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Estefany Carolina Ghisio

Aplicação da nanotecnologia como ferramenta de *drug delivery* no estudo de terapias moleculares e farmacológicas para do câncer de pele

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Orientadora: Prof. Dra Márcia Rosângela Wink

Coorientadora: Dra Karina Paese

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RESUMO

O câncer de pele é um dos tipos de câncer mais comuns e os tipos mais frequentes são o carcinoma escamoso, o carcinoma basocelular e omelanoma. Esta patologia ocorre através de um crescimento anormal das células da pele e se detectada precocemente pode ser tratada de forma eficaz. Apesar da melhora nos desfechos dos pacientes, aumentando as taxas de sobrevida global, os tratamentos apresentam algumas particularidades, como respostas diferenciadas em diferentes tipos de tumor e aquisição de resistênciaao tratamento pelo paciente e alguns efeitos adversos. Dessa forma, a nanotecnologia se apresenta como uma alternativa visando o aprimoramento dos tratamentos antitumorais. Esta abordagem pode aumentar a biodisponibilidade das moléculas terapêuticas em alvos específicos reduzindo os efeitos adversos do tratamento resultando em uma maior eficáciaterapêutica. O objetivo deste trabalho foi realizar uma revisão na literatura acerca da utilização da nanotecnologia associada a diferentes terapias e aplicadas no câncer de pele. Além disso, esse trabalho teve como objetivo desenvolver uma nanopartícula polimérica de Eudragit® RS 100 com estabilidade eletrostática. Acerca da revisão de trabalhos que utilizaram a nanotecnologia como ferramente de drug delivery para tratamento de cancer depele, os resultados encontrados apresentaram resultados promissores de inibição da disseminacao tumoral, reducao de taxas de metastases e melhora na taxa de sobrevida global nos experimentos *in vivo*. Enquanto isso, no objetivo experimental dessa dissertação, obteve-se uma nanocápsula polimérica com resultados com características monomodais e homogêneas indicando um potencial veículo farmacológico promissor quando associado a novos agentes terapêuticos ou a dacarbazina, no entanto necessitando de maistestes para demonstrar seu potencial uso.

Palavras chave: Câncer de pele; melanoma; drug delivery; nanotecnologia; nanopartículas poliméricas;

ABSTRACT

Skin cancer is one of the most common types of cancer and the most commontypes are squamous cell carcinoma, basal cell carcinoma and melanoma. Thispathology occurs through an abnormal growth of skin cells and if detected earlyit can be treated effectively. Despite the improvement in patient outcomes,increasing overall survival rates, the treatments have some particularities, suchas differentiated responses in different types of tumor and acquisition ofresistance to treatment by the patient and some adverse effects. In this way,nanotechnology presents itself as an alternative aimed at improving antitumor treatments. This approach can increase the bioavailability of therapeuticmolecules on specific targets, reducing the adverse effects of treatmentresulting in greater therapeutic efficacy. The objective of this work was to reviewthe literature on the use of nanotechnology associated with different therapiesand applied to skin cancer. Furthermore, this work aimed to develop a polymeric nanoparticle of Eudragit® RS 100 with electrostatic stability.Regarding the review of studies that used nanotechnology as a drug deliverytool for the treatment of skin cancer, the results found showed promising resultsin inhibiting tumor dissemination, reducing metastases rates and improving theoverall survival rate in in vivo experiments. Meanwhile, in the experimental objective of this dissertation, a polymeric nanocapsule was obtained with resultswith monomodal and homogeneous characteristics indicating a promising potential pharmacological vehicle when associated with new therapeutic agentsor dacarbazine, however requiring further tests to demonstrate its potential use. Key words: skin cancer; melanoma; drug delivery; nanotechnology; polymericnanoparticles

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1 REFERENCIAL TEÓRICO

1.1 Câncer de pele não Melanoma

Conceitualmente, o câncer é um grupo de doenças onde se tem um crescimento descontrolado de algumas células anormais, que, caso não tenha um controle efetivo de sua propagação, pode acarretar à morte. O câncer de pele não melanoma, dentre os tipos de câncer, representa mais de um milhão (5,8%) de novos casos e 65 mil mortes (0,7%) no mundo (SUNG *et al.*, 2021).No Brasil, esse tipo de câncer representa uma parcela de cerca de 30% de todos os tumores identificados e segundo dados publicados, 176.930 novos casos e 2.616 mortes foram registradas em 2019 (FERLAY *et al.*, 2019). Dessa forma, apesar da maior incidência do câncer de pele não melanoma, há uma menor mortalidade devido ao seu alto potencial de cura, quando detectado e tratado precocemente.

O diagnóstico do câncer de pele é realizado através de análise histopatológica da lesão e pode ser subclassificado de acordo com características específicas. Os subtipos mais frequentes são o carcinoma basocelular e o carcinoma epidermóide, ambos originados de células basais ouescamosas da epiderme da pele. O câncer de pele basocelular é o maisincidente e com desenvolvimento lento, e majoritariamente, na face, cabeça e pescoço (MITCHELL TC, KARAKOUSIS G, 2020) (Figura 1).

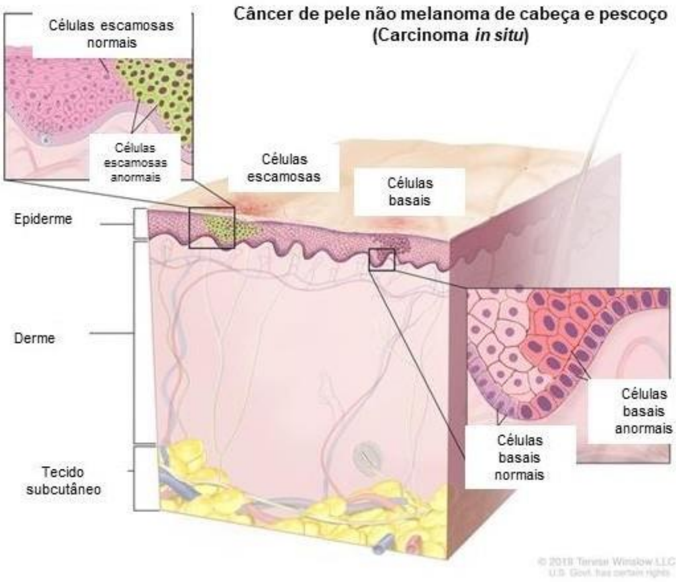

Figura 1: Demonstração das camadas da pele epiderme, derme e tecido subcutâneo e a localização das células escamosas e basais. Adaptado de Terese Winslow (2019)

1.1.1 Diagnóstico

Inicialmente, o médico responsável deve realizar a anamnese adequada examinando a superfície de pele de forma detalhada utilizando, para isto, as regras denominadas, “ABCDE”. Essa regra se baseia em: (A) Assimetria da lesão, (B) Bordas irregulares, (C) Coloração variável (vários tons de marrom, preto, cinza, branco, vermelho ou azul), (D) Diâmetro maior ou igual a 6 mm e (E) Evolução, na qual uma lesão que está mudando de forma, cor, tamanho ou o surgimento de novas lesões (KITTLER, 2021). Juntamente a essa análise, o histórico clínico completo do paciente é avaliado para observar outras lesões e fatores de risco que o indivíduo possa estar exposto. A lesão deve ser avaliadapela técnica de dermatoscopia. Esta técnica utiliza uma luz polarizada cruzada para evidenciar algumas características importantes no diagnóstico do câncer de pele, indispensáveis para que o dermatologista possa determinar se a lesão é passível de biópsia excisional (YOUNG et al., 2021). O diagnóstico definitivo é feito pela biópsia e análise histopatológica, pois são necessários para a confirmação a presença de atipias melanocíticas e desarranjo da arquitetura dotecido (ELMORE et al., 2017).

O estadiamento TNM, o T refere-se ao tamanho do tumor e disseminação, sendo que, no melanoma, os principais pontos avaliados são a espessura do tumor (medida de Breslow, quanto menor, melhor o prognóstico), o índice mitótico e a ulceração

(Figura 2). O N indica se há disseminação linfonodal ou metástase em trânsito e o M se existe metástase para outros órgãos (BRESLOW, 1970; DICKSON; GERSHENWALD, 2011).

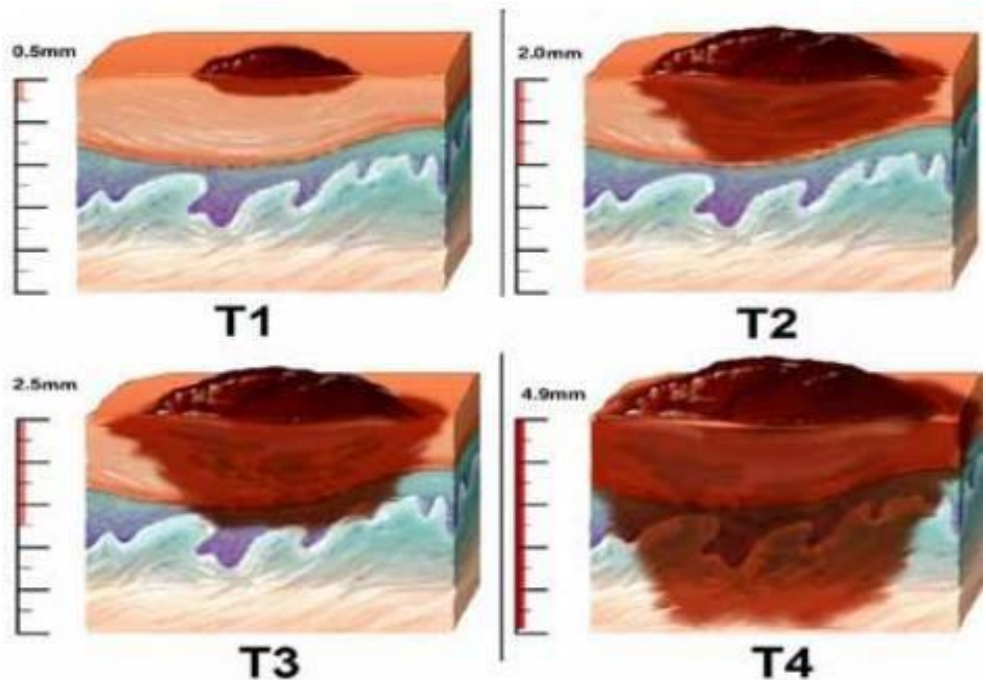

Figura 2: Extensão vertical do tumor primário, segundo a classificação TNM, no qual em T1 a lesão possui menos de 0,5mm, em T2 até 2mm, em T3 até 2,5mm e T4 acometendo todas as camadas da pele atingindo 4mm. Fonte: NCCN (2011).

1.1.2 Estratégias Terapêuticas

Após a realização do diagnóstico por um médico, a primeira linha de tratamento disponível para o câncer de pele não melanoma é a ressecção cirúrgica das lesões. A excisão cirúrgica proporciona um prognóstico favorável ao paciente, possuindo taxas de cura de aproximadamente 90%. Para ospacientes não elegíveis à excisão, deve-se considerar inicialmente o tratamento utilizando radioterapia (FANIA *et al.*, 2021).

Nos tumores provenientes de células escamosas, além da radioterapia, háa opção de quimioterapia com o uso de cisplatina com 5-fluoracil e carboplatinaou outros tratamentos atualmente avaliados em ensaios clínicos. Ainda, em certos casos, pode-se utilizar imunoterápicos inibidores de receptor do fator de crescimento epidérmico (EGFR) como cetuximab. (COMBALIA; CARRERA,2020; FANIA *et al.*, 2021).

Nos tumores provenientes de células basais, quando há a necessidade de terapias sistêmicas as opções disponíveis incluem inibidores da via hedgehog de diferenciação celular como vismodegib, sonidegib para lesões que não são acessíveis para cirurgia ou radioterapia e cemiplomab (BASSET-SEGUIN; HERMS, 2020).

1.2 Câncer de pele Melanoma

O melanoma é responsável por apenas 3% dos cânceres de pele e é considerado um problema de saúde pública devido à sua alta mortalidade nos pacientes acometidos. Tal mortalidade é devido à alta taxa de formação de metástases nos pacientes e, principalmente, pela resistência às terapias atualmente disponíveis (INCA, 2020).

O câncer de pele melanoma tem origem nos melanócitos, células que produzem melanina, localizadas em meio às células basais da epiderme (Figura 3), e assim o tumor pode aparecer em qualquer parte da pele ou mucosas. Essa patologia tende a ser mais frequente em adultos caucasianos, principalmente devido à sua fotoproteção reduzida em decorrência da menor produção de melanina. Também, unindo o grau de fotoproteção dos indivíduos,o histórico de exposição prolongada e repetida aos raios ultravioleta e histórico,familiar ou pessoal, de câncer são fatores de risco para o aparecimento do melanoma (Brenner et al, 2008; Chao et al, 2017; Padovese et al, 2017).

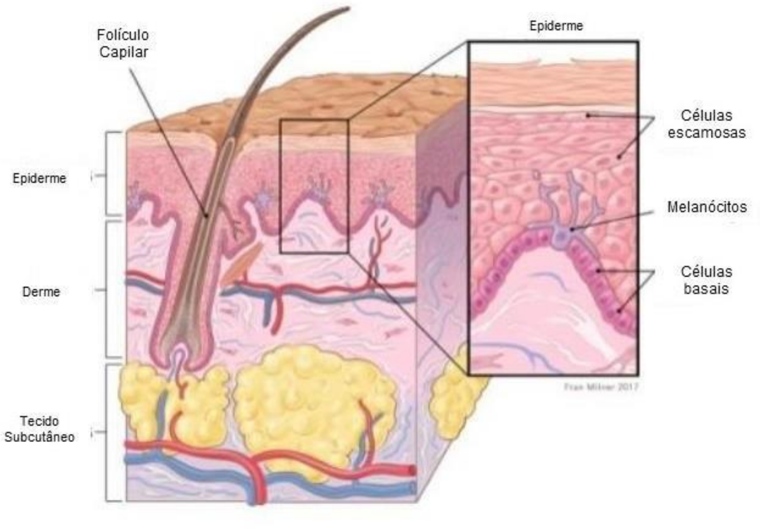

Figura 3: Demonstração das camadas da pele epiderme e derme, da localização dos melanócitos (células produtoras do pigmento protetor melanina, localizadas na camada basal da epiderme). Fonte: Adaptado de Sociedade Americana de Câncer (2016).

De acordo com o Instituto Nacional de Câncer, os dados para 2020 de novos casos de câncer de pele do tipo melanoma, no Brasil, ficaram em torno de 8.450 casos, sendo que 4.200 em homens e 4.250 em mulheres (Ministério da Saúde, 2020). Dentre o número de óbitos por causa do melanoma, foram de1.978 em 2019, sendo 1.159 em homens (Atlas de Mortalidade por Câncer – SIM, 2019). Em 2016, 1.773 pessoas morreram no Brasil dessa doença, correspondendo a um índice de mortalidade de 0,66 em cada 100.000 habitantes (Ministério da Saúde do Brasil, 2018).

Em 2021, houve 101.280 novos casos de melanoma *in situ* e com probabilidade de se desenvolver para forma invasiva, principalmente em homens, com a probabilidade de 1 para cada 27, em relação às mulheres, com chances de 1 em 40 (SUNG *et al.*, 2021). Entre todos os tipos de câncer, o melanoma é o mais agressivo e possui o pior prognóstico, devido a altas taxase potencial de metástase e resistência dos tratamentos disponíveis, consequentemente tendo uma alta taxa de mortalidade (VANNI *et al.*, 2020).

1.2.1 Estratégias Terapêuticas

O tratamento para melanoma é definido após a confirmação histopatológica e o estadiamento patológico do tumor (SCHADENDORF, D., FISHER, D., GARBE, 2015). As opções terapêuticas podem ser a excisão cirúrgica, quimioterapia, radioterapia, terapia fotodinâmica (PDT), imunoterapia e terapia alvo. Para pacientes de estadiamento I-IIIB, a ressecção cirúrgica é o tratamento primário, na qual o tamanho da excisão varia de acordo com a classificação do tumor (DOMINGUES et al., 2018). Já nas opções farmacológicas, ocorrem eventos adversos como toxicidade gastrointestinal e reações imunes, além de apresentarem eficiência reduzida em certos casos, o que pode acarretar resistência perante as terapias (PASQUALI et al., 2018; VAN ZEIJL et al., 2017).

A quimioterapia é uma opção para os casos mais avançados, como estadiamento IV, os quais a ressecção cirúrgica não é mais possível. Atualmente, a imunoterapia é o padrão ouro, ou primeira linha de tratamento utilizada para os casos metastáticos ou tumores irressecáveis. Pembrolizumab e Nivolumab são estratégias de monoterapia que utilizam anti-PD1, inibidores da proteína PD-1, proteína na qual reconhece as células tumorais. O anti-PD1 impede a interação entre o PD-1 da superfície do linfócito com o PD-L1 da superfície da célula tumoral.

A mutação BRAF V600E é encontrada em aproximadamente 60% dospacientes com e para esses casos há também as combinações alvo de agentes inibidores de quinase como as combinações de Vemurafenib/coimetinib e Encorafenib/binimetinib e Debrafanib/trematinib (TATE *et al.*, 2019; VANNI *et al.*, 2020).

1.3 Nanotecnologia aplicada aos distúrbios de pele

Nas últimas décadas, o uso da nanotecnologia para tratamento e diagnóstico de doenças ganhou espaço em aplicações biomédicas (EL- SAYED; KAMEL, 2020). Uma das vantagens da conexão entre a nanotecnologia e as ciências biomédicas é o *drug delivery*, que tem como objetivo, o desenvolvimento de estratégias para maior eficácia de agentes terapêuticos, a melhoria da segurança e biodisponibilidade desses fármacos em alvos específicos e redução de efeitos adversos nos pacientes (COIT *et al.*,2019; GHEIBI HAYAT; DARROUDI, 2019).

Os nanomaterias são elementos com dimensões de cerca de $1x10^{-9}$ metros,ou seja, são partículas muito pequenas, sendo classificadas devido a características específicas. As nanopartículas são partículas sintéticas, queapresentam diâmetro na escala nanométrica, produzidas com polímeros, lipídios e metais com características físico-químicas específicas. Comoexemplos temos os lipossomas, as nanopartículas lipídicas sólidas, micelas, nanoesferas, nanocápsulas, entre outras estruturas utilizadas para o *drug delivery* (MARCATO; DURÁN, 2008; ZHU; LIAO, 2015).

Essas nanopartículas podem modificar as propriedades farmacocinéticas das substâncias carreadas em seu interior e, por consequência, podem aumentar o direcionamento do fármaco para as células tumorais de forma específica e eficiente (GOLDBERG, 2015). Na administração tópica cutânea, as nanopartículas possuem benefícios terapêuticos devido à facilidade de penetração nas barreiras da pele, assim a ação de agentes terapêuticos é facilitada, reduzindo efeitos adversos, devido a velocidade de liberação controlada e ao direcionamento dos agentes químicos (ASLAN et al., 2013). Além disso, as nanopartículas promovem a permanência da formulação na pele por muitas horas e previnem a degradação dos fármacos encapsulados (ALEXIS et al., 2008). Por isso, visando melhorar a eficácia terapêutica e aumentar a captação das nanopartículas pelas células, tem-se estudado a associação de peptídeos, anticorpos e moléculas pequenas na superfície das nanopartículas ocasionando o direcionamento ativo dessas nanoestruturas para o tratamento de células específicas, uma estratégia conhecida no desenvolvimento de nanovacinas (GHEIBI HAYAT; DARROUDI, 2019; GU et al., 2007). As nanovacinas são uma nova geração de vacinas que utilizam nanopartículas (NPs) como carreadores e/ou adjuvantes, pois devido ao tamanho semelhante entre as NPs e os patógenos, o sistema imunológico pode ser bem estimulado, resultando em respostas imunes celulares e humorais desencadeadas (GHEIBI HAYAT; DARROUDI, 2019).

Atualmente, no entanto, são limitados os recursos terapêuticos que envolvam nanopartículas já aprovados pelas agências regulatórias. De forma mais recente no mundo, os avanços na nanotecnologia se apresentaram na forma de testes rápidos de diagnóstico e, talvez mais importante, em vacinas desenvolvidas rapidamente contra o SARS-CoV-2 (SALAMANCA- BUENTELLO; DAAR, 2021). Já se tratando de câncer, os primeiros medicamentos contra o câncer, baseados em nanotecnologia, são o Abraxane® (aprovado em 2005) com Paclitaxel e albumina associado indicado para tratamento de câncer de mama, pancreático e pulmão, e o Doxil® (aprovado em 2007) contendo doxorubicina para tratamento de câncer deovário, câncer de mama, mieloma múltiplo e sarcoma de Kaposi (WICKI et al., 2015).

2 JUSTIFICATIVA

O câncer de pele possui alta incidência e dependendo do subtipo possui também uma alta mortalidade. Embora já existam novas terapias direcionadasa alvos e imunoterapia, muitos pacientes não respondem ou tornam-se resistentes a esses tratamentos, ou ainda há um nicho populacional muito grande que não se beneficia. Portanto, se houvesse uma alternativa que garantisse uma melhor eficácia, com menos efeitos adversos e uma terapia mais direcionada, especialmente para melanoma, mais pacientes poderiam ser beneficiados com o tratamento. Visando isto, esse trabalho buscou unir os benefícios que a nanotecnologia traz para o *delivery* de fármacos, bem como os efeitos que um fármaco já conhecido e utilizado proporciona, de forma a oportunizar uma alternativa mais eficiente e menos tóxica aos pacientes com melanoma. Como proposta, a nanopartícula polimérica desenvolvida no trabalho apresentou potencial zeta positivo, o que a tornará promissora para administração pela via tópica cutânea, devido a interação com as cargas residuais negativas presentes na superfície da pele e, consequente, aumento do tempo de permanência do fármaco na pele.

3 OBJETIVOS

3.1 Objetivo Geral

Revisar o uso de nanotecnologia para tratamento de câncer de pele, e desenvolver e caracterizar físico-quimicamente substâncias anti-tumorais em solução e nanoencapsulada para tratamento de melanoma.

3.2 Objetivos Específicos

- Realizar uma revisão sistemática e identificar as diferentes estratégiasde uso da nanotecnologia em relação ao tratamento do câncer de pele;

- Desenvolver suspensões de nanocápsulas poliméricas de Eudragit® RS100, sem revestimento com polissorbato 80, contendo dacarbazina ou pirimidobenzimidazóis 1 e 4;

- Validar o método analítico por cromatografia líquida de alta eficiência com detecção UV para a quantificação dos pirimidobenzimidazóis e dacarbazina nanoencapsulada e livre;

- Caracterizar físico-quimicamente as formulações quanto ao diâmetromédio, índice de polidispersão, potencial zeta, número de partículas por mL, pH, teor de fármaco, eficiência de encapsulação emorfologia.

4 ARTIGO DE REVISÃO (MANUSCRITO EM PREPARO)

Anexo III

5 ARTIGO DE DADOS (MANUSCRITO EM PREPARO)

PHYSICAL STABILITY AND IN VITRO ANALYSIS OF POLYMERIC NANOPARTICLES AS THERAPEUTIC STRATEGY FOR THE TREATMENT OF MELANOMA

Estefany Carolina Ghisio[1], Silvia Guterres[2], Adriana Pohlmann[2], Karina Paese[2], Márcia Rosângela Wink[1*]

[1]Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA);
[2]Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS)

*Corresponding Author: Márcia Rosângela Wink

Departamento de Ciências Fisiológicas, Fundação Universidade Federal de Ciências da Saúde de Porto Alegre

Rua Sarmento Leite, 245 Sala 304

Centro, Porto Alegre, RS

Brazil - 90050-170

Phone: + 555133038762

E-mail: marciawink@yahoo.com.br

ABSTRACT

Skin cancer is one of the most common types of cancer and the most common types are squamous cell carcinoma, basal cell carcinoma and melanoma. Melanoma has a high rate of metastasis formation in patients and, mainly, by resistance to currently available therapies. Variations in patients have some specifics treatments, such as differentiated responses in tumor resistance and acquisition of resistance to treatment by the patient and overall differences in resistance. In this way, nanotechnology presents itself as an alternative to antitumor treatments. In this paper we developed a polymeric nanoparticle of Eudragit® RS 100 with monomodal and homogeneous diameter distribution were obtained with a physical stabilization mechanism probably associated with electrostatic repulsion. Because of that, there is a perspective of associating a drug to this nanostructure and to produce a new delivery vehicle.

KEY WORDS: Polymeric nanoparticles, Skin cancer, Melanoma, Drug delivery, Nanotechnology

INTRODUCTION

Melanoma cancer originates from melanocytes, cells located on the basal layer of the epidermis that are the players of the melanin production. so the tumor appears on the skin or mucosa(1). Melanoma is one of the most aggressive because has high metastatic potential and resistance to the current available treatments, resulting in a high mortality rate(2). About 1,280 new cases of melanoma in situ of the skin and the probability of developing invasive cancer in 2021 (3). Whether diagnosed in the early stages of the disease, the excisional surgery is effective. In metastatic or unresectable stages, the preferred regimens in first-line therapy are immunotherapies with anti PD-1 monotherapy (pembrolizumab and nivolumab) and combination target therapy if BRAF V600-activating mutation, when the patient is not eligible for any of these recommended therapies is used cytotoxic therapy (dacarbazine, temozolomide, paclitaxel, etc) considering each case (4).

In the last decades, the use of nanotechnology as treatment and diagnosis has gained space in biomedical applications (5). Nanomaterials are elements nanoscale sized ($1x10^{-9}$ meter) and this organized systems are produced from polymers, lipids or metals nanoscale sized with physical and chemical specific characteristics (6).

The connection between nanotechnology with biomedical sciences has the objective of development of pharmaceutical formulations carrying therapeutic agents and approaches with higher efficacy, the improvement of safety and bioavailability of therapeutic agents in target sites and reduction of adverse side effects presented by patients (7,8). For example, these structures are modified by the substances that they are carrying and, thus, increase the positioning of the molecules into the tumoral site in a more efficient and specific manner (11). In this line, to increase the nanoparticles uptake by the cells, there are reports evaluating the association of peptides, antibodies and small molecules on the nanoparticles surface causing the active direction of these nanostructures for specific treatments, such as the development of nanovaccines (8,12).

In this paper, we evaluated the effect of an innovative polymeric nanocapsules produced with cationic polymer and stabilization by electrostatic repulsion in human melanoma cell line was selected (SK-MEL-103), a human fibroblast lineage (MRC5), a human keratinocyte lineage (HaCaT) and primary culture of mesenchymal stem cells (MSC).

MATERIALS AND METHODS

Materials

Eudragit RS 100® (Degussa – Darmstadt, Germany), polysorbate 80 and capric/caprylic triglycerides were obteined from Brasquim (Porto Alegre, Brazil). Acetone, ethanol, and acetonitrile from Sigma-Adrich (Saint Louis, USA).

Production of polymeric nanoparticles

The nanocapsules were obtained by the interfacial deposition of a preformed polymer method using Eudragit® RS100 as the polymeric wall and capric/caprylic triglycerides were used to obtain the oily core(9,10). An organic phase containing 100 mg of the polymer Eudragit RS100, 330µl of capric/caprylic triglycerides and 27 mL of acetone was injected into an aqueous phase containing 53 mL of water Milli-Q® water. After 10 minutes, the solvents were eliminated and the suspension was concentrated under reduced pressure using a rotary evaporator (Büchi R-114, Switzerland). The final volume was adjusted to 10 mL. The formulation was named NC.

Characterization of polymeric nanoparticles

The nanocapsules were characterized in terms of size, polydispersity, zeta potential, drug content and encapsulation efficiency. The average diameter (D[4,3]) and SPAN (polydispersity) value were determined by laser diffraction (Mastersizer® 2000, Malvern Instruments, Malvern, UK), applying the refraction index of Eudragit® RS100 (1.38); the z-average was determined by photon correlation scattering (Zetasizer® NanoZS Malvern Instruments) after dilution in filtered ultrapure water (1:500 v/v). The zeta potential was determined by electrophoretic mobility (Zetasizer® NanoZS Malvern Instruments) after dilution in NaCl 10 mM (1:500 v/v) aqueous solution. Also, with Nanoparticle Tracking Analysis we could evaluate the size distribution and the particle number density (number of particles per mL) based on the Brownian motion of the particles (NanoSight LM10 and NTA 2.0 Analytical Software,NanoSight Ltd).

Cell culture

For this study, a human melanoma cell line was selected (SK-MEL-103), a human fibroblast lineage (MRC5), a human keratinocyte lineage (HaCaT) and primary culture of mesenchymal stem cells (MSC) which were cultured in Dulbecco's Modified Eagle Medium (DMEM-High glucose) supplemented with 10% FBS and antibiotics (100 U/mL of penicillin and 100 µg/mL of strepto) and kept at 37°C in a umid atmosphere containing 5% CO_2. The treatment was carried out through a curve of concentration of the nanocapsule suspensions, where from $2{,}6x10^9$ to $2{,}08x10^{10}$ particles.

Viability assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed with polymeric nanoparticles 24h and 72h as recommended by the International Organization for Standardization 10993-5 and 10993-12 for Biological Evaluation of Medical Devices. Cells were seeded in 96 well cell culture plates at a density of 3×10^3 cells/well and 10×10^3, respectively. MTT incubation was carried out for 3.5h at 37°C, then 50 µl dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. SpectraMax Microplate Reader (Molecular Devices Corporation, USA) at 560 nm was used for measuring optical density. Results are presented in percentage and analyzed by two-way ANOVA.

RESULTS AND DISCUSSION

The formulations showed monomodal and homogeneity size distribution (Figure 1) without aggregate of particles in the aqueous phase. NC had D[4,3] and SPAN values of 157 nm and 1.320, the size was in the nanometric range.

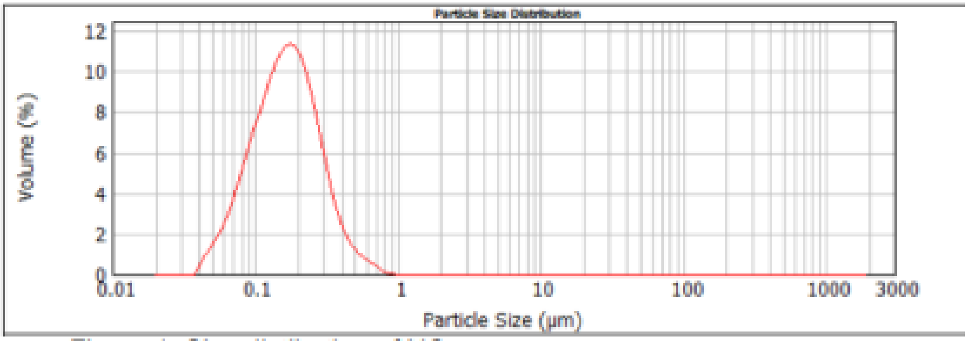

Figure 1: Size distibution of NC

Eudragit® RS 100 has already been described for creating nanocapsules for different types of administration and with potential for sustained release of local drugs for exemple cutaneous(10), vaginal(12), ocular(13)(14) and buccal mucosa(15) that shows its biocompatibility and mucoadevisity. This material is a co-polymer of poly (ethylacrylate, methyl-methacrylate and chlorotrimethyl-ammonioethyl methacrylate), contains 4.5-6.8% of quaternary ammonium groups(16), and it confers a cationic characteristic to the nanoparticle and ammonium groups make the co-polymer low permeable (17,18). Therefore, the zeta potential values were +32,77 mV (Table I), this high positive value guarantees the physical stability of the nanoparticles.

Table 1: $D_{[4,3]}$ z-avarage, zeta potential, span and PDI of the NC formulation (n = 3).

Sample	$D_{[4,3]}$ (nm)	z-avarage (nm)	Zeta potential (Mv)	Span	PDI
NC	138	177	+32.,77	1.320	0.044

The nanoparticle tracking analysis (NTA) with the liquid colloidal formulations confirmed the monomodal nanometric size distributions with average diameter of 172 nm. Also, the particle number density of colloidal formulations with the values of particle number density (concentration of particles per volume) were e $1,04 \times 10^{13}$ for NC.

This study aimed to investigate the NC cytotoxicity in MRC5, HaCaT and SK-MEL-103 cell lineages and mesenchymal stem cells (MSC). After exposure to curve of concentration of the nanocapsule suspensions, where from $2,6 \times 10^{9}$ to $2,08 \times 10^{10}$ particles, the overall growth pattern cells was reduced when investigated 24 and 72h after particle addition but without statistical significance.

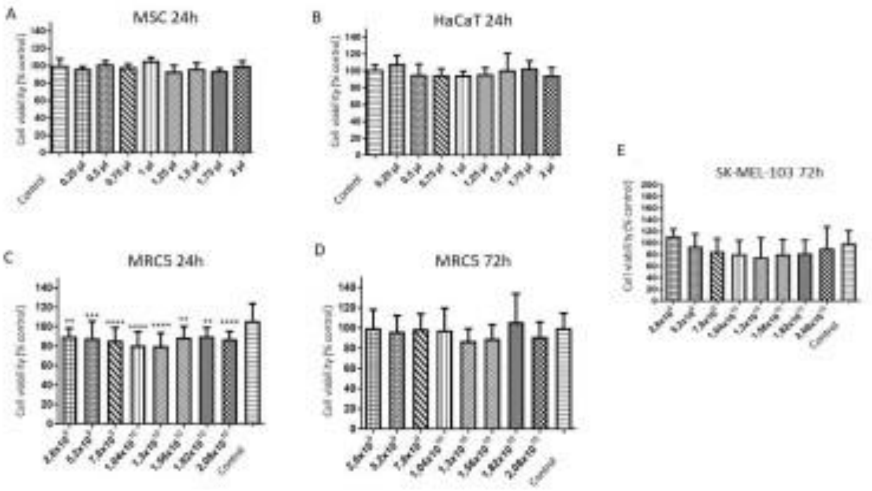

Figura 2: Results of various concentrations treatments of NC on cell viability in the Mesenchymal stem cells (MSC) and HaCaT, MRC5 and SK-MEL-103 cell lineages(n=3).

CONCLUSION

The developed polymeric nanocapsules demonstrated appropriate physicochemical characteristics allowing to continue the experiments with this innovative structure without polysorbate 80 in the nanocapsules surface, which was kept stable physically due to electrostatic repulsion. Biocompatible properties were verified in vitro, demonstrating viability of the structures when tested with human fibroblasts and human melanoma cell lines. However, it is necessary to continue the investigation finalizing the physicochemical characterization, evaluation of in vitro release and cutaneous permeation and cellviability assay.

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DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interests regarding this publication.

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6 DISCUSSÃO E RESULTADOS PRELIMINARES

6.1 Elaboração das Nanocápsulas a partir da literatura

Citotoxicidade, biodistribuição e biodisponibilidade, parâmetros de estabilidade físico-química como pH e a via de administração de interesse devem ser avaliados em nanopartículas quando desenvolvemos essessistemas para câncer de pele. Além disso, na presença de fármacos, deve-se sempre avaliar o tratamento usual do fármaco em solução comparado aofármaco nanoencapsulado. O uso de diferentes estruturas estabelecidas, processos de fabricação e composições para nanopartículas e as diferentes abordagens mostraram os avanços recentes da nanotecnologia e uma potencial abordagem terapêutica do câncer de pele.

No artigo de revisão elaborado apresentado anteriormente vimos os resultados de diferentes tipos de nanopartículas para tratamento de melanoma,carcinoma basal e escamoso que mostraram através de abordagens *in vitro* e *in vivo*, a melhora da taxa de sobrevida, redução no crescimento tumoral e efeitos colaterais. O melanoma é o tipo de câncer de pele mais discutido nos artigos, possivelmente devido ao seu alto índice de metástase e mortalidade. Para este tipo de câncer, as nanopartículas foram dos mais diversos tipos, comdestaque para as de material polimérico e quanto aos tratamentos associados, existem vários contendo medicamentos já utilizados para outros tipos de câncer ou novas substâncias. Em relação as vias de administração, observou- se a estratégia da via tópica para possível tratamento adjuvante no local da lesão, demonstrando uma interessante via de estudo para esse tipo tumoral.

Dessa forma, que demonstrou-se que a nanomedicina abriu um caminho demuitas possibilidades para pesquisar e projetar fármacos e sistemas de liberação de fármacos para o tratamento do melanoma. Beaseado nisso, elaborou-se a presente dissertação com o objetivo experimental de desenvolver e caracterizar físico-quimicamente substâncias anti-tumorais em solução e nanoencapsulada para tratamento de melanoma.

6.2 Nanopartículas Promissoras

O Eudragit® RS 100 já foi descrito para a criação de nanocápsulas para diferentes tipos de administração e com potencial de liberação sustentada de fármacos locais para, por exemplo, mucosas cutânea (CONTRI et al., 2014), vaginal (FRANK et al., 2014), ocular (QIN et al., 2021) e bucal (DI PRIMA; CONIGLIARO; DE CARO, 2019) que mostra sua biocompatibilidade e mucoadesidade. Este material é um copolímero de poli (etilacrilato, metil- metacrilato e clorotrimetil-amôniaetil metacrilato), contém 4,5-6,8% de grupos quaternários de amônio (JOSHI, 2013), e confere uma característica catiônica às nanopartículas e os grupos amônio tornam o copolímero - polímero pouco permeável (NIKAM et al., 2011).

Assim, nesse presente trabalho avaliou-se a citotoxicidade de uma formulação de nanocápsulas poliméricas de Eudragit® RS 100 pela metodologia de MTT. Os resultados obtidos sinalizaram que as nanoformulações sem a substância em estudo encapsulada, diminuíram a viabilidade celular das linhagens de melanoma humano (SK-MEL-103) e fibroblasto humano (MRC5) (Figura 4A-D), demonstrando uma possível citotoxicidade das nanocápsulas sem o fármaco.

Em relação a linhagem de MRC5, em 2Em 24 horas, houve diminuição de 10,31% a 33,40% de células viáveis, apresentando um $p < 0,0001$ (****), perante a concentração máxima (4A). Na análise em 72 horas, houve diminuição de 15,26% a 35,76% apresentando $p < 0,0001$ (****) em ambas concentrações (4b).

Em relação a linhagem de SK-MEL-103, em 24 horas, houve diminuição de 50,22% a 53,36% de células viáveis, apresentando um $p < 0,0001$ (****) (4C). Na análise em 72 horas, houve diminuição de 41,58% a 69,45% apresentandop $< 0,0001$ (****) (4D). Em 24 horas houve diminuição de 15,94% a 18,66% de células viáveis, apresentando um $p < 0,0001$ (****) perante a concentração máxima (4E). Na análise em 72 horas, não houve diminuição estatisticamente significativa (4F). Em 72 horas, quando a linhagem foi submetida ao tratamentosem polissorbato 80, houve uma variação de células viáveis de 5,25 a 8,95% sem uma significância estatística (4G).

Criou-se a hipótese de que algum componente da estrutura da nanocápsula polimérica seria citotóxico para as linhagens avaliadas, ou a própria estrutura em si. Dessa forma, fez-se necessária a adaptação dessa nanoestrutura para que a mesma interferisse o menos possível na viabilidade celular, permitindo que o efeito fosse predominantemente do composto composto desejado para análise.

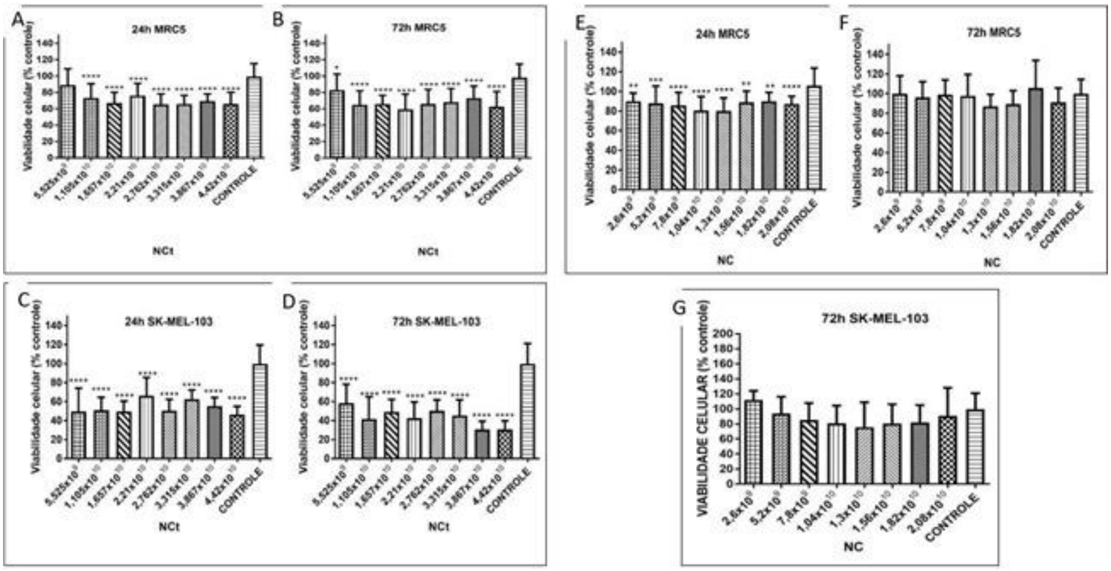


Figura 4 : **A e B**: Avaliação da viabilidade celular da linhagem MRC5 perante ao tratamento com Nanocápsulas com polissorbato 80 (NCt) de 5,525x10^{9} a 4,42x10^{10} partículas. **C e D**: Viabilidade celular da linhagem SK-MEL-103 perante ao tratamento com a NCt de 5,525x10^{9} a 4,42x10^{10} partículas por poço. **E e F**: Avaliação da viabilidade celular da linhagem MRC5 perante ao tratamento com a nanoformulação sem polissorbato 80 (NC) de 2,6x10^{9} a 2,08x10^{10} partículas. **G:** Avaliação da viabilidade celular da linhagem SK-MEL-103 perante ao tratamento com a nanoformulação sem polissorbato 80 (NC) de 2,6x10^{9} a 2,08x10^{10} partículas. (N = 3).

Sendo assim, criou-se uma nova proposta de trabalho alterando um dos componentes da estrutura da nanopartícula polimérica já descrita (CONTRI *et al.*, 2011). A proposta a ser testada se baseou na produção de uma formulação inovadora sem o revestimento das nanocápsulas com polissorbato 80, um tensoativo que está associado a estabilidade estérica das partículas, ou seja evitando agregações que possam vir a diminuir a estabilidade física das formulações. Por fim, os resultados iniciais se demonstraram promissores, pois ao retirar o tensoativo da nanoestrutura, as características físico-químicas analisadas se mantiveram conforme preconizada para uma formulação nanotecnológica – monomodal e homogênea. Associado a estas características, ressaltou-se o elevado valor de potencial zeta da formulação que garantiu sua estabilidade eletrostática, como descrito no manuscrito do artigo de dados presente nesta dissertação. Ademais, os testes iniciais in vitro demonstraram uma baixa citotoxicidade da nanocápsula sem o fármaco, frente a linhagem MRC5 e a linhagem SK-MEL-103 (Figura 4E-G).

6.3 Nanocápsulas poliméricas associadas a novos compostos Anti-Tumorais

A possibilidade de produzir nanocápsulas sem a presença desse tensoativo permitiu a continuação do trabalho. Inicialmente, a suspensão de nanocápsulas poliméricas sem o tensoativo (NC) foi testada em outros tipos celulares como linhagem de queratinócitos humanos (HaCaT) e células mesenquimaisprimárias de prepúcio (MSCs) (Número do Parecer de aprovação do CEP: 3.214.091), não demonstrando uma diferença estatística significativa frente ao controle, ou seja, para essas células as nanocápsulas não foram citotóxicas (Figura 5).

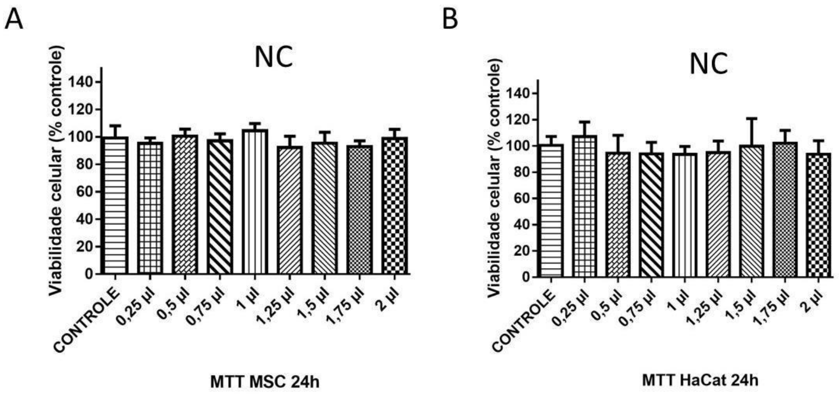


Figura 5: Avaliação da viabilidade celular da linhagem células mesenquimas primária (MSC) perante e linhagem de queratinócitos (HaCaT) ao tratamento com a nanoformulação sem polissorbato 80 (NC) de 2,6x109 a 2,08x1010 partículas sem significância estatística. (N=3)

Em 2019, o grupo do Laboratório de Biologia Celular da UFCSPA emparceria com a Universidade Federal de Santa Maria (UFSM) estava avaliando o potencial anti-tumoral para melanoma humano de novos compostos pirimidobenzimidazóis (ZANATTA *et al.*, 2008) (Patente BR10201901345). Comisso, viu-se a oportunidade de utilizar as nanopartículas poliméricas comoveículo para esses compostos. O melanoma foi a patologia escolhida, devidoao alto poder metastático e por não dispor de um tratamento que garanta um significante impacto na sobrevida dos pacientes acometidos, fazendo-se necessário a descoberta de novas estratégias terapêuticas (HUANG et al., 2021).

Por fim, o projeto de dissertação inicial tinha como objetivo avaliar o potencial anti-tumoral de novos compostos pirimidobenzimidazóis (PBZs) em solução e nanoencapsuladoas, visando oportunizar opções de tratamento, bem como desenvolver, validar e caracterizar novas formulações de nanocápsulas eavaliar a possibilidade de novos veículos que facilitem a distribuição e o efeito desses compostos no tratamento de melanomas.

Os compostos PBZs 1 foram associados às nanocápsulas, demonstrando resultados prévios promissores, com potencial anti-tumoral para melanoma (não publicados). Assim, as partículas foram produzidas, como descrito no manuscrito de dados, e os PBZs associados ao núcleo destes nanossistemas. Realizou-se a caracterização físico-química, demonstrando características de distribuição de diâmetro monomodal e homogênea, além do potencial zeta positivo como previsto, devido a natureza do polímero utilizado, garantindo a estabilidade física da formulação, devido a repulsão eletrostática (Tabela 1).

Tabela 1: Caracterização das nanopartículas poliméricas associadas ao PBZ 1 (NC-PBZ-1).

Formulação	**D4,3 (nm)**	**z-average (nm)**	**Span**	**PDI**	**Potencial zeta (mV)**
NC-PBZ-1	180	185	1,2	0,11	+32,63

O comprimento de onda de 250 nm do PBZ 1 foi encontrado através de varredura de espectrofotometria. Assim, iniciou-se a etapa para desenvolver e validar o método analítico de quantificação por cromatografia líquida de alta eficiência com detecção no ultravioleta (CLAE-UV), utilizando uma coluna C18 Merck com pré-coluna do mesmo fabricante, como fase estacionária. A fase móvel, foi determinada como sendo modo isocrático de metanol (grau CLAE) e água ultrapura (80:20). A análise da pré formulação obteve-ve um método linear, exato e específico para o PBZ 1 (Figura 6).

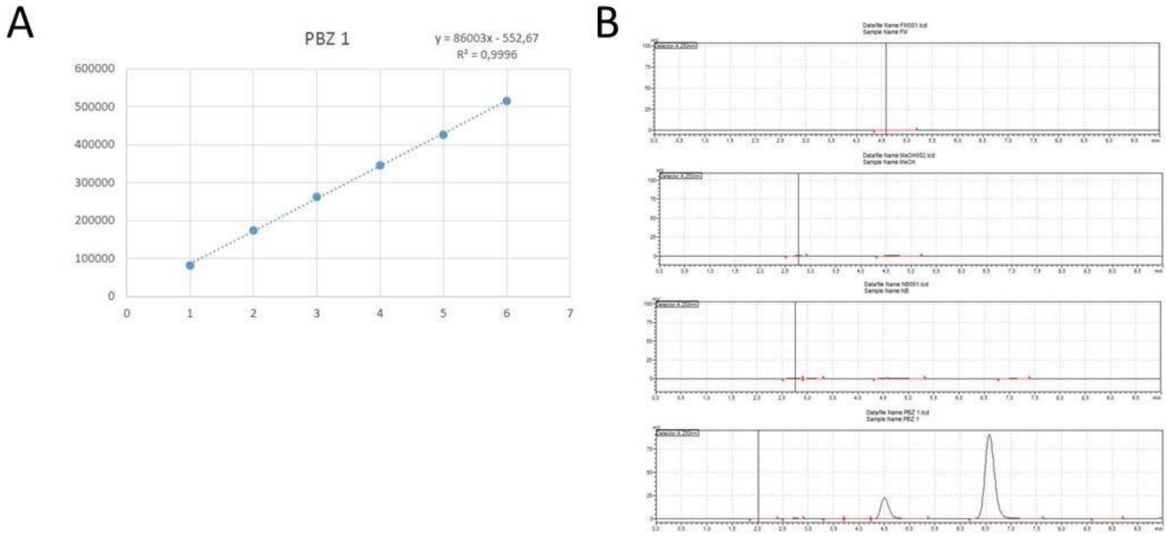


Figura 6: Validação do método analítico por cromatografia líquida de alta eficiência com detecção no ultravioleta (CLAE-UV) de linearidade (A) e especificidade (B) para caracterização da presença do PBZ 1 na amostra (N=3).

6.4 Dacarbazina associada à Nanocápsulas Poliméricas

Outra estratégia para a utilização das nanopartículas poliméricas foi a nanoencapsulação da dacarbazina (DTIC), um agente alquilante, que é utilizado como terapia única ou combinada com outras substâncias, imunoterapias e terapias alvo para o tratamento de melanoma. A dacarbazina e o seu análogo oral, a temozolomida, possuem respostas de maioria transitória, correspondendo a uma taxa de remissão entre 5% e 15%. E apenas 1%-2% possuem resposta a longo prazo. Dessa forma, estima-se em 8 meses o tempomédio de sobrevida desses pacientes tratados com esses quimioterápicos,sendo que menos de 10% sobrevivem 5 anos a partir do diagnóstico (KIM *et al.*, 2010).

As nanopartículas possuem benefícios terapêuticos devido à facilidade de penetração nas barreiras da pele, adicionalmente facilita a ação de agentes terapêuticos reduzindo os efeitos adversos devido à liberação controlada e o direcionamento de agentes químicos a tecidos de interesse (BAZAK et al., 2015). Além disso, as nanopartículas promovem o aumento da permanência daformulação nas diferentes camadas da pele e previnem a metabolização dos fármacos encapsulados (FRANK et al., 2014).

Até o momento, o que se sabe na literatura, é que a dacarbazina teve sua nanoencapsulação por diferentes métodos, como um nanocristal contendo esse quimioterápico e teve a boa eficiência de encapsulação. As nanopartículas foram transportadas por endocitose para as células da linhagemde melanoma humano A375 (HOU et al., 2019). Outra estratégia utilizada foi uma camada de hidróxidos de metais contendo DTIC como estratégia de *drug delivery* na linhagem A375. Ainda, outro estudo usando uma nanopartícula contendo dacarbazina para uso tópico, demonstrou sucesso ao produzir um creme eficiente para auxiliar no tratamento de melanoma (HAFEEZ; KAZMI, 2017).

Diante disso, iniciamos o processo de desenvolvimento de uma formulação de nanocápsulas poliméricas de Eudragit® RS 100 sem polissorbato 80 na superfície e contendo a dacarbazina para investigar inicialmente a possibilidade de utilização desse veículo farmacológico para uso tópico desse agente alquilante. O método analítico para a quantificação do fármaco foi cromatografia líquida de alta eficiência, adaptado de LIU et al., 2017. A coluna foi a mesma utilizada anteriormente, C18 Merck com pré-colunado mesmo fabricante. A fase móvel constituiu-se de uma solução de água ultrapura e metanol (grau CLAE) com 0,1% de ácido trifluoroacético (0,1%, 75:25) com vazão de 0,7 ml/min.

Nos resultados analisados obteve-se linearidade, especificidade, precisão e exatidão (Figura 7 e Tabela 2 e 3). No entanto, quando associada à nanopartícula não houve precisão e teor reprodutível adequado, assim a elaboração das partículas foi adaptada e modificou-se o solvente orgânico de acetona para etanol se tornando uma perspectiva desse trabalho a readaptação do solvente na elaboração da partícula.

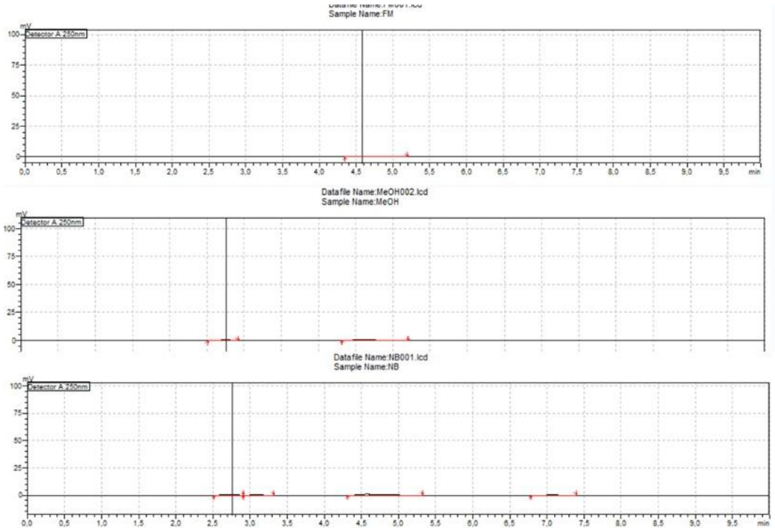
Tabela 2: Exatidão da formulação de dacarbazina associada a nanocápsulas poliméricas.

Ponto	Concentração (µg/mL)	CV (%)
5	5.54 ± 0.1937	3.4942
7.5	8.11 ± 0.1665	2.0523
10	10.54 ± 0.3451	3.2737

Tabela 3: Precisão da formulação de dacarbazina associada a nanocápsulas poliméricas.

Ponto Médio	Concentraçãomédia (µg/mL)	CV (%)
7.5 (intradia)	5.2494 ± 0.0887	1.6889
7.5 (interdia)	6.9406 ± 0.0584	0.8419

A



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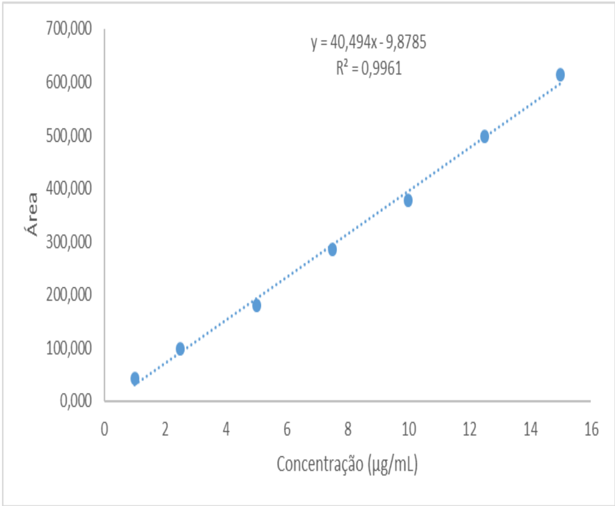

Figura 7: Validação do método analítico por cromatografia líquida de alta eficiência com detecção no ultravioleta (CLAE-UV) de linearidade (7B) e especificidade (7A) para detecção da dacarbazina (N=3).

7 PERSPECTIVAS

A formulação de nanocápsulas poliméricas propostas, tendo como material estruturante o Eudragit® RS 100 e sem o revestimento com polissorbato 80, demonstrou-se um veículo para *drug delivery* promissor, oferecendo características físico-químicas adequadas e estabilidade. Dessa forma, após osestudos iniciais com essa estrutura inovadora, temos como perspectiva finalizara caracterização físico-química, validação do método analítico para quantificação da dacarbazina associada às nanocápulas, realizar novas extrações de células primárias, como melanócitos, para avaliar a citotoxicidade das nanocápsulas poliméricas. Ademais, analisaremos a estabilidade da nanoformulação contendo o fármaco, realizaremos testes de lavabilidade, permeação cutanea e liberação *in vitro*.

8 CONCLUSÃO

Baseado na busca na literatura, foram encontrados resultados com diversas estratégias, utilizando diferentes vias e formas de administração, tecnologias e tipos de tratamentos. Assim, a estratégia de utilização da nanotecnologia para possíveis terapêuticas tem se mostrado um caminho promissor para melhorar aeficácia dos tratamentos para câncer de pele. No entanto, se tratando deestudos clínicos, ainda há uma quantidade muito pequena. Isso demonstra que os estudos, apesar de promissores, são muito iniciais e não chegaram em sua maioria na aplicabilidade e utilização do estudo em humanos.

Neste trabalho desenvolveu-se uma formulação nanotecnológica inovadora por meio da produção de nanocápsulas poliméricas de Eudragit® RS 100, sem revestimento com polissorbato 80. A metodologia foi de produção adaptada e validada conforme recomendações internacionais, assim a partir da retirada do tensoativo da superfície das nanocápsulas, se obteve formulações com distribuição de diâmetro monomodal e homogêneo com mecanismo de estabilização física provavelmente associado a repulsão eletrostática comodemonstrado no manuscrito de dados em preparo.

Novas hipóteses foram geradas com o objetivo de associar um fármaco a essa nanoestrutura e para produzir um novo veículo de administração. Foram testadas novas substâncias com potenciais antitumorais para melanoma comoo PBZ 1 e o quimioterápico dacarbazina.

As nanocápsulas desenvolvidas possuem potencial para serem explorados em uma série de associações, uma vez que apresentam propriedades físico- químicas interessantes e reprodutíveis. Dessa forma, fica visto mais uma vez a natureza versátil das nanopartículas e sua capacidade de ser reinventada, permitindo abordagens que sejam otimizadas a cada contexto clínico, gerando a cada nova descoberta, resultados de maior excelência.

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ANEXOS

Anexo I

Normas da Revista

Seguindo as recomendações da coordenação do PPG - Biociências, optou-sepor inserir apenas o link para as normas online da revista (Guia para Autores).

https://www.elsevier.com/journals/advanced-drug-delivery-reviews/0169-409X/guide-for-authors

Anexo II – Lattes do aluno

LATTES DO ALUNO

Estefany Carolina Ghisio

Endereço para acessar este CV: http://lattes.cnpq.br/3795010885395346
ID Lattes: **3795010885395346**
Última atualização do currículo em 10/03/2022

Biomédica pela Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) habilitada em Farmacologia, Patologia Clínica (Análises Clínicas) e Biologia Molecular. Especialista em Análises Clínicas pela UFRGS e Mestrado em andamento no Programa de Pós Graduação em Biociências da UFCSPA, atuando na linha de pesquisa de desenvolvimento de nanoformulações para aplicações farmacológicas em melanoma. Trabalhou como Assistente de Pesquisa e Coordenadora Não Cega no Escritório de Projetos e Parcerias Estratégicas do Centro de Pesquisa Clínica do Hospital de Clínicas de Porto Alegre em estudos clínicos de Infectologia e Pneumologia. Atualmente é Site Management Coordinator na IQVIA. **(Texto informado pelo autor)**

Identificação

Nome	Estefany Carolina Ghisio
Nome em citações bibliográficas	GHISIO, E. C.
Lattes iD	http://lattes.cnpq.br/3795010885395346

Endereço

Endereço Profissional	Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, Laboratório de Biologia Celular. Rua Sarmento Leite Centro Histórico 90050170 - Porto Alegre, RS - Brasil Telefone: (51) 33039000

Formação acadêmica/titulação

2019	Mestrado em andamento em BIOCIÊNCIAS (Conceito CAPES 4). Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil. Orientador: Márcia Rosângela Wink. Coorientador: Karina Paese.
2018 - 2020	Especialização em Análises Clínicas. (Carga Horária: 450h). Universidade Federal do Rio Grande do Sul, UFRGS, Brasil. Título: NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS ASSOCIATED WITH IMMUNOTHERAPY AS A STRATEGY TO TREAT SKIN MELANOMA.
2015 - 2018	Graduação em Biomedicina. Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil. Título: CARACTERIZAÇÃO E AVALIAÇÃO DA CITOTOXICIDADE DE DUAS NOVAS NANOFORMULAÇÕES EM LINHAGEM DE FIBROBLASTO HUMANO (MRC5) E EM LINHAGEM DE MELANOMA HUMANO (SK-MEL-103). Orientador: Márcia Rosângela Wink.

Formação Complementar

2021 - 2021	Good Clinical Practice. (Carga horária: 6h). NIDA Clinical Trials, NIDA, Estados Unidos.
2021 - 2021	Planejamento Estratégico em Saúde. (Carga horária: 30h). Hospital Sírio-Libanês, SIRIO-LIBANÊS, Brasil.
2021 - 2021	Elaborando um protocolo de ensaio clínico randomizado: considerações estatí. (Carga horária: 10h). Hospital Moinhos de Vento, HMV, Brasil.

Anexo III – Artigo de revisão em preparo

NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS AS A STRATEGY TO TREAT SKIN CANCER

Estefany Carolina Ghisio[1], Iago Carvalho Schultz[1], Alicce Reis[1], Jéssica Gonçalves Azevedo[1], Diogo André Pilger[2], Karina Paese[2], MárciaRosângela Wink[1*]

[1] Laboratório de Biologia Celular da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA);

[2] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS)

*Corresponding Author: Márcia Rosângela Wink

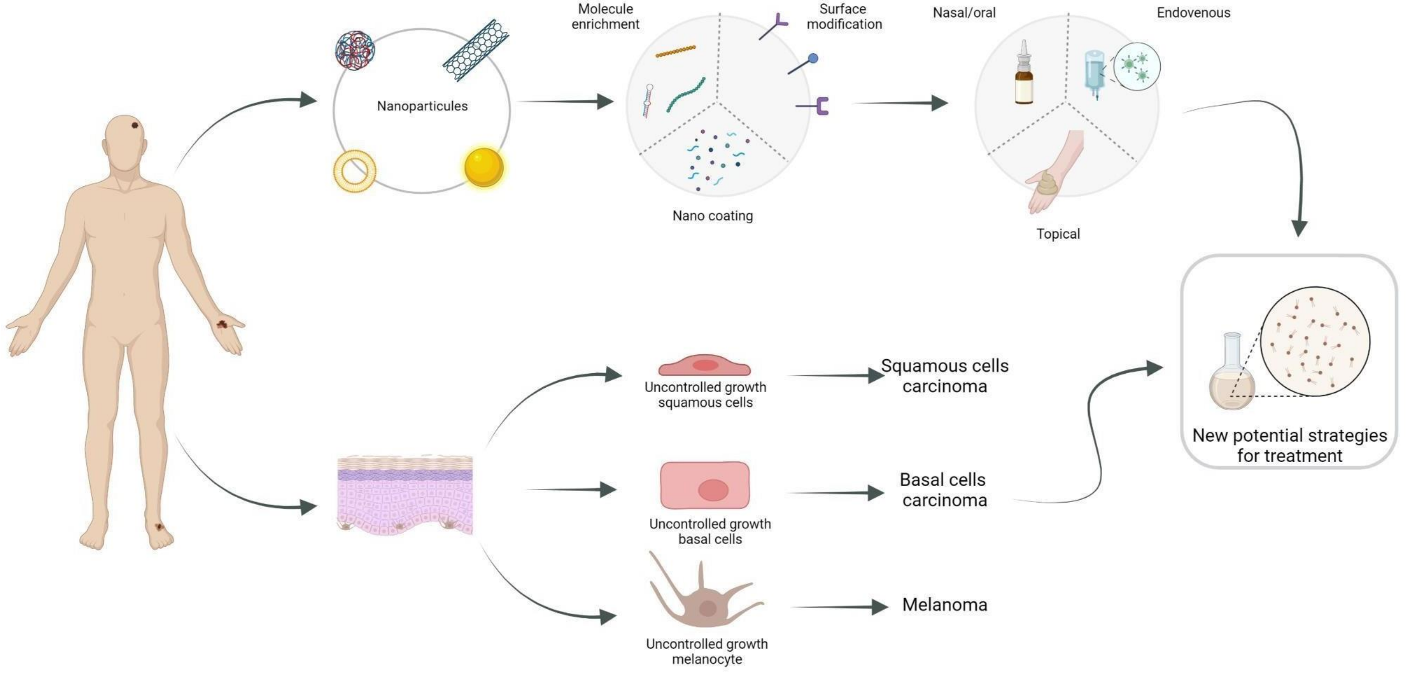
Departamento de Ciências Fisiológicas, Fundação Universidade Federal de Ciências da Saúde de

Porto AlegreRua Sarmento Leite, 245 Sala 304   Centro, Porto Alegre, RS Brazil - 90050-170

Phone: + 555133038762

E-mail: marciawink@yahoo.com.br

Graphical abstract



Abstract

Skin cancer is one of the most common types of cancer and the most frequent types are squamous cell carcinoma, basal cell carcinoma and melanoma. This pathology occurs through an abnormal growth of specific-epidermal cells and the warly diagnosis could improve it`s efficacy of treatment. Despite the improvement in the patient outcomes, increasing the overall survival rates, the treatments have some particularities such asdifferent responses on different types of tumor and acquisition of treatment resistance by the patient and some adverse effects. Also, one of the biggest challenges of tumoral treatment is the transposition of physiological barriers. Therefore, nanotechnology has emerged as an alternative aiming the improvement of antitumor treatments. The nanotechnology approach can increase the bioavailability of the therapeutic molecules in specific targets, such as cancer cells, reducing the treatment adverse effects, which is translated into a higher therapeutic efficacy. The objective ofthis paper was to review studies using nanotechnology associated with different therapies applied to treat in skin cancer. Articles were found withseveral different strategies, using different routes and administration, technologies and types of treatments. Nanoparticles such as polymeric, lipidicand inorganic composition tested at *in vitro* or *in vivo* skin cancer models showed an increase in drug uptake by cells, inhibition of tumor spread, reduction of the metastasis rates and improved the overall survival rates of illness animals. Therefore, the strategy of using nanotechnology for possible therapeutic has shown to be a promising way to improve the efficacy of treatments to skin cancers.

Keywords: nanotechnology, nanoparticles, skin cancer, melanoma, squamous carcinoma, basal carcinoma

Introduction

Cancer is a disease characterized by an uncontrolled growth of abnormal cells(1). Among them, skin cancer is one of the most common types of cancer being divided into non-melanoma and melanoma types. In 2020, over one million of patients have been diagnosed with skin cancercausing 64.000 deaths worldwide(2).

The sub classification of skin cancer is due to the cellular origin of the tumor (Figure 1). The non-melanoma types are composed of basal cell carcinoma and squamous cell carcinoma. Both non-melanoma types originate in the epidermis from the basal cells or squamous cells respectively. The basal cell carcinoma is the most common and it develops slowly and usually on the face, head and neck, all body parts frequentlyexposed to UV radiation(3). The melanoma skin cancer emerges due to a neoplastic transformation and uncontrolled proliferation that are the players of the melanin production.

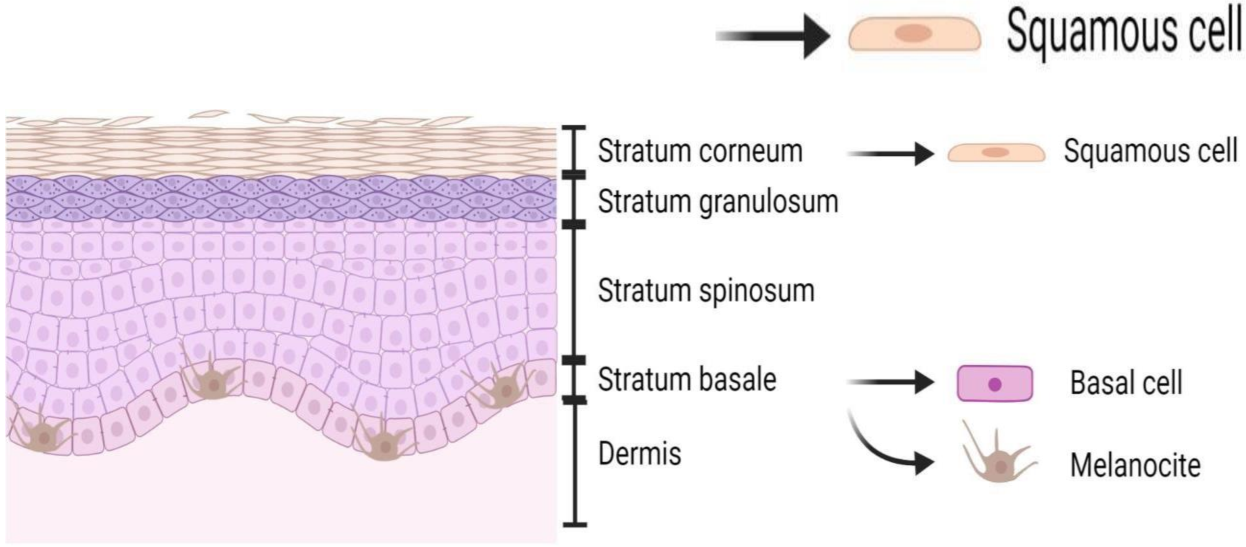

Squamous cell
Stratum corneum
Squamous cell
Stratum granulosum
Stratum spinosum
Stratum basale
Basal cell
Dermis
Melanocite

Figure 1: Layers of the skin and its cells cells. Being basal cells, squamous cells and melanocytes precursors of basal carcinoma, squamous carcinoma and melanoma, respectively.

In 2021, 101,280 new cases of melanoma in situ of the skin were diagnosed and the probability of developing invasive cancer in men was about 1 in 27 compared to women who have a probability of 1 in 40 (2). Among all types of skin cancer, melanoma is the most aggressive and has the worst prognosis due to its high metastatic potential and resistance to the current available treatments, resulting in a high mortality rate (4).

After being diagnosed by a physician, the available treatment for basal cell and squamous cell carcinoma is the surgical excision with 4 to 8 mm of depth. In squamous cell skin cancer, the patients with cases of curative surgery that are not feasible the therapy options with radiotherapy are cisplatin and clinical trials treatments and in certain cases EGFR inhibitors (cetuximab), cisplatin with 5-fluorouracil and carboplatin. When the systemic therapy is used alone, the preferred regimens are immune checkpoint inhibitors (cemiplimab-rwlc and pembrolizumab) and clinical trials, in certain circumstances if the patient is ineligible or progressed can consider the use of EGFR inhibitors, capecitabine, cisplatin, cisplatin with 5fluorouracil and carboplatin (NCCN guideline squamous cell skin cancer). In basal cell skin cancer, when a systemic therapy is needed, the options include hedgehog pathway inhibitors (vismodegib, sonidegib) and cemiplimab-rwlc in patients previously treated with the previous treatment without success (NCCN guideline basal cell skin cancer).

About melanoma skin cancer, whether diagnosed in the early stages of the disease, the excisional surgery is effective. In metastatic or unresectable stagombining the target therapy if BRAF V600-activating mutation. When patients are not eligible for any of these recommended therapies, it is used cytotoxic therapy (dacarbazine, temozolomide, paclitaxel, etc), considering each case (NCCN guideline melanoma cutaneous).

In the last decades, the use of nanotechnology as treatment and diagnosis has gained space in biomedical applications (5). The connection between nanotechnology and biomedical sciences has the objective to improve the development of therapeutic agents, increasing bioavailability intarget sites, the efficacy and the safety, therefore, the reduction of adverse side effects presented by patients (6,7).

Nanomaterials are elements nanoscale sized ($1x10^{-9}$ meter) that are classified due to specific characteristics. The nanoparticles are organized systems produced from polymers, lipids or metals nanoscale sized with physical and chemical specific characteristics. Some examples are liposomes, solid lipidic nanoparticles, micelles, nanospheres, nanocapsules among others used for drug delivery (8,9). These structures can be modified by the substances they carry, especially if they are on the surface, thus, increase the positioning of the molecules into the tumoral site in a more efficient and specific manner (10). In this line, to increase the nanoparticles uptake by the cells, there are reports evaluating the association of peptides, antibodies and small molecules on the nanoparticles surface causing the active direction of these nanostructures for specific treatments, such as the development of nanovaccines (7,11).

Therefore, considering the challenges of the current available treatments for skin cancer, especially for advanced melanoma cases, in addition to the promising use of nanotechnology the main objective of this systematic review was to perform a screening articles with original data and non-reviews available in the NCBI PubMed platform that used nanoparticles for the treatment of skin cancer. For the research, the MESHterms nanotechnology, skin cancer, meloma, basal carcinoma, squamous carcinoma, immunotherapy, nanoparticles were used. As inclusion criteria, the publication date was considered, between 2017 and 2021, and articles that used polymeric, lipid or metallic nanoparticles tested in skin cancer cells. Articles outside the pre-defined period, articles from literature reviews and that were not considered in the English language.

Results and discussion

Nanoparticles

Nanoparticles are an extensive class of nanomaterials often defined as having at least one dimension ranging from 1-100nm. Based on their chemical composition, these nanosystems can be classified into organic and inorganic nanoparticles, although there are many hybrid nanoparticles currently being developed and evaluated in order to obtain more versatile nanomaterials(12). Among the organic nanoparticles, there are the polymeric nanoparticles, which have specific properties like their small size and can be differentiated by their shape. Polymeric nanocapsules are composed of an oil in which the drug is dissolved and a polymeric wall that can control the release profile of the substance inside, while nanospheres are made of a polymeric network in which the substance can be either inside or on its surface (13). One of the most popular polymers used in nanoparticle synthesis is PLGA—poly(lactide-co-glycolide), a biodegradable polymer FDA-approved as a delivery system, whose composition allows its use in the control of release (14). Its structure, as expected of all nanoparticles, protects the content inside, for example the antigens, which have the limitation of being chemically unstable and hardly internalized by the cells. In addition, it provides protection against enzymatic degradation and maintains antigenic bioactivity(15).

Regarding lipidic nanoparticles, liposomes are the most widely investigated and were the earliest nano-scale drug approved for clinical use (16). These nanoparticles can carry both hydrophobic and hydrophilic drugs and their lipid layer structure can be modified in order to improve therapeutic drug delivery (17). As an example, the synthesis of doxorubicin-loaded liposomes decorated with a T-cell receptor mimicking scFv antibodies directed against melanoma-associated antigen A1, the authors reported that these decorated liposomes were able to effectively target antigen-positive melanoma(18).

Inorganic nanoparticles have a central core composed of inorganic materials, such as metals and metal oxides, and seem promising delivery systems due to their higher surface area to volume ratio, when compared to other types of nanoparticles(17).They are emerging as good delivery systems due to their easily done surface functionalization, which enables the modification of the surface properties and represents a manner of improving biocompatibility and cellular internalization of the nanoparticles(19). As an example, a recent study developed amino-functionalized mesoporous silica nanoparticles (MSN-NH^2), assuming that the functionalization could improve the encapsulation of drugs through hydrogen bonding or electrostatic interactions. Results suggested that MSN-NH^2 had good stability, possibly due to the presence of amino groups on the surface of the nanoparticles(20).

Although many metals are currently being used for inorganic nanoparticles development, gold has gained special attention due to the gold nanoparticles' surface negative charge(21), which gives them tunable features(20). The physicochemical properties of nanoparticles, such as their size, shape and chemical structure, influence the cellular uptake, which involves regulated mechanisms to overcome the cell plasma membrane. Therefore, the gold nanoparticles malleable properties represent an advantage when compared to other types of nanoparticles and the spherical shape of AuNPS is able to enhance the cellular uptake efficiency. In another study, 5-Fluorouracil loaded gold nanoparticles capped with CTAB (5-FU/CTAB-GNPs) presented a spherical shape and size around 16-150 nm. *In vivo* studies confirmed the topical 5-FU/CTAB-GNPs potential toimprove drug efficacy against skin cancer(22). Besides that, biological factors, such as cell type, affect the nanoparticle uptake efficiency (23). Forexample, dendritic cells, an important cell to anti-tumor immunity, internalize particles <500 nm in diameter. In addition, the hydrophobicity is animportant characteristic for an efficient binding and uptake of particles by dendritic cells (24).

Drug delivery to melanoma

Drug delivery to melanoma has many studies with different structures and different routes of administration that had promising results with substances already approved in FDA or with new substances (Table 1). Amino-functionalized mesoporous silica nanoparticles (MSN-NH2) were used with 5-FU and dexamethasone for a synergistic effect. The results showed a significant increase in toxicity compared to free drug, optimized skin permeability and a significant increase in the penetration when using these nanoparticles (25). Inhalable administration is an interesting strategy for local and systemic action. 5-FU polymeric nanoparticles using chondroitin sulfate as a structuring polymer were used to treat metastatic melanoma. The nanoparticles were formulated by two innovative inhalable powder formulations produced by spray-drying. The study investigated their cytotoxic action on melanoma cancer cells and both showed a cytotoxic effect in theese cells as expected (26).

An innovative strategy developed with Fe^3O^4 nanoparticles incorporating polycaprolactone (PCL) fibers based bandages using parental/doxorubicin was tested *in vivo* on skin tumors developed in BALB/c mice. Results showed no sign of inflammation and recurrence of cancer post-therapy (27). Another successful strategy was an immunoliposome developed decorated with TCR-mimicking scFv antibodies. It effectively and selectively targets antigen-positive melanoma promoting a significant antitumor response, when administered intravenously as a result there was tumor growth inhibition (18). To enhance the efficiency of DOX delivery, a diosgenin nanoparticles (NPs) platform was developed, to be use as a therapy to cutaneous melanoma. The authors observed that the biodistribution could efficiently accumulate in tumor sites through enhancing permeation and retention. In addition the *in vivo* experiment revealed that there was no heart toxicity and significant inhibition of tumor metastasis by induction of apoptosis (28).

Nanoparticle	**Terapeutical strategy**	***In vitro/in vivo***	**Dosage**	**Results**	**Ref**

Imiquimod is a compound approved for topical treatment of superficial basal cell carcinoma (29). It showed a good strategy to melanoma, when associated with the polymer Eudragit® L100 for topical administration being capable of improving imiquimod skin permeation in vivo. Its chemopreventive activity as well as antiangiogenic effect represent a promising alternative for the management of malignant skin lesions (30). This compound also was used in nanovaccines of phospholipid micelles loaded zinc-doped iron oxide magnetic nanoparticles (MNPs) that combined TLR ligation using polyIC as TLR3 and imiquimod (R837) as TLR7 agonist, in combination with the model antigen ovalbumin (OVA). The combination of TLR agonists a strong and synergistic activation of innate immune response in the lymph nodes (LNs) without systemic release ofpro-inflammatory cytokines (31). For these nanovaccines actions the immunostimulating effect is expected to be a better approach.

Unusual drugs for melanoma treatments such as Lemivudine (32), Paclitaxel (PTX) (33,34), Indomethacin (35), Itraconazole (36), Plumbagin and

Docetaxel (DTX) (37) were nanoencapsulated. Other studies presented new compounds with anti-tumor potential for melanoma, such as diphenyl diselenide [(PhSe)2(38), sodium diethyldithiocarbamate (DETC) (39) and 4-nitrochalcone (4NC), Salinomycin(40) and 10hydroxycamptothecin (HCPT)(41).

Table 1: Summary of nanoparticles combined with therapeutic strategies for melanoma.

Mesoporous silica nanoparticles (MSN-NH2)	5-Fluorouracil (5and FU) Dexamethasone (DEX)	HT-144 melanoma cells, male Wistar rats	380.2 µg/cm2 of 5-FU MSN-NH2-gel vs. 30.9 µg/cm2 of free 5-FU-gel and 590.4 µg/cm2 of DEX MNS-NH2-gel vs. and 55.5 µg/cm2 of free DEX-gel; 50 µg/mL:125 µg/mL 5-FU: DEX	Greater skin permeability, the toxicity was enhanced and a synergetic effect relative to free drug combination.	(25)
Mesoporous silica nanoparticles	Indomethacin	Melanoma cell line B16F10; female C57BL/6	IND (2.5 and 5.0 mg/kg b.w.) and MSNPs+IND (16.66 and 33.33 mg/kg	Inhibited tumor growth by up to 70.09% and decreased the frequency of mitosis in tumor tissues, which was up to 37.95% lower compared to the IND groups.	(35)
Fe3O4 magnetic nanoparticles and its surface modification with layered double hydroxide (LDH)	Lamivudine	Mel-Rm and A-549 cancer cell line	0.0µg to 160µg.	Able to inhibit Mel-Rm and A549 cells proliferation in a dose-dependent manner.	(32)

Lactoferrin nanoparticles (LfNPs)	5-Fluorouracil	B16F10 cells	For cellular uptake studies, free 5FU and 5-FU-LfNPs equivalent to 100 µg of 5-FU were used. For toxicity studies, free 5-FU or 5-FU-LfNPs ranging from 5 to 50 µg ml-1 were used.	Showed enhanced celullar uptake compared to free 5-FU, cytotoxic effect corresponding to 2.7times fold in melanoma cells (B16F10).	(42)
Chondroitin sulfate Nanoparticles	5-Fluorouracil	Melanoma cancer cells (A2058 and A375)	0,1g/mL 5FU-NS and 5FU-MS	Cytotoxic effect of 4.3 and 1.7 times greater than the drug 5-FU pure.	(26)
Albumin nanoparticles (ANPs) coated with macrophage plasma membranes (RANPs)	Paclitaxel (PTX)	Melanoma cell line B16F10; female C57BL/6	*In vitro* PTX concentrations in the investigated formulations were 1, 2, 3, 4, 5 and 6µg/mL. ANPs/Did, and RANPs/Did doses of 0.015 mg/kg. For *in vivo* antitumor efficacy test, ANPs/PTX, and RANPs/PTX were used in an equivalent dose of 1 mg/kg PTX.	Increased cytotoxicity and apoptosis rates at all concentrations evaluated, enhanced internalization efficiency in B16F10 cells, improved antitumor efficacy	(33)

Coating layer of didodecyldimethylammonium bromide (DDAB)	Itraconazole	HaCat, SK-Mel-5, A431	3 µg/mL NB-ITZ	Increase of almost 20% the reduction of the viability of the cancer cells treated.	(36)
Organosilica nanoparticles	Dabrafenib and Trametinib	A375 and SK-Mel 28; in vivo	5.36 µM drug and 10.0 µM Pc	Cell viability decreased to 10.0% and 6.2% for A375 and SKMEL-28 cells, respectively.	(43)
Beeswax nanoparticles (BNs)*	Sodium diethyldithiocarbamate (DETC) and 4-nitrochalcone (4NC)	B16F10 cancer cells	5 µg/mL free DETC, free 4NC, BNs-DETC and BNs-4NC; (2.0DETC + 1.04NC µg/mL)	Simultaneous encapsulation led to a synergic effect of DETC and 4NC on B16F10 cells, decreasing the cell viability from 46% (DETC BNs) and 54% (4NC BNs) to 64% (DETC+4NC BNs). When the B16F10 cells were exposed to DETC+4NCS-BNs, the cell viability decreased to 57.9%.	(39)
NPs based on the conjugation of biomaterials, including sodium alginate, cholesterol, and folic acid	Metformin (MET) and Doxorubicin (DOX)	Human A375 and SK-MEL-28 cells; C57BL/6J mice and male BALB/C-NU mice	NPs (250 lg/mL) or an equivalent of Cy5 were injected in tumor bearing mice. For *in vitro* (10 mM), DOX (10 lM), and a combination of both drugs (MET: 5mM, DOX: 5 lM) were used.	FCA NPs were able to co-deliver a dual-drug combination of metformin and doxorubicin into melanoma tumors and inhibit the tumor progression.	(44)

Polymeric nanocapsules	diphenyl diselenide [(PhSe)2	SK-Mel-103, SKMel-28, B16F10 cell line	*In vitro* concentration range of 0.7–200 μM. SK-Mel-28 and B16F10 cells were treated with samples at concentrations of 10–100 μM. For skin permeation studies, around 0.5g of (PhSe)2 from HGs (HG NC (PhSe)2 and HG (PhSe)2) were spread on the skin surface.	Skin permeation showed the nano-based hydrogel promoted enhanced compound content in dermis layer, indicating higher permeation than free compound.	(38)
Polymer-prodrug based on diosgenin nanoparticles (NPs)	Doxorubicin (DOX) and diosgenin	B16F10 cell line, male BALB/c mice	Gradient concentrations of DOX from 0.01 to 5.0 μg/mL, and the gradient concentrations of NPs ranged from 1 to 100 μg/mL were used for *in vitro* experiments. For *in vivo* experiments, NPs (13.3 mg/kg), DOX·HCl (5 mg/kg) or DOX/NPs (5 mg/kg) were used.	*In vivo* inhibit tumor metastasis, as well as reduce the original tumor volume and weight by inducing apoptosis. Enhanced inhibitory effect on tumor proliferation and migration, when compared to NPs or free DOX.	(28)
Copolymer nanoparticles	Paclitaxel (PTX)	A875 human melanoma cells; A875 cells-bearing nude mice	PTX-loaded CA-PLGA-TPGS, PLGA-TPGS and PLGA NPs concentrations of 0.25, 2.5, and 25 μg/mL, respectively. PTX-free CA-PLGA-TPGS NPs with the same NPs dose were used as well.	Cellular uptake efficiency of CA-PLGA-TPGS NPs was maximum. PTX-loaded CA-PLGA-TPGS NPs showed antitumor effects *in vitro* and in vivo.	(34)

Lipid-polymer nanoparticles with anti-CD20 aptamers (CD20-SA-NPs)	Salinomycin	Human melanoma cell lines WM266-4 and A375, WM2664 cells bearing BALB/c nude mice	For *in vitro* experiments, 50 μg/mL equivalent salinomycin concentration were used. For *in vivo* experiments, salinomycin 5 mg·kg1·d-1, iv, for 60 days were used.	Cellular uptake of CD20-SA-NPs was significantly enhanced, when compared free salinomycin. CD20-SA-NPs, presented higher cytotoxicity and selective cytotoxic effects indicated by the higher concentration of CD20+ cells in melanoma cell lines. *In vivo* experiments showed higher inhibition of melanoma growth.	(40)
PLA/PVA/DITC/ ZnPc nanoparticles	Dacarbazine and zinc phthalocyanine + phototherapy	HMEC-1 and melanoma MV3 cells; male Balb/C mice	100 μg PLA/PVA/DITC/ZnPC	The number of cancer cells was reduced by ~80% within the first 24h, compared to control and was dose-dependent.	(45)
Chitosan nanoparticles	10 hydroxycamptothecin (HCPT)	Melanoma cell lines B16F10 and B16F10; female C57BL/6 tumor bearing mice	For *in vitro* cytotoxicity assay, free HCPT and NPs/HCPT solutions with equivalent HCPT concentrations ranging from 0 to 10.0mgml−1 were used. For *in vivo* studies, free HCPT and NPs/HCPT at equivalent doses of 5mg/kg every	*In vitro* studies indicated NPs/HCPT presented higher cytotoxicity against melanoma cells,when compared to free drug counterpart. *In vivo*studies showed that NPs/HCPT were able to significantly inhibit tumor progression, presenting greater antitumor efficacy than free HCPT.	(41)
			3 days for four times were administered in mice.		

Polymeric nanoparticles	Docetaxel (DTX)	Female Kunming genealogy mice bearing melanoma B16F10 cells	0.5, 1, 5, 10, 50, 100 µg/mL	Greater antitumor effect when compared to free drug at equivalent concentrations.	(37)
Lipid Nanoformulation	Dacarbazine and All-trans-retinoic acid (ATRA)	B16F10 cancer cells	25 µg/ml equivalents of free ATRA, DBZ, D-LNF, and RD-LNF formulations	The combination of DBZ + ATRA contributed to the apoptosis of cancer cells and could inhibit the proliferative inhibition of B16F10 melanoma cells.	(46)
Immunoliposome	Doxorubicin + Tcell receptor (TCR)like antibody (scFv G8 and Hyb3)	Melanoma antigen A1 (MAGE-A1) presented by human leukocyte antigen A1 (M1/A1)	10 µm Doxorubicin	Significant tumor cell kill at 10 µM doxorubicin (DXR). Two to five times higher binding and internalization of these immunoliposomes The accumulation of targeted liposomes was 2- to 2.5-fold and 6.6-fold enhanced when compared with nontargeted liposomes and free drug, respectively.	(18)

Drug delivery to basal and squamous carcinoma

Basal cell carcinoma is the most common type of skin cancer, represented by 8 out of 10 cases and appears especially in the face, head and neck and usually the lesion can be completely removed, when the treatment is necessary, can be used topical chemotherapy, often with 5fluorouracil (47–49). In addition to conventional treatments, the literature presents strategies for nanoencapsulation of the substance 5-Fluorouracil with different strategies (Table 2). Rata et al (50), contributed with new strategies of antitumor therapy and developed poly (N-vinylpyrrolidonealt-itaconic anhydride) nanocapsules with AS1411 aptamer on the surface. The nanocapsules were incorporated into gel to provide suitable treatment of the skin lesions to release the 5-FU at the site of the tumor in a controlled manner. This study showed *in vivo* a cytotoxic effect similar to that induced by free 5-FU with a protective effect of the gel when topically administered. Another strategy was a new chitosan and sodium tripolyphosphate (TPP) nanoparticle and when it was tested *in vitro* with A375 basal cell carcinoma cell-line showed the possibility of enhanced delivery of 5-Fluorouracil for this type of skin cancer(51).

Squamous carcinoma occurs in the in the middle layer of the epidermis and represents 2 out of 10 cases of skin cancers. These pathology is related with UV exposure and can be removed completely, but sometimes can grow into deeper layers and in these cases. 5-FU can also be used and studies have proposed new strategies employing nanoparticles (47,52). Gold nanoparticles capped with cetyltrimethylammonium bromide (CTAB) loaded with 5-FU, when tested *in vivo* for topic administration showed a decrease on tumor volume and the possibility of reduction of systemic side effects. Doxorubicin has been tested with liposomes *in vitro* in human skin carcinoma cells (A431) and showed a significant decrease in cell viability, when combined with Celocoxib. DOX also was tested *in vivo* with iron oxide magnetic nanoparticles showing cytoxicity, with the delivery of therapeutic doses of this substance able to inhibit tumor growth. Another alternative strategy that was proposed was a Liposomal with a hydrophobic compound named Avicequinone-B. It induced apoptosis in this type of skin cancer offering a perspective of topical use for treatment(53).

The interference RNA technique can be an alternative used built on mesoporous silica nanoparticles (MSNPs) and applied topically, the results *in vivo* showed tumor volume decreased about three times compared to control (54). In alternative to these treatments, other substances have been studied and tested for this pathology like curcumin that was tested in human epidermoid squamous cell carcinoma cell line (A431) and the viability of the cells treated was significantly decreased and *in vivo* the dermal delivery were able to improve the skin penetration and deposition of curcumin (55). Another natural product is a medicinal plant of Asia called Cratoxylum formosum associated with ZnO spherical nanoparticles that demonstrated high cytotoxicity *in vitro* with A431. Other alternative particles were used like virus-like nanoparticles that successfully delivered apeptide that could target skin cancer cells(56). A decrease in cell viability was observed in SCC cells and melanoma cells with another strategy using solid lipid nanoparticles formulation containing itraconazole with a coating layer of didodecyldimethylammonium bromide or gelatin nanoparticles(57).

Table 2: Summary of nanoparticles combined with terapeutical strategies for basal and squamous cells carcinoma.

Nanoparticle	**Terapeutical strategy**	**Type of cancer**	***In vitro/in vivo***	**Dosage**	**Results**	**Ref**
Chitosan nanoparticles	5-Fluorouracil (5-FU)	Basal cell carcinoma	A375 basal cell carcinoma cell line	For *in vitro* studies, 5-FU loaded chitosan nanoparticles samples at equivalent concentrations ranging from 1 μg/ml to 5000 μg/ml were used.	Chitosan nanoparticles loaded with 5-FU presented greater cytotoxic effects than free drug at 10, 50 and 100 μg/ml concentration.	(51)
Gold Nanoparticles capped with cetyltrimethylammonium bromide (CTAB)	5-Fluorouracil	Squamous carcinoma	Full thickness C57BL/6 mice dorsal skin*, A431 tumor-bearing mice	1g sample of 5-FU/CTAB-GNPs gel and cream, equivalent to 10 mg of 5-FU; gel and cream containing 1% w/w free drug.	5-FU/CTAB-GNP incorporated into cream had significantly enhanced drug flux and *in vivo* studies results showed achieved 6.8- and 18.4-fold lower tumor volume when compared to the untreated control.	(22)

Thermo-responsive fluorescent polymer (TFP) conjugated on the surface of iron oxide magnetic nanoparticles (MNPs)	Doxorubicin	Squamous carcinoma	A431 and G361 skin cancer cells, C57BL6 mice injected with B16F10 skin cancer cells	TFP-MNPs concentration of 50, 100 and 100µg/mL. Free DOX concentration of 37 nM for A431, 32 nM for G361, and 258 nM for PC3 cells, and equivalent amounts of DoxTFP-MNPs for *in vitro* studies. In *in vivo* studies, Free Dox concentration was 40 µg/ml, 200 µl and Dox-TFP-MNPs was 0,8 mg/ml, 200 µl.	Higher cellular uptake of TFP-MNPs than LNCaP, *in vitro* studies showed that Dox-TFP-MNPs presented citotoxicity against cancer cells under simulated hyperthermia conditions. *In vivo* the growth was decreased.	(58)
Aptamer-functionalized polymeric nanocapsules	5-Fluorouracil	Basal cell carcinoma	TE 354.T human basal carcinoma cell line, 30 male albino mice and chicken skin membrane*	For *in vitro* cytotoxicity assay, different doses of 5-FU were used, ranging from 25 to 200µg/mL. For ex vivo studies, 5-FU loaded nanocapsules, gel formulation containing 5-FU loaded nanocapsules and simple gel formulation containing 5FU at equivalent amounts of 5FU of 10% were used.	Formulations containing 5-FU loaded nanocapsules presented greater diffusion properties, when compared to free drug. In addition, the formulations exhibited considerable cytotoxicity in the *in vitro* experiments.	(50)
Liposomes	Avicequinone-B (Naphtho[2,3b]furan-4,9dione)	Squamous carcinoma	HSC-1 cells	Avicequinone-B 2 µg/ml (dissolved in PBS) and freeform Avicequinone-B 2 µg/ml	Cytotoxicity on HSC-1 cells by decreasing the mitochondrial membrane potential, incrising cytosolic cytochrome-c level and cleaved caspase 8 level, as well as induction of apoptosis. On the other hand, free-form Avicequinone-B had no significant effect on cell viability.	(53)

Lipid nanoparticles (LNs)*	5-Fluorouracil	Squamous carcinoma	A431 epidermoid carcinoma cells and normal human keratinocytes cells (HaCat)	Hydrogel formulation enriched with NLC loaded with 5-FU 0,1% (w:w) and hydrogel formulation containing pure 5FU 0,1% (w:w); 10-4M	Anticancer effect on A431 epidermoid carcinoma cells, when compared to free drug counterpart and decreased cytotoxicity against keratinocyte cells. The amount of drug that penetrated into rat skin was significantly higher when using the NLC loaded with 5-FU enriched hydrogel, when compared to 5-FU hydrogel.	(59)
Liposomes	Doxorubicin and Celecoxib	Squamous carcinoma	human skin carcinoma (A431) cells	Doxorubicin 5 μM, Celecoxib ranging from 100 μM to 50 μM, and Dox-L (5 μM) + Cele-L (50, 75, 100μM) at a ration of 1:10	Liposomes containing doxorubicin at 5 μM and celecoxib at 75 μM did not present cytotoxicity against human skin carcinoma cells (A431), but the drug combination reduced cell viability in >99%.	(60)

Immunotherapy and nanoparticles

Nanotechnology can be used to get better results from general and personalized cancer immunotherapies, this technology can guide certain therapeutic agents to specific sites in the body through their application and improve their bioavailability and stability (61) . Min et al (62), used PLGA nanoparticles transporting these proteins to antigen-presenting cells (APCs) to promote the cancer immunity and showed that antigencapturing nanoparticles were capable of potentiate an immune response in vivo. These particles also promoted more infiltrating CD8+ T cells when compared with the mice that did not receive the treatment and could elicit systemic T-cell activation. Yin et al (63) constructed a thermosensitive nanoparticle to co-deliver chemotherapeutic drug doxorubicin and cytokine IFN γ. They showed a synergistic effect of immunotherapy and chemotherapy by prolonging the circulation time of DOX. Consequently, *in vivo* there was the inhibition of the tumor progression, due to the effect of IFN γ at high dosage that could up-regulate the expression of PD-L1. It is possible to observe that the thermosensitive modified structure had the role of sustainably delivering the cytokine without losing bioactivity.

PLGA was used by Kim et al (64) to formulate a PLGA nanoparticle that encapsulated two promising TLR7/8 bi-specific imidazoquinolinebased esters (racemic mixture '522' and its S-configured stereoisomer '528'). They chose these agonist molecules due to the power to induce high levels of pro-inflammatory cytokines release and promote an increase in T cell activation. The combination of antigen and agonist induced a strong protective immune response in melanoma and a delay in primary tumor growth, reducing chances of metastasis on the B16F10-OVA mice, when co-administered with tumor-specific antigen. Ordikhani et al (65) made a delivery of anti–PD-1 antibody encapsulated in PLGA nanoparticles (anti–PD-1 NPs) and significantly improved its antitumor effect in the B16-F10 murine melanoma model. PLGA nanoparticles were used by Yaman et al (66) loaded with an FDA-approved melanoma chemotherapeutic drug, Trametinib. T-cell membrane camouflaged Trametinib loaded PLGA NPs displayed high stability, hemo- and cyto-compatibility. *In vivo* biodistribution studies displayed the therapeutic capabilities of these NPs with more than a twofold increase in the tumor retention, when compared to the uncoated and non-specific membrane coated groups. Clemente et al (67) tried two types of polymer nanoparticles, i.e. cross-linked β-cyclodextrin nanosponges (CDNS) and poly(lactic-co-glycolic acid) (PLGA) NPs. The authors showed *in vivo* that the treatment of C57BL6/J mice with the two nanoformulations inhibits the growth of established subcutaneous B16-F10 tumors, involving both anti-angiogenic and immunoregulatory effects.

Another polymer used in drug delivery is poly (β-amino esters) that exhibit characteristics like biodegradable, hydrophilic and diferente kinetic profiles and tunable charge density(68). Zhang et al (69), described that nanoparticles formulated with mRNAs encoding interferon regulatory factor 5, in combination with its activating kinase IKKβ, were able to reverse the immunosuppressive, tumor-supporting state of Tumorassociated macrophages (TAMs), and reprogram them to a phenotype that induces anti-tumor immunity and promotes tumor regression. Guevara et al(70), described a new lipopolyplex vector (multi-LP), incorporating the immune adjuvant α-galactosylceramide (α-GalCer) and a multivalent cationic lipid. This multi-LP vaccine induced a significant therapeutic effect against a highly malignant B16-F10 melanoma tumor.

Deng et al(71), designed a novel and efficient CRISPR-Cas9 genome editing system delivered by cationic copolymer aPBAE to downregulate PD-L1 expression on tumor cells, via specifically knocking out Cyclin-dependent kinase 5 (Cdk5) gene *in vivo* and effective inhibited tumor growth in murine melanoma.

Wilson et al (72) tested poly(beta-amino ester) (PBAE) nanoparticles to deliver CDNs to the cytosol leading to robust immunity. Zou et al (73), reduced immunosuppression in the tumor microenvironment with PBAE-loaded PD-L1 antagonist nano-vaccines to delay melanoma development. They also found that PBAE nano-vaccines can target mature DCs, consequently boosting antigen-specific cytotoxic T lymphocyte activity against melanoma.

Saeed et al (18) used smallest fragments of antibodies (25–30 kDa) for potentially reduce immunogenicity.Their results indicate that the immunoliposome formulations resulted in antigen-specific killing, DXR-L scFvs accumulated in higher amounts in kidneys, spleen, lungs, and heart. Finally, they showed that DXR-L scFvs inhibited tumor growth significantly when compared with other groups. Li e Han (74) have designed a PEI-siRNA complex loaded in the aqueous core of the liposomal nanoparticles. The results showed a significant reduction in the cell viability and a synergistic anticancer effect of siRNA-PD-L1 and IMT in the induction of apoptosis of melanoma cells.

Kang et al (75) utilized another strategy with T-cell-membrane-coated nanoparticles that exhibited a high therapeutic efficacy and immune checkpoint blockade in melanoma treatment, also demonstrated in the treatment of lung cancer in an antigen nonspecific manner. Song et al (76) tested spherical poly lactic-co-glycolic acid nanoparticles with antigen-presenting cells (APCs) and demonstrated their greater resistance to phagocytosis and stronger functionality to expand antigen-specific T cells caused inhibition of tumor growth.

Table 3: Summary of nanoparticles combined with immunotherapy strategies for melanoma.

Nanoparticle	**rapeutical strategy**	***vitro/invivo***	**Dosage**	**Results**	**Ref**
PLGA nanoparticles	Antigencapturing	melanoma cell line B16F10;	AC-NPs (2 mg in 100 µl PBS)was intraperitoneally injected into the	Capable of potentiate an immune response *in vivo* and, also, these particles promoted more infiltrating CD8+ T	(77)
		female C57BL/6	animals on days 5, 8 and 11. AC-NPs (2 mg in 100 µl PBS) were injected into the primary tumours on days 10, 11 and 12	cells when compared with the mice that did not received the treatment and could elicit systemic T-cell activation	
PLGA nanoparticles	Doxorubicin (DOX) and interferon γ (IFNγ)	melanoma cell line B16F10; female C57BL/6	2.5 mg/kg	Synergistic effect *in vivo* inhibited the progression of tumors due to the effect of IFN γ at high dosage could upregulated the expression of PD-L	(63)

PLGA nanoparticles	TLR7/8 bi-specific imidazoquinoli ne-based esters (racemic mixture '522' its Sconfigu red stereoisomer '528')	melanoma cell line B16F10OVA; female C57BL/6 mice	Free528 (1 µg) or 528NPs (1mg, equivalent to 1 µg of Free528) in a 100 µl volume.	Promoted an increased T cell activation occurs with the combination of antigen and agonist, induced a strong protective imune reponse in melanoma and a delayed primary tumor growth and chances of metastasis on the B16F10-OVA cells and mice when co-administered with tumor-specific antigen.	(64)
PLGA nanoparticles	Anti–PD-1	melanoma cell line B16F10; female C57BL/6	3.6 µg anti–PD-1 mAb per 100µl of NPs injection/mouse	Significantly improved its antitumor effect in the B16F10 murine melanoma model.	(65)
PLGA nanoparticles	Trametinib	melanoma cell lines, DM6 and 1520; female C57BL/6	injecting 0.5 × 106 DM-6 cellsin 150 µl of Matrigel	High stability, hemo- and cyto-compatibility and *in vivo* biodistribution increase in the tumor retention compared to the uncoated and non-specific membrane coated groups.	(66)

PLGA nanoparticles	CD47-Fc, H-2Kb/TRP2180188-Ig dimers, and anti-CD28	melanoma cell line B16F10; female C57BL/6	1 mg NPs per mouse per time point	Resistance to phagocytosis and stronger functionality to expand antigen-specific T cells caused inhibition oftumor growth	(76)
Cross-linked β-cyclodextrin nanosponges (CDNS) and PLGA nanoparticles	ICOS (a T-cell costimulatory molecule)	melanoma cell line B16F10; female C57BL/6	100 µg	Showed *in vivo* experiments that treatment of C57BL6/J mice into the two nanoformulations inhibits the growthof established subcutaneous B16-F10 tumors, involvedboth anti-angiogenic and immunoregulatory effects.	(67)
PbAE nanoparticles	transcribed mRNA encoding M1polarizing transcription factors	melanoma cell line B16F10; female C57BL/6	30 µg mRNA suspended inPBS	Supporting state of Tumor-associated macrophages (TAMs) and Reprogram them to a phenotype that induces anti-tumor immunity and promotes tumor regression.	(69)
PbAE nanoparticles	lipopolyplex vector (multiLP), incorporating	melanoma cell line B16F10; female C57BL/6	20 µg	Induced a significant therapeutic effect against a highly malignant B16-F10 melanoma tumor.	(70)

	the immune adjuvant αgalactosylceramide (α-GalCer) and a multivalent cationic lipid				
PbAE nanoparticles	CRISPR-Cas9	melanoma cell line B16F10; female C57BL/6	5 mg plasmid for each injection	Downregulate PD-L1 expression on tumor cells via specifically knocking out Cyclin-dependent kinase 5 (Cdk5) gene *in vivo* and effective inhibited tumor growthin murine melanoma.	(71)
PbAE nanoparticles	Cyclic dinucleotides (CDNs)	melanoma cell line B16F10; female C57BL/6	2 μg PBAE+CDN	Cytosolic delivery method for STING agonists synergizes with checkpoint inhibitors and attenuated tumor cells.	(72)
PbAE nanoparticles	PD-L1 antagonist	melanoma cell line B16F10; female C57BL/6	100 μg Trp -2, 10 μg MPLA per mouse	Reduced immunosuppression in the tumor microenvironment to delay melanoma development and boosting antigen-specific cytotoxic T lymphocyte activity against melanoma.	(73)

Liposomes	Doxorubicin and a T-cell receptor	human melanoma, patient-	4 mg/kg DXR-Ls and DXR-L scFvs	Reduced immunogenicity and antigen-specific killing, DXR-L scFvs accumulated higher in kidneys, spleen,	(74)

	(TCR)-like antibody (scFvG8 and Hyb3)	derived cell lines were used: two M1+/A1+ (MZ2Mel43 and G43) and two M1−/A1+ (Mel78 and Mel2A), with all cell lines being HLAA1+; female C57BL/6		lungs, and heart, so showed that DXR-L scFvs inhibited tumor growth significantly compared with other groups	
Liposomes	siRNA-PD-L1 (siPD-L1) and imatinib (IMT)	melanoma cell line B16F10; female C57BL/6	IMT was administered at a fixed dose of 5 mg/kg and siRNA was administered at 500 μg/kg onevery 3rd for three times	Reduction in the viability and a synergistic anticancer effect of siRNA-PD-L1 and IMT in the induction ofapoptosis of melanoma cells	(75)

Clinical trials in skin cancer with the use of NPs

Currently, there are three clinical trials registered in the clinicaltrials.gov evaluating the use of nanoparticles through topical administration on skin malignancies, NCT03101358 (completed), NCT00821964 (completed) and NCT04138342 (recruiting). The first trial evaluated the cutaneous metastasis originated from non-melanoma skin cancers and use as interventional approach, the SOR007 (Uncoated Nanoparticle Paclitaxel) Ointment in three different concentrations. The NCT00821964 evaluated the application of a topical formulation containing imiquimod together with Abraxane in advanced breast cancer patients with cutaneous metastasis. The NCT04138342 will evaluate also in breast cancer patients with cutaneous metastasis, the use of CdS/ZnS core-shell type quantum dots with carboxylic acid-functionalized (QDs-COOH) nanoparticles decorated with VELD. The trials with the treatment of breast cancer metastasis the evaluated treatment is for skin cancer that is metastasis to the breast (Table 4).

Table 4: Summary of clinical trials registered on clinicaltrials.gov which evaluates the administration of treatments with nanoparticles through topical route in skin cancer.

Identifier	Status	Allocation	Phase	Startdate	End date	Type of cancer	Nanoparticle	Terapeutical strategy
NCT00821964	Completed	N/A	II	2009	2018	Male Breast Cancer Recurrent Breast Cancer Skin Metastases Stage IV Breast Cancer	aclitaxel albumin-stabilized nanoparticle fo rmulation	imiquimod; Abraxane; laboratory biomarker analysis; RNA analysis; immunoenzyme technique

NCT03101358	Completed	NonRandomized	I/II	2017	2020	Cutaneous Metastasis	Uncoated Nanoparticle Paclitaxel	SOR007 Ointment
NCT04138342	Recruiting	Randomized	I	2019	ongoing	Breast Cancer Skin Cancer Skin Diseases	QDs-COOH-VELD	Quantum dots coated with veldoreotide; Topical approved placebo

Conclusion

Recent progress in nanotechnology-based application in drug delivery systems or as a therapy strategy for skin cancer has been summarized in this review. However, when it comes to clinical studies, there is still a very small amount. This demonstrates that the studies, although promising, are very early and have not reached the applicability and use of the study in humans for the most part. The precise delivery of antitumor drugs at the targeted tumor site is one of the big problems associated with cancer therapy, besides the side effects in the patients. Cytotoxicity, biodistribution and bioavailability, physicochemical stability parameters such as pH and the administration route of interest must be evaluated in nanoparticles when we develop these systems for skin cancer. In addition, when drugs are present, the usual treatment of the drug in solution compared to the nanoencapsulated drug should always be evaluated. The use of different structures, manufacturing processes and compositions for nanoparticles and the different approaches showed the recent advances in nanotechnology and established a potential skin cancer therapy approach.

In this review we saw the results of different types of nanoparticles for treatment of melanoma, basal and squamous carcinoma that showed through *in vitro* and *in vivo* approaches, the improvement of the survival rate, reduction in tumor growth and side effects. Melanoma is the type of skin cancer most discussed in the articles, possibly due to its high rate of metastasis and mortality. For this type of cancer, the nanoparticles were of the most diverse types, highlighting the polymeric one and regarding associated treatments, there are several containing drugs already used for other types of cancer or new substances. In relation to the other subtypes, squamous cell carcinoma was the one that presented more studies related to 5-fluoracil nanoencapsulation and the most used particles were liossomes.

In the case of nanotechnology associated with immunotherapy, melanoma was present in the studies for this strategy and mostly using polymeric nanoparticles. The results showed that is possible to develop a personalized therapy playing an exclusive role in targeted delivery of immunotherapics. However, the limitation for this strategy is the situation of each patient, requiring a personalized treatment for each case. We can conclude that nanomedicine has opened an infinite way of possibilities to search and design drug and drug delivery systems for treatment of melanoma. Therefore, nanotechnology should emerge as a critical area of research in the 21st century for different strategies of cancer treatments.

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Conflict of interest

The authors declare no conflict of interest.

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