federal de Alfenas
Biblioteca Central

Andrade, João Lucas Corrêa de.
Potencial prognóstico e terapêutico do receptor de quimiocinas CCR5 na carcinogênese / João Lucas Corrêa de Andrade. - Alfenas, MG, 2023.
87 f. : il. -

Orientador(a): Carine Ervolino de Oliveira.
Dissertação (Mestrado em Ciências Biológicas) - Universidade Federal de Alfenas, Alfenas, MG, 2023.
Bibliografia.

1. Quimiocina. 2. Receptor CCR5. 3. Câncer. 4. Antagonistas dos Receptores CCR5. I. Oliveira, Carine Ervolino de, orient. II. Título.

Ficha gerada automaticamente com dados fornecidos pelo autor.

JOÃO LUCAS CORRÊA DE ANDRADE

POTENCIAL PROGNÓSTICO E TERAPÊUTICO DO RECEPTOR DE QUIMIOCINAS CCR5 NA CARCINOGÊNESE

A Banca examinadora abaixo-assinada aprova a Dissertação/Tese apresentada como parte dos requisitos para a obtenção do título de Mestre em Ciências Biológicas pela Universidade Federal de Alfenas. Área de concentração: Biologia Celular, Molecular e Estrutural das doenças agudas e crônicas.

Aprovada em: 28 de fevereiro de 2023.

Profa. Dra. Carine Ervolino de Oliveira
Instituição: Universidade Federal de Alfenas - UNIFAL-MG

Profa. Dra. Nilva de Karla Cervigne Furlan
Instituição: Faculdade de Medicina de Jundiai - FMJ

Profa. Dra. Marina Lara de Carli Dias
Instituição: Universidade Federal de Alfenas - UNIFAL-MG

Documento assinado eletronicamente por **Carine Ervolino de Oliveira**, **Professor do Magistério Superior**, em 23/03/2023, às 10:55, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Marina Lara de Carli Dias**, **Professor do Magistério Superior**, em 23/03/2023, às 12:34, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **NILVA DE KARLA CERVIGNE FURLAN**, **Usuário Externo**, em 24/03/2023, às 09:12, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

A autenticidade deste documento pode ser conferida no site https://sei.unifal-mg.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **0951634** e o código CRC **7E4E066E**.

AGRADECIMENTOS

A Deus, por ter permitido que eu tivesse saúde e determinação para superar os momentos difíceis durante a realização deste trabalho.

Aos meus pais, Abel Soares de Andrade e Dalva Stela Corrêa, pelo amor incondicional, apoio, incentivo e por acreditarem no meu potencial. Sou imensamente grato por tudo que fazem por mim. É uma felicidade poder compartilhar com vocês esse momento tão importante para mim. Foi um trabalho árduo, de muitas superações ao longo desses anos, mas conseguimos. Conquistamos essa vitória juntos. Sem vocês, nada disso seria possível. Amo vocês!

À minha noiva, Priscila Souza Cabral Tuci, pela paciência, incentivo, por sempre acreditar no meu potencial e por estar presente nos momentos que mais precisei de ajuda. Não há palavras que expressam o quanto sou grato por ter você em minha vida. Essa conquista é nossa! Amo você!

À minha orientadora, Profª. Drª Carine Ervolino de Oliveira, exemplo de dedicação, seriedade e integridade. Sou imensamente grato pela oportunidade, acolhida, amizade, competência e eficiência com que conduziu a minha orientação. Lembrarei com muito carinho de cada momento de aprendizado, conselhos e críticas construtivas, importantes para o meu amadurecimento e desenvolvimento profissional. Obrigado por confiar na minha capacidade, principalmente nos momentos em que eu mesmo não acreditava que fosse capaz.

À minha vó, Silvia Máxima Corrêa, e a minha vó do coração Teresa Souza Gomes, pelo amor incondicional, incentivo, apoio e o mais importante, pelas orações. Vocês são meu maior exemplo de superação e fé.

À minha tia, Denise Aparecida Corrêa, meu exemplo de dedicação, superação e resiliência. Sua força me inspira e me dá coragem para enfrentar os maiores desafios. Muito obrigado por ter me apoiado nessa jornada.

À minha sogra, Andréa Cabral, e ao meu sogro, Adeilton da Silva, por todo apoio, conselhos, boas risadas e incentivo. Vocês são muito especiais para mim. Sou muito grato por tudo que fazem por mim. Fico feliz em poder compartilhar esse momento com vocês.

Aos funcionários do Departamento de Patologia e Parasitologia, Maria Ângela, Matheus, Laiane e Enilda, por todo carinho, gentileza e solicitude durante todos esses anos. É sempre uma alegria estar na presença de vocês. Muito obrigado pelas conversas, boas risadas e por todo incentivo. Sempre irei me lembrar de vocês com muito carinho.

Ao Prof. Dr. Ricardo Della Coletta e à Profª. Drª Nilva Karla Cervigne, pela contribuição e auxílio na elaboração deste projeto.

Aos Prof. Dr. João Adolfo Costa Hanemann e Prof. Dr. Leonardo Amaral dos Reis, pela oportunidade e acolhida na clínica de Estomatologia da UNIFAL. Agradeço imensamente pelos ensinamentos que contribuíram grandiosamente para a minha formação acadêmica e pessoal.

À Profª. Drª. Lívia Máris Ribeiro Paranaíba, pela oportunidade de participar do projeto de assistência odontológica aos pacientes oncológicos. Agradeço também por todo apoio, atenção e pelo conhecimento compartilhado.

À Profª. Drª Lívia de Figueiredo Diniz Castro, pela gentileza e paciência por ter me ensinado técnicas importantes para os meus experimentos em modelo animal.

Ao Prof. Dr. Rômulo Dias Novaes, pela gentileza em auxiliar na elaboração da minha revisão sistemática.

À secretária do Programa de Pós-Graduação em Ciências Biológicas, Martha Priscila Azevedo, por toda ajuda durante esses anos.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001

À Universidade Federal de Alfenas – UNIFAL/MG, na pessoa de seu reitor, Prof. Dr. Sandro Amadeu Cerveira.

Ao Programa de Pós-Graduação em Ciências Biológicas – UNIFAL/MG, na pessoa de seu coordenador, Prof. Dr. Masaharu Ikegaki.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão de minha bolsa de mestrado.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pelo auxílio financeiro concedido (APQ-00319-18).

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico - (CNPq), pelo incentivo financeiro (421895/2018-7).

RESUMO

O objetivo deste estudo foi avaliar o potencial efeito na inibição do receptor de quimiocinas CCR5 no desenvolvimento e progressão do câncer. Esta dissertação foi elaborada na forma de dois artigos, o primeiro uma revisão sistemática e, o segundo, um estudo *in vitro*. Na revisão sistemática, além de avaliar as abordagens utilizadas para inibir o CCR5, bem como seus efeitos no desenvolvimento e progressão do câncer, também foi avaliada a qualidade dos estudos pré-clínicos em animais. A revisão sistemática foi realizada de acordo com as diretrizes PRISMA usando uma pesquisa estruturada nas bases de dados PubMed/MEDLINE, Scopus, Web of Science e Embase, recuperando e analisando 21 estudos originais. Para a análise do risco de viés e da qualidade metodológica dos estudos, foi utilizada a ferramenta desenvolvida pela SYRCLE (Systematic Review Center for Laboratory Animal Experimentation). Resultados promissores foram identificados após inibição de CCR5 em diferentes tipos de câncer, os quais foram associados, principalmente a redução do tamanho tumoral. Entretanto, os mecanismos subjacentes a esta redução foram bastante variáveis entre os estudos. Além disso, a maioria dos experimentos utilizou Maraviroc como inibidor de CCR5. Ao analisar a qualidade metodológica dos estudos, foram identificados potenciais riscos de viés nos diferentes domínios avaliados. Assim, esta revisão fornece suporte objetivo para delimitar estudos futuros com maior rigor metodológico, fornecendo evidências inequívocas sobre o impacto da inibição do CCR5 no desenvolvimento e progressão do câncer. Tendo em vista a relevância dos resultados encontrados e a escassez de estudos sobre a inibição de CCR5 no contexto da carcinogenese oral, foi realizado um estudo *in vitro*. No estudo *in vitro*, foi avaliada a expressão de CCR5 em diferentes linhagens celulares (CAL27, SCC4, SCC9, SCC15, SCC25 e HSC3) de carcinoma de células escamosas oral (CCEO) e os efeitos de sua inibição pelo tratamento com Maraviroc (MVC). A maior expressão de CCR5 foi detectada nas linhagens celulares SCC15 e SCC25 que, portanto, foram selecionadas para ensaios funcionais. Os resultados deste estudo demonstraram que, o tratamento com MVC resultou em diminuição significativa da proliferação celular e migração de células SCC15 e SCC25 de maneira dependente da concentração e tempo de exposição ao tratamento. Esses resultados sugerem que o tratamento com MVC desempenha um papel importante na biologia tumoral dos CCEOs e pode

representar uma nova estratégia para o tratamento do câncer oral. Contudo, novos estudos são necessários para entender melhor os mecanismos associados ao tratamento de CCEO com MVC.

Palavras-chave: Quimiocina; Receptor CCR5; Câncer; Antagonistas dos Receptores CCR5.

ABSTRACT

The aim of this study was to evaluate the potential effect of CCR5 chemokine receptor inhibition on cancer development and progression. This dissertation was prepared in the form of two articles, the first a systematic review and the second an *in vitro* study. In the systematic review, in addition to evaluating the approaches used to inhibit CCR5, as well as their effects on the development and progression of cancer, the quality of preclinical studies in animals was also evaluated. The systematic review was performed according to PRISMA guidelines using a structured search in PubMed/MEDLINE, Scopus, Web of Science and Embase databases, retrieving and analyzing 21 original studies. For the analysis of the risk of bias and the methodological quality of the studies, the tool developed by SYRCLE (Systematic Review Center for Laboratory Animal Experimentation) was used. Promising results were identified after CCR5 inhibition in different types of cancer, which were mainly associated with a reduction in tumor size. However, the mechanisms underlying this reduction were quite variable between studies. In addition, most of the experiments used Maraviroc as a CCR5 inhibitor. When analyzing the methodological quality of the studies, potential risks of bias were identified in the different domains evaluated. Thus, this review provides objective support to delimit future studies with greater methodological rigor, providing unequivocal evidence on the impact of CCR5 inhibition on cancer development and progression. In view of the relevance of the results found and the scarcity of studies on CCR5 inhibition in the context of oral carcinogenesis, an *in vitro* study was carried out. In the *in vitro* study, the expression of CCR5 in different cell lines (CAL27, SCC4, SCC9, SCC15, SCC25 and HSC3) of oral squamous cell carcinoma (OSCEC) and the effects of its inhibition by treatment with Maraviroc (MVC) were evaluated. . The highest expression of CCR5 was detected in cell lines SCC15 and SCC25 which, therefore, were selected for functional assays. The results of this study demonstrated that treatment with MVC resulted in a significant decrease in cell proliferation and migration of SCC15 and SCC25 cells, depending on the concentration and time of exposure to the treatment. These results suggest that MVC treatment plays an important role in the tumor biology of CCEOs and may represent a new strategy for the treatment of oral cancer. However, further studies are needed to better understand the mechanisms associated with the treatment of CCEO with MVC.

Keywords: Chemokine; CCR5 Receptor; Cancer; CCR5 Receptor Antagonists.

LISTA DE FIGURAS

LISTA DE TABELAS

ARTIGO 1 - Impact of CCR5-inhibitors on cancer development: A systematic review of preclinical evidence

LISTA DE ABREVIATURAS E SIGLAS

CAPES:	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
UNIFAL-MG:	Universidade Federal de Alfenas
CNPq:	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CCEO:	Carcinoma de células escamosas oral
CCR5:	Receptor 5 de quimiocinas CC
FDA:	Food and Drug Administration
HIV:	Vírus da imunodeficiência humana (do inglês, *Human Immunodeficiency Virus*)
MVC:	Maraviroc
PBS:	Tampão fosfato-salino (do inglês, *Phosphate Buffered Saline*)
Treg:	Células T reguladoras (do ingles, Regulatory T cells)
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SYRCLE:	Systematic Review Center for Laboratory Animal Experimentation
PROSPERO:	International Prospective Register of Systematic Reviews
PICO:	P= Problem, I= Intervention, C= Comparison and O= outcome
RevMan:	Review Manager
RoB:	Risk of Bias
MSC:	Mesenchymal stem cells
BM:	Bone marrow
NOD/SCID:	Nonobese diabetic/severe combined immunodeficient
NSG:	NOD/SCID gamma chain deficient
FVB:	Friend leukemia virus B
IgG:	Immunoglobulin G
ddH_2O:	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
AU:	Arbitrary units
ALT:	Alanine aminotransferase
AP:	Alkaline phosphatase
CCL:	CC motif chemokine ligand
CXCL:	CXC motif chemokine ligand
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
CDE:	Choline deficient diet supplemented with ethionine
NT:	Not treated
T:	Treated
IC:	Inhibitory concentration
BrdU:	Bromodeoxyuridine

SUMÁRIO

1 INTRODUÇÃO GERAL

De acordo com as estimativas globais, são diagnosticados cerca de 19,3 milhões de casos de câncer por ano em todo o mundo (GLOBOCAN, 2020). Além disso, o câncer é a primeira ou segunda principal causa de morte antes dos 70 anos em 112 de 183 países e ocupa o terceiro ou quarto lugar em outros 23 países (SUNG *et al.*, 2021).

No geral, a alta taxa de mortalidade de pacientes com câncer está relacionada ao seu diagnóstico tardio associado às opções de tratamento limitadas e pouco efetivas. Além disso, a escassez de marcadores prognóstico dificulta o estabelecimento de um protocolo de tratamento adequado e individualizado para pacientes com câncer (ALQAHTANI *et al.*, 2019; CHEN *et al.*, 2021; SORIA *et al.*, 2019; WOODARD; JONES; JABLONS, 2016). Sendo assim, é evidente a necessidade de estudos para a identificação não só de novos marcadores prognóstico, mas também de novas opções de tratamento para o câncer.

Neste contexto, diferentes estudos têm relatado que a interação das quimiocinas com seus receptores é responsável por ativar vias de sinalização importantes para a proliferação, migração e invasão de células tumorais através de diversos mecanismos (KORBECKI *et al*., 2020; MOLLICA POETA *et a*l. , 2019). Em particular, o receptor de quimiocinas CCR5 demonstrou estar envolvido em diferentes estágios do desenvolvimento e progressão do câncer, sugerindo que sua inibição representa um importante alvo para o tratamento antineoplásico (CASAGRANDE *et al*., 2019; HUANG *et al*., 2020; JIAO *et al*., 2018; NIE *et al*., 2019; PERVAIZ *et al*., 2015; SICOLI *et al*., 2014; TANABE *et al*., 2016; WANG *et al*., 2017)

Sendo assim, vários pesquisadores têm investigado o impacto dos inibidores de CCR5 (Maraviroc, Leronlimabe, Anibamina, Composto 18, Composto 38, TAK-779 e Anticorpo anti-CCR5) no desenvolvimento de diferentes tipos de câncer (Arnatt CK *et al*, 2013; Jiao X *et al*, 2021; Menu E *et al*, 2006; Sicoli D *et al*, 2014; Zhang F *et al*, 2012; Zhou Q *et al*, 2020). Embora resultados promissores tenham sido relatados, a eficácia e a segurança desses tratamentos, bem como a qualidade dos estudos que os apresentaram, precisam ser analisadas antes de sua aplicação na prática clínica.

Assim, o objetivo do presente estudo foi avaliar não apenas a qualidade dos estudos pré-clínicos em animais, mas também as abordagens que foram usadas

para inibir o CCR5, bem como seus efeitos no desenvolvimento do câncer. Além disso, tendo em vista a escassez de estudos sobre o papel de CCR5 no contexto da carcinogenese oral, também foi avaliada a expressão deste receptor em diferentes linhagens celulares de carcinoma de células escamosas e os efeitos de sua inibição pelo tratamento com maraviroc. Esta dissertação foi elaborada na forma de dois artigos conforme as determinações da UNIFAL-MG, sendo o primeiro uma revisão sistemática e o segundo artigo, um estudo *in vitro*.

ARTIGO 1 - Impact of CCR5-inhibitors on cancer development: A systematic review of preclinical evidence

Impact of CCR5-inhibitors on cancer development: A systematic review of preclinical evidence

João Lucas Corrêa de Andrade[1], Bruno Augusto Linhares Almeida Mariz[2], Ricardo Della Coletta[2,3], Nilva Karla Cervigne[4], Rômulo Dias Novaes[1], Carine Ervolino de Oliveira[1,5]

[1]Postgraduate Program in Biological Sciences, Institute of Biomedical Sciences, Federal University of Alfenas, Alfenas, MG, Brazil.
[2]Department of Oral Diagnosis, School of Dentistry, University of Campinas, Piracicaba, SP, Brazil
[3]Graduate Program in Oral Biology, School of Dentistry, University of Campinas, Piracicaba, SP, Brazil.
[4]Graduate Program in Health Sciences, Faculty of Medicine of Jundiaí (FMJ), Jundiaí, SP, Brazil
[5]Departament of Pathology and Parasitology, Federal University of Alfenas (UNIFAL-MG), Alfenas, MG, Brazil.

Running title: Cancer and CCR5-inhibitors

Corresponding author: Carine Ervolino de Oliveira, Institute of Biomedical Sciences, Department of Pathology and Parasitology, Federal University of Alfenas, Rua Gabriel Monteiro da Silva, 700, Alfenas, zip-code: 37130-000, Minas Gerais, Brazil. Phone/Fax: +55 (35) 3701-9563. E-mail: carineusp@yahoo.com.br

ABSTRACT

In view of the role of the CCR5 chemokine receptor in tumor development and progression, several researchers have investigated the effects of its inhibition in different types of cancer. Although promising results have been reported, the efficacy and safety, as well as the quality of the studies that present them, need to be analyzed before their application in clinical practice. Thus, the aim of this study was to evaluate approaches that have been used to inhibit CCR5 and its effects on cancer development. In addition, we also assessed the methodological quality of preclinical animal studies. This systematic review was performed according to PRISMA guidelines, retrieving and analyzing 21 original studies. To analyze the risk of bias and the quality of preclinical studies, the SYRCLE tool (Systematic Review Center for Laboratory Animal Experimentation) was used. Despite the wide methodological variability found in the reviewed studies, some common characteristics were observed. Most experiments (85.71%; n= 18) used immunosuppressed mice in their induction models. Furthermore, the response to treatment of CCR5 inhibition was primarily assessed by measuring tumor size in most studies (90.47%; n=19). Maraviroc was the most used CCR5 inhibitor in the experiments (76.19%; n=16). Our results provided significant evidence that CCR5 inhibition represents an important target for cancer treatment. On the other hand, by mapping the risk of bias across all investigated studies, this review provides objective support to delimit future studies with greater methodological rigor, providing unequivocal evidence on the impact of CCR5 inhibition on cancer development and progression.

Keywords: Chemokine receptor; CCR5; Cancer; malignant neoplasms; Systematic review

1 INTRODUCTION

Cancer represents a serious public health problem worldwide due to its high incidence, prevalence and mortality (SUNG *et al.*, 2021). Annually, 19.3 million new cases of cancer are expected worldwide and an estimated 9.96 million deaths from this disease (GLOBOCAN, 2020). These results are associated with the limited and ineffective options of available anticancer treatments. In addition, the absence of prognostic markers in most types of cancer hinders the therapeutic planning (ALQAHTANI *et al.*, 2019; CHEN *et al.*, 2021; SORIA *et al.*, 2019; WOODARD; JONES; JABLONS, 2016). Thus, it extremely necessary not only to search new prognostic markers but also to identify new therapeutic options.

In this context, several studies have reported the role of chemokines as well as their receptors in different stages of tumor development and progression (NAGARSHETH; WICHA; ZOU, 2017; OZGA; CHOW; LUSTER, 2021; SARVAIYA *et al.*, 2013). In particular, the chemokine receptor CCR5 has been shown to be an important target of study due its contribution on different steps of tumor growth and development (ARNATT, C K *et al.*, 2013; JIAO *et al.*, 2018; VELASCO-VELÁZQUEZ *et al.*, 2012; ZHANG *et al.*, 2012).

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) (OPPERMANN, 2004; VELASCO-VELÁZQUEZ; XOLALPA; PESTELL, 2014). This receptor is expressed and 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 (ALDINUCCI; BORGHESE; CASAGRANDE, 2020). Furthermore, CCR5 is also highly expressed in different types of cancer (CASAGRANDE *et al.*, 2019; MENU *et al.*, 2006; SALES *et al.*, 2014; SINGH *et al.*, 2018; VELASCO-VELÁZQUEZ *et al.*, 2012).

Different studies have shown not only indirect effects of CCR5 by promoting the migration of different cell types to the tumor microenvironment, but also its direct effects when expressed by the cancer cells (DE OLIVEIRA *et al.*, 2017; JIAO *et al.*, 2018; MENCARELLI *et al.*, 2013; NIE *et al.*, 2019; TANABE *et al.*, 2016). Accordingly, its high expression has been associated with poor prognosis in breast,

colorectal, renal and gastric cancer patients (JIAO *et al.*, 2018; MENCARELLI *et al.*, 2013; NISHIKAWA *et al.*, 2019; ZHOU *et al.*, 2020).

This potential role in carcinogenesis has been explored by several studies investigating the impact of CCR5-inhibitors on cancer development (ARNATT, C K *et al.*, 2013; JIAO *et al.*, 2021; MENU *et al.*, 2006; SICOLI *et al.*, 2014; ZHANG *et al.*, 2012; ZHOU *et al.*, 2020). Although promising results have been reported in different types of cancer, the efficacy and safety of these treatments, as well as the quality of the studies that presented them, need to be analyzed before their application in clinical practice.

So, considering the role of chemokine receptors in the pathogenesis and progression of tumor lesions and that currently available evidence is fragmented, the present study uses a systematic review framework to investigate the impact of CCR5 inhibitors on cancer treatment in animal preclinical models. In addition to mapping the cancer types and CCR5 inhibitors investigated, the dosimetry characteristics and the effect of these inhibitors on histopathological, biochemical and immunological outcomes, as well as tumor progression and survival rates were investigated. 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.

2 METHODS

2.1 GUIDING QUESTION AND DEFINITIONS

Our guiding question was structured considering the PICO (P= Problem, I= Intervention, C= Comparison and O= outcome) strategy (ERIKSEN; FRANDSEN, 2018). Thus, the following guiding question was adopted in this review: Could animals with cancer and treated with CCR5 inhibitors exhibit improved histopathological, biochemical and immunological outcomes, as well as reduced tumor progression and mortality rates compared to untreated animals? We defined a structured methodological protocol to answer this question, which was registered in the PROSPERO (International Prospective Register of Systematic Reviews) database (register number CRD42023368156).

To guide the definitions adopted in this review, the terms "cancer" and "tumor

progression" were defined as follows: Cancer development refer to a multistep process in which cells gradually become malignant through a progressive series of alterations involving mutation which progressively increasing its capacity for proliferation, survival, invasion, and metastasis (COOPER, 2000). Tumor progression refer to phenotypic (morphological, molecular, and functional) changes in an already-formed neoplastic lesion (CONTI, 2010).

2.2 SEARCH STRATEGY AND RESEARCH ALGORITHM

To retrieve research records, four different electronic databases were used, which consisted of two levels of search: (i) direct search in electronic databases and (ii) indirect screening of reference lists of all studies identified in the direct search (ALTOÉ *et al.*, 2019). 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. Thus, structured search filters stratified into three complementary levels was used as follows: (i) disease (cancer), (ii) intervention (CCR5 inhibitors) and (iii) type of study. Initially, filters were developed for the PubMed/Medline search engine using standardized descriptors obtained from the MeSH (Medical Subject Headings) thesaurus and relevant indexing keywords related to the topic. The descriptors and keywords were combined by Boolean operators (AND/OR), as well as the search algorithms [MeSH Terms] and [Title/Abstract] (FELIZARDO *et al.*, 2018). The same search strategy was adapted to the Embase, Scopus and Web of Science databases using the specific syntax and search algorithms recognized by the search machine associated to each database (e.g., de,ab,ti, TITLE-ABS-KEY and TS=, respectively). The search limit "NOT [MEDLINE]/lim" was applied in Embase to exclude duplicate studies on Medline. A similar strategy was applied in Scopus, using the search limit “AND NOT (INDEX (medline)”. Chronological limits were not adopted (MARCELINO *et al.*, 2022). The complete search strategy and the results found are described in table 1.

2.3 PRISMA WORKFLOW AND RECORDS SCREENING

The PRISMA workflow was applied considering two research strategies: (i) primary search in four comprehensive electronic databases (PubMed-Medline,

Embase, Scopus and Web of Sciences), and (ii) secondary search through manual screening of the reference list of all relevant studies retrieved in the primary search (MOHER *et al.*, 2009; PAGE *et al.*, 2021).

In this review, only animal model studies that investigated the potential effect of CCR5 blockade on tumor development and progression were included. Initially, all search records retrieved in electronic databases were loaded into the Mendeley Reference Management Program (Mendeley, London, Westminster, 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 our search strategy is presented in figure 1.

2.4 ELIGIBILITY CRITERIA AND INTER-RATER AGREEMENT

Studies were considered eligible and included when: (i) preclinical studies (ii) investigations based on specific inhibition of the CCR5 chemokine receptor, (iii) cancer studies, (iv) investigations of the direct and/or indirect effect of CCR5 inhibition on cancer development. Studies were considered irrelevant and excluded when: (i) exclusively investigating *in vitro* or human systems, (ii) secondary research (e.g. literature reviews, editorials, letters, notes and conference abstracts), (iii) gray literature (studies that were not formally published or peer-reviewed), (iv) absence of untreated control group, (v) studies with combined treatments where was not possible to isolate the effect of CCR5 receptor inhibition, (vi) studies where there was knowdown and/or silencing of the CCR5 gene. All exclusion criteria were equally applied in the primary and secondary search strategies.

Eligibility criteria were analyzed by 2 independent researchers (J.L.C.A. and C.E.O.), minimizing selection bias. Thus, both evaluators removed duplicated registers, screened titles and abstracts. Disagreements were analyzed by refereeing a third expert researcher (N.K.C.) at all stages of manuscript selection. Concluded the PRISMA-based selection, the results of inclusion and exclusion obtained by the evaluators was used to calculate the inter-rater agreement based on the kappa coefficient (MCHUGH, 2012).

2.5 DATA EXTRACTION

Data extraction from pre-clinical *in vivo* studies was categorized as follows: (i) publication characteristics: authors, year of publication, and country in which the study was conducted; (ii) Experimental model: Animal species, lineage, sex and age; (iii) Specific treatment: CCR5 specific inhibition/blocking drug, concentration, frequency, time and rout of treatment; (iv) Disease model: only cancer; and (v) Reproductive outcomes: inhibition of tumor growth and/or cell proliferation, promotion of cell apoptosis, metastasis inhibition, metastasis remission, tumor size decrease, inhibition of antineoplastic treatment resistance and blockade of angiogenesis.

2.6 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 was originally adjusted for specific aspects of bias that have a relevant impact on animal intervention studies. The SYRCLE's tool is structured into ten topics, which are related to potential sources of bias, such as: (i) selection, (ii) performance, (iii) detection, (iv) friction, (v) reporting and (vi) additional sources of bias not covered by other domains (HOOIJMANS *et al.*, 2014). The overall and individual result obtained from the SYRCLE's strategy was graphically expressed using the Review Manager (RevMan) software, version 5.3

3 RESULTS

3. 1 CHARACTERISTICS AND GEOGRAPHIC DISTRIBUTION OF INCLUDED STUDIES

Our primary search strategy recovered 412 records, of which 16 studies were selected following the application of our eligibility criteria. From the secondary search, 5 additional studies were identified in all reference lists and were included in the systematic review (Figure1). The search filters applied to all electronic databases and a flowchart with the complete PRISMA-based strategy is shown in Table 1 and Figure 1, respectively. The Kappa coefficient ($\kappa = 0.947$) indicated an almost perfect

agreement between the two independent reviewers who conducted the direct and indirect search for relevant studies (Table 2). Most studies identified (33.33% *n* = 7) were developed from United States of America (USA), followed by China (14.29% n = 3), Italy (9.52% n = 2), Spain (9.52% n = 2), Japan (9.52% n = 2), Germany (9.52% n = 2), Belgium (4.76% n = 1), Canada (4.76% n = 1) and Egypt (4.76% n = 1).

3.2 CHARACTERISTICS OF ANIMAL MODELS

There was a predominance of studies (85.71%; n= 18) that used mice as an animal model, while only 3 (14.29%) studies used rats. The main lineage used in these studies was NUDE (28.57%; n=6 mice and 9.53%; n=2 rats). Moreover, 5 (23.81%) studies reported NOD/SCID mice, 3 (14.29%) studies reported C57 mice, 3 (14.29%) studies reported Balb/c mice, 1 (4.76%) study reported using FVB mice, and 1 (4.76%) study used WAG rat (Table 3). Most studies (42.86%; n=9) used only female mice, 7 (33.33%) studies reported only male mice use, 2 (9.52%) studies used both male and female mice and 3 (14.29%) studies did not specify the sex of the animal used (Table 3). There was no animals age standardization. In 10 (46.61%) studies, 8-week-old animals were used, however, only 3 studies have this age well established, while the other 7 studies showed variation between the ages of the animals (5-8 weeks; 8-12 weeks). Two (9.52%) studies did not inform the animal age (Table 3).

3.3 CHARACTERISTICS OF TUMOR MODELS

Breast cancer (n= 6, 28.57%) was the most prevalent cancer type reported, followed by colorectal and prostate cancer (14.29%; n=3 studies each), pancreatic and gastric cancer (9.52%; n=2 studies each). Multiple myeloma, Hodgkin's lymphoma, liver, kidney and lung cancer were reported in 1 study (4.76%) each (Table 3). Most studies used the injection of cancer cells by different routes, including subcutaneous (42.85%; n= 9), intravenous (28.57%; n= 6), intraperitoneal and intracardiac (4.76%; n= 1 each). One study (4.76%) did not specify the route through by the tumor cells were injected was specified. The remain studies used tumor tissue orthotopic implantation (n= 4, 19.04%) and cancer induction by choline-deficient diet supplemented with ethionine (CDE) (n=1, 4.76%) (Table 3).

In most studies (66.67%; n=14), the treatment started immediately after the confirmation of tumor presence. In one specific study of these, additional trials were also performed starting treatment 10 days after and 5 days before cancer cell injection. Although the treatment has been started after cancer cells injections, the interval between tumor induction and initiation of treatment was quite variable in 6 studies (28.57%), as shown in the table 4. In addition, only 1 study (4.76%) started treatment 5 days before tumor cell injection (Table 4).

3.4 CHARACTERISTICS OF THE TREATMENT PROTOCOLS

Table 4 presents the main characteristics of the treatments protocol addressed in each study. To assess the effects of CCR5 inhibition on cancer, most studies (71.43%; n=15) used Maraviroc (MVC). Only 1 (4.76%) study performed independent trials to evaluated not only MVC but also Leronlimab effects on CCR5 inhibition in cancer. The treatment was also performed with TAK-779 in 2 (9.52%) studies, Compound 18 and Anti-CCR5 antibody in 1 (4.76%) study each. Independent assays with Anibamine and Compound 38 was reported in 1 (4.76%) study only (Table 4). The dose and frequency of treatment were quite heterogeneous. The main route for drug administration was intraperitoneal (47.62%; n= 9), followed by oral (28.57% n= 06) and intravenous route (9.52%; n=2). Only one study (4.76%) administered the treatment not only intraperitoneally, but also orally. Intratumoral injection, subcutaneous and systemic routes were reported in 1 (4.76%) study each (Table 4). The vehicle used in the control group was not informed in 5 (23.80%) studies. In the remains studies, DMSO (28.57%; n=6), saline solution (9.52%; n=2), water (14.28%; n=3), isotype control antibody (n=3), PBS (4.76%; n=1) or 5% mannitol (4.76%; n=1) were used in the control group (Table 4).

3.5 EFFECT OF CCR5 INHIBITION ON CANCER OUTCOMES

A wide variety of outcomes associated with CCR5 inhibition were observed, however, the main outcome reported was tumor size reduction. The reduction of primary tumors was related to different mechanisms including the reduction of the migration of fibroblasts, macrophages, mesenchymal stem cells and Treg cells to the tumor microenvironment (CASAGRANDE *et al.*, 2019; NIE *et al.*, 2019; NISHIKAWA

et al., 2019; TAN *et al.*, 2009; TANABE *et al.*, 2016); reduced cancer cell proliferation (ARNATT, Christopher K. *et al.*, 2013; ZAZO *et al.*, 2020; ZHANG *et al.*, 2012); suppression of DNA Methyltransferase 1 (WANG *et al.*, 2017); cancer cell necrosis (ZHOU *et al.*, 2020); and cellular apoptosis and decreased proliferation in a CDE diet-induced model (Table 5) (OCHOA-CALLEJERO *et al.*, 2013). Of studies that analyzed the reduction in size of metastatic tumors, only one clarified its reduction by intratumoral necrosis and decrease of cancer cell proliferation (MENCARELLI *et al.*, 2013). In contrast, one study reported that CCR5 inhibition did not affect tumor size (HALVORSEN *et al.*, 2016). Moreover, only one study did not assess tumor growth (Table 5) (MENU *et al.*, 2006).

Among other effects reported, there are increased animal survival (CASAGRANDE *et al*., 2019; JIAO *et al*., 2021; OCHOA-CALLEJERO *et al*., 2013), maintenance of body weight (CASAGRANDE *et al*., 2019; MENCARELLI *et al*., 2013; OCHOA-CALLEJERO *et al*., 2013), remission of metastases (PERVAIZ *et al*., 2021), reduction of metastatic tumor burden (SICOLI *et al*., 2014), reduction in the number of macroscopic tumors (OCHOA-CALLEJERO *et al*., 2013; VELASCO-VELÁZQUEZ *et al*., 2012), besides to the reduction of bone lesions (Table 5) (MENU *et al.*, 2006).

The microstructural outcomes were related to the reduction of metastatic cells (HALVORSEN *et al*., 2016; VELASCO-VELÁZQUEZ *et al*., 2012), decreased cell migration to the bone marrow (BM) and lower angiogenesis (MENU *et al*., 2006), decrease in the number of microscopic tumors and percentage of hepatic fibrotic area (Table 5) (OCHOA-CALLEJERO *et al*., 2013).

Among immunological findings, a reduction in the rate of leukocyte destruction was observed, as well as the reduction of growth factors and cytokines in primary tumors (Table 6) (CASAGRANDE *et al.*, 2019; HALVORSEN *et al.*, 2016; OCHOA-CALLEJERO *et al.*, 2013; TAN *et al.*, 2009; TANABE *et al.*, 2016; ZHOU *et al.*, 2020). Furthermore, only 1 study reported a biochemical outcome related to decreased levels of markers of liver damage, which include transaminases, alkaline phosphatase and bilirubin (Table 6) (OCHOA-CALLEJERO *et al.*, 2013).

3.6 RISK OF BIAS BY SYRCLE TOOL

The results of the bias analysis based on the SYRCLE tool are detailed in Table 7 and summarized in Figure 2. No reviewed study met all methodological

quality criteria, indicating potential risks of bias in different domains evaluated. 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 (Table 7). 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 (76.19; n=16), selective results (71.42; n=15), other potential sources of bias (90.47; n=19), the route of administration and the type of treatment adopted (95.23%, n=20), induction model (95.23%, n=20) and the tests used in each study (71.42, n = 15) were the best evaluated domains (Table 7).

4 DISCUSSION

In view of the high incidence and mortality rates associated with various cancers worldwide, CCR5 inhibitors are potentially relevant for the development of more efficient anticancer therapies (ARNATT, C K *et al.*, 2013; JIAO *et al.*, 2021; MENU *et al.*, 2006; SICOLI *et al.*, 2014; ZHANG *et al.*, 2012; ZHOU *et al.*, 2020). Thus, we propose to evaluate not only the quality of preclinical animal studies but also the approaches that have been used to inhibit CCR5 as well as their effects on cancer development.

Most scientific evidences about the antineoplastic effects of CCR5 inhibition was produce in studies performed in USA and China. Accordingly, this result reflects the map of the scientific landscape related to cancer research, which highlights these countries as responsible for the largest production volume in this area (CABRAL; DA GRAÇA DERENGOWSKI FONSECA; MOTA, 2018). A reasonable explanation for the increased knowledge generation in these countries is the substantial increase in investment in this research field. In addition, China’s increased publications over time has been also attribute to the efforts to deal with the leading cause of death in this country since 2010 (CABRAL; DA GRAÇA DERENGOWSKI FONSECA; MOTA, 2018; XIA *et al.*, 2022).

Although most studies have shown wide methodological variability, there was a predominance of studies that used immunosuppressed mice as animal model. However, neither sex nor age of the animals was standardized in the studies

analyzed. In fact, the pre-clinical *in vivo* study of the drugs in small animal models, especially in inbred mice, has become an essential step for cancer research (YANG, 2021). Mice are good animals to create tumor models because they are low cost, have a short reproductive cycle, exhibit high tumor growth rates, and can be easily genetically modified (CHULPANOVA *et al.*, 2020). However, the obvious problem of these models is the high failure rate observed in human clinical trials after promising results obtained in mouse models (CHULPANOVA *et al.*, 2020; TIAN *et al.*, 2020). In this context, the use of immunocompromised mice xenografted with human cancer cells become a valuable model for anticancer drug efficacy investigation by simulate the physiology of cancer patients (SAJJAD *et al.*, 2021; TIAN *et al.*, 2020; YANG, 2021). Among the advantages also related to these models are included the fact that tumor is derived exclusively from Homo sapiens, the easy reproducibility to obtain and monitor the growth of a homogeneous tumor mass in almost any part of the body, the rapid tumor development and the possibility to study a specific type of cancer (SAJJAD *et al.*, 2021; TIAN *et al.*, 2020; YANG, 2021). Therefore, the predominance of the use of athymic nude mice (T-cell-deficient) or severe combined immunodeficiency (T/B/NK cell deficiency and macrophage tolerance for human cells) NOD/SCID mice in most studies that we retrieved represented good choice.

The parameter of sex was largely ignored across most studies analyzed. Excluding reproductive cancer models, most studies have used only male or female animals. Only few studies have not ignored sex disparity and analyzed the treatment response not only in male but also in female mice. The negligence of sex disparities in preclinical models reduce significantly cancer research robustness (HAUPT *et al.*, 2021). It is extremely important to analyze treatment response in both sex because even non-reproductive cancers progression is influenced by sex hormones (CLOCCHIATTI *et al.*, 2016). Hence, to avoid risks of bias due inherent to sex differences, including vulnerabilities to genomic damage, metabolism, immune defense, response to treatment, among others, it is mandatory to carry out preclinical studies in animals of both sexes in order to provide robust foundations for new cancer therapies (HAUPT *et al.*, 2021).

The onset of most cancers takes place in middle and old age (LI; HAIDER; BOUTROS, 2022). However, most preclinical studies we analyzed ignored the impact of aging on cancer by selecting young animals aged 5-12 weeks, equivalent to humans aged approximately 12 to 30 years (CHATSIRISUPACHAI; LAGGER; DE

MAGALHÃES, 2022; DUTTA; SENGUPTA, 2016). In addition to the accumulation of somatic mutations with age, tissue microenvironment changes during aging contribute to tumor initiation, progression and metastasis, and even affect the treatment effectiveness (CHATSIRISUPACHAI; LAGGER; DE MAGALHÃES, 2022; LI; HAIDER; BOUTROS, 2022). Thus, this lack of consistency regarding aging among preclinical studies clearly impairs the reproducibility of findings as well as the optimization of treatment strategies (LI; HAIDER; BOUTROS, 2022). So, to investment in rigorously designed animal models that account inherent influences of lineage, sex and age of the animals for tumor biology is essential not only to avoid misinterpretation risk and bias, but also to boost the ability of preclinical work to predict drug effects in humans.

The main types of cancer in which the effects of CCR5 blockade have been studied reflect the incidence and mortality landscape by this disease worldwide. In fact, with the exception of breast cancer, colorectal, prostate and gastric cancers just do not occur as frequent as lung ones (SUNG *et al.*, 2021). Although most studies have used the injection of cancer cells to establish tumors, the routes were quite variable including subcutaneous, intravenous, intraperitoneal and intracardiac or even not informed. This preclinical model induced by cancer cell transplantation, especially into immunosuppressed mice, is a valuable approach for clinical predictability of anticancer drugs in humans (SAJJAD *et al.*, 2021). Although there is no a perfect model, tumor grafts retain genomic alterations similar to primary human tumors as well as gene expression pattern, mimicking at least some features of human cancer (KNUDSEN *et al.*, 2018). 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 (GOODSPEED *et al.*, 2016) and consequently increase fail chance to reproducibility of the results in clinical trials.

We found that most studies prioritized analysis of a single CCR5-inhibitor among which MVC was predominant, followed by TAK-779, compound 18, Leronlimab, anti-CCR5 antibody, Anibamine, and compound 38. Among these, only MVC and the humanized monoclonal antibody leronlimab are already approved by the Food and Drug Administration (FDA) for the treatment of patients infected with human immunodeficiency virus type 1 (HIV-1). These drugs' ability to selectively inhibit CCR5, in addition to its excellent safety profiles and clinical efficacy, has

spurred investigation about its effects in cancers with high expression of this receptor (FÄTKENHEUER *et al.*, 2008; KAPLON; REICHERT, 2018). The predominance of studies with MVC is justified by reduced cost and time of its repositioning compared with drug development such as Anibamine that is a natural CCR5 antagonist, isolated from *Aniba panurensis*, from which a series of analogues were also developed including compound 38 and compound 18 that were evaluate in prostate cancer models (ARNATT, Christopher K. *et al.*, 2013; ZHANG *et al.*, 2012). Only one study used the anti-ccr5 antibody to evaluate CCR5 inhibition effects on kidney cancer (ZHOU *et al.*, 2020). On the other hand, despite have been used in two reports, TAK-779 (a quaternary ammonium derivative, also developed to prevent the entry of the HIV virus into human cells via CCR5) is not released for clinical use yet (BABA *et al.*, 1999; SHIRAISHI *et al.*, 2000).

Dosage schedules and drug delivery methods were also quite heterogeneous, impairing the comparison of results among themselves, as well as their reproducibility. The main route for drug administration was intraperitoneal, followed by oral, intravenous, intratumoral injection, subcutaneous and systemic. The choice by these invasive methods for drug administration may hinder the drug absorbance and drug metabolism (SAJJAD *et al.*, 2021). On the other hand, another concern is related to the two studies that reported oral drug treatment without specifying whether delivery was performed by gavage or drinking water limiting the dose-dependent responses comparation due undefined amount of medication ingested at *ad libitum* exposure model (MENCARELLI *et al.*, 2013; SICOLI *et al.*, 2014). In addition, the robustness of five studies was also impaired due lack of control-related information including dose and/ or vehicle used.

Response to CCR5 inhibition treatment was primarily assessed by tumor size measurement. Although most studies reported a tumor size reduction, the mechanisms underlining to this was quite variable or unclarified. In fact, the linear measurements of tumor size form the basis for assessing treatment response in many clinical trials of anticancer therapeutics. However, due to some technical aspects that limit the volumetric evaluation, a joint effort should have been made not only to qualify the tumor volume, but also to clarify its underlying mechanisms (GOLDMACHER; CONKLIN, 2012). Only a few CCR5-induced hallmarks of cancer, including cancer cell homing to metastatic sites, enhancing pro-inflammatory and pro-metastatic immune phenotype, and enhancing DNA repair, providing aberrant cell

survival and resistance to DNA-damaging agents (JIAO *et al.*, 2019), were occasionally analyzed in specific studies. In addition to reduced migration of fibroblasts, macrophages, mesenchymal stem cells, and Treg cells into microenvironment, primary tumors reduced were attributed to suppression of DNA Methyltransferase 1, lower proliferation, and induction of apoptosis of the tumor cells themselves. The level of liver damage markers were taking in account in only one study (OCHOA-CALLEJERO *et al.*, 2013). Among the studies related to metastatic tumors, only one clarified its reduction by intratumoral necrosis and decrease of cancer cell proliferation. The remaining studies disregarded the evaluation of the metastatic tumor or did not identify its reduction. All these variations on experimental design may account, at least in part, for failure of cancer therapies to achieve efficacy in clinical phase trials (GOLDMACHER; CONKLIN, 2012; TIAN *et al.*, 2020).

Considering a critical interpretation of the evidence, most of the reviewed studies presented potential risks of bias in the different domains evaluated. In general, the methodological quality was questionable in the articles due to the absence of detailed information about the allocation and randomization of the animals, baseline characteristics, blindness of the evaluation of the results and of the interventions that the animals received and evaluation of the results. Unfortunately, despite methodological advances and the availability of more sensitive and specific analytical tools, elements of bias are continually replicated. On the other hand, we observed that the best evaluated domains were data on incomplete results, reporting of selective results, other potential sources of bias, route of administration and type of treatment adopted, induction model and the statistical tests used in each study. It is important to emphasize that these bias elements do not indicate flaws in the experimental protocols, they only indicate limitations in the research report. Thus, it was clear the need preclinical cancer models standardization with focus on bias-reducing methods not only to improve the quality of the reports but also to decrease possible speculations about false-positive results. Hence, new well designed preclinical studies backed by evidence of their ability to predict clinical success or even failure of CCR5 inhibition in cancer are needed.

5 CONCLUSION

Our results provided significant evidence that CCR5 inhibition represents an

important target for cancer treatment. The use of immunosuppressed mice was an important approach to investigate the effectiveness of CCR5 inhibitors, since these animals have the ability to simulate the physiology of cancer patients. Interestingly, MVC was the most used CCR5 inhibitor in the experiments and was responsible for significant antineoplastic effects such as reduction in tumor size, remission of metastases and increased animal survival. The use of MVC represents an important strategy given the reduced cost and time that its repositioning has in relation to other CCR5 inhibitors that are not yet approved for use in clinical practice. Furthermore, by mapping the risk of bias across all investigated studies, this review provide objective support to delimit future studies with greater methodological rigor, accounting unequivocal evidence on the impact of CCR5 inhibition on cancer development and progression.

FUNDING

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, Brasília, Brazil (421895/2018-7), Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG, Minas Gerais, Brazil (APQ-00319-18) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

6 REFERENCES

ALDINUCCI, Donatella; BORGHESE, Cinzia; CASAGRANDE, Naike. The ccl5/ccr5 axis in cancer progression. **Cancers**, [*s. l.*], v. 12, n. 7, p. 1–30, 2020.

ALQAHTANI, Ali *et al.* Hepatocellular Carcinoma: Molecular Mechanisms and Targeted Therapies. **Medicina**, [*s. l.*], v. 55, n. 9, p. 526, 2019. Disponível em: https://www.mdpi.com/1648-9144/55/9/526.

ALTOÉ, Luciana Schulthais *et al.* Does antibiotic use accelerate or retard cutaneous repair? A systematic review in animal models. **PLOS ONE**, [*s. l.*], v. 14, n. 10, p. e0223511, 2019. Disponível em: https://dx.plos.org/10.1371/journal.pone.0223511.

ARNATT, Christopher K. *et al.* Design, syntheses, and characterization of pharmacophore based chemokine receptor CCR5 antagonists as anti prostate cancer agents. **European Journal of Medicinal Chemistry**, [*s. l.*], v. 69, p. 647–658, 2013. Disponível em: http://dx.doi.org/10.1016/j.ejmech.2013.09.004.

BABA, Masanori *et al.* A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. **Proceedings of the National Academy of Sciences of the United States of America**, [*s. l.*], v. 96, n. 10, p. 5698–5703, 1999.

CABRAL, Bernardo Pereira; DA GRAÇA DERENGOWSKI FONSECA, Maria; MOTA, Fabio Batista. The recent landscape of cancer research worldwide: a bibliometric and network analysis. **Oncotarget**, [*s. l.*], v. 9, n. 55, p. 30474–30484, 2018. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.25730.

CASAGRANDE, Naike *et al.* CCR5 antagonism by maraviroc inhibits hodgkin lymphoma microenvironment interactions and xenograft growth. **Haematologica**, [*s. l.*], v. 104, n. 3, p. 564–575, 2019. Disponível em: http://www.haematologica.org/lookup/doi/10.3324/haematol.2018.196725.

CHATSIRISUPACHAI, Kasit; LAGGER, Cyril; DE MAGALHÃES, João Pedro. Age-associated differences in the cancer molecular landscape. **Trends in Cancer**, [*s. l.*], v. 8, n. 11, p. 962–971, 2022. Disponível em: https://doi.org/10.1016/j.trecan.2022.06.007.

CHEN, Xin *et al.* Cell death in pancreatic cancer: from pathogenesis to therapy. **Nature Reviews Gastroenterology and Hepatology**, [*s. l.*], v. 18, n. 11, p. 804–823, 2021.

CHULPANOVA, Daria S. *et al.* Mouse Tumor Models for Advanced Cancer Immunotherapy. **International Journal of Molecular Sciences**, [*s. l.*], v. 21, n. 11, p. 4118, 2020. Disponível em: https://www.mdpi.com/1422-0067/21/11/4118.

CLOCCHIATTI, Andrea *et al.* Sexual dimorphism in cancer. **Nature Reviews Cancer**, [*s. l.*], v. 16, n. 5, p. 330–339, 2016. Disponível em: http://www.nature.com/articles/nrc.2016.30.

CONTI, C.J. Mechanisms of Tumor Progression. *In*: COMPREHENSIVE TOXICOLOGY. [*S. l.*]: Elsevier, 2010. v. 18, p. 335–347. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1553838917301069.

COOPER, Geoffrey M. The Development and Causes of Cancer. **The Cell: A Molecular Approach**, [*s. l.*], v. 2nd editio, p. 743, 2000.

DE OLIVEIRA, Carine Ervolino *et al.* CCR5-Dependent Homing of T Regulatory Cells to the Tumor Microenvironment Contributes to Skin Squamous Cell Carcinoma Development. **Molecular Cancer Therapeutics**, [*s. l.*], v. 16, n. 12, p. 2871–2880, 2017. Disponível em: https://aacrjournals.org/mct/article/16/12/2871/92237/CCR5-Dependent-Homing-of-T-Regulatory-Cells-to-the.

DUTTA, Sulagna; SENGUPTA, Pallav. Men and mice: Relating their ages. **Life Sciences**, [*s. l.*], v. 152, p. 244–248, 2016. Disponível em: http://dx.doi.org/10.1016/j.lfs.2015.10.025.

ERIKSEN, Mette Brandt; FRANDSEN, Tove Faber. The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: a systematic review. **Journal of the Medical Library Association**, [*s. l.*], v. 106, n. 4, p. 420–431, 2018. Disponível em: http://jmla.pitt.edu/ojs/jmla/article/view/345.

FÄTKENHEUER, Gerd *et al.* Subgroup Analyses of Maraviroc in Previously Treated R5 HIV-1 Infection. **New England Journal of Medicine**, [*s. l.*], v. 359, n. 14, p. 1442–1455, 2008. Disponível em: http://www.nejm.org/doi/abs/10.1056/NEJMoa0803154.

FELIZARDO, Amanda Aparecida *et al.* Could age and aging change the host response to systemic parasitic infections? A systematic review of preclinical evidence. **Experimental Gerontology**, [*s. l.*], v. 104, n. January, p. 17–27, 2018. Disponível em: https://doi.org/10.1016/j.exger.2018.01.022.

GLOBOCAN. **Cancer Tomorrow.** [*S. l.*], 2020. Disponível em: https://gco.iarc.fr/tomorrow/en/dataviz/isotype?types=0&single_unit=500000. Acesso em: 20 dez. 2022.

GOLDMACHER, Gregory V.; CONKLIN, James. The use of tumour volumetrics to assess response to therapy in anticancer clinical trials. **British Journal of Clinical Pharmacology**, [*s. l.*], v. 73, n. 6, p. 846–854, 2012. Disponível em: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2125.2012.04179.x.

GOODSPEED, Andrew *et al.* Tumor-Derived Cell Lines as Molecular Models of Cancer Pharmacogenomics. **Molecular Cancer Research**, [*s. l.*], v. 14, n. 1, p. 3–13, 2016. Disponível em: https://aacrjournals.org/mcr/article/14/1/3/89618/Tumor-Derived-Cell-Lines-as-Molecular-Models-of.

HALVORSEN, E. C. *et al.* Maraviroc decreases CCL8-mediated migration of CCR5+ regulatory T cells and reduces metastatic tumor growth in the lungs. **OncoImmunology**, [*s. l.*], v. 5, n. 6, p. e1150398, 2016. Disponível em: http://dx.doi.org/10.1080/2162402X.2016.1150398.

HAUPT, Sue *et al.* Sex disparities matter in cancer development and therapy. **Nature Reviews Cancer**, [*s. l.*], v. 21, n. 6, p. 393–407, 2021. Disponível em: http://www.nature.com/articles/s41568-021-00348-y.

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.

JIAO, Xuanmao *et al.* CCR5 Governs DNA Damage Repair and Breast Cancer Stem Cell Expansion. **Cancer Research**, [*s. l.*], v. 78, n. 7, p. 1657–1671, 2018. Disponível em: http://cancerres.aacrjournals.org/lookup/doi/10.1158/0008-5472.CAN-17-0915.

JIAO, Xuanmao *et al.* Leronlimab, a humanized monoclonal antibody to CCR5, blocks breast cancer cellular metastasis and enhances cell death induced by DNA damaging chemotherapy. **Breast Cancer Research**, [*s. l.*], v. 23, n. 1, p. 11, 2021. Disponível em: https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-021-01391-1.

KAPLON, Hélène; REICHERT, Janice M. Antibodies to watch in 2018. **mAbs**, [*s. l.*], v. 10, n. 2, p. 183–203, 2018. Disponível em: https://www.tandfonline.com/doi/full/10.1080/19420862.2018.1415671.

KNUDSEN, Erik S. *et al.* Pancreatic cancer cell lines as patient-derived avatars: genetic characterisation and functional utility. **Gut**, [*s. l.*], v. 67, n. 3, p. 508–520, 2018. Disponível em: https://gut.bmj.com/lookup/doi/10.1136/gutjnl-2016-313133.

LI, Constance H.; HAIDER, Syed; BOUTROS, Paul C. Age influences on the molecular presentation of tumours. **Nature Communications**, [*s. l.*], v. 13, n. 1, p. 208, 2022. Disponível em: https://www.nature.com/articles/s41467-021-27889-y.

MARCELINO, Rodolfo C. *et al.* The emerging risk of microplastics and nanoplastics on the microstructure and function of reproductive organs in mammals: A systematic review of preclinical evidence. **Life Sciences**, [*s. l.*], v. 295, n. February, p. 1–9, 2022.

MCHUGH, Mary L. Interrater reliability: The kappa statistic. **Biochemia Medica**, [*s. l.*], v. 22, n. 3, p. 276–282, 2012. Disponível em: http://www.biochemia-medica.com/en/journal/22/3/10.11613/BM.2012.031.

MENCARELLI, Andrea *et al.* CCR5 antagonism by maraviroc reduces the potential for gastric cancer cell dissemination. **Translational Oncology**, [*s. l.*], v. 6, n. 6, p. 784–793, 2013.

MENU, Eline *et al.* Role of CCR1 and CCR5 in homing and growth of multiple myeloma and in the development of osteolytic lesions: a study in the 5TMM model. **Clinical & experimental metastasis**, Netherlands, v. 23, n. 5–6, p. 291–300, 2006.

MOHER, David *et al.* Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. **PLOS Medicine**, [*s. l.*], v. 6, n. 7, p. e1000097,

2009. Disponível em: https://dx.plos.org/10.1371/journal.pmed.1000097.

NAGARSHETH, Nisha; WICHA, Max S.; ZOU, Weiping. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. **Nature Reviews Immunology**, [*s. l.*], v. 17, n. 9, p. 559–572, 2017. Disponível em: http://dx.doi.org/10.1038/nri.2017.49.

NIE, Y *et al.* Breast Phyllodes Tumors Recruit and Repolarize Tumor-Associated Macrophages via Secreting CCL5 to Promote Malignant Progression, Which Can Be Inhibited by CCR5 Inhibition Therapy. **Clinical cancer research**, [*s. l.*], v. 25, n. 13, p. 3873–3886, 2019.

NISHIKAWA, Gen *et al.* Bone marrow-derived mesenchymal stem cells promote colorectal cancer progression via CCR5. **Cell Death and Disease**, [*s. l.*], v. 10, n. 4, p. 264, 2019. Disponível em: http://www.nature.com/articles/s41419-019-1508-2.

OCHOA-CALLEJERO, Laura *et al.* Maraviroc, a CCR5 Antagonist, Prevents Development of Hepatocellular Carcinoma in a Mouse Model. **PLoS ONE**, [*s. l.*], v. 8, n. 1, p. e53992, 2013. Disponível em: https://dx.plos.org/10.1371/journal.pone.0053992.

OPPERMANN, Martin. Chemokine receptor CCR5: Insights into structure, function, and regulation. **Cellular Signalling**, [*s. l.*], v. 16, n. 11, p. 1201–1210, 2004.

OZGA, Aleksandra J.; CHOW, Melvyn T.; LUSTER, Andrew D. Chemokines and the immune response to cancer. **Immunity**, [*s. l.*], v. 54, n. 5, p. 859–874, 2021. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1074761321000352.

PAGE, Matthew J. *et al.* The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. **PLOS Medicine**, [*s. l.*], v. 18, n. 3, p. e1003583, 2021. Disponível em: https://dx.plos.org/10.1371/journal.pmed.1003583.

SAJJAD, Humna *et al.* Cancer models in preclinical research: A chronicle review of advancement in effective cancer research. **Animal Models and Experimental Medicine**, [*s. l.*], v. 4, n. 2, p. 87–103, 2021. Disponível em: https://onlinelibrary.wiley.com/doi/10.1002/ame2.12165.

SALES, Kurt J. *et al.* CCR5 expression is elevated in cervical cancer cells and is up-regulated by seminal plasma. **MHR: Basic science of reproductive medicine**, England, v. 20, n. 11, p. 1144–1157, 2014. Disponível em: https://academic.oup.com/molehr/article-lookup/doi/10.1093/molehr/gau063.

SARVAIYA, Purvaba J. *et al.* Chemokines in tumor progression and metastasis. **Oncotarget**, [*s. l.*], v. 4, n. 12, p. 2171–2185, 2013. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.1426.

SHIRAISHI, Mitsuru *et al.* Discovery of Novel, Potent, and Selective Small-Molecule CCR5 Antagonists as Anti-HIV-1 Agents: Synthesis and Biological Evaluation of Anilide Derivatives with a Quaternary Ammonium Moiety. **Journal of Medicinal Chemistry**, [*s. l.*], v. 43, n. 10, p. 2049–2063, 2000. Disponível em:

https://pubs.acs.org/doi/10.1021/jm9906264.

SICOLI, Daniela *et al.* CCR5 receptor antagonists block metastasis to bone of v-Src oncogene-transformed metastatic prostate cancer cell lines. **Cancer Research**, [*s. l.*], v. 74, n. 23, p. 7103–7114, 2014.

SINGH, Santosh Kumar *et al.* CCR5/CCL5 axis interaction promotes migratory and invasiveness of pancreatic cancer cells. **Scientific Reports**, [*s. l.*], v. 8, n. 1, p. 1323, 2018. Disponível em: http://dx.doi.org/10.1038/s41598-018-19643-0.

SORIA, Francesco *et al.* Molecular markers in bladder cancer. **World Journal of Urology**, [*s. l.*], v. 37, n. 1, p. 31–40, 2019. Disponível em: https://doi.org/10.1007/s00345-018-2503-4.

SUNG, Hyuna *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. **CA: A Cancer Journal for Clinicians**, [*s. l.*], v. 71, n. 3, p. 209–249, 2021. Disponível em: https://onlinelibrary.wiley.com/doi/10.3322/caac.21660.

TAN, Marcus C. B. *et al.* Disruption of CCR5-Dependent Homing of Regulatory T Cells Inhibits Tumor Growth in a Murine Model of Pancreatic Cancer. **The Journal of Immunology**, [*s. l.*], v. 182, n. 3, p. 1746–1755, 2009. Disponível em: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.182.3.1746.

TANABE, Yamato *et al.* Blockade of the chemokine receptor, CCR5, reduces the growth of orthotopically injected colon cancer cells via limiting cancerassociated fibroblast accumulation. **Oncotarget**, [*s. l.*], v. 7, n. 30, p. 48335–48345, 2016. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.10227.

TIAN, Huimin *et al.* Humanized Rodent Models for Cancer Research. **Frontiers in Oncology**, [*s. l.*], v. 10, n. September, p. 1–11, 2020. Disponível em: https://www.frontiersin.org/article/10.3389/fonc.2020.01696/full.

VELASCO-VELÁZQUEZ, Marco *et al.* CCR5 antagonist blocks metastasis of basal breast cancer cells. **Cancer Research**, [*s. l.*], v. 72, n. 15, p. 3839–3850, 2012.

VELASCO-VELÁZQUEZ, Marco; XOLALPA, Wendy; PESTELL, Richard G. The potential to target CCL5/CCR5 in breast cancer. **Expert Opinion on Therapeutic Targets**, [*s. l.*], v. 18, n. 11, p. 1265–1275, 2014. Disponível em: http://www.tandfonline.com/doi/full/10.1517/14728222.2014.949238.

WANG, H C *et al.* Tumor-Associated Macrophages Promote Epigenetic Silencing of Gelsolin through DNA Methyltransferase 1 in Gastric Cancer Cells. **Cancer immunology research**, [*s. l.*], v. 5, n. 10, p. 885–897, 2017.

WOODARD, Gavitt A; JONES, Kirk D; JABLONS, David M. Lung cancer staging and prognosis. *In*: CANCER TREATMENT AND RESEARCH. [*S. l.: s. n.*], 2016. v. 170, p. 47–75. Disponível em: http://link.springer.com/10.1007/978-3-319-40389-2_3.

XIA, Changfa *et al.* Cancer statistics in China and United States, 2022: profiles,

trends, and determinants. **Chinese Medical Journal**, [*s. l.*], v. 135, n. 5, p. 584–590, 2022. Disponível em: https://journals.lww.com/10.1097/CM9.0000000000002108.

YANG, Quan-en. Human cancer xenografts in immunocompromised mice provide an advanced genuine tumor model for research and drug development-A revisit of murine models for human cancers. **Biochimica et Biophysica Acta (BBA) - General Subjects**, [*s. l.*], v. 1865, n. 8, p. 129929, 2021. Disponível em: https://doi.org/10.1016/j.bbagen.2021.129929.

ZAZO, Sandra *et al.* Autocrine CCL5 effect mediates trastuzumab resistance by ERK pathway activation in HER2-positive breast cancer. **Molecular Cancer Therapeutics**, United States, v. 19, n. 8, p. 1696–1707, 2020.

ZHANG, Feng *et al.* Structure activity relationship studies of natural product chemokine receptor CCR5 antagonist anibamine toward the development of novel anti prostate cancer agents. **European Journal of Medicinal Chemistry**, France, v. 55, p. 395–408, 2012.

ZHOU, Q *et al.* CCR5 blockade inflames antitumor immunity in BAP1-mutant clear cell renal cell carcinoma. **Journal for immunotherapy of cancer**, [*s. l.*], v. 8, n. 1, 2020.

Table 1. 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.

Table 1 (continuation).

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.

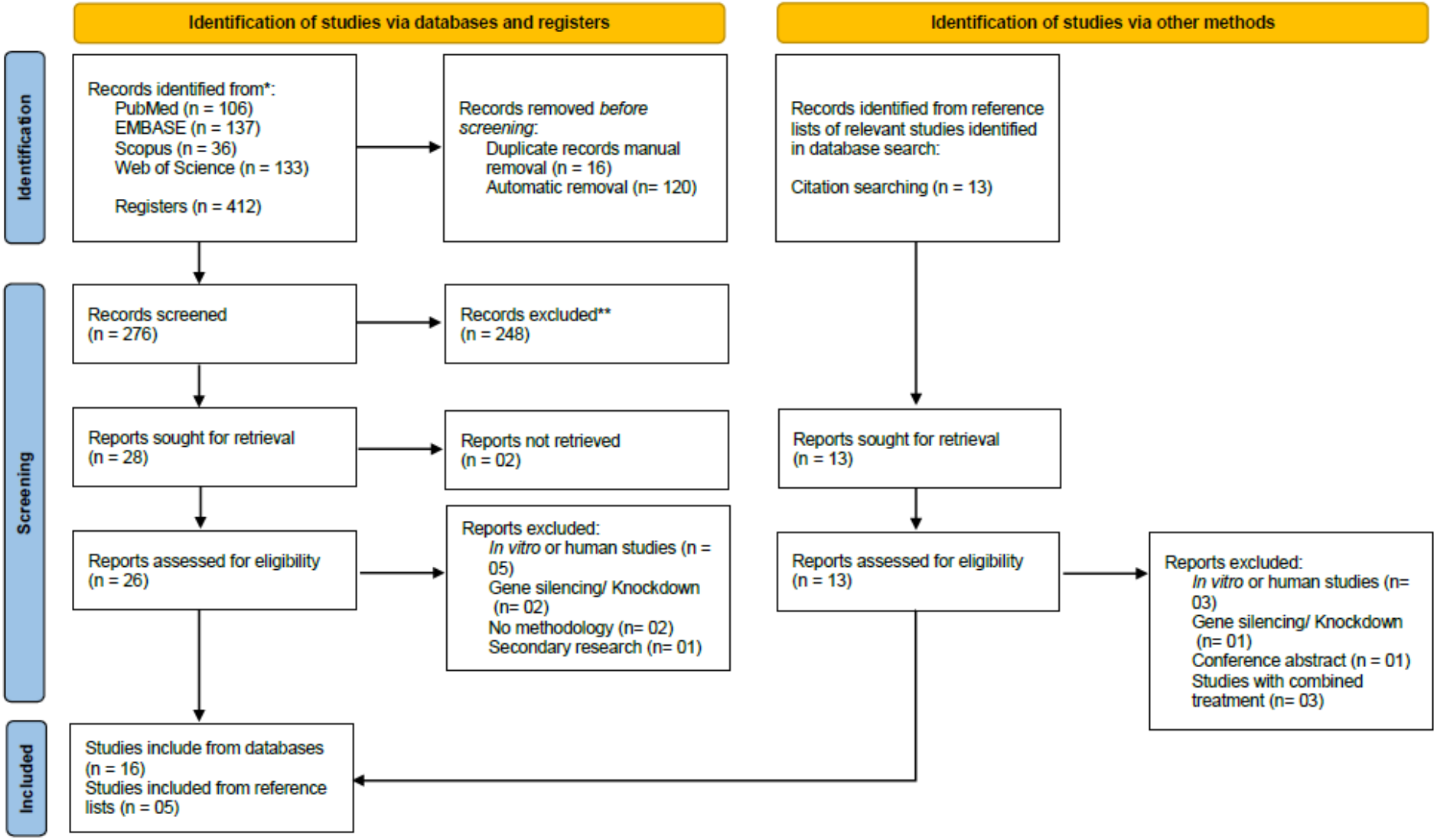


Figure 1. PRISMA flow diagram describing the stages of selection of eligible studies

*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org

Table 2. Results of the PRISMA-based study selection used to quantify Cohen's kappa coefficient (κ) to measure inter-rater reliability of primary search strategy

Kappa calculation		Researcher 1		
		Paper included	Paper excluded	Total
Researcher 2	Paper included	19	02	21
	Paper excluded	0	391	391
	Total	19	393	412

Statistical calculator: https://www.graphpad.com/quickcalcs/kappa1/

Number of observed agreements: 410 (99.51% of the observations)
Number of agreements expected by chance: 373.9 (90.76% of the observations)

Kappa= 0.947
SE of kappa = 0.037
95% confidence interval: From 0.875 to 1.000

"One way to interpret kappa is with this scale (1):

- Kappa < 0: No agreement
- Kappa between 0.00 and 0.20: Slight agreement
- Kappa between 0.21 and 0.40: Fair agreement
- Kappa between 0.41 and 0.60: Moderate agreement
- Kappa between 0.61 and 0.80: Substantial agreement
- Kappa between 0.81 and 1.00: Almost perfect agreement."

1. Landis, J.R.; Koch, G.G. (1977). The measurement of observer agreement for categorical data. Biometrics. 33 (1): 159-174. https://doi.org/10.2307%2F2529310

Table 3. Characteristics of tumor induction models in animals

Author	Specie/Lineage	Age	Sex	Induction model/cell line	Type of cancer
MENU *et al., 2006*	C57BL/KaLwRij mice	8-10 weeks	(-)	IV injection of multiple myeloma 5T2MM cells	Multiple myeloma
TAN *et al., 2009*	C57BL/6 mice	6 weeks	(-)	SC injection of pan02 murine pancreatic adenocarcinoma cell line on the left thigh	Pancreatic 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

(Continued)

Table 3. (Continued)

Author	Specie/Lineage	Age	Sex	Induction model/cell line	Type of 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 mice[a] NSG mice[b]	4 weeks	Female[a] Male[b]	Injection of classic Hodgkin lymphoma L-540 cells into the flank[a] Injection of classic Hodgkin lymphoma L-428 cells into the flank[b]	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

From: Author
(-) 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 4. General characteristics of CCR5 blocking/inhibition interventions performed in the studies

Author	CCR5 inhibitor	Control group	Treated group	Route of administration	Treatment period
MENU *et al., 2006*	TAK-779	DMSO or the vehicles	150 µg of TAK779 dissolved in 100 µl of 5% mannitol	IP	1x day/ 2 days
TAN *et al., 2009*	TAK-779	5% mannitol	150 µg of TAK779 dissolved in 100 µl of 5% mannitol	SC (in the flank)	Test 1: 1× day / 7 days Test 2: 1x day/ 5 days
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

(Continued)

Table 4. (Continued)

Author	CCR5-inhibitor	Control group	Treated group	Route of administration	Treatment period
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[(A)] Test 2: MVC 03 mg/kg[(B)] Test 3: MVC 10 mg/kg[(C)]	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)

(Continued)

Table 4. (Continued)

Author	CCR5 inhibitor	Control group	Treated group	Route of administration	Treatment period
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 ddH_2O 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(D)	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.

From: Auhtor
(?) Incomplete information
(MVC) Maraviroc; (IgG) Immunoglobulin G; (ddH_2O) 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
(#)Administration through drinking water; (A) Treatment protocol described in the material and methods; (B, C) Treatment protocol described in

figure 6 C; [(D)] For treatment purposes, a mixture of maraviroc (25 mg/kg/rat), KolliphorR EL (cremophor EL) as emulsifier (100 µl/rat) and double distilled autoclaved water was prepared (500 µl/rat)

Table 5. Macro and microstructural outcomes of the studies included in this systematic review

Author	CCR5-inhibitor	Outcomes
		Macrostructural findings
MENU *et al., 2006*	TAK779	**Osteolytic lesions (Tibiae and femur) (5T2MM)** NT: 5.97 ± 0.88 T: 4.88 ± 1.50 **Trabecular bone area (%) (5T2MM)** NT: 2.36 ± 1.07 T: 4.28 ± 1.14
TAN *et al., 2009*	TAK779	**Tumor volume/mm³** NT: 126.79 ± 10.59 T: 97.36 ± 8.30
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*	Maraviroc	**Mice with metastatic tumors (%)** NT: 84.69 T: 50.81* **Tumor area (μm²) x10^4** NT: 8.96 ± 1.41 T: 3.43 ± 1.14* **Tumor growth - Lung metastasis (x10^8 p/s/cm²/sr)** NT: 9.82 ± 4.87 T: 0.90 ± 0.00 **Growth of established metastasis (x10^9 p/s/cm²/sr)** NT: 0.68 ± 0.07 T: 0.74 ± 0.05
MENCARELLI *et al., 2013*	Maraviroc	**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

(Continued)

Table 5. Continued

Author	CCR5-inhibitor	Outcomes Macrostructural findings
MENCARELLI *et al., 2013*	Maraviroc	**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*	Maraviroc	**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*	Maraviroc	**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*	Maraviroc	**Tumor volume/mm³** NT: 1234.72 ± 77.77 T: 1098.61 ± 68.05

(Continued)

Table 5. (Continued)

Author	CCR5-inhibitor	Outcomes
		Macrostructural findings
TANABE *et al., 2016*	Maraviroc	**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*	Maraviroc	**Tumor volume/mm³** NT: 3033.81 ± 212.56 T: 1391.30 ± 502.41*
JIAO *et al., 2018*	Maraviroc	**Tumor volume/lung (×10^7 p/sec/cm^2/sr)** NT: 10.05 ± 4.4 T: 3.01 ± 1.16
NISHIKAWA *et al., 2019*	Maraviroc	**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*	Maraviroc	**Tumor volume/mm^3 (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*	Maraviroc	**Median survival (%) (L540 cells)** NT: 13 days T: 15.5 days **Tumor volume/mm^3 (L-540 cells)** NT: 880 ± 88 T: 435 ± 75* **Tumor volume/mm^3 (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

(Continued)

Table 5. Continued

Author	CCR5-inhibitor	Outcomes Macrostructural findings
PERVAIZ *et al., 2019*	Maraviroc	**Tumor growth (x10^{9}p/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
HUANG *et al., 2020*	Maraviroc	**Liver weight (g)** NT: 13.75 ± 3.03 T: 9.13 ± 1.92
ZAZO *et al., 2020*	Maraviroc	**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*	Maraviroc	**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: maraviroc	**Tumor size - Lung metastasis** **(x 10^9 p/s/cm^2/sr)** **(Leronlimab)** NT: 860 × 10^6 T: 3.7 × 10^6 **(Maraviroc)** NT: 860 × 10^6 T: 0.4x × 10^6 **Treatment start at 7 weeks:** **Survival (%) - (Leronlimab)** NT: 0.00 T: 28.6

(Continued)

Table5. Continued

Author	CCR5-inhibitor	Outcomes Microstructural findings
MENU *et al., 2006*	TAK779	**Migration of cells towards the BM (%)** **(5T2MM)** NT: 11.60 ± 1.69 T: 8.08 ± 1.04* **(5T33MM)** NT: 7.34 ± 0.97 T: 5.30 ± 0.53* **% positive cells in the BM** **(5T2MM)** NT:54.44 ± 3.18 T: 53.73 ± 4.94 **Microvessel density** **in the tibiae and femur (N^{o})** **(5T2MM)** NT: 26.93 ± 01.09 T: 21.02 ± 0.43*
VELASCO-VELÁZQUEZ *et al., 2012*	Maraviroc	**Lung colonization** **(Cells per field)** NT: 9.05 ± 1.03 T: 5.16 ± 0.87
OCHOA-CALLEJERO *et al., 2013*	Maraviroc	**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*	Maraviroc	**Tumor cells in the lungs (N^{o}) x10^{7}** NT: 8.00 ± 0.46 x10^{7} T: 5.89 ± 0.51 x10^{7}*

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

Table 6. Biochemical and immunological outcomes of the studies included in this systematic review

Author	CCR5-inhibitor	Biochemical and immunological findings
TAN *et al., 2009*	TAK779	**Leucocytes distribution:** **CD4+Foxp3+ Treg cells** NT: 74 ± 0,5% T: 49 ± 0,02%*
OCHOA-CALLEJERO *et al., 2013*	Maraviroc	**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*	Maraviroc	**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*	Maraviroc	**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

(Continued)

Table 6. (Continued)

Author	CCR5-inhibitor	Biochemical and immunological findings
TANABE *et al., 2016*	Maraviroc	**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* **$CD11b^{+}Gr\text{-}1^{+}$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*	Maraviroc	**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 10^5 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

(Continued)

Table 6. (Continued)

Author	CCR5-inhibitor	Biochemical and immunological findings
ZHOU *et al., 2020*	Anti-CCR5 antibody	**Cell number (per 10^5 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 10^5 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

(Continued)

Table 6. (Continued)

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 10^5 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:** **($IFN\gamma^+$)** 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

From: Author

(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 7. Risk of bias in all original preclinical studies according to the Syrcle's quality index1

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. Were the pharmacological treatment route of administration clearly described?	12. Were the *in vivo* cancer induction model sclearly described?	13. Were statistical tests appropriate and clearly described?	14. **Individual score (%)**
MENU *et al., 2006*	?	?	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	46.15
TAN *et al., 2009*	?	?	?	?	?	?	?	Yes	No	Yes	Yes	Yes	Yes	38.46
ZHANG *et al., 2012*	?	?	?	?	?	?	?	Yes	No	Yes	Yes	Yes	No	30.76
VELASCO-VELÁZQUEZ *et al., 2012*	?	No	?	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	38.46
MENCARELLI *et al., 2013*	Yes	Yes	Yes	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	69.23
OCHOA-CALLEJERO *et al., 2013*	Yes	No	Yes	Yes	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	69.23
ARNATT *et al.,* 2013	?	?	?	?	?	?	?	No	No	No	Yes	Yes	No	15.38
SICOLI *et al., 2014*	?	?	?	?	?	?	?	No	Yes	Yes	Yes	Yes	No	30.76

(Continued)

Table 7. (Continued)

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. Were the pharmacological treatment route of administration clearly described?	12. Were the *in vivo* cancer induction model sclearly described?	13. Were statistical tests appropriate and clearly described?	14. **Individual score (%)**
HALVORSEN *et al., 2016*	?	No	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	46.15
TANABE *et al., 2016*	?	No	?	?	?	?	?	?	No	Yes	Yes	Yes	Yes	30.76
WANG *et al., 2017*	?	No	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	46.15
JIAO *et al., 2018*	?	No	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	46.15
NISHIKAWA *et al., 2019*	Yes	Yes	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	61.53
NIE *et al., 2019*	?	Yes	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	53.84
CASAGRANDE *et al., 2019*	?	Yes	?	?	?	?	?	Yes	Yes	Yes	Yes	No	No	38.46
PERVAIZ *et al., 2019*	Yes	No	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	53.84
HUANG *et al., 2020*	?	No	?	?	?	?	?	Yes	No	Yes	Yes	Yes	Yes	38.46
ZAZO *et al., 2020*	Yes	Yes	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	61.53

(Continued)

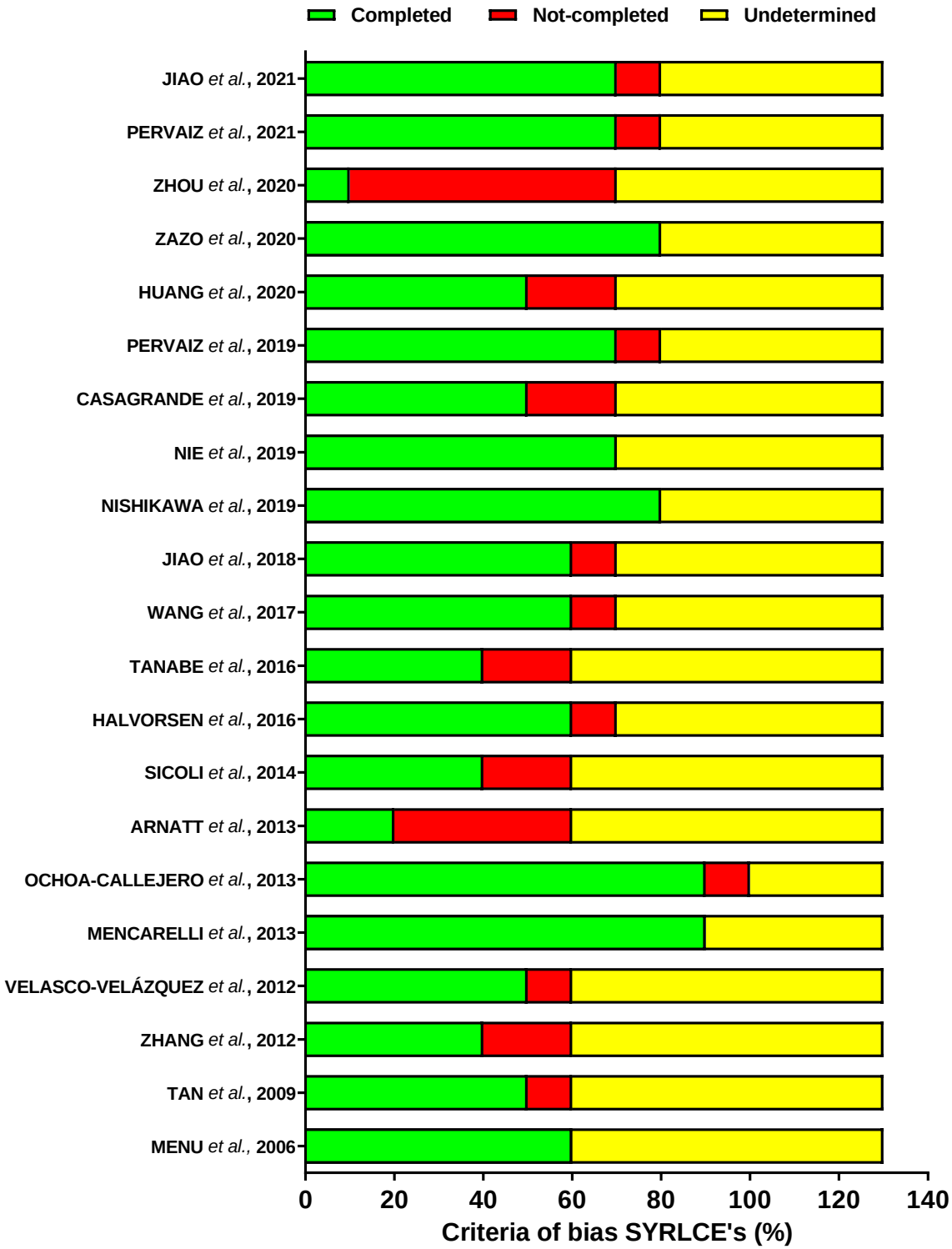
Table 7. (Continued)

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. Were the pharmacological treatment route of administration clearly described?	12. Were the *in vivo* cancer induction model sclearly described?	13. Were statistical tests appropriate and clearly described?	14. **Individual score (%)**
ZHOU *et al., 2020*	?	No	?	?	?	?	?	No	No	No	No	Yes	No	7.69
PERVAIZ *et al., 2021*	Yes	No	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	Yes	53.84
JIAO *et al., 2021*	Yes	Yes	?	?	?	?	?	Yes	Yes	Yes	Yes	Yes	No	53.84
Total score / Yes (n)	7	6	2	1	0	0	0	16	15	19	20	20	15	
Total score (%)	33.33	28.57	9.52	4.76	0	0	0	76.19	71.42	90.47	95.23	95.23	71.42	

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.

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



Based on the SYRCLE's risk of bias tool for animal studies. The dotted line indicates the average score obtained for all studies reviewed.

ARTIGO 2 – Treatment with the CCR5-inhibitor maraviroc suppresses proliferation and migration of oral squamous cell carcinoma

Treatment with the CCR5-inhibitor maraviroc suppresses proliferation and migration of oral squamous cell carcinoma

João Lucas Corrêa de Andrade[1], Luan Col Oenning[2], Lucilene Lopes-Santos[2], Lívia Máris Ribeiro Paranaiba Dias[1,3], Íris Sawazaki-Calone[4], Fábio Haach Téo[5,6], Ricardo Della Coletta[5,6,] Nilva Karla Cervigne[2,7,8], Carine Ervolino de Oliveira[1,3]

[1]Postgraduate Program in Biological Sciences, Federal University of Alfenas (UNIFAL-MG), Alfenas, MG, Brazil.

[2]Laboratory of Molecular Biology and Cell Culture (LBMCC), Faculty of Medicine of Jundiaí (FMJ), Jundiaí, SP, Brazil.

[3]Departament of Pathology and Parasitology, Federal University of Alfenas (UNIFAL-MG), Alfenas, MG, Brazil.

[4]Department of Oral Pathology and Oral Medicine, Dentistry School, Western Paraná State University, Cascavel, PR, Brazil.

[5]Departmentof Oral Diagnosis, School of Dentistry, University of Campinas, Piracicaba, SP, Brazil.

[6]Graduate Program in Oral Biology, School of Dentistry, University of Campinas, Piracicaba, SP, Brazil.

[7]Graduate Program in Health Sciences, Faculty of Medicine of Jundiaí (FMJ), Jundiaí, SP, Brazil.

[8]Department of Internal Medicine, Faculty of Medicine of Jundiaí (FMJ), São Paulo, SP, Brazil.

Corresponding author: Carine Ervolino de Oliveira, Federal University of Alfenas, Rua Gabriel Monteiro da Silva, 700, Alfenas, zip-code: 37130-000, Minas Gerais, Brazil. Phone/Fax: +55 (35) 3701-9563. E-mail: carineusp@yahoo.com.br

ABSTRACT

Oral squamous cell carcinoma (OSCC) represents more than 90% of malignant neoplasms of the oral cavity. Its late diagnosis associated with limited and low effective treatment options are responsible for significant morbidity and mortality rates. Thus, it becomes extremely necessary to identify new therapeutic options. In view of the role of the chemokine receptor CCR5 in the development and progression of cancer, its inhibition has been speculated as a new therapeutic target. Promising results have been reported with the use of Maraviroc (MVC) in several types of cancer, however, its effects on OSCC are still unclear. Thus, we evaluated the CCR5 expression in OSCC cell lines with different malignant potentials. We also investigated the *in vitro* effects of MVC on the proliferation and migration of cell lines with highest CCR5 expression levels. Cell proliferation and migration were analyzed by BrdU incorporation and Scratch assay, respectively. The highest expression of CCR5 was detected in SCC15 and SCC25 cell lines which, therefore, were selected for functional assays. It was observed that CCR5 inhibition with MVC treatment successfully impaired the proliferation and migration in a time and dose-dependent manner. Although significant results were observed at 24 and 48 hours of treatment, the most significant effects in reducing the proliferation and migration of SCC15 and SCC15 occurred at concentrations of 288uM and 465uM after 48 h. Altogether, these results suggest that CCR5 plays an important role in the OSCC development and its inhibition with MVC may represent a new treatment option for oral cancer.

Keywords: Oral squamous cell carcinoma; CCR5; Maraviroc; Treatment

1 INTRODUCTION

Oral squamous cell carcinoma (OSCC) accounts for more than 90% of malignant neoplasms of the oral cavity (IRIMIE *et al.*, 2018), with more than 370,000 new cases diagnosed each year worldwide and an estimate of approximately 178,000 deaths (GLOBOCAN, 2020). It represents one of the most frequent disease in men after the fifth decade of life, and it is highly associated with tobacco and alcohol consumption (ALI *et al.*, 2017; MARICHALAR-MENDIA *et al.*, 2010; SARODE *et al.*, 2020; WARNAKULASURIYA, 2010). However, epidemiological studies have shown an increasing incidence in young adults, aged less than 40 years (LYDIATT *et al.*, 2017; YOU; HENRY; ZEITOUNI, 2019).

Despite advances in scientific investigations, the overall survival rate of patients with OSCC is less than 60% at 5 years (LIU, L. *et al.*, 2019). The low survival rate is mainly associated with late diagnosis, facilitating disease recurrence and the development of metastases, when therapeutic options are limited and less effective (FURY; PFISTER, 2011). Furthermore, the absence of prognostic markers makes difficult to establish a treatment protocol appropriated and individualized, contributing to significant morbidity and mortality rates (ALMANGUSH *et al.*, 2017; CERVINO *et al.*, 2019). Therefore, it becomes extremely necessary not only to search for new prognostic markers, but also to identify new therapeutic options.

Thus, several studies report the role of the chemokine receptor CCR5 in the development and progression of different types of cancers (DE OLIVEIRA *et al.*, 2017; HUANG *et al.*, 2020; JIAO *et al.*, 2018; NIE *et al.*, 2019; TANABE *et al.*, 2016). It has been demonstrated that CCR5 may have an indirect role by promoting the migration of different cell types to the tumor microenvironment, or direct effects when it is expressed by the cancer cells themselves (CASAGRANDE *et al.*, 2019; NISHIKAWA *et al.*, 2019; PERVAIZ *et al.*, 2021; ZHOU *et al.*, 2020). In addition, the high expression of this receptor was related to poor prognosis in several types of cancer (JIAO *et al.*, 2018; MENCARELLI *et al.*, 2013; SINGH *et al.*, 2018; ZHOU *et al.*, 2020), suggesting that CCR5 inhibition may represent an new target to cancer treatment.

In this context, maraviroc (MVC) is a specific CCR5 inhibitor that is already approved by the Food and Drug Administration (FDA) for the treatment of patients infected with the human immunodeficiency virus (HIV) (GAGLIARDINI *et al.*, 2014;

WOOLLARD; KANMOGNE, 2015). The MVC ability to selectively inhibit on CCR5, associated with its excellent safety and efficacy profile, has attracted the interest of researchers about its antineoplastic effects (HUANG *et al.*, 2020; JIAO *et al.*, 2018; TANABE *et al.*, 2016; VELASCO-VELÁZQUEZ *et al.*, 2012). In colorectal and pancreatic cancer studies, MVC was responsible for promoting cell cycle arrest and inducing apoptosis of cancer cells (HUANG *et al.*, 2020; PERVAIZ *et al.*, 2015). The reduction of different tumor lesions such as breast, stomach, Hodgkin's lymphoma was also associated with MVC treatment (CASAGRANDE *et al.*, 2019; MENCARELLI *et al.*, 2013; VELASCO-VELÁZQUEZ *et al.*, 2012; WANG *et al.*, 2017). MVC antineoplastic effects were also assigned to angiogenesis blockade (SAX *et al.*, 2016), inhibition of resistance to antineoplastic treatment (JIAO *et al.*, 2018), inhibition of fibroblast recruitment into the tumor microenvironment (TANABE *et al.*, 2016) and metastasis remission (PERVAIZ *et al.*, 2021; SICOLI *et al.*, 2014).

Although promising results have been reported with the use of MVC in different types of cancers, there are no studies about MVC effects in OSCC development. Thus, we evaluated the expression of CCR5 in different OSCC cell lines with different malignant potential and effects of its inhibition by treatment with MVC.

2 MATERIALS AND METHODS

2.1 CELL CULTURE

Human oral cancer cell lines CAL27, SCC4, SCC9, SCC15 and SCC25, originally isolated from squamous cell carcinoma of the human tongue, were obtained from American Type Culture Collection (ATCC). These cells were grown in DMEM plus Ham's F12 medium (DMEM/ F-12), supplemented with 10% fetal bovine serum (FBS, Life Technologies, Inc.), penicillin (100 U/ml), streptomycin (100 µg/ml) and 0.4 µg/ml hydrocortisone. HSC-3, a human squamous cell carcinoma cell line of the tongue (JCRB 0623; Osaka National Institute of Health Sciences, Japan), was cultured in DMEM/F-12 medium (Invitrogen, USA) supplemented with 10% FBS, 50 µg/ml ascorbic acid (Sigma-Aldrich, USA), 400 ng/ml hydrocortisone (Sigma-Aldrich, USA), penicillin (100 U/ml) and streptomycin (100 µg/ml). HaCat, an immortalized but not transformed epithelial cell line, was maintained in DMEM containing 10% FBS,

penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

2.2 REAL-TIME QUANTITATIVE PCR (QRT-PCR)

Total RNA from cell lines was isolated with TRIzol Reagent method according to the manufacture's protocol (Invitrogen, USA). Following DNase I treatment in order to eliminate genomic DNA contamination, 1 µg of total RNA per sample was used to generate cDNA using Oligo-dT (Invitrogen, USA) and reverse transcriptase (Superscript II RT enzyme, Invitrogen, USA). The resulting cDNAs were subjected to qPCR using the primers and SYBR Green PCR master mix (Applied Biosystems, USA) in the StepOnePlus Real Time PCR (Applied Biosystems, USA). Gene expression was determined using the delta-delta CT method (LIVAK; SCHMITTGEN, 2001) and the housekeeping gene PPIA (cyclophilin A) was used as reference gene for data normalization. The sequences of primers for RT-PCR were: forward CCR5, 5′-GGCAGTTCTCCAGGCTATTTGT-3′; and reverse CCR5, 5′-GGAGGCCAAAGACACAGATCA-3′, forward PPIA, 5′-GCTTTGGGTCCAGGAATGG-3′; and reverse PPIA, 5′-GTTGTCCACAGTCAGCAATGGT-3′. All reactions were performed in triplicate.

2.3 CITOTOXICITY TEST

Cell viability was evaluated using MTT (tetrazolium bluethiazol-3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium) assay. Briefly, cells were plated in 96-well plates at a density of 5×10^4 per well in 100 µl of media containing 10% FBS, cell cycling-synchronized by the absence of FBS for 24 h, and then treated or not treated with MVC for 24 and 48 h. One hundred (100) µl of a solution containing 90% medium and 10% 5 mg MTT/mLdiluted in PBS was added and incubated for 4 h at 37°C. Next, 100 µl of SDS 10% solution was added to each well with the MTT solution and incubated in the dark in a plate shaker for 20 min. After 16 h, the absorbance was measured at 550 nm using a spectrofluorometer and plate luminometer (VarioScan Lux,Thermo Fisher).

2.4 CELL PROLIFERATION ASSAY

Cells were plated in 96-well plates at a density of 2×10^4 cells per well in 100 µl of media containing 10% FBS. After 24 h, the cells were washed with PBS and cultured in serum-free media for an additional 24 h. Following serum starvation, the media were replaced by media with and without MVC containing 10% FBS. Proliferation rates were determined 24 h and 48 h after incubation by measuring BrdU incorporation into DNA using the cell proliferation ELISA BrdU (colorimetric) kit (Abcam, UK). The experiment was performed three independent times in tripiclates. The absorbance was measured at 450 nm using a spectrofluorometer and plate luminometer (VarioScan Lux,Thermo Fisher)

2.5 MIGRATION ASSAY

To analyze the movement of the cell towards the artificial space, a scratch was created in a monolayer of confluent cells plated at $5x10^4$ in 12-well plates. When the cell monolayer reached confluence, a scratch was made with a tip of (time 0). Following 24 and 48 h the images were recorded by the camera coupled to the inverted microscope (Nikon, Eclipse TS100) using the Motic Image Plus 2.0 software.

2.6 STATISTICAL ANALYSIS

All *in vitro* assays were performed at least three times in triplicates or quadruplicates. The data were analysed using Mann-Whitney U test or one-way analysis of variance (ANOVA) with post-hoc comparisons based on the Tukey's multiple comparisons test were applied. These analyses were performed using GraphPad Prism Software version 5.0 and the level of significance considered was 5% ($p\leq0.05$).

3 RESULTS

CCR5 IS OVEREXPRESSED IN THE OSCC CELL LINES

The expression of CCR5 in the OSCC cell lines was analyzed by qPCR. CCR5 mRNA levels were significantly higher in the SCC15 ($p<0.05$) and SCC25

($p<0.05$) cell lines compared to HaCat, an immortalized but non-transformed epithelial cell line (Figure 1). Thus, SCC15 and SCC25 cell lines were selected to perform *in vitro* functional assays to investigate the role of MVC in oral cancer development.

3.2 CCR5 BLOCKADE TREATMENT LED TO A DECREASE IN VIABILITY AND PROLIFERATION OF OSCC CELLS

We determined the sublethal inhibitory concentration in 25% (IC_{25}) of dead cells, which included MVC doses of 215 μM for SCC15 and 450 μM for SCC25. The IC_{50} values for MVC in SCC15 cells was 288 μM and for SCC25 cells 465 μM. Exposure to different concentrations of MVC inhibited the proliferation of SCC15 and SCC25 cells in a concentration and time-dependent manner. After 24 hours, there was a significant decrease in the proliferation of SCC15 and SCC25 with MVC treatment at IC of 288 μM and 465 μM, respectively (Figure 2). Furthermore, the proliferation of SCC25 cells was also affected at dose 450 μM after 24 h of incubation (Figure 2). In contrast, although significant results were observed in IC_{25} and IC_{50} concentrations after 48 h, IC_{50} (288 μM and 465 μM) had most significant effects in decreasing proliferation of both OSCC cells. Thus, it is suggested that IC_{50} needs a longer time to have its effect in SCC25 cells (Figure 2).

3.3 CCR5 BLOCKADE TREATMENT INHIBITS THE MIGRATION OF OSCC CELLS

The Scratch wound assay showed significantly migration decrease of SCC15 and SCC25 cells within 24 h of MVC treatment at dose 215 μM and 450 μM, respectively (Figure 3). After 48 h of treatment, the most significant reduction in the migration rate of SCC15 and SCC25 cells was observed at the MVC concentrations of 288 μM and 465 μM compared with respective untreated controls (Figure 3).

4 DISCUSSION

The late diagnosis, limited and low effective treatment options are responsible for significant OSCC morbidity and mortality rates (FURY; PFISTER, 2011). Thus, considering the promising results obtained by treating different types of cancer with

MVC and, above all, the advantages of repositioning drugs already approved for clinical practice, we decided to evaluate its effects on oral carcinogenesis (CASAGRANDE *et al.*, 2019; HUANG *et al.*, 2020; JIAO *et al.*, 2018; TANABE *et al.*, 2016; WANG *et al.*, 2017).

Our results demonstrated that OSCC cell lines SCC15 and SCC25 showed highest CCR5 expression. Corroborating these findings, high expression of CCR5 was detected not only in cell lines but also in human tumor samples including squamous cell carcinoma of the esophagus, oral cavity, oropharynx and larynx, in melanoma, breast and colorectal cancer (DA SILVA *et al.*, 2017; DOMINGUETI *et al.*, 2019; GONZÁLEZ-ARRIAGADA *et al.*, 2018; KODAMA *et al.*, 2020; LIU, J. *et al.*, 2019; VELASCO-VELÁZQUEZ *et al.*, 2012).

Cell proliferation is an essential feature for tumor development and progression (HANAHAN; WEINBERG, 2011). In our experiments, we observed that MVC treatment impaired the proliferation of SCC15 and SCC25 cell lines. Similar effects have been seen in inhibition of the proliferation of gastric, colorectal, pancreatic and lymphoblastic leukemia cancer cells (HUANG *et al.*, 2020; MENCARELLI *et al.*, 2013; PERVAIZ *et al.*, 2015; ZI *et al.*, 2017). Specifically, in colorectal and pancreatic cancer cells this effect was attributed to the arrest in G1 phase due down-regulation of cell cycle related genes including cyclin and cyclin-dependent kinases (CDKs) (HUANG *et al.*, 2020; PERVAIZ *et al.*, 2015). On the other hand, in lymphoblastic leukemia cells, the antiproliferative mechanism was related to blocking JAK phosphorylation in a concentration range similar to that required to inhibit STAT3 activity (ZI *et al.*, 2017). However, MCV was not effective in inhibiting the proliferation of breast cancer cells (ZAZO *et al.*, 2020).

Because preventing the migration of cancer cells to metastatic sites is a desirable characteristic in an anticancer agent (WU *et al.*, 2021), we have further investigated whether treatment with MVC prevented it on OSCC cells. Again, results from these experiments demonstrate that CCR5 blockage for 24 or 48 hours effectively reduced the cellular migration. Although, the effects of MVC have been ignored in most *in vitro* assays, CCR5 high expression has been associated with increased migration of breast and pancreas cancer cells (SINGH et al., 2018; VELASCO-VELÁZQUEZ et al., 2012).

5 CONCLUSION

In summary, we have shown that CCR5 is expressed in SCC15 and SCC25 cell lines and that its antagonism by maraviroc inhibits cell proliferation and migration *in vitro*. These results suggest that MVC treatment plays an important role in the tumor biology of OSCCs and may be a potential target for individualized treatment of patients with oral cancer with high CCR5 expression. Since these results support a path toward the clinical use of CCR5 antagonists as novel treatments for OSCC, properly designed preclinical studies are required to evaluate whether the present observations extend to clinical settings.

FUNDING

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, Brasília, Brazil (421895/2018-7), Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG, Minas Gerais, Brazil (APQ-00319-18) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

6 REFERENCES

ALDINUCCI, Donatella; BORGHESE, Cinzia; CASAGRANDE, Naike. The ccl5/ccr5 axis in cancer progression. **Cancers**, [*s. l.*], v. 12, n. 7, p. 1–30, 2020.

ALI, Johar *et al.* Genetic etiology of oral cancer. **Oral Oncology**, [*s. l.*], v. 70, p. 23–28, 2017. Disponível em: http://dx.doi.org/10.1016/j.oraloncology.2017.05.004.

ALMANGUSH, Alhadi *et al.* Prognostic biomarkers for oral tongue squamous cell carcinoma: A systematic review and meta-analysis. **British Journal of Cancer**, [*s. l.*], v. 117, n. 6, p. 856–866, 2017.

ALQAHTANI, Ali *et al.* Hepatocellular Carcinoma: Molecular Mechanisms and Targeted Therapies. **Medicina**, [*s. l.*], v. 55, n. 9, p. 526, 2019. Disponível em: https://www.mdpi.com/1648-9144/55/9/526.

ALTOÉ, Luciana Schulthais *et al.* Does antibiotic use accelerate or retard cutaneous repair? A systematic review in animal models. **PLOS ONE**, [*s. l.*], v. 14, n. 10, p. e0223511, 2019. Disponível em: https://dx.plos.org/10.1371/journal.pone.0223511.

ARNATT, Christopher K. *et al.* Design, syntheses, and characterization of pharmacophore based chemokine receptor CCR5 antagonists as anti prostate cancer agents. **European Journal of Medicinal Chemistry**, [*s. l.*], v. 69, p. 647–658, 2013. Disponível em: http://dx.doi.org/10.1016/j.ejmech.2013.09.004.

BABA, Masanori *et al.* A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. **Proceedings of the National Academy of Sciences of the United States of America**, [*s. l.*], v. 96, n. 10, p. 5698–5703, 1999.

CABRAL, Bernardo Pereira; DA GRAÇA DERENGOWSKI FONSECA, Maria; MOTA, Fabio Batista. The recent landscape of cancer research worldwide: a bibliometric and network analysis. **Oncotarget**, [*s. l.*], v. 9, n. 55, p. 30474–30484, 2018. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.25730.

CASAGRANDE, Naike *et al.* CCR5 antagonism by maraviroc inhibits hodgkin lymphoma microenvironment interactions and xenograft growth. **Haematologica**, [*s. l.*], v. 104, n. 3, p. 564–575, 2019. Disponível em: http://www.haematologica.org/lookup/doi/10.3324/haematol.2018.196725.

CERVINO, Gabriele *et al.* Molecular Biomarkers Related to Oral Carcinoma: Clinical Trial Outcome Evaluation in a Literature Review. **Disease Markers**, [*s. l.*], v. 2019, p. 1–11, 2019. Disponível em: https://www.hindawi.com/journals/dm/2019/8040361/.

CHATSIRISUPACHAI, Kasit; LAGGER, Cyril; DE MAGALHÃES, João Pedro. Age-associated differences in the cancer molecular landscape. **Trends in Cancer**, [*s. l.*], v. 8, n. 11, p. 962–971, 2022. Disponível em: https://doi.org/10.1016/j.trecan.2022.06.007.

CHEN, Xin *et al.* Cell death in pancreatic cancer: from pathogenesis to therapy.

Nature Reviews Gastroenterology and Hepatology, [*s. l.*], v. 18, n. 11, p. 804–823, 2021.

CHULPANOVA, Daria S. *et al.* Mouse Tumor Models for Advanced Cancer Immunotherapy. **International Journal of Molecular Sciences**, [*s. l.*], v. 21, n. 11, p. 4118, 2020. Disponível em: https://www.mdpi.com/1422-0067/21/11/4118.

CLOCCHIATTI, Andrea *et al.* Sexual dimorphism in cancer. **Nature Reviews Cancer**, [*s. l.*], v. 16, n. 5, p. 330–339, 2016. Disponível em: http://www.nature.com/articles/nrc.2016.30.

CONTI, C.J. Mechanisms of Tumor Progression. *In*: COMPREHENSIVE TOXICOLOGY. [*S. l.*]: Elsevier, 2010. v. 18, p. 335–347. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1553838917301069.

COOPER, Geoffrey M. The Development and Causes of Cancer. **The Cell: A Molecular Approach**, [*s. l.*], v. 2nd editio, p. 743, 2000.

DA SILVA, Janine Mayra *et al.* Relevance of CCL3/CCR5 axis in oral carcinogenesis. **Oncotarget**, [*s. l.*], v. 8, n. 31, p. 51024–51036, 2017. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.16882.

DE OLIVEIRA, Carine Ervolino *et al.* CCR5-Dependent Homing of T Regulatory Cells to the Tumor Microenvironment Contributes to Skin Squamous Cell Carcinoma Development. **Molecular Cancer Therapeutics**, [*s. l.*], v. 16, n. 12, p. 2871–2880, 2017. Disponível em: https://aacrjournals.org/mct/article/16/12/2871/92237/CCR5-Dependent-Homing-of-T-Regulatory-Cells-to-the.

DOMINGUETI, CB *et al.* Prognostic value of immunoexpression of CCR4, CCR5, CCR7 and CXCR4 in squamous cell carcinoma of tongue and floor of the mouth. **Medicina Oral Patología Oral y Cirugia Bucal**, [*s. l.*], v. 24, n. 3, p. e354–e363, 2019. Disponível em: http://www.medicinaoral.com/medoralfree01/aop/22904.pdf.

DUTTA, Sulagna; SENGUPTA, Pallav. Men and mice: Relating their ages. **Life Sciences**, [*s. l.*], v. 152, p. 244–248, 2016. Disponível em: http://dx.doi.org/10.1016/j.lfs.2015.10.025.

ERIKSEN, Mette Brandt; FRANDSEN, Tove Faber. The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: a systematic review. **Journal of the Medical Library Association**, [*s. l.*], v. 106, n. 4, p. 420–431, 2018. Disponível em: http://jmla.pitt.edu/ojs/jmla/article/view/345.

FÄTKENHEUER, Gerd *et al.* Subgroup Analyses of Maraviroc in Previously Treated R5 HIV-1 Infection. **New England Journal of Medicine**, [*s. l.*], v. 359, n. 14, p. 1442–1455, 2008. Disponível em: http://www.nejm.org/doi/abs/10.1056/NEJMoa0803154.

FELIZARDO, Amanda Aparecida *et al.* Could age and aging change the host response to systemic parasitic infections? A systematic review of preclinical evidence. **Experimental Gerontology**, [*s. l.*], v. 104, n. January, p. 17–27, 2018.

Disponível em: https://doi.org/10.1016/j.exger.2018.01.022.

FURY, Matthew G; PFISTER, David G. Current Recommendations for Systemic Therapy of Recurrent and/or Metastatic Head and Neck Squamous Cell Cancer. **Journal of the National Comprehensive Cancer Network**, [*s. l.*], v. 9, n. 6, p. 681–689, 2011. Disponível em: https://jnccn.org/view/journals/jnccn/9/6/article-p681.xml.

GAGLIARDINI, Roberta *et al.* Safety and therapeutic efficacy of the switch to maraviroc+darunavir/ritonavir in HIV/HCV coinfected patients: initial results from GUSTA study. **Journal of the International AIDS Society**, [*s. l.*], v. 17, n. Suppl 3, p. 19818, 2014. Disponível em: http://doi.wiley.com/10.7448/IAS.17.4.19818.

GLOBOCAN. **Cancer Tomorrow.** [*S. l.*], 2020. Disponível em: https://gco.iarc.fr/tomorrow/en/dataviz/isotype?types=0&single_unit=500000. Acesso em: 20 dez. 2022.

GOLDMACHER, Gregory V.; CONKLIN, James. The use of tumour volumetrics to assess response to therapy in anticancer clinical trials. **British Journal of Clinical Pharmacology**, [*s. l.*], v. 73, n. 6, p. 846–854, 2012. Disponível em: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2125.2012.04179.x.

GONZÁLEZ-ARRIAGADA, Wilfredo A. *et al.* Clinicopathological significance of chemokine receptor (CCR1, CCR3, CCR4, CCR5, CCR7 and CXCR4) expression in head and neck squamous cell carcinomas. **Journal of Oral Pathology & Medicine**, [*s. l.*], v. 47, n. 8, p. 755–763, 2018. Disponível em: https://onlinelibrary.wiley.com/doi/10.1111/jop.12736.

GOODSPEED, Andrew *et al.* Tumor-Derived Cell Lines as Molecular Models of Cancer Pharmacogenomics. **Molecular Cancer Research**, [*s. l.*], v. 14, n. 1, p. 3–13, 2016. Disponível em: https://aacrjournals.org/mcr/article/14/1/3/89618/Tumor-Derived-Cell-Lines-as-Molecular-Models-of.

HALVORSEN, E. C. *et al.* Maraviroc decreases CCL8-mediated migration of CCR5+ regulatory T cells and reduces metastatic tumor growth in the lungs. **OncoImmunology**, [*s. l.*], v. 5, n. 6, p. e1150398, 2016. Disponível em: http://dx.doi.org/10.1080/2162402X.2016.1150398.

HANAHAN, Douglas; WEINBERG, Robert A. Hallmarks of Cancer: The Next Generation. **Cell**, [*s. l.*], v. 144, n. 5, p. 646–674, 2011. Disponível em: http://dx.doi.org/10.1016/j.cell.2011.02.013.

HAUPT, Sue *et al.* Sex disparities matter in cancer development and therapy. **Nature Reviews Cancer**, [*s. l.*], v. 21, n. 6, p. 393–407, 2021. Disponível em: http://www.nature.com/articles/s41568-021-00348-y.

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.

HUANG, Huiying *et al.* The CCR5 antagonist maraviroc causes remission of

pancreatic cancer liver metastasis in nude rats based on cell cycle inhibition and apoptosis induction. **Cancer Letters**, [*s. l.*], v. 474, n. January, p. 82–93, 2020. Disponível em: https://doi.org/10.1016/j.canlet.2020.01.009.

IRIMIE, Alexandra *et al.* Current Insights into Oral Cancer Epigenetics. **International Journal of Molecular Sciences**, [*s. l.*], v. 19, n. 3, p. 670, 2018. Disponível em: http://www.mdpi.com/1422-0067/19/3/670.

JIAO, Xuanmao *et al.* CCR5 Governs DNA Damage Repair and Breast Cancer Stem Cell Expansion. **Cancer Research**, [*s. l.*], v. 78, n. 7, p. 1657–1671, 2018. Disponível em: http://cancerres.aacrjournals.org/lookup/doi/10.1158/0008-5472.CAN-17-0915.

JIAO, Xuanmao *et al.* Leronlimab, a humanized monoclonal antibody to CCR5, blocks breast cancer cellular metastasis and enhances cell death induced by DNA damaging chemotherapy. **Breast Cancer Research**, [*s. l.*], v. 23, n. 1, p. 11, 2021. Disponível em: https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-021-01391-1.

JIAO, Xuanmao *et al.* Recent Advances Targeting CCR5 for Cancer and Its Role in Immuno-Oncology. **Cancer Research**, [*s. l.*], v. 79, n. 19, p. 4801–4807, 2019. Disponível em: https://aacrjournals.org/cancerres/article/79/19/4801/638294/Recent-Advances-Targeting-CCR5-for-Cancer-and-Its.

KAPLON, Hélène; REICHERT, Janice M. Antibodies to watch in 2018. **mAbs**, [*s. l.*], v. 10, n. 2, p. 183–203, 2018. Disponível em: https://www.tandfonline.com/doi/full/10.1080/19420862.2018.1415671.

KNUDSEN, Erik S. *et al.* Pancreatic cancer cell lines as patient-derived avatars: genetic characterisation and functional utility. **Gut**, [*s. l.*], v. 67, n. 3, p. 508–520, 2018. Disponível em: https://gut.bmj.com/lookup/doi/10.1136/gutjnl-2016-313133.

KODAMA, Takayuki *et al.* CCL3–CCR5 axis contributes to progression of esophageal squamous cell carcinoma by promoting cell migration and invasion via Akt and ERK pathways. **Laboratory Investigation**, [*s. l.*], v. 100, n. 9, p. 1140–1157, 2020. Disponível em: http://dx.doi.org/10.1038/s41374-020-0441-4.

LI, Constance H.; HAIDER, Syed; BOUTROS, Paul C. Age influences on the molecular presentation of tumours. **Nature Communications**, [*s. l.*], v. 13, n. 1, p. 208, 2022. Disponível em: https://www.nature.com/articles/s41467-021-27889-y.

LIU, Jie *et al.* High expression of CCR5 in melanoma enhances epithelial-mesenchymal transition and metastasis via TGFβ1. **The Journal of Pathology**, [*s. l.*], v. 247, n. 4, p. 481–493, 2019. Disponível em: https://onlinelibrary.wiley.com/doi/10.1002/path.5207.

LIU, Lian *et al.* Progress in targeted therapeutic drugs for oral squamous cell carcinoma. **Surgical Oncology**, [*s. l.*], v. 31, n. September, p. 90–97, 2019. Disponível em: https://doi.org/10.1016/j.suronc.2019.09.001.

LIVAK, Kenneth J.; SCHMITTGEN, Thomas D. Analysis of Relative Gene Expression

Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. **Methods**, [*s. l.*], v. 25, n. 4, p. 402–408, 2001. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1046202301912629.

LYDIATT, William M. *et al.* Head and neck cancers-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. **CA: A Cancer Journal for Clinicians**, [*s. l.*], v. 67, n. 2, p. 122–137, 2017. Disponível em: http://doi.wiley.com/10.3322/caac.21389.

MARCELINO, Rodolfo C. *et al.* The emerging risk of microplastics and nanoplastics on the microstructure and function of reproductive organs in mammals: A systematic review of preclinical evidence. **Life Sciences**, [*s. l.*], v. 295, n. February, p. 1–9, 2022.

MARICHALAR-MENDIA, Xabier *et al.* Oral cancer and polymorphism of ethanol metabolising genes. **Oral Oncology**, [*s. l.*], v. 46, n. 1, p. 9–13, 2010. Disponível em: http://dx.doi.org/10.1016/j.oraloncology.2009.09.005.

MCHUGH, Mary L. Interrater reliability: The kappa statistic. **Biochemia Medica**, [*s. l.*], v. 22, n. 3, p. 276–282, 2012. Disponível em: http://www.biochemia-medica.com/en/journal/22/3/10.11613/BM.2012.031.

MENCARELLI, Andrea *et al.* CCR5 antagonism by maraviroc reduces the potential for gastric cancer cell dissemination. **Translational Oncology**, [*s. l.*], v. 6, n. 6, p. 784–793, 2013.

MENU, Eline *et al.* Role of CCR1 and CCR5 in homing and growth of multiple myeloma and in the development of osteolytic lesions: a study in the 5TMM model. **Clinical & experimental metastasis**, Netherlands, v. 23, n. 5–6, p. 291–300, 2006.

MOHER, David *et al.* Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. **PLOS Medicine**, [*s. l.*], v. 6, n. 7, p. e1000097, 2009. Disponível em: https://dx.plos.org/10.1371/journal.pmed.1000097.

NAGARSHETH, Nisha; WICHA, Max S.; ZOU, Weiping. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. **Nature Reviews Immunology**, [*s. l.*], v. 17, n. 9, p. 559–572, 2017. Disponível em: http://dx.doi.org/10.1038/nri.2017.49.

NIE, Y *et al.* Breast Phyllodes Tumors Recruit and Repolarize Tumor-Associated Macrophages via Secreting CCL5 to Promote Malignant Progression, Which Can Be Inhibited by CCR5 Inhibition Therapy. **Clinical cancer research**, [*s. l.*], v. 25, n. 13, p. 3873–3886, 2019.

NISHIKAWA, Gen *et al.* Bone marrow-derived mesenchymal stem cells promote colorectal cancer progression via CCR5. **Cell Death and Disease**, [*s. l.*], v. 10, n. 4, p. 264, 2019. Disponível em: http://www.nature.com/articles/s41419-019-1508-2.

OCHOA-CALLEJERO, Laura *et al.* Maraviroc, a CCR5 Antagonist, Prevents Development of Hepatocellular Carcinoma in a Mouse Model. **PLOS ONE**, [*s. l.*], v. 8,

n. 1, p. e53992, 2013. Disponível em: https://dx.plos.org/10.1371/journal.pone.0053992.

OPPERMANN, Martin. Chemokine receptor CCR5: Insights into structure, function, and regulation. **Cellular Signalling**, [*s. l.*], v. 16, n. 11, p. 1201–1210, 2004.

OZGA, Aleksandra J.; CHOW, Melvyn T.; LUSTER, Andrew D. Chemokines and the immune response to cancer. **Immunity**, [*s. l.*], v. 54, n. 5, p. 859–874, 2021. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S1074761321000352.

PAGE, Matthew J. *et al.* The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. **PLOS Medicine**, [*s. l.*], v. 18, n. 3, p. e1003583, 2021. Disponível em: https://dx.plos.org/10.1371/journal.pmed.1003583.

PERVAIZ, Asim *et al.* Antineoplastic effects of targeting CCR5 and its therapeutic potential for colorectal cancer liver metastasis. **Journal of Cancer Research and Clinical Oncology**, [*s. l.*], v. 147, n. 1, p. 73–91, 2021. Disponível em: https://link.springer.com/10.1007/s00432-020-03382-9.

PERVAIZ, Asim *et al.* CCR5 blockage by maraviroc induces cytotoxic and apoptotic effects in colorectal cancer cells. **Medical Oncology**, [*s. l.*], v. 32, n. 5, p. 158, 2015. Disponível em: http://dx.doi.org/10.1007/s12032-015-0607-x.

SAJJAD, Humna *et al.* Cancer models in preclinical research: A chronicle review of advancement in effective cancer research. **Animal Models and Experimental Medicine**, [*s. l.*], v. 4, n. 2, p. 87–103, 2021. Disponível em: https://onlinelibrary.wiley.com/doi/10.1002/ame2.12165.

SALES, Kurt J. *et al.* CCR5 expression is elevated in cervical cancer cells and is up-regulated by seminal plasma. **MHR: Basic science of reproductive medicine**, England, v. 20, n. 11, p. 1144–1157, 2014. Disponível em: https://academic.oup.com/molehr/article-lookup/doi/10.1093/molehr/gau063.

SARODE, Gargi *et al.* Epidemiologic aspects of oral cancer. **Disease-a-Month**, [*s. l.*], v. 66, n. 12, p. 100988, 2020. Disponível em: https://doi.org/10.1016/j.disamonth.2020.100988.

SARVAIYA, Purvaba J. *et al.* Chemokines in tumor progression and metastasis. **Oncotarget**, [*s. l.*], v. 4, n. 12, p. 2171–2185, 2013. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.1426.

SAX, Michael John *et al.* Cancer cell CCL5 mediates bone marrow independent angiogenesis in breast cancer. **Oncotarget**, [*s. l.*], v. 7, n. 51, p. 85437–85449, 2016. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.13387.

SHIRAISHI, Mitsuru *et al.* Discovery of Novel, Potent, and Selective Small-Molecule CCR5 Antagonists as Anti-HIV-1 Agents: Synthesis and Biological Evaluation of Anilide Derivatives with a Quaternary Ammonium Moiety. **Journal of Medicinal Chemistry**, [*s. l.*], v. 43, n. 10, p. 2049–2063, 2000. Disponível em: https://pubs.acs.org/doi/10.1021/jm9906264.

SICOLI, Daniela *et al.* CCR5 receptor antagonists block metastasis to bone of v-Src oncogene-transformed metastatic prostate cancer cell lines. **Cancer Research**, [*s. l.*], v. 74, n. 23, p. 7103–7114, 2014.

SINGH, Santosh Kumar *et al.* CCR5/CCL5 axis interaction promotes migratory and invasiveness of pancreatic cancer cells. **Scientific Reports**, [*s. l.*], v. 8, n. 1, p. 1323, 2018. Disponível em: http://dx.doi.org/10.1038/s41598-018-19643-0.

SORIA, Francesco *et al.* Molecular markers in bladder cancer. **World Journal of Urology**, [*s. l.*], v. 37, n. 1, p. 31–40, 2019. Disponível em: https://doi.org/10.1007/s00345-018-2503-4.

SUNG, Hyuna *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. **CA: A Cancer Journal for Clinicians**, [*s. l.*], v. 71, n. 3, p. 209–249, 2021. Disponível em: https://onlinelibrary.wiley.com/doi/10.3322/caac.21660.

TAN, Marcus C. B. *et al.* Disruption of CCR5-Dependent Homing of Regulatory T Cells Inhibits Tumor Growth in a Murine Model of Pancreatic Cancer. **The Journal of Immunology**, [*s. l.*], v. 182, n. 3, p. 1746–1755, 2009. Disponível em: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.182.3.1746.

TANABE, Yamato *et al.* Blockade of the chemokine receptor, CCR5, reduces the growth of orthotopically injected colon cancer cells via limiting cancerassociated fibroblast accumulation. **Oncotarget**, [*s. l.*], v. 7, n. 30, p. 48335–48345, 2016. Disponível em: https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.10227.

TIAN, Huimin *et al.* Humanized Rodent Models for Cancer Research. **Frontiers in Oncology**, [*s. l.*], v. 10, n. September, p. 1–11, 2020. Disponível em: https://www.frontiersin.org/article/10.3389/fonc.2020.01696/full.

VELASCO-VELÁZQUEZ, Marco *et al.* CCR5 antagonist blocks metastasis of basal breast cancer cells. **Cancer Research**, [*s. l.*], v. 72, n. 15, p. 3839–3850, 2012.

VELASCO-VELÁZQUEZ, Marco; XOLALPA, Wendy; PESTELL, Richard G. The potential to target CCL5/CCR5 in breast cancer. **Expert Opinion on Therapeutic Targets**, [*s. l.*], v. 18, n. 11, p. 1265–1275, 2014. Disponível em: http://www.tandfonline.com/doi/full/10.1517/14728222.2014.949238.

WANG, H C *et al.* Tumor-Associated Macrophages Promote Epigenetic Silencing of Gelsolin through DNA Methyltransferase 1 in Gastric Cancer Cells. **Cancer immunology research**, [*s. l.*], v. 5, n. 10, p. 885–897, 2017.

WARNAKULASURIYA, Saman. Living with oral cancer: Epidemiology with particular reference to prevalence and life-style changes that influence survival. **Oral Oncology**, [*s. l.*], v. 46, n. 6, p. 407–410, 2010. Disponível em: http://dx.doi.org/10.1016/j.oraloncology.2010.02.015.

WOODARD, Gavitt A; JONES, Kirk D; JABLONS, David M. Lung cancer staging and

prognosis. *In*: CANCER TREATMENT AND RESEARCH. [*S. l.: s. n.*], 2016. v. 170, p. 47–75. Disponível em: http://link.springer.com/10.1007/978-3-319-40389-2_3.

WOOLLARD, Shawna M.; KANMOGNE, Georgette D. Maraviroc: A review of its use in hivinfection and beyond. **Drug Design, Development and Therapy**, [*s. l.*], v. 9, p. 5447–5468, 2015. Disponível em: https://www.dovepress.com/maraviroc-a-review-of-its-use-in-hiv-infection-and-beyond-peer-reviewed-article-DDDT.

WU, Jia-shun *et al.* Plasticity of cancer cell invasion: Patterns and mechanisms. **Translational Oncology**, [*s. l.*], v. 14, n. 1, p. 100899, 2021. Disponível em: https://doi.org/10.1016/j.tranon.2020.100899.

XIA, Changfa *et al.* Cancer statistics in China and United States, 2022: profiles, trends, and determinants. **Chinese Medical Journal**, [*s. l.*], v. 135, n. 5, p. 584–590, 2022. Disponível em: https://journals.lww.com/10.1097/CM9.0000000000002108.

YANG, Quan-en. Human cancer xenografts in immunocompromised mice provide an advanced genuine tumor model for research and drug development-A revisit of murine models for human cancers. **Biochimica et Biophysica Acta (BBA) - General Subjects**, [*s. l.*], v. 1865, n. 8, p. 129929, 2021. Disponível em: https://doi.org/10.1016/j.bbagen.2021.129929.

YOU, E.L.; HENRY, M.; ZEITOUNI, A.G. Human Papillomavirus–Associated Oropharyngeal Cancer: Review of Current Evidence and Management. **Current Oncology**, [*s. l.*], v. 26, n. 2, p. 119–123, 2019. Disponível em: https://www.mdpi.com/1718-7729/26/2/4819.

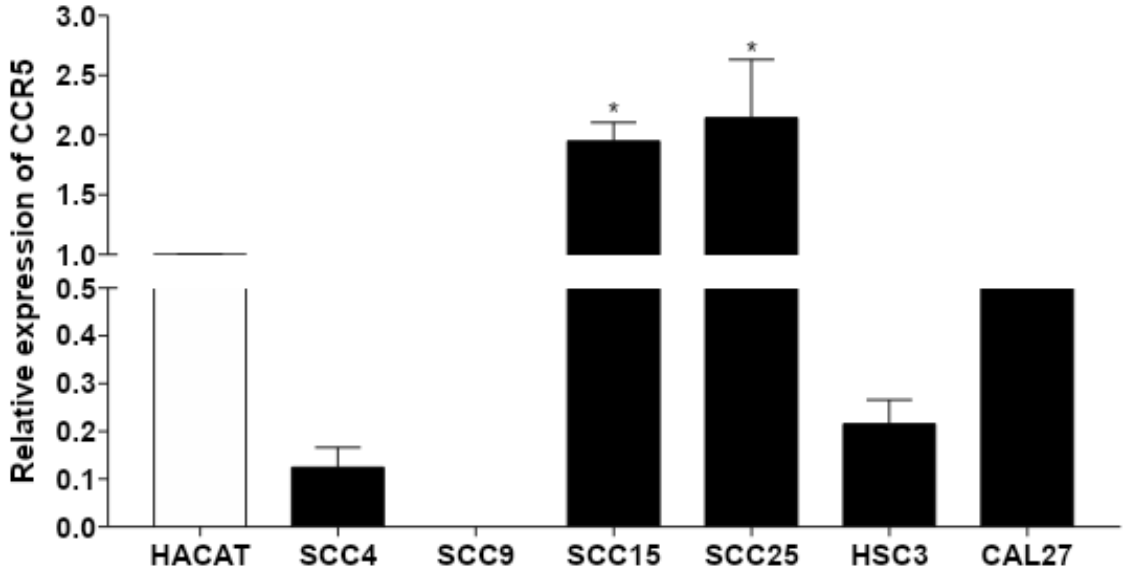
ZAZO, Sandra *et al.* Autocrine CCL5 effect mediates trastuzumab resistance by ERK pathway activation in HER2-positive breast cancer. **Molecular Cancer Therapeutics**, United States, v. 19, n. 8, p. 1696–1707, 2020.

ZHANG, Feng *et al.* Structure activity relationship studies of natural product chemokine receptor CCR5 antagonist anibamine toward the development of novel anti prostate cancer agents. **European Journal of Medicinal Chemistry**, France, v. 55, p. 395–408, 2012.

ZHOU, Q *et al.* CCR5 blockade inflames antitumor immunity in BAP1-mutant clear cell renal cell carcinoma. **Journal for immunotherapy of cancer**, [*s. l.*], v. 8, n. 1, 2020.

ZI, Jie *et al.* Treatment with the C-C chemokine receptor type 5 (CCR5)-inhibitor maraviroc suppresses growth and induces apoptosis of acute lymphoblastic leukemia cells. **American journal of cancer research**, [*s. l.*], v. 7, n. 4, p. 869–880, 2017. Disponível em: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L615846581.

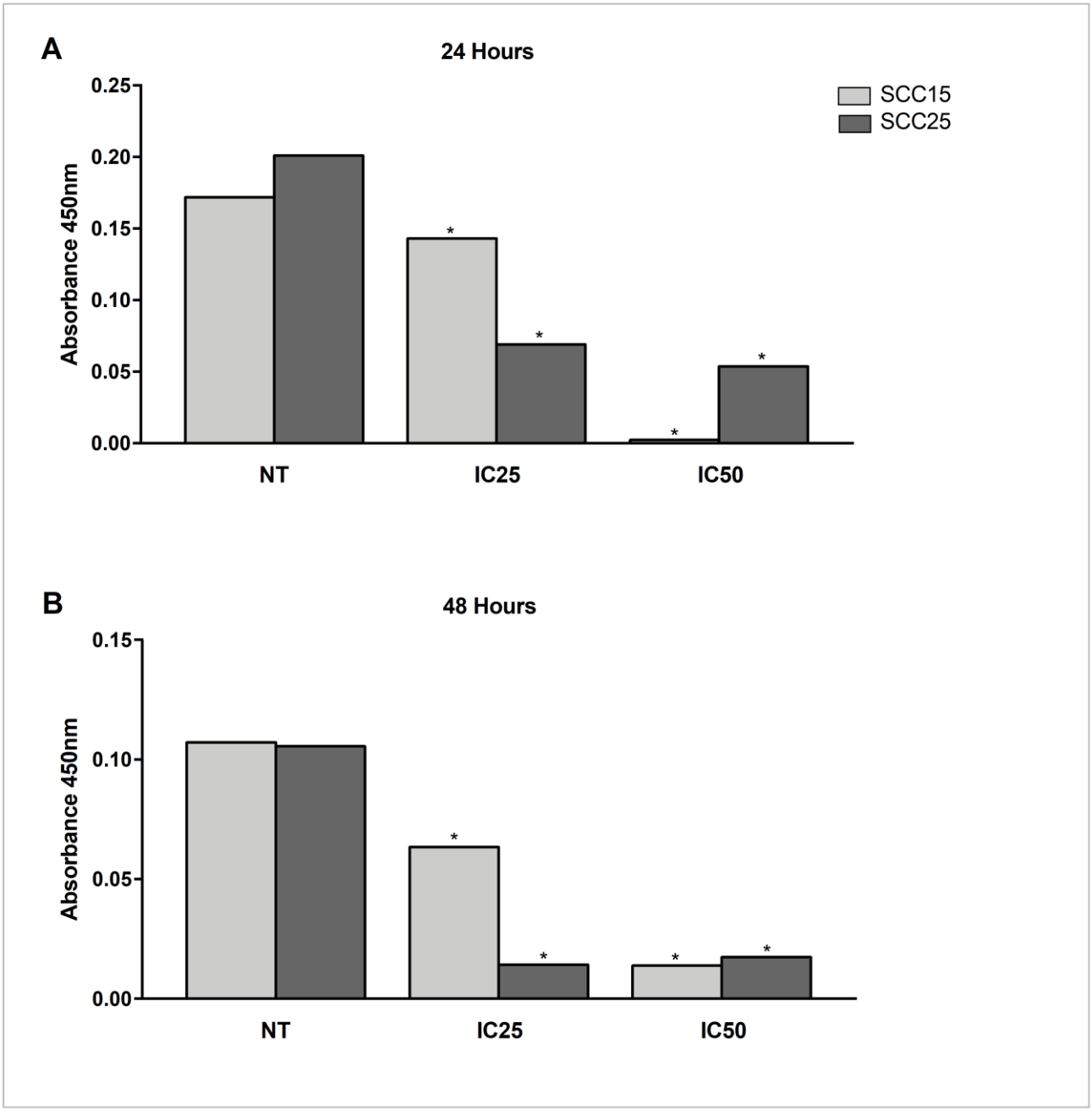
Figure 1. CCR5 expression in OSCC cell lines



From: Author

The levels of CCR5 mRNA in OSCC cell lines were assessed by real-time PCR. Expression was normalized to the average value of the immortalized but not transformed epithelial HaCat cell line. Data showed a clear CCR5 overexpression in SCC15 and SCC25 cell lines (*$p<0.05$)

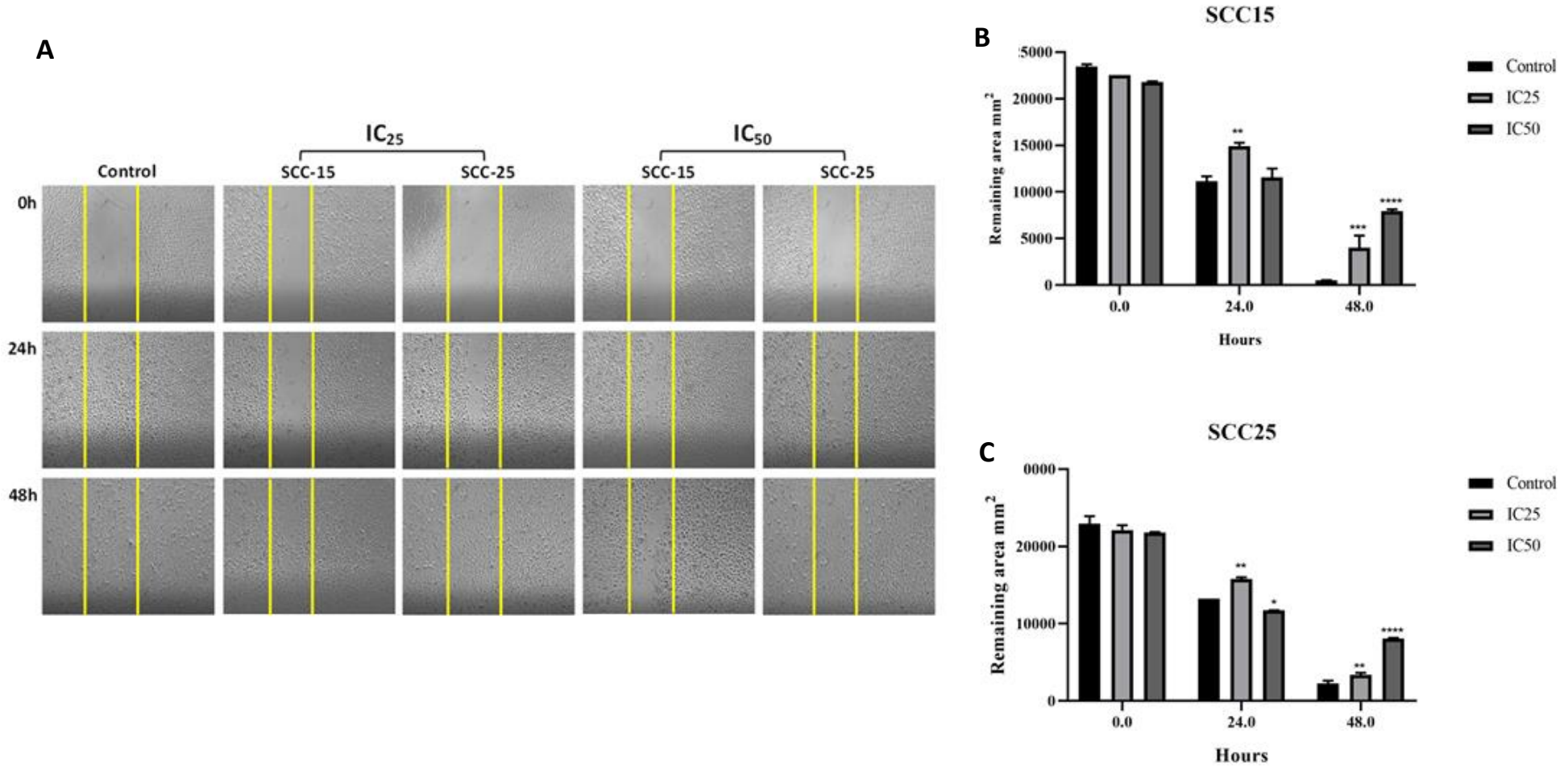
Figure 2. MVC impaired cell proliferation of OSCC cells



From: Author

Treatment with MVC inhibited the proliferation of SCC15 and SCC25 cells compared with non-treated (NT) controls, as revealed by bromodeoxyuridine (BrdU) incorporation index after (A) 24 and (B) 48 hours. The graphs represent a experimental triplicate analysis and were statistically imposed using ANOVA followed by Tukey's test, where *$p < 0.05$.

Figure 3. MVC impaired cell migration of OSCC cells



From: Author

Photomicrographs of cell lines were taken at 0, 24, and 48 h after wounding (40X) and the average width of the lacunae was measured (A). Migration of SCC15 and SCC25 cells treated with MVC was significantly decreased, in comparison with non-treated cells (control) for both 24 and 48 h of treatment (B). The graphs compile experimental analyzes of quantification in triplicate of the remaining area, and the results were obtained by One-way ANOVA followed by the Tukey test, where *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0,0001.