"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY BUCHAREST DOCTORAL SCHOOL

PHARMACY

FORMULATION OF SMART SYSTEMS USED IN CUTANEOUS NEOPLASTIC CONDITIONS

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Skin cancer is one of the most frequently diagnosed neoplasms globally, with a significant increase in incidence in recent decades. This condition, primarily classified as melanoma and non-melanoma, represents a major public health challenge. Melanoma is known for its aggressiveness and high metastatic potential, while non-melanoma skin cancers, though less lethal, are much more common and can significantly affect patients' quality of life. The main risk factors include excessive exposure to ultraviolet radiation, genetic predisposition, and environmental factors. In this context, prevention is essential, but developing effective treatments remains a priority in medical research.

Taxanes, such as docetaxel and paclitaxel, have revolutionized oncology treatments due to their unique mechanism of action, which involves stabilizing cellular microtubules, inhibiting cell division, and inducing apoptosis in tumor cells. These substances have been successfully implemented in the treatment of various cancers, including breast, prostate, and lung cancer. However, their use is limited by low water solubility and high systemic toxicity, underscoring the need for innovation in pharmaceutical formulation. Thus, minimizing systemic side effects and improving bioavailability are major objectives of current pharmaceutical research.

Advances in nanotechnology have opened new perspectives in optimizing the release of complex active pharmaceutical ingredients such as taxanes. Nanoscale lipid structures, such as nanostructured lipid carriers (NLCs) and transfersomes, have demonstrated the ability to increase the permeability and bioavailability of active substances, especially in topical applications. These systems allow efficient penetration of the stratum corneum barrier, ensuring controlled and prolonged drug release directly at the tumor site. In the case of skin cancer, the use of these technologies could revolutionize therapeutic approaches, offering more targeted treatments with reduced side effects.

This doctoral thesis is structured into two main sections: a general-theoretical part and a section dedicated to personal contributions.

The general-theoretical part is organized into three chapters, covering essential topics related to skin cancer treatment through the use of nanotechnologies for administering poorly water-soluble pharmaceutical ingredients, improving therapeutic efficacy by using lower doses, and reducing systemic side effects by precisely targeting the drugs. These aspects are

crucial for improving the quality of life of cancer patients and increasing treatment compliance.

Chapter 1 provides a comprehensive review of skin cancer, one of the most common and dangerous dermatological conditions, highlighting the alarming increase in its global incidence. The major types of skin cancer, such as melanoma and non-melanoma (basal cell carcinoma and squamous cell carcinoma), are discussed alongside the main risk factors, including genetic predisposition and ultraviolet radiation exposure. Additionally, available therapeutic options are analyzed, from surgical interventions and radiation therapy to innovative therapies such as photodynamic therapy and topical applications, highlighting both the benefits and limitations.

Chapter 2 explores the use of taxanes, particularly paclitaxel and docetaxel, which have become standard treatments for various types of cancer due to their ability to stabilize cellular microtubules and inhibit cell division. Despite their efficacy, low water solubility and systemic side effects limit the use of these agents, necessitating the development of new pharmaceutical formulation strategies. This chapter also examines the clinical challenges associated with taxane use, such as hematological and neurological toxicities, and how pharmaceutical innovations can help reduce these risks by improving solubility and safety profiles.

Chapter 3 focuses on the use of nanotechnologies, particularly lipid nanostructures, for developing innovative pharmaceutical formulations aimed at treating skin cancer. The integration of taxanes, such as docetaxel, into nanostructured lipid carriers (NLCs) and transfersomes offers a promising solution for improving the stability and bioavailability of these therapeutic agents. These formulations allow for controlled and prolonged drug release at the tumor site, while protecting healthy tissues and reducing systemic side effects. The chapter also discusses the technical challenges encountered in developing these formulations and the potential benefits of their transdermal application in oncology, opening new perspectives for safer and more efficient cancer therapies.

This structure enables a comprehensive approach to the subject, providing a detailed theoretical analysis and proposing innovative solutions to current challenges in skin cancer treatment.

The personal contributions section is structured into chapters 4-6, representing the core of the experimental research conducted for this thesis.

Chapter 4 presents the working hypotheses and general objectives of the thesis, which establish the essential directions for the investigations carried out.

Chapter 5 is dedicated to the development and evaluation of nanostructured lipid particles loaded with docetaxel for skin cancer treatment. By applying Quality by Design principles, critical parameters influencing desired outcomes, such as the stability and physicochemical properties of the formulated nanostructures, were identified. Rigorous experiments evaluated the performance of these nanostructured systems, with a focus on encapsulation efficiency and controlled release of active substances. In this context, advanced formulations designed to optimize the bioavailability of poorly soluble drugs were tested, and the molecular interactions between the nanostructure components and encapsulated active substances were studied. Controlled release studies were complemented by precise analytical methods, such as high-performance liquid chromatography, to quantify the effectiveness of the proposed systems. The detailed characterization of these systems included complex physicochemical analyses, such as electron microscopy and other advanced structural analysis techniques of nanomaterials. This chapter highlights the potential of these innovative technologies to improve drug delivery and therapeutic efficacy, contributing to the development of safer and more effective treatments.

Chapter 6 focuses on the optimization of transfersomes loaded with docetaxel to improve the transdermal delivery of this antineoplastic agent. Experimental design was used to identify and optimize key parameters, such as particle size, zeta potential, and encapsulation efficiency, which are essential for developing a delivery system capable of maximizing dermal retention and minimizing systemic side effects. The obtained structures were analyzed for their structural properties and evaluated for selective cytotoxicity on melanoma cells compared to normal umbilical cells, demonstrating the formulations' potential to effectively target cancer cells.

The studies included in this section also investigated rheological behavior and in vitro permeation to determine the release mechanism of docetaxel from transfersomes incorporated into a hydrogel. This research focused on the development, optimization, and detailed characterization of two types of pharmaceutical formulations for the treatment of skin cancer: nanostructured lipid carriers (NLCs) and transfersomes, both loaded with docetaxel. By

applying rigorous experimental design methodologies and the One-Factor-At-a-Time method, critical parameters influencing characteristics such as particle size, zeta potential, encapsulation efficiency, and recovery after filtration were identified—factors critical for developing safe and effective delivery systems for clinical use. This systematic approach allowed the selection of formulations with optimal performance, validated by the obtained physicochemical characteristics.

The experimental studies confirmed the consistency of particle sizes and the well-defined morphology of the optimized formulations, highlighting their potential for advancing to clinical trials. X-ray diffraction analyses and advanced imaging techniques, such as scanning electron microscopy (SEM) and cryo-transmission electron microscopy (cryo-TEM), confirmed the integrity and optimal structure of the formulations, demonstrating the robustness of the manufacturing process. The in vitro release kinetics studies showed that both types of formulations, transfersomes and NLCs, can ensure sustained release of docetaxel, maintaining therapeutic concentrations in the skin layers, thus amplifying the therapeutic effect on cancer cells without affecting healthy cells.

A central aspect of the research was the detailed evaluation of the efficacy of these formulations on cancer cells, with particular attention to minimizing the impact on normal cells. Experimental results showed that both delivery systems exert a strong and selective cytotoxic effect on SK-MEL-24 melanoma cells, underscoring their significant therapeutic potential in targeted skin cancer treatment. Transfersomes and NLCs demonstrated the ability to maintain effective local concentrations of docetaxel, enhancing the antitumor effect by inhibiting cancer cell proliferation and promoting apoptosis, contributing to the reduction of tumor mass.

A notable result of the research was the minimal impact on normal HUVEC cells, demonstrating the high selectivity of the formulations. While systemic administration of docetaxel is often associated with severe toxicity, the use of these targeted delivery systems significantly reduced adverse effects on healthy cells. This remarkable selectivity supports the potential of these formulations to offer an extended therapeutic range and improved safety profile, essential for their adoption in clinical practice.

The specific structure of transfersomes and NLCs not only enhances therapeutic efficacy but also improves treatment tolerability, reducing the risk of adverse effects

associated with conventional cancer therapies. This essential characteristic increases patient comfort and treatment adherence, critical factors for the long-term success of therapy.

In conclusion, the studies conducted demonstrate that transfersome and NLC formulations loaded with docetaxel hold significant potential in advancing treatments for skin cancer. Comparatively, NLCs exhibited high encapsulation efficiency (over 90%) and sustained docetaxel release for approximately 48 hours, aspects that confer a significant therapeutic advantage. These formulations not only inhibit cancer cell proliferation but also protect healthy tissues, opening new opportunities for safer and more efficient therapies in dermatologic oncology.

The methods used for preparing these formulations are easily scalable, suggesting promising industrial applicability and an efficient path for large-scale production. Transdermal application, with minimized systemic exposure, can facilitate accelerated regulatory approval and may be easily accepted by patients due to its non-invasive nature and reduced side effect profile.

The results of this research highlight the potential of transfersomal gel and NLC formulations loaded with docetaxel for optimizing skin cancer treatments, justifying further preclinical and clinical investigations.

Selective bibliography:

- Khan NH, Mir M, Qian L, Baloch M, Ali Khan MF, Rehman A-u, et al. Skin cancer biology and barriers to treatment: Recent applications of polymeric micro/nanostructures. Journal of Advanced Research. 2022;36:223-47.
- Leiter U, Keim U, Garbe C. Epidemiology of Skin Cancer: Update 2019. In: Reichrath J, editor. Sunlight, Vitamin D and Skin Cancer. Cham: Springer International Publishing; 2020. p. 123-39.
- An S, Kim K, Moon S, Ko K-P, Kim I, Lee JE, et al. Indoor Tanning and the Risk of Overall and Early-Onset Melanoma and Non-Melanoma Skin Cancer: Systematic Review and Meta-Analysis. Cancers [Internet]. 2021; 13(23).
- Feller L, Khammissa RAG, Kramer B, Altini M, Lemmer J. Basal cell carcinoma, squamous cell carcinoma and melanoma of the head and face. Head & Face Medicine. 2016;12(1):11.
- Dinu-Pirvu CE, Cocos FI, Anuta V, Ghica MV, Popa L. Plant Secondary Metabolites: Natural Compounds as Cosmetic Ingredients and Their Potential Activity in Skin Cancer. In: Sharma AK, Sharma A, editors. Plant Secondary Metabolites: Physico-Chemical Properties and Therapeutic Applications. Singapore: Springer Nature Singapore; 2022. p. 121-39.
- Dawes SM, Tsai S, Gittleman H, Barnholtz-Sloan JS, Bordeaux JS. Racial disparities in melanoma survival. Journal of the American Academy of Dermatology. 2016;75(5):983-91.
- Wu S, Cho E, Li W-Q, Weinstock MA, Han J, Qureshi AA. History of Severe Sunburn and Risk of Skin Cancer Among Women and Men in 2 Prospective Cohort Studies. American Journal of Epidemiology. 2016;183(9):824-33.
- Collins L, Quinn A, Stasko T. Skin Cancer and Immunosuppression. Dermatol Clin. 2019;37(1):83-94.
- Yeung H Fau - Balakrishnan V, Balakrishnan V Fau - Luk KMH, Luk Kmh Fau - Chen SC, Chen SC. Risk of Skin Cancers in Older Persons Living With HIV: A Systematic Review. J Assoc Nurses AIDS Care. 2019;30(1)(1552-6917 (Electronic)).
- Chang AY, Doiron P, Maurer T. Cutaneous malignancies in HIV. Curr Opin HIV AIDS. 2017;12(1):57-62.
- Rollison Dana E, Viarisio D, Amorrortu Rossybelle P, Gheit T, Tommasino M. An Emerging Issue in Oncogenic Virology: the Role of Beta Human Papillomavirus

Types in the Development of Cutaneous Squamous Cell Carcinoma. Journal of Virology. 2019;93(7):10.1128/jvi.01003-18.

- Nichols AJ, Gonzalez A, Clark ES, Khan WN, Rosen AC, Guzman W, et al. Combined Systemic and Intratumoral Administration of Human Papillomavirus Vaccine to Treat Multiple Cutaneous Basaloid Squamous Cell Carcinomas. JAMA Dermatology. 2018;154(8):927-30.
- John V, Ana MSM. Ocular surface biopsies of patients with xeroderma pigmentosum in the United Kingdom: a retrospective observational case series. British Journal of Ophthalmology. 2021;105(9):1222.
- Lova Navarro M, Vera Casaño A Fau - Benito López C, Benito López C Fau - Fernández Ballesteros MD, Fernández Ballesteros Md Fau - Godoy Díaz DJ, Godoy Díaz Dj Fau - Crespo Erchiga A, Crespo Erchiga A Fau - Romero Brufau S, et al. Transient neonatal zinc deficiency due to a new autosomal dominant mutation in gene SLC30A2 (ZnT-2). Pediatric dermatology. 2014;31(2)(1525-1470 (Electronic)):251-2.
- Leachman SA, Lucero OM, Sampson JE, Cassidy P, Bruno W, Queirolo P, et al. Identification, genetic testing, and management of hereditary melanoma. Cancer and Metastasis Reviews. 2017;36(1):77-90.
- Shay JW. Role of Telomeres and Telomerase in Aging and Cancer. Cancer Discov. 2016;6(6):584-93.
- Boscolo-Rizzo P, Da Mosto MC, Rampazzo E, Giunco S, Del Mistro A, Menegaldo A, et al. Telomeres and telomerase in head and neck squamous cell carcinoma: from pathogenesis to clinical implications. Cancer and Metastasis Reviews. 2016;35(3):457-74.
- Flies EJ, Mavoa S, Zosky GR, Mantzioris E, Williams C, Eri R, et al. Urban-associated diseases: Candidate diseases, environmental risk factors, and a path forward. Environment International. 2019;133:105187.
- Schmitt J, Haufe E, Trautmann F, Schulze HJ, Elsner P, Drexler H, et al. Is ultraviolet exposure acquired at work the most important risk factor for cutaneous squamous cell carcinoma? Results of the population-based case–control study FB-181. British Journal of Dermatology. 2018;178(2):462-72.

- Loras A, Gil-Barrachina M, Marqués-Torrejón MÁ, Perez-Pastor G, Martinez-Cadenas C. UV-Induced Somatic Mutations Driving Clonal Evolution in Healthy Skin, Nevus, and Cutaneous Melanoma. Life [Internet]. 2022; 12(9).
- Downs NJ, Axelsen T, Schouten P, Igoe DP, Parisi AV, Vanos J. Biologically effective solar ultraviolet exposures and the potential skin cancer risk for individual gold medalists of the 2020 Tokyo Summer Olympic Games. Temperature (Austin). 2020;7(1):89-108.
- Didona D, Paolino G, Bottoni U, Cantisani C. Non Melanoma Skin Cancer Pathogenesis Overview. Biomedicines [Internet]. 2018; 6(1).
- Ouhtit A, Konrad Muller H, Gorny A, Ananthaswamy HN. UVB-induced experimental carcinogenesis: dysregulation of apoptosis and p53 signalling pathway. Redox Report. 2000;5(2-3):128-9.
- Moan J, Grigalavicius M Fau - Baturaite Z, Baturaite Z Fau - Dahlback A, Dahlback A Fau - Juzeniene A, Juzeniene A. The relationship between UV exposure and incidence of skin cancer. Photodermatology, photoimmunology & photomedicine. 2015;31(1)(1600-0781 (Electronic)):26-35.
- Hart PH, Norval M. Ultraviolet radiation-induced immunosuppression and its relevance for skin carcinogenesis. Photochemical & Photobiological Sciences. 2018;17(12):1872-84.
- Ruan L, Cheng S-P, Zhu Q-X. Dietary Fat Intake and the Risk of Skin Cancer: A Systematic Review and Meta-Analysis of Observational Studies. Nutrition and Cancer. 2020;72(3):398-408.
- Black HS, Rhodes LE. Potential Benefits of Omega-3 Fatty Acids in Non-Melanoma Skin Cancer. Journal of Clinical Medicine [Internet]. 2016; 5(2).
- Park MK, Li WQ, Qureshi AA, Cho E. Fat Intake and Risk of Skin Cancer in U.S. Adults. Cancer Epidemiol Biomarkers Prev. 2018;27(7):776-82.
- 29. Danciu C, Soica C, Antal D, Alexa E, Ghiulai R, Pavel I, et al. Natural Compounds in the Chemoprevention of Malignant Melanoma. Anti-Cancer Agents in Medicinal Chemistry. 2017;17.
- Muenyi CS, Ljungman M, States JC. Arsenic Disruption of DNA Damage Responses—Potential Role in Carcinogenesis and Chemotherapy. Biomolecules [Internet]. 2015; 5(4):[2184-93 pp.].

- Maiorino A, Simone C, Perino F, Caldarola G, Peris K. Melanoma and non-melanoma skin cancer in psoriatic patients treated with high-dose phototherapy. The Journal of dermatological treatment. 2016;27:1-5.
- Archier E, Devaux S, Castela E, Gallini A, Aubin F, Le Maitre M, et al. Carcinogenic risks of Psoralen UV-A therapy and Narrowband UV-B therapy in chronic plaque psoriasis: a systematic literature review. Journal of the European Academy of Dermatology and Venereology : JEADV. 2012;26 Suppl 3:22-31.
- Stenz NA, Stampf S, Arnold AW, Cozzio A, Dickenmann M, Gaide O, et al. Skin Cancer Development in Solid Organ Transplant Recipients in Switzerland (Swiss Transplant Cohort Study). Dermatology. 2021;237(6):970-80.
- Rizvi SMH, Aagnes B, Holdaas H, Gude E, Boberg KM, Bjørtuft Ø, et al. Long-term Change in the Risk of Skin Cancer After Organ Transplantation: A Population-Based Nationwide Cohort Study. JAMA Dermatology. 2017;153(12):1270-7.

LIST OF PUBLISHED SCIENTIFIC PAPERS

ISI articles with impact factor

1. **Cocoș, F.-I.**; Anuța, V.; Popa, L.; Ghica, M.V.; Nica, M.-A.; Mihăilă, M.; Fierăscu, R.C.; Trică, B.; Nicolae, C.A.; Dinu-Pîrvu, C.-E. Development and Evaluation of Docetaxel-Loaded Nanostructured Lipid Carriers for Skin Cancer Therapy. *Pharmaceutics* 2024, *16*, 960, https://doi.org/10.3390/pharmaceutics16070960, ISSN 1999-4923, **(IF=4,9/WoS).** https://www.mdpi.com/1999-4923/16/7/960 (Capitolul 5)
2. **Cocoș, F.-I.**; Prisada, R.-M.; Anuța, V.; Popa, L.; Ghica, M.V.; Fierăscu, R.C.; Nicolae, C.A.; Dinu-Pîrvu, C.-E. A Quality-by Design Approach of Docetaxel Transfersomal Formulations. *Farmacia* 2024, *5,* 1077, https://doi.org/10.31925/farmacia.2024.5.10 , **(IF=1,4/WoS).** (Capitolul 6)

ISI articles with impact factor developed during the doctoral studies– co-author

1. Nica, M.-A.; Anuța, V.; Nicolae, C.A.; Popa, L.; Ghica, M.V.; **Cocoș, F.-I.**; Dinu-Pîrvu, C.-E. Exploring the Potential of Deep Eutectic Solvents to Enhance Solubility of Poorly Water-Soluble Drugs. *Pharmaceuticals* 2024, 17(10), 1316, https://doi.org/10.3390/ph17101316, **(IF=4,3/WoS).** https://www.mdpi.com/1424-8247/17/10/1316

Book chapter in international paper

1. C.E. Dinu-Pîrvu, **F.I. Cocoș**, V. Anuta, M.V. Ghica, L. Popa, (**Capitolul 5**), 2022, p. 121-139, Plant secondary metabolites: natural compounds as cosmetic ingredients and their potential activity in skin cancer, In: Plant Secondary Metabolites. Physico-Chemical Properties and Therapeutic Applications, edyted by Anil Kumar Sharma, Ajay Sharma, *Springer*, *Singapore*, DOI: https://doi.org/10.1007/978-981-16-4779-6_5, ISBN 978-981-16-4778-9. https://link.springer.com/chapter/10.1007/978-981-16-4779-6_5?error=cookies_not_supported&code=9267f001-2ef2-41f6-b8fb-4a97f8d40f6b (Capitolul 1 și 2)

Papers Presented as Oral Communications and Posters at Various Scientific Events

1. **F.I. Cocoș**, C.E. Dinu-Pîrvu, V. Anuța, L. Popa, M.V. Ghica, M. Nica, Nanostructuri lipidice utilizate în tratamentul cancerului de piele, lucrare comunicare

orală prezentată în cadrul *Aniversării a 100 de ani de la înființarea Învățământului Universitar Farmaceutic autonom în România – Sărbătorirea Centenarului Facultății de Farmacie din București*, 12-15 iunie 2023, București, volum de rezumate, p. 85, ISSN 2457-3027.