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# Exploring the anti-tumor effect of silver nanoparticles in oral and skin cancer in vivo: Systematic review and meta-analysis

**Explorando o efeito antitumoral das nanopartículas de prata em câncer oral e de pele in vivo: Revisão sistemática e meta-análise**

**Exploración del efecto antitumoral de las nanopartículas de plata en el cáncer oral y cutáneo in vivo: Revisión sistemática y metaanálisis**

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**Abstract**
This study aimed to analyze the in vivo anti-tumor effect of silver nanoparticles (AgNPs) against oral and skin cancer. The PRISMA guidelines were followed, and a registration in PROSPERO was performed. A search of databases was carried out in September 2023. The data were analyzed using the inverse variance method with a random effect model. All studies assessed anti-tumor efficacy against melanoma, and 21 days after treatment with AgNPs showed a reduction in tumor volume (SMD -6.58; PI [-9.82, -3.34]). Administration of AgNPs in injectable and topical form was effective in reducing tumors, erythema and preventing metastases without causing side effects. We concluded that AgNPs showed antitumor action against mice melanoma by reducing tumor volume and preventing cell proliferation and metastasis.
**Keywords:** Silver Compounds; Melanoma; Antineoplastic Agents.

**Resumo**
Este estudo teve como objetivo analisar o efeito antitumoral in vivo das nanopartículas de prata (AgNPs) contra o câncer oral e de pele. Foram seguidas as diretrizes PRISMA e realizado registro no PROSPERO. A pesquisa nos bancos de dados foi realizada em setembro de 2023. Os dados foram analisados usando o método de variância inversa com um modelo de efeito aleatório. Todos os estudos avaliaram a eficácia antitumoral contra o melanoma, e 21 dias após o tratamento com AgNPs mostraram uma redução no volume do tumor (SMD -6,58; PI [-9,82, -3,34]). A administração de AgNPs na forma injetável e tópica foi eficaz na redução de tumores, eritema e prevenção de metástases sem causar efeitos colaterais. Concluiu-se que as AgNPs apresentaram ação antitumoral contra o melanoma de camundongos, reduzindo o volume do tumor e prevenindo a proliferação celular e a metástase.
**Palavras-chave:** Compostos de Prata; Melanoma; Agente Antitumoral.

**Resumen**
Este estudio tuvo como objetivo analizar el efecto antitumoral in vivo de las nanopartículas de plata (AgNPs) contra el cáncer oral y de piel. Se siguieron las directrices PRISMA y se realizó un registro en PROSPERO. Se realizó una búsqueda en bases de datos en septiembre de 2023. Los datos se analizaron mediante el método de la varianza inversa con un modelo de efectos aleatorios. Todos los estudios evaluaron la eficacia antitumoral frente al melanoma, y 21 días después del tratamiento con AgNPs se observó una reducción del volumen tumoral SMD -6,58; PI [-9,82, -3,34]). La administración de AgNPs en forma inyectable y tópica fue eficaz para reducir los tumores, el eritema y prevenir las metástasis sin causar efectos secundarios. Concluimos que las AgNPs mostraron acción antitumoral contra el melanoma en ratones al reducir el volumen tumoral y prevenir la proliferación celular y la metástasis.
**Palabras clave:** Compuestos de Plata; Melanoma; Agente Antitumoral.

## 1. Introduction

Among the most aggressive oral and skin tumors, melanoma (ME), which originates from melanocytes, is the most aggressive due to its rapid proliferation and production of metastases to other organs (Zheng et al., 2023). Mucosal ME of the head and neck may have a rare primary incidence in the mucosa of the membranes of the oral, nasal, and sinus cavities, pharynx, and larynx (Rashid et al., 2023; Sergi et al., 2023), or from the proliferation of metastases originating in cutaneous melanoma in other regions of the body (Bai et al., 2023). This cancer has irregular borders, variable coloration, and rapid evolution, with a high incidence and lethality rate among men and women (Bai et al., 2023). Risk factors include chronic exposure to ultraviolet (UV) light, genetic factors, and white skin color (Siegel et al., 2023).

Surgical excision, radiotherapy, chemotherapy, and immunotherapy can be used to treat this condition (Wang et al., 2022). These treatments have limitations related to high cost, tumor volume, involvement of other organs, a limited dose of chemotherapy with the possibility of cell resistance, and the induction of serious side effects (Zheng et al., 2023).

Early diagnosis increases the success rate of treatment and patient survival, but it is difficult to achieve due to the variability in the clinical appearance of melanoma and the incidence in internal organs (Wang et al., 2022). To overcome the failure of late diagnosis, methods have been developed using lipid nanoparticles (NP) (Sivadasan et al., 2023) and metallic NP (Li et al., 2023) that optimize tumor localization, image acquisition, and immunodiagnosis. Among these, the use of AgNPs is highlighted for its theranostic potential through its physical property of surface plasmon resonance, which facilitates contrast for imaging and intercalation with antibodies, biomarkers, and drugs that have anti-tumor action (Shabatina et al., 2023).

AgNPs, initially used as antimicrobials in the biomedical and industrial fields (Silva et al., 2023), showed an innate anti-tumor effect in vitro through the production of reactive oxygen species (ROS) that induce tumor cell apoptosis (Shabatina et al., 2023); anti-inflammatory action that reduces local vascular permeability; and anti-angiogenic potential that induces tumor death and prevents metastasis formation (Twilley et al., 2022).

To assess the anti-tumor efficacy of AgNPs against the wide variety of tumors that affect the oral cavity and skin, we selected melanoma due to its high incidence, lethality, limitations of conventional treatments that may not be effective depending on the stage of the tumor, and induction of severe side effects (Zheng et al., 2023). The theranostic potential of AgNPs due to their antitumor and anti-inflammatory effects and the possibility of intercalation with other compounds could be an alternative for antitumor and metastasis prevention (Twilley et al., 2022). This study aimed to analyze the in vivo anti-tumor effect of silver nanoparticles (AgNPs) against oral and skin cancer. The investigation of pre-clinical studies and answering the question "Do silver nanoparticles (AgNPs) show antitumor action in vivo against oral and skin cancer?" could clarify the current state of the art and encourage the development of studies that validate their use for the treatment of oral and skin cancer in safe doses.

## 2. Methodology

This systematic review was structured according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021) and registered in the International Prospective Register of Systematic Reviews (PROSPERO - CRD42023483515).

The question was conducted using the PICOS strategy: 1. population (mice of both sexes of different ages and weights); 2. intervention (administration of silver nanoparticles); 3. comparison (placebo or no treatment); 4. outcome (anti-tumor or tumor-reducing effect); and 5. type of study (in vivo animal model).

A search was performed in the PubMed/MEDLINE, Lilacs, Web of Science, Scopus, and Google Scholar databases on September 18, 2023 with the combination of the terms (("Silver Nanoparticles" OR "AgNPs") AND ("Anti-cancer" OR

"Tumor" OR "Antitumor" OR AND ("In Vivo" OR "Mice" OR "Mouse" OR "Rats")). No language or date restrictions were applied.

The articles were manually selected by one researcher (J.M.C.S). In the first stage, duplicate articles were removed in EndNote, and the selection was carried out by analyzing titles and abstracts in Rayann (Qatar Computing Research Institute) (Ouzzani et al., 2016). The second stage was carried out by reading the full text, and the results were analyzed by the supervisor (A.C.R).

Eligibility criteria included: 1. in vivo studies in animal models (mice of both sexes and of different ages and weights) that evaluated the anti-tumor activity of AgNPs against oral and skin tumors; 2. studies that evaluated tumor reduction in volume, size, and weight; 3. studies that administered AgNPs topically or injectably; and 4. publications in peer-reviewed journals. The exclusion criteria included: 1. in vitro studies, human clinical trials, observational studies, review articles, case reports, letters to the editor, short communications, patents, conference abstracts, book chapters, and editorials; 2. evaluation of other silver-based compounds for the treatment of melanoma; 3. studies that, in addition to the administration of AgNPs, included another variable such as the application of light and temperature; and 4. studies that evaluated the anti-tumor action of AgNPs against other types of cancer.

The SYRCLE tool for animal studies was used to assess the risk of bias of the included studies (Hooijmans et al., 2014). Based on the analysis of the domains, the articles were classified as "low risk", "high risk," or "uncertain risk" of bias. The certainty of the evidence (antitumor action in reducing melanoma volume after in vivo treatment with AgNPs) was determined using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (Zeng et al., 2021).

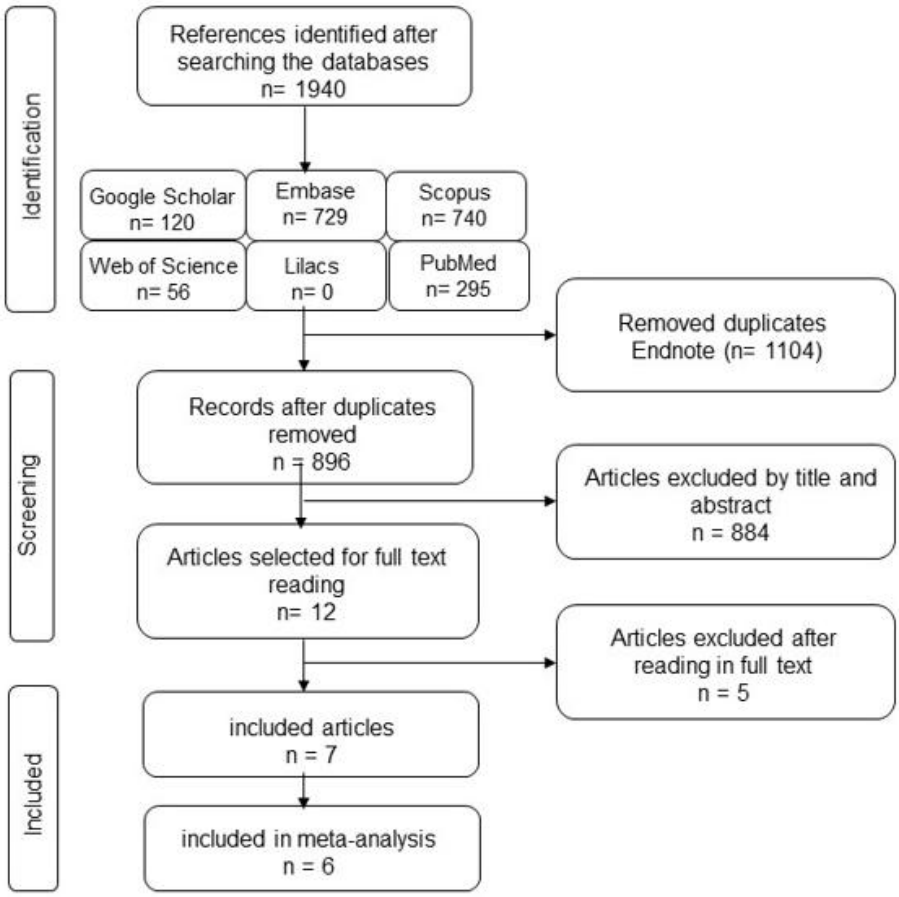
The data was extracted qualitatively in a table containing the following information: 1. Authors, year, country, and Journal; 2. Size and concentration of AgNPs and sintezys processes; 3. Method; 4. Results; and 5. Conclusion.

Data analysis (tumor reduction after application of AgNPs compared to placebo or no treatment) was performed using Review Manager Web (RevMan Web, The Cochrane Collaboration). Quantitative data was grouped as continuous variables based on the mean, standard deviation, and total number of mice included in the groups evaluated. Quantitative data in figures were collected using WebPlotDigitizer software, version 4.6 (Ankit Rohatgi, USA) (Drevon et al., 2017), and the data presented with median and confidence interval were transformed into mean and standard deviation (Luo et al., 2018). The inverse variance method was used for the random effect (RE) model, and the results were reported with a standardized mean difference (SMD) ($a = 0.05$; calculation of prediction interval; heterogeneity tested with the Q test and reported with the $I^2$ index), as recommended by Cochrane. Due to the small number of studies included, it was not possible to perform Egger's test; however, the data was plotted on a funnel plot for qualitative analysis.

## 3. Results

A total of 295 studies were found in PubMed, 740 in Scopus, 729 in Embase, 56 in Web of Science, and 0 in Lilacs. A complementary search in Google Scholar yielded 26900 references, of which the first 120 were selected. Of the 1940 studies found, 1044 duplicates were excluded using EndNote. After the initial selection of 896 studies by title and abstract, 12 studies were selected for full-text reading, and 7 were included in this review (Figure 1).

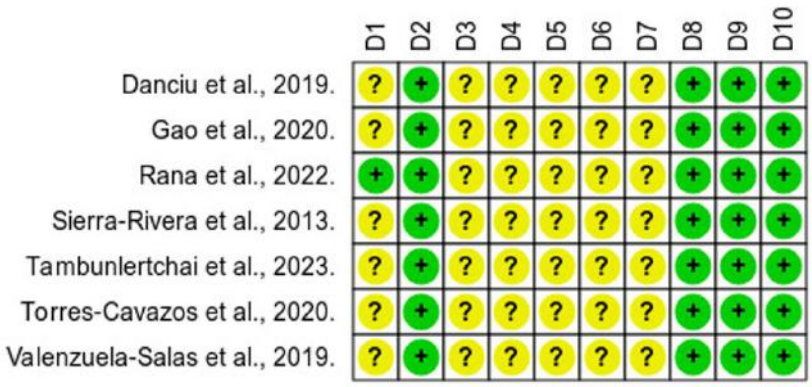
**Figure 1 -** Systematic review flowchart.




Source: Adapted by Page, et al. (2021).

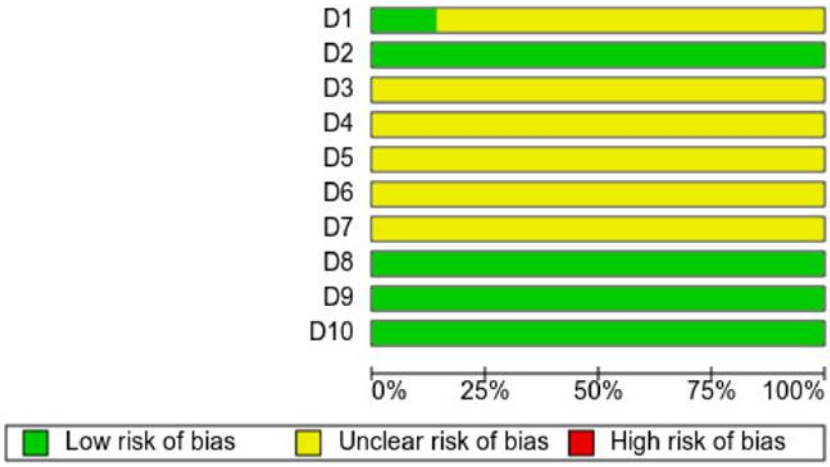
The risk of bias is shown in Figures 2 and 3. Of the 7 included studies, 6 presented an uncertain risk of bias because they did not mention the sequence of allocation of the rats (D1) Tambunlertchai et al., 2023; Sierra Rivera et al., 2013; Torres-Cavazos et al., 2020; Valenzuela-Salas et al., 2019; Danciu et al., 2019; Gao et al., 2020). All of the studies presented an uncertain risk of bias because they did not report adequate allocation blinding (D3), randomized placement of the animals during the experiment (D4), investigators' knowledge of the treatment administered to the participants (D5), randomized selection of animals for outcome assessment (D6) and outcome assessment (D7). Low risk of bias was observed for similarity at baseline and adjustment for confounding factors (D2), treatment of outcomes (D8), study reports free from individual selection of outcomes (D9), and absence of factors that could generate high risk of bias for outcomes (D10) (Tambunlertchai et al., 2023 (Tambunlertchai et al., 2023; Rana et al., 2022; Sierra Rivera et al., 2013; Torres-Cavazos et al., 2020; Valenzuela-Salas et al., 2019; Danciu et al., 2019; Gao et al., 2020). The overwall of certainty of the evidence showed that the results were highly recommended.

**Figure 2 -** Risk of bias summary by SYRCLE's tool.




Source: Created by the authors using Cochrane's RevMan Web.

**Figure 3 -** Risk of bias graph by SYRCLE's tool.




Source: Created by the authors using Cochrane's RevMan Web.

This review included pre-clinical studies in animal models using male and female (C57BL/6) black mice and female albino mice (BALB/c), aged between 6 and 14 weeks and weighing between 18 and 25 g (Table 1). The melanoma-type tumor was induced in the animals by subcutaneous injection with murine melanoma cells B16 4A5 (1×106 cells) and B16 F10 (1×106 cells), and alternating injection with carcinogenic substances DMBA (7,12-dimethylbenz[a]anthracene - 500 nmol/100 µl) and TPA (12-O-tetradecanoylphorbol-13-acetate - 1.7 nmol/100 µl) (Tambunlertchai et al., 2023; Rana et al., 2022; Sierra Rivera et al., 2013; Torres-Cavazos et al., 2020; Valenzuela-Salas et al., 2019; Danciu et al., 2019; Gao et al., 2020).

After the melanoma induction protocols (palpable lesion), treatment was started with AgNPs produced by green methods with betulin and dopamine and by conventional chemical. AgNPS were used in different concentrations (0.02mM, 5 mg/kg and at 20%) and sizes ranging from 5 to 35 nm. NP has been included in PEG hydrogels for injection and Pluronic F127 for topical application, in suspensions for injection and combined with other products for injection such as chitosan, nisin, 5-FU, dichloroacetate and polyvinylpyrrolidone (PVP). The treatment varied between the number of doses (1 to 6 doses/day) and time period (2 to 28 days) (Tambunlertchai et al., 2023; Rana et al., 2022; Sierra Rivera et al., 2013; Torres-Cavazos et al., 2020; Valenzuela-Salas et al., 2019; Danciu et al., 2019; Gao et al., 2020).

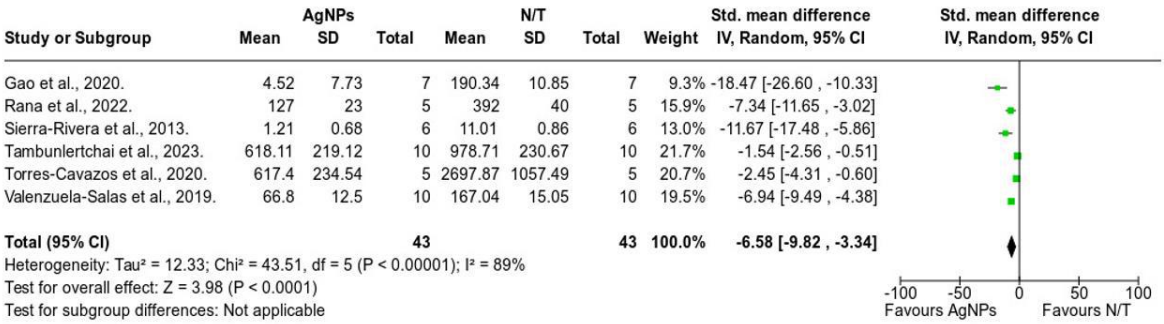
Antitumor activity was measured after treatment with AgNPs by non-invasive methods to measure melanin content, skin erythema and hydration, and body weight, as well as surgical methods for measuring size and volume, body weight, tumor

weight and tumor regression. Treatment of melanoma with injectable hydrogels containing AgNPs associated or not with betulin was effective in reducing the melanin content of the tumor and erythema; however, only the hydrogel containing AgNPs did not induce dehydration in the mice (Tambunlertchai et al., 2023; Rana et al., 2022; Sierra Rivera et al., 2013; Torres-Cavazos et al., 2020; Valenzuela-Salas et al., 2019; Danciu et al., 2019; Gao et al., 2020).

Another AgNPs-based hydrogel for topical application after surgical excision of the tumor showed effective antitumor activity by completely inhibiting the appearance of cancer cells. Tumor volume reduction was observed after the injection of pure AgNPs in a dose-dependent manner, coated with PVP and combined with dichloroacetate, chitosan, nisin, and 5-FU. Valenzuela-Salas et al. (2019) also reported that AgNPs were more effective in reducing tumor volume compared to cisplatin (an anti-tumor drug), and Sierra-Rivera et al. (2013) observed that AgNPS inhibited metastasis formation.

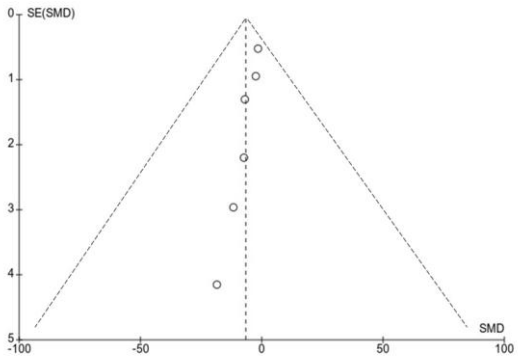
Were included 6 studies, with 86 participants, divided into 2 groups that evaluated the reduction in melanoma volume after 21 days of treatment with AgNPs compared to placebo or no treatment (Figure 4) (Tambunlertchai et al., 2023; Rana et al., 2022; Sierra Rivera et al., 2013; Torres-Cavazos et al., 2020; Valenzuela-Salas et al., 2019; Gao et al., 2020). After AgNPs treatment, a significant reduction in melanoma volume was observed (SMD -6,58, PI [-9.82 , -3.34]), and despite the heterogeneity between the results ($I^2$ = 91%), the overall effect was consistent with tumor reduction (Z = 2.55; P = 0.0001). Sensitivity analysis showed that after removing the study by Gao et al. (2020), Sierra-Rivera et al. (2013), Torres-Cavazos et al. (2020) and Tambunlertchai et al. (2023), heterogeneity became low (I2 = 0%) and the magnitude of the effect was increased (SMD -7.04, PI [-9.24, -4.84]; P < 0.0001). However, we assumed heterogeneity after a qualitative assessment of the distribution in the funnel plot (Figure 5), which indicated low publication bias.

**Figure 4 -** Forest-plot of melanoma volume reduction after treatment with AgNPs and placebo over 21 days.




Source: Created by the authors using Cochrane's RevMan Web.

**Figure 5 -** Funnel Plot of the included studies.




Source: Created by the authors

**Table 1 -** Anti-tumor effect of AgNPs in the treatment of melanoma in mice.

| Author/ Year/ Journal | AgNPs | Methods | Results | Conclusion |
|---|---|---|---|---|
| Danciu et al., 2019. Romania and Germany.<br>European Journal of Pharmaceutics and Biopharmaceutics. | PEG-AgNPs 0,02 mM (25 nm) e PEG-AgNPs-Betulin (75 nm).<br>Green synthesis with Betulin. | *Population:* 24 female mice/4 (C57BL), 12 to 14 weeks old, and weight 23 ± 2 g.<br>*Protocol*: subcutaneous induction of melanoma with murine B16 4A5 cells ($1\times10^6$ cells/100 µL of PBS/mice); and treatment with 6 doses over 2 days.<br>*Groups:* G1 (control group), G2 (inoculated group; no intervention), G3 (PEG-AgNPs, 10 mg/kg), and G4 (PEG-AgNPs-B, 10 mg/kg).<br>*Evaluation*: During 28 days non-invasively with the Multiprobe Adapter System (MPA5, Courage-Khazaka, Germany): 1. melanin content; 2. erythema; and 3. skin hydration. | 1. The melanin content was increased for G2, G3, and G4, and a similar reduction was observed for G3 and G4.<br>2. Erythema showed a higher reduction for G4, followed by G3.<br>3. The melanoma groups showed a reduction in hydration, and, over time, G3 and G2 showed a similar reduction, and G4 showed less hydration. | Treatment with PEG-AgNPs associated with or not with betulin was effective in tumor reduction. AgNPs-B were more effective in reducing erythema. Pure AgNPs showed less induction of dehydration. |
| Gao et al., 2020. China. Nano. | F127-AgNPs hydrogel (5 mg/kg) with a size of 5 nm.<br>Green synthesis with dopamine. | *Population:* 14 female mice/2 (C57BL), 6 to 8 weeks old, weight not specified.<br>*Protocol*: subcutaneous induction of melanoma with murine B16 F10 cells ($1\times10^6$ cells), resection of the tumor (200 $mm^3$), and gel application to the wound at 0, 2, and 4 days.<br>*Groups*: G1 (inoculated group; no intervention) and G2 (F127-AgNP hydrogel).<br>*Evaluation*: During 15 days of non-invasively measuring 1. body weight; 2. tumor volume; and invasively measuring 3. Histological. | 1. There was no change in body weight between the groups.<br>2. G1 showed tumor growth of 540 $mm^3$ after 15 days, and no growth was observed for G2.<br>3. In G2, no tumor cells were found in the skin tissue after treatment. | The hydrogel containing AgNPs showed anti-tumor effect *in vivo* without causing weight loss in the rats, suggesting its low toxicity. |
| Rana et al., 2022. India. International Journal of Pharmaceutics. | AgNPs with a size of 14.91 nm; CHI-AgNPs (38.09 nm); nisin-CHI-AgNPs (61.67 nm); 5-FU/CHI-AgNPs (45.08 nm); and 5-FU/nisin-CHI-AgNPs (72.39 nm). | *Population*: 30 female/6 (BALB/c) mice, age not specified, weighing 25–30 g.<br>*Protocol*: subcutaneous induction of melanoma with DMBA 2x day (500 nmol/100 µl) or TPA 3x day (1.7 nmol/100 µl) for 20 weeks and treatment for 4 weeks.<br>*Groups*: G1 (control group), G2 (untreated melanoma), G3 (CHI-AgNPs; 3.5 mg/kg), G4 (Nisin-CHI-AgNPs 0.63 mg/kg), G5 (5-FU/CHI-AgNPs 0.75 mg/kg), and G6 (5-FU/nisin-CHI-AgNPs 0.63 and 0.75 mg/kg of nisin and 5-FU, respectively).<br>*Evaluation*: 1. mean tumor volume and 2. mean tumor weight. | 1. A reduction in tumor volume was observed for groups G3 (10.90%), G4 (19.05%), G5 (37.69%), and G6 (68.34%).<br>2. A reduction in the average tumor load was observed for groups G3 (8.89%), G4 (29.44%), G5 (55.78%), and G6 (82.39%). | The association of AgNPs with products such as chitosan, 5-FU, and nisin shows anti-tumor action in reducing pre-formed melanomas. The combination of all the products showed higher efficacy. |

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| Sierra-Rivera et al., 2013. Mexico. African Journal of Microbiology Research. | AgNPs 10 nm. | *Population*: 12 male and 2 female mice (C57BL), 6 weeks old, and weighing 18 to 25g.<br>*Protocol*: subcutaneous induction of melanoma with murine B16 F10 cells ($5\times10^6$ cells) over 8 days and a single injection around the tumor for 21 days.<br>*Groups*: G1 (inoculated group; no intervention) and G2 (AgNPs 1000 mg/kg).<br>*Evaluation*: After 21 days: 1. body weight; 2. tumor weight; 3. tumor volume; and 4. metastasis formation. | 1. G1 (25.00 ± 2.78 g) and G2 (21.30 ± 1.14 g).<br>2. G1 (4.97 ± 0.31 g) and G2 (0.5 ± 0.22 g)<br>3. G1 (11.01 ± 0.86 mm3) and G2 (1.21 ± 0.68 mm3)<br>4. G1 presented metastases in muscle, peritoneal cavity, intestine, and liver, and no metastases were reported for G2. | AgNPs were effective in reducing tumor size and volume and preventing the formation of metastases. |
| Torres-Cavazos et al., 2020. Mexico.<br>Journal of Nanomaterials. | Dichloroacetate-loaded silver nanoparticles, separate and combined. Concentration not specified. | *Population*: 10 female mice/2 (C57BL), 6–10 weeks old, weight 23 g.<br>*Protocol*: subcutaneous induction of melanoma with murine B16 F10 cells ($1\times10^6$ cells) over 7 days and a single injection around the tumor for 21 days.<br>*Groups*: G1 (inoculated group, no intervention) and G2 (AgNPs 28 mg/kg), G3 (DCA 50 mg/kg), and G4 (AgNPs + DCA, 28 mg/kg and 50 mg/kg, respectively, on alternate days).<br>*Evaluation*: After 21 days, 1. tumor regression | There was similar tumor regression for groups G2, G3 and G4. | AgNPs, either alone or in combination with DCA, are effective anti-tumor agents for tumor reduction. |
| Valenzuela-Salas et al., 2019. Mexico.<br>Oxidative Medicine and Cellular Longevity. | AgNPs (35 ± 15 nm): commercial formulation Argovit™ (AgNPs coated with PVP, highly dispersed in water, 200 mg/mL). | *Population*: 60 female/6 mice (C57BL/6JNHsd), 8-10 weeks old, weight 20 g.<br>*Protocol*: subcutaneous induction of melanoma with murine B16 F10 cells ($1\times10^6$ cells) over 12 days and a single injection around the tumor every 3 days for 21 days.<br>*Groups*: G1 (inoculated group, no intervention), G2 (cisplatin, 2 mg/kg), G3 (PVP, 12 mg/kg), G4 (AgNPs, 3 mg/kg), G5 (AgNPs, 6 mg/kg), and G6 (AgNPs, 12 mg/kg).<br>*Evaluation*: After 21 days, tumor volume was observed. | Lower tumor volumes in G4, G5, and G6 were observed (722–837 mm3) compared to G1 (1,500 mm3), G2 (1,704 mm), and G3 (31,890 mm3), which were similar 9 days after the start of treatment (1,500 mm3). On the 11th day of treatment, G4, G5, and G6 showed a similar tumor increase (837–1,142 mm3), and an increase was observed for G2 (3,500 mm3) and G3 (9,500 mm3).<br>At the end of the experiment, the mice treated with 3 or 12 mg/kg of AgNPs survived 19, 15, and 13 days longer than those treated with G1, G2, and G3, respectively. | AgNPs showed effective dose-dependent anti-tumor action compared to cisplatin and no treatment. |

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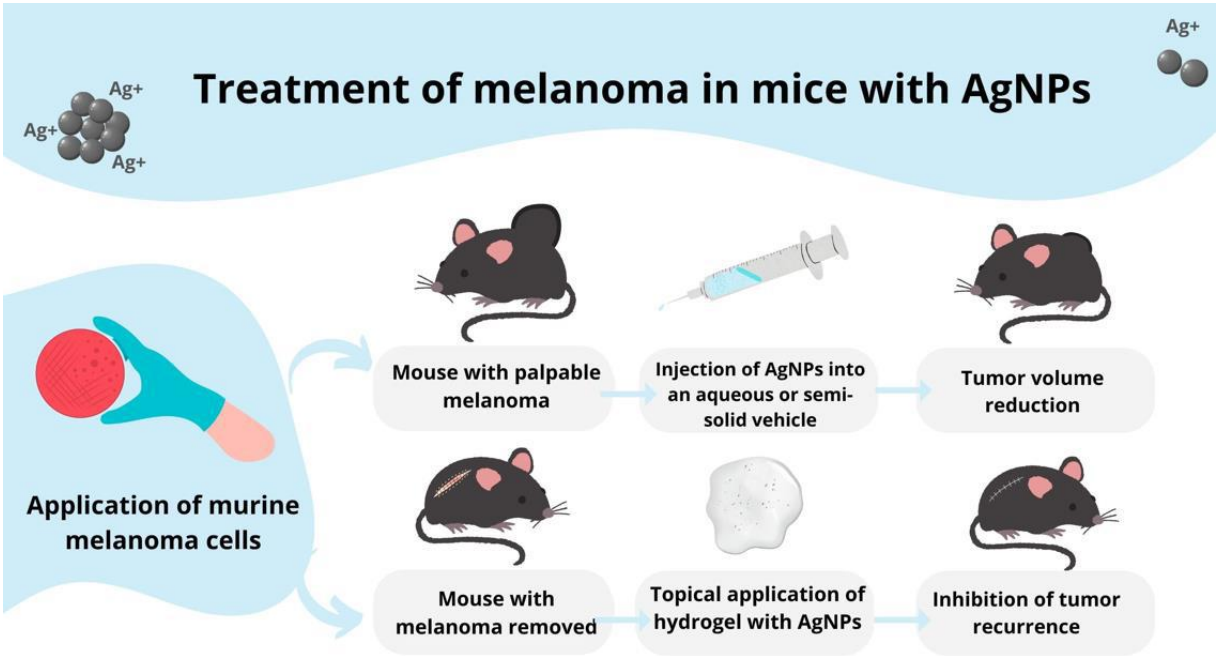
| Tambunlertchai et al., 2023. USA.<br>European Journal of Pharmaceutics and Biopharmaceutics. | AgNPs (10.60 ± 4.93 nm) coated with PVP in 0.2 M succinate buffer vehicle (100 µL). | *Population*: 30 female mice/3 (C57BL/6J), 6-8 weeks old and weight 18-20 g.<br>*Protocol*: subcutaneous induction of melanoma with murine B16 F10 cells ($1\times10^6$ cells) in 7 days and 7 injections into the tumor (2 mg/kg).<br>*Groups*: G1 (inoculated group – without intervention), G2 (AgNPs), G3 (AgNPs + RSQ).<br>*Evaluation*: After 21 days, assessed 1. Weight of the mice, 2. Volume of the tumor. | 1. No weight loss was observed in G1 rats;<br>2. Tumor volume reduction was more effective for G3 compared to G2. | AgNPs showed effective anti-tumor action; however, higher action was observed when combined with RSQ. |

Source: Prepared by the authors.

## 4. Discussion

Although the anti-tumor effect of silver has been studied for many years [50], to the present day, this material is still being studied to determine the best form of application and dose in order to achieve positive results. This systematic review evaluated the anticancer efficacy of AgNPs after inducing melanoma in mice (Figure 6). AgNPs were initially used for product development in the form of solutions, creams (Parveen et al., 2021), gels (Silva et al., 2023; and tissue dressings (Alamer et al., 2022) due to their broad-spectrum of antimicrobial action and aid in tissue healing. It has also been applied to hygiene products (Bansod et al., 2015), paints (Alali et al., 2023), and medical equipment (Kaabipour et al., 2021) to prevent the accumulation of microorganisms and infections. Further studies have shown that NP in low concentrations has anti-inflammatory and anti-tumor potential in a single compound, and it is believed that this synergy will allow the development of anti-tumor treatments and prevention of metastases without inducing side effects like conventional chemotherapeutic drugs (Wang et al., 2022).

**Figure 6 -** Injectable and topical treatment with AgNPs reduces tumor volume and inhibits the growth of new tumor cells.



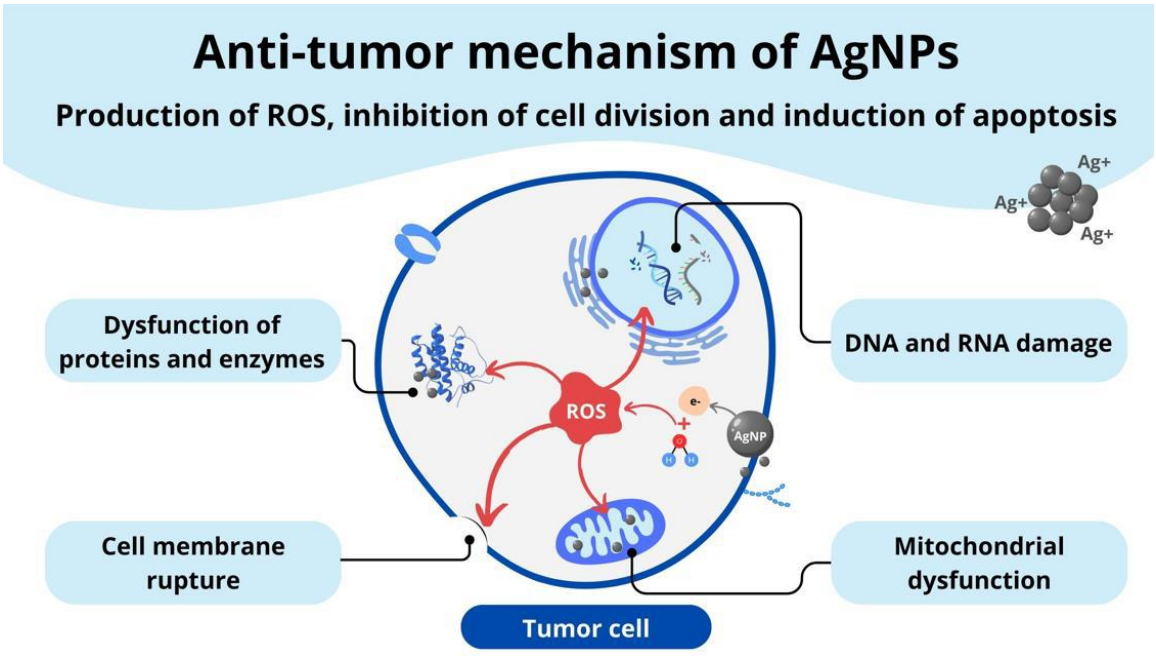

Source: Prepared by the authors.

The search for new treatments for invasive and non-invasive (in situ) melanoma is stimulated by its high incidence, the metastasis formation in organs of the lymphatic, digestive, nervous, respiratory, and mucosal systems, and the high mortality rate in the world (Zheng et al., 2023). Current treatments include surgical excision of the tumor with or without chemotherapy and radiotherapy (Zheng et al., 2023). However, surgical and radiotherapy treatments may not be successful due to the size and location of the tumor and the involvement of other organs (Zheng et al., 2023). Chemotherapy treatment has limitations such as high cost, dose-dependent toxicity, side effects that reduce patients' quality of life, and cellular resistance to the drug (Miranda et al., 2020). Tumor cells can develop mechanisms of resistance to chemotherapeutic drugs by expressing P-glycoprotein, MDR1, and ABCB1 proteins, which act as multidrug resistance (MDR) transporters and expel the drug into the extracellular environment (Miranda et al., 2020).

As an antitumor alternative, AgNPs have been applied to murine melanoma, and a time-dependent decrease in the tumor was observed due to their direct application [34–40] and the enhanced permeability and retention (EPR) effect (Shinde et al., 2022). EPR occurs due to the solid tumor exhibiting higher blood vascular permeability and decreased lymphatic draining, which allows for higher accumulation of AgNPs and an efficient antitumor effect (Shinde et al., 2022). The selective accumulation of NP in the tumor mass shows a promising alternative for reducing cancer and its accumulation in other organs, and subsequent surgical excision of the tumor could remove the AgNPs to avoid prolonged release of ROS into the organism.

The accumulation of NP in the tumor generates ROS (O2-, H2O2 and HO-) through direct electron donation to molecular oxygen or indirectly with other molecules present, inducing oxidative stress and apoptosis (Canaparo et al., 2021). Tumor cells are more susceptible to oxidative stress due to the prior production of ROS by their metabolism, which, added to the accumulation of AgNPs, promotes cell death (Canaparo et al., 2021). In addition, NP induces tumor apoptosis due to the production of membrane damage, electron leakage, activation of cytochrome C-dependent pathways and caspases, and inhibits the energy chain of oxidative phosphorylation (mitochondria) and glycolysis (cytoplasm) (Arruda et al., 2022). The selective damage to tumor cells caused by the injection of NPs and their accumulation has the potential to treat cancer with less chance of inducing systemic side effects such as those caused by chemotherapy (Figure 7).

**Figure 7 -** Anti-tumor mechanisms of AgNPs.




Source: Prepared by the authors.

The anti-tumor efficacy of AgNPs without inducing systemic effects was observed in comparison to chemotherapy in mice. Valenzuela-Salas et al. (2019) reported that the injection of cisplatin (2 mg/kg) showed inferior anti-tumor action compared to the administration of AgNPs (3-12 mg/kg) and resulted in the death of the animals 7 days after the treatment protocol due to the toxicity of the drug. Cisplatin belongs to the class of alkylating chemotherapeutic drugs that add alkyl groups (carbon and hydrogen) to the DNA of tumor cells, preventing their division and inducing cell death [21,71–73]. In addition, weight loss and loss of appetite were observed in mice during treatment with the chemotherapeutic drug [38], which commonly induces nausea, vomiting, bone marrow suppression, and gastrointestinal, liver, and kidney problems in oncological patients (Li et al., 2023). The pre-clinical evidence of non-induction of adverse effects and survival of mice after treatment with AgNPs shows their therapeutic potential and encourages clinical investigation of their pharmacokinetic and pharmacodynamic mechanisms and long-term effects.

AgNPs show enhanced biocompatibility for biomedical applications when synthesized by green methods using botanical components, microorganisms, and other biomolecules to reduce silver (Alkhalaf et al., 2020). These methods allow the preparation of homogeneous NP and the adjustment of physicochemical parameters such as size, distribution, metal charge content, surface, and attachment of biomolecules or drugs to enhance their therapeutic potential. Gao et al. (2020) synthesized AgNPs using dopamine, formulated hydrogels, and applied them after surgical excision of the tumor. After 15 days, they observed no local or systemic growth of tumor cells due to the pro-apoptotic effect of tumor cells, induction of tissue healing, and inhibition of angiogenesis to prevent metastasis migration. The use of hydrogels as a vehicle for topical application of NP is advantageous due to their adherence to tissues and the controlled and prolonged release of the active ingredient, which promotes an effective anti-tumor effect due to their direct contact with the target area (Gao et al., 2020).

Danciu et al. (2019) also formulated AgNPs-based hydrogels obtained from the terpene betulin due to its pro-apoptotic potential in tumor cells and anti-inflammatory properties (modulation of cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) enzymes) and reduction of pro-inflammatory molecules (Schwiebs et al., 2017). The hydrogel (Hg) containing AgNPs synthesized from betulin (AgNPs-B) and Hg-AGNPs-B with the addition of the terpene in the formulation showed similar antitumor activity; however, the second showed a higher reduction of erythema (Danciu et al., 2019). AgNPs also show anti-inflammatory action in vivo due to the activation of pathways that produce anti-inflammatory molecules and reduce pro-inflammatory ones (Danciu et al., 2019). The use of green NP combined or not with other compounds suggests a promising alternative by combining the anti-tumor and anti-inflammatory actions of different compounds to reduce local inflammation, angiogenesis, and vascular permeability, with consequent prevention of metastasis.

AgNPs can also be combined with anti-tumor drugs such as dichloroacetate (DCA) [37], nisin, and 5-fluorouracil (5-FU) [35] for application in melanoma (Rana et al., 2022; Sierra Rivera et al., 2013). DCA inhibits tumor growth by inhibiting the enzyme pyruvate dehydrogenase kinase (PDK), which promotes an increase in intracellular pyruvate and directs metabolism towards oxidative phosphorylation (a pathway incompatible with tumor cells), in addition to producing ROS and inducing apoptosis (Rana et al., 2022; Sierra Rivera et al., 2013). Nisin, an antibiotic produced by species of Lactobacillus spp. and Streptococcus spp., alters membrane integrity and induces apoptosis in cancer cells [83–85]. Fluoropyrimidine class (5-FU) interferes with cell division by binding to DNA and RNA and inducing transcription errors (Rana et al., 2022; Sierra Rivera et al., 2013). However, one of the main justifications for replacing the long-term use of chemotherapeutic drugs (DCA and 5-FU) is the induction of serious side effects and antibiotics (nisin) that induce gastrointestinal disorders and microbial resistance, which limits their indications (Rana et al., 2022; Sierra Rivera et al., 2013). An alternative to reducing the dose of these drugs is to intercalate them with AgNPs, which also have an anti-tumor mechanism, in order to develop efficient therapies that reduce the possibility of causing toxic effects to patients.

The injectable application of suspensions and hydrogels containing AgNPs showed anti-tumor activity by reducing the volume of the melanoma developed in the mice, inhibiting the metastasis, reducing the erythema associated with inflammation, and inducing no side effects. In addition, the topical application of AgNPs-based hydrogels after surgical excision was effective in inhibiting the development of new tumors. When AgNPs were associated with other chemotherapeutic and/or anti-tumor drugs, the synergistic action of the drugs promoted a higher anti-melanoma effect.

This systematic review had the following limitations: 1. Heterogeneity among the included studies as indicated by the meta-analyses; 2. Heterogeneity among the method of synthesis, size, concentration, and delivery vehicle of AgNPs; 3. Differences among the sex, age, weight, and species of mice evaluated; and 4. Uncertain risk of bias in the primary studies related to the method of randomization for intervention and analysis of results. This review, limited to the study of oral and skin melanomas, showed that AgNPs have an antitumor effect with consistency between the individual results of the authors and in combination by means of meta-analysis. Considering the search for treatments with lower side effects and the technological development in obtaining drugs, new primary studies could improve the level of statistical significance for the outcome observed in this review, as well as suggest more robust literature on the anticancer action of NP and the development of studies investigating the parameters of dose, administration, absorption, accumulation, metabolism, excretion, and their long-term effects in order to determine effective treatments. effects in order to determine effective treatments.

## 5. Conclusion

AgNPs administered in pure form or combined with chemotherapeutic drugs showed an anti-tumor effect against induced melanoma in rats by reducing tumor volume and preventing cell proliferation and metastasis. Possible anti-tumor mechanisms include induction of ROS, damage to cell membranes, inhibition of metabolic bioenergetics, and interference in cell division by binding to DNA and RNA.

## References

Alali, A., Hosseini-Abari, A., Bahrami, A., & Yazdan Mehr, M. (2023). Biosynthesis of Copper Oxide and Silver Nanoparticles by Bacillus Spores and Evaluation of the Feasibility of Their Use in Antimicrobial Paints. *Materials (Basel)*, 16: 4670. https://doi.org/10.3390/ma16134670

Alamer, F. A., & Beyari, R. F. (2022). Overview of the Influence of Silver, Gold, and Titanium Nanoparticles on the Physical Properties of PEDOT:PSS-Coated Cotton Fabrics. *Nanomaterials*, 12:1609. https://doi.org/10.3390/nano12091609

Alkhalaf, M. I., Hussein, R. H., & Hamza, A. (2020). Green synthesis of silver nanoparticles by Nigella sativa extract alleviates diabetic neuropathy through anti-inflammatory and antioxidant effects. *Saudi J Biol Sci*, 27:2410–2419. https://doi.org/10.1016/j.sjbs.2020.05.005

Sanfelice, R. A. S., Silva, T. F., Tomiotto-Pellissier, F. *et al.* (2022). Biogenic silver nanoparticles reduce Toxoplasma gondii infection and proliferation in RAW 264.7 macrophages by inducing tumor necrosis factor-alpha and reactive oxygen species production in the cells. *Microbes Infect*, 24:104971. https://doi.org/10.1016/j.micinf.2022.104971

Bai, H., Bosch, J. J., & Heindl, L. M. (2023). Current management of uveal melanoma: A review. *Clin Exp Ophthalmol*, 51:484–494. https://doi.org/10.1111/ceo.14214

Bansod, S. D., Bawaskar, M. S., Gade, A. K., & Rai, M. K. (2015). Development of shampoo, soap and ointment formulated by green synthesised silver nanoparticles functionalised with antimicrobial plants oils in veterinary dermatology: Treatment and prevention strategies. *IET Nanobiotechnology*, 9:165–171. https://doi.org/10.1049/iet-nbt.2014.0042

Canaparo, R., Foglietta, F., Limongi, T., & Serpe L. (2021). Biomedical applications of reactive oxygen species generation by metal nanoparticles. *Materials (Basel)*, 14:1–14. https://doi.org/10.3390/ma14010053

Danciu, C., Pinzaru, I., Coricovac, D. *et al*. (2019). Betulin silver nanoparticles qualify as efficient antimelanoma agents in in vitro and in vivo studies. *Eur J Pharm Biopharm*, 134:1–19. https://doi.org/10.1016/j.ejpb.2018.11.006

Drevon, D., Fursa, S. R., & Malcolm, A.L. (2017) Intercoder Reliability and Validity of WebPlotDigitizer in Extracting Graphed Data. *Behav Modif*, 41:323–339. https://doi.org/10.1177/0145445516673998

Gao, H., Fan, P., Xu, Q. *et al.* (2015). In Vitro and in Vivo Antitumor Activity of Silver Nanoparticles on B16 Melanoma. *Nano*, 15:1–14. https://doi.org/10.1142/S1793292020501635

Hooijmans, C. R., Rovers, M. M, De Vries, R. B. M., *et al.* (2014). SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*, 14:1–9. https://doi.org/10.1186/1471-2288-14-43

Kaabipour, S., & Hemmati, S. (2021). A review on the green and sustainable synthesis of silver nanoparticles and one-dimensional silver nanostructures. *Beilstein J Nanotechnol*, 12:102–136.

Li, F., Zheng, Z., Chen, W. *et al.* (2023). Regulation of cisplatin resistance in bladder cancer by epigenetic mechanisms. *Drug Resist Updat*, 68:100938. https://doi.org/10.1016/j.drup.2023.100938

Li, Y., Liao, Q., Hou, W., & Qin, L. (2023). Silver-Based Surface Plasmon Sensors: Fabrication and Applications. *Int J Mol Sci*, 24: 4142. https://doi.org/10.3390/ijms24044142

Luo, D., Wan, X., Liu, J., & Tong T. (2018). Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res*, 27:1785–1805. https://doi.org/10.1177/0962280216669183

Miranda, R. R., Sampaio, I., & Zucolotto, V. (2022). Exploring silver nanoparticles for cancer therapy and diagnosis. *Colloids Surf B Biointerfaces*, 210:112254. https://doi.org/10.1016/j.colsurfb.2021.112254

Ouzzani, M., Hammady, H., Fedorowicz, Z., & Elmagarmid, A. (2016). Rayyan-a web and mobile app for systematic reviews. *Syst Rev*, 5:1–10. https://doi.org/10.1186/s13643-016-0384-4

Page, M. J., McKenzie, J. E., Bossuyt, P. M., *et al.* (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, 29:372. https://doi.org/10.1136/bmj.n71

Parveen, A., Kulkarni, N., Yalagatti, M., Abbaraju, V., & Deshpande, R. (2018). In vivo efficacy of biocompatible silver nanoparticles cream for empirical wound healing. *J Tissue Viability*, 27:257–261. https://doi.org/10.1016/j.jtv.2018.08.007

Rana, K., Kumar, P. S., Chauhan, S., & Preet, S. (2022). Anticancer therapeutic potential of 5-fluorouracil and nisin co-loaded chitosan coated silver nanoparticles against murine skin cancer. *Int J Pharm*, 620:121744. https://doi.org/10.1016/j.ijpharm.2022.121744

Rashid, S., Shaughnessy, M., & Tsao, H. (2023). Melanoma classification and management in the era of molecular medicine. *Dermatol Clin*, 41:49–63. https://doi.org/10.1016/j.det.2022.07.017

Schwiebs, A., & Radeke, H. H. (2017). Immunopharmacological Activity of Betulin in Inflammation-associated Carcinogenesis. *Anticancer Agents Med Chem*, 18:645–651. https://doi.org/10.3390/plants10122663

Sergi, M. C., Filoni, E., Triggiano, G. *et al*. (2023). Mucosal Melanoma: Epidemiology, Clinical Features, and Treatment. *Curr Oncol Rep*, 1:0123456789. https://doi.org/10.1007/s11912-023-01453-x

Shabatina, T. I., Vernaya, O. I., Shimanovskiy, N. L., & Melnikov, M. Y. (2023). Metal and Metal Oxides Nanoparticles and Nanosystems in Anticancer and Antiviral Theragnostic Agents. *Pharmaceutics*, 15:118115. https://doi.org/10.3390/pharmaceutics15041181

Shinde, V. R., Revi, N., Murugappan, S., Singh, S. P., & Rengan, A. K. (2022). Enhanced permeability and retention effect: A key facilitator for solid tumor targeting by nanoparticles. *Photodiagnosis Photodyn Ther*, 39:102915. https://doi.org/10.1016/j.pdpdt.2022.102915

Siegel, R. L., Miller, K. D., Wagle, N. S., & Jemal, A. (2023). Cancer statistics, 2023. *CA Cancer J Clin*, 73:17–48. https://doi.org/10.3322/caac.21763

Sierra Rivera, C. A., Franco Molina, M. A., Mendoza Gamboa, E. *et al.* (2013). Potential of colloidal or silver nanoparticles to reduce the growth of B16F10 melanoma tumors. *African J Microbiol* 7:2745–2750. https://doi.org/10.5897/AJMR12.1968

Silva, J. M. C., Teixeira, A. B., & Reis, A. C. (2023). Silver-based gels for oral and skin infections: antimicrobial effect and physicochemical stability. *Future Microbiol*, 18:985-996. https://doi.org/10.2217/fmb-2023-0034

Sivadasan, D., Ramakrishnan, K., Mahendran, J. *et al.* (2023). Solid Lipid Nanoparticles: Applications and Prospects in Cancer Treatment. *Int J Mol Sci*, 24: 6199. https://doi.org/10.3390/ijms24076199

Tambunlertchai, S., Geary, S. M., Naguib, Y. W., & Salem, A. K. (2023). Investigating silver nanoparticles and resiquimod as a local melanoma treatment. *Eur J Pharm Biopharm*, 183:1–12. https://doi.org/10.1016/j.ejpb.2022.12.011

Torres-Cavazos, Z., Franco-Molina, M.A., Santana-Krímskaya, S. E. *et al*. (2020). In Vivo Evaluation of the Antitumor and Immunogenic Properties of Silver and Sodium Dichloroacetate Combination against Melanoma. *J Nanomater*, 741019. https://doi.org/10.1155/2020/3741019

Twilley, D., Thipe, V. C., Kishore, N. *et al*. (2022). Antiproliferative Activity of Buddleja saligna (Willd.) against Melanoma and In Vivo Modulation of Angiogenesis. *Pharmaceuticals (Basel)*, 15:1497. https://doi.org/10.3390/ph15121497

Valenzuela-Salas, L. M., Girón-Vázquez, N. G., García-Ramos, J. C. *et al*. (2019). Antiproliferative and antitumour effect of nongenotoxic silver nanoparticles on melanoma models. *Oxid Med Cell Longev*, 25:4528241. https://doi.org/10.1155/2019/4528241

Wang, H., Tran, T. T., Duong, K. T. *et al*. (2022). Nguyen T, Le UM. Options of Therapeutics and Novel Delivery Systems of Drugs for the Treatment of Melanoma. *Mol Pharm*, 4487–4505. https://doi.org/10.1021/acs.molpharmaceut.2c00775

Zeng, L., Brignardello-Petersen, R., & Guyatt, G. (2021). When applying GRADE, how do we decide the target of certainty of evidence rating? *Evid Based Ment Health*, 24:121–123. https://doi.org/10.1136/ebmental-2020-300170

Zheng, D. X., Soldozy, S., Mulligan, K. M. *et al*. (2023). Epidemiology, management, and treatment outcomes of metastatic spinal melanoma. *World Neurosurg*, 18:100156. https://doi.org/10.1016%2Fj.wnsx.2023.100156