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MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

ACTINIC KERATOSIS TREATMENT – FROM CURRENT GUIDELINES TO PROMISING DRUG DELIVERY SYSTEMS

Trabalho submetido por
Juliana Costa Rodrigues
para a obtenção do grau de Mestre em Ciências Farmacêuticas

Novembro de 2024

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À minha família e amigos pela constante motivação

Esta conquista também é vossa

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Abstract

Due to its high prevalence and ability to evolve into skin cancer, Actinic keratosis (AK) is one of the most frequent reasons that leads patients to the dermatologist. Due to his privileged position within the community, the pharmacist is an important player in promoting health literacy, therefore it is important to raise awareness on this specific disease and to have a detailed knowledge on the available treatments. The current AK treatment guidelines refer to lesion-directed destruction (destructive therapies) or/combined with field treatments, that regard topical drugs.

Topical drug delivery offers several advantages which favour patient compliance such as direct access for localized delivery to the disease site, absence of first-pass metabolism, fewer adverse side effects, non-invasive profile. For this reason, topical drugs such as 5-fluorouracil, imiquimod and ingenol mebutate are widely used in AK management. There are however disadvantages concerning the topical route, namely its limited efficacy in removing all lesions in a short period of time. Therefore, there is the need to develop new topical medicines able to surpass these limitations.

There are currently 158 clinical trials registered in the clinicaltrial.gov database investigating new topical drug products for AK treatment. Furthermore, there are also numerous academic studies that present novel drug delivery systems, such as liposomes, nanoparticles, polymeric micelles, among others, which may be useful in AK. These systems are design to improve drug stability, enhance skin penetration, provide controlled release and increase the drug concentration in the target site.

Taking this background into account, the aim of this monograph is to conduct a review of the available treatments for AK. To achieve so, besides addressing the drugs mentioned in the AK management guidelines, this work will also focus on the drugs being currently tested in clinical trials, as well as on promising drug delivery systems.

Keywords: Actinic keratosis, topical drugs, clinical trials, drug delivery systems.

Resumo

Devido à sua elevada prevalência e capacidade de evoluir para cancro de pele, a queratose actínica (QA) é uma das razões mais frequentes de consulta dermatológica. Na sua posição privilegiada, o farmacêutico desempenha um papel importante na promoção da literacia em saúde, sendo crucial sensibilizar para esta patologia e dominar os tratamentos disponíveis. As diretrizes atuais para a QA referem a destruição direcionada da lesão (terapias destrutivas) e/ou combinada com tratamentos de campo, que envolvem medicamentos tópicos.

A administração tópica apresenta vantagens que favorecem a adesão do paciente, como o tratamento direto na lesão, ausência de metabolismo de primeira passagem, menos efeitos adversos e perfil não invasivo. Por esta razão, medicamentos tópicos como 5-fluorouracilo, imiquimode e mebutato de ingenol são amplamente utilizados no tratamento da QA. No entanto, existem desvantagens relacionadas à via tópica, nomeadamente a sua eficácia limitada na remoção de todas as lesões num curto período de tempo. Portanto, há a necessidade de desenvolver novos medicamentos tópicos capazes de superar estas limitações.

Atualmente, existem 158 ensaios clínicos registados na base de dados clinicaltrials.gov a investigar novos produtos de medicamentos tópicos para o tratamento da QA. Além disso, existem também numerosos estudos académicos que apresentam novos sistemas de administração de medicamentos, como lipossomas, nanopartículas, micelas poliméricas, entre outros, que podem ser úteis na QA. Estes sistemas são concebidos para melhorar a estabilidade do medicamento, aumentar a penetração na pele, fornecer libertação controlada e aumentar a concentração do medicamento no local alvo.

Tendo em conta este contexto, o objetivo desta monografia é realizar uma revisão dos tratamentos disponíveis para a QA. Para tal, além de abordar os medicamentos mencionados nas diretrizes de tratamento da QA, este trabalho também se concentrará nos medicamentos que estão atualmente em teste em ensaios clínicos, bem como em sistemas promissores de administração de medicamentos.

Palavras-chave: Queratose actínica, medicamentos tópicos, ensaios clínicos, sistemas de vectorização de fármacos

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List of abbreviations

5-ALA	5-Aminolevulinic acid
5-FU	5-fluorouracil
AFXL	Ablative fractional laser resurfacing
AK	Actinic keratosis
ALA	Aminolevulinic acid
API	Active Pharmaceutical Ingredient
BCC	Basal cell carcinoma
BMIMBr	1-Butyl-3-methylimidazolium bromide
CO2	Carbon dioxide
DIA	Drug-in-adhesive
EGFR	Epidermal growth factor receptor
EPR	Enhanced permeability and retention
Gd	Gadolinium
GnRs	Gold nanorods
GUV	Giant unilamellar vesicles
HPMC	Hydroxypropyl methylcellulose
HPV	Human papilloma virus
HSV	Herpes simplex virus
ILV	Intraluminal vesicles
IMQ	Imiquimod
iSCC	Invasive squamous cell carcinoma
KC	Keratinocyte carcinomas
KIN	Keratinocyte intraepidermal neoplasia
LA	Laser ablation
LUV	Large unilamellar vesicles
MLV	Multiamellar vesicles
Mn	Manganese
MVB	Multivesicular bodies
NEs	Nanoemulsions
NLC	Nanostructured lipid carriers
NMSC	Non-melanoma skin cancer
NP	Nanoparticles

NSAID	Nonsteroidal anti-inflammatory drug
OLV	Oligolamellar vesicles
PDT	Photodynamic therapy
QA	Queratose actínica
RCTs	Randomized controlled trials
ROS	Reactive oxygen species
SC	Stratum corneum
SCC	Squamous cell carcinoma
SLN	Solid lipid nanoparticles
SUV	Unilamellar vesicles
TLR-7	Toll-like receptor 7
UV	Ultraviolet

I. Introduction

1. Actinic Keratosis

Actinic keratosis (AK) represents the earliest manifestation of non-melanoma skin cancer. The primary difference between non-melanoma skin cancer (NMSC) and melanoma skin cancer lies in the type of cells they originate from, being that non-melanoma skin cancer develops on skin that's exposed to the sun and this type of cancer has a high cure rate. On the other hand, non-melanoma skin cancer arises from basal cells, a type of keratinocyte found at the bottom of the epidermis, which leads to the development of basal cell carcinoma (BCC). Furthermore, non-melanoma skin cancer may also develop from squamous cells, which regard keratinocytes in the epidermis. In this situation, the patient will develop instead, squamous cell carcinoma (SCC). Regarding melanoma skin cancers, these occur when abnormal cells in the skin start to grow and divide in an uncontrolled way. These changes normally manifest in melanocytes, the cells responsible for melanin production, as depicted in **Figure 1** (Cancer Research UK, 2022, 2024).

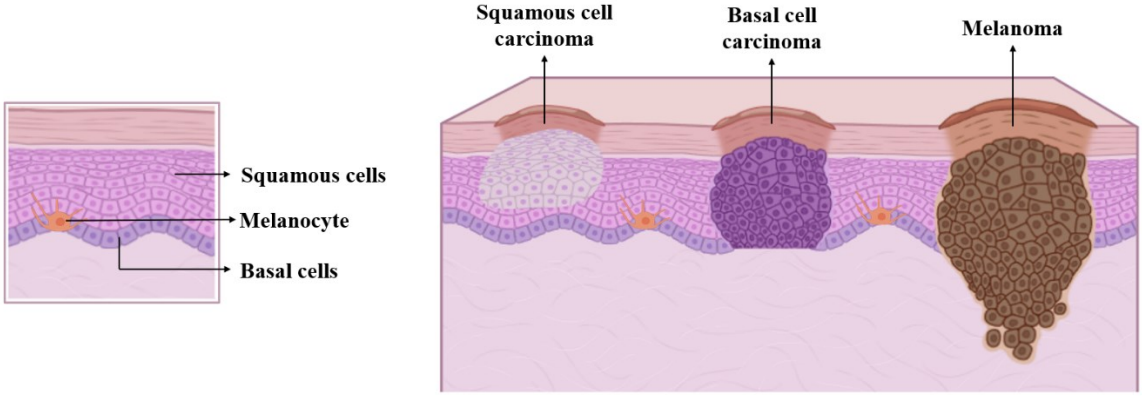

Figure 1 – Types of skin cancer. Adapted from (NCCN, 2021)

AK is characterized by rough, scaly patches on the skin, being the main etiological factor of the disease the exposure to prolonged ultraviolet (UV) radiation. The lesions

may present various sizes and display a sandpaper-like texture, which is often more easily felt than seen. For these reasons, the diagnosis may be challenging (Casari et al., 2018).

The most common anatomical areas where AK lesions appear are the face, ears, neck, scalp, chest, backs of hands, forearms or lips. Patients are usually asymptomatic, but some may complain about itching, burning, or a splinter-like sensation in the affected skin area (Casari et al., 2018).

AK lesions are problematic because of their potential to progress into invasive squamous cell carcinoma (iSCC), which is a very concerning health issue. As the main etiological factor of AK concerns UV prolonged light exposure and since the disease often manifests in older individuals, AK represents a serious public health concern in countries like Portugal (Casari et al., 2018). Therefore, a timely diagnosis coupled with the prescription of an adequate treatment is crucial to ensure the patient´s quality of life.

In the following sections, we will discuss the prevalence of actinic keratosis, its causes, associated symptoms, and available treatment options. Understanding these aspects is crucial to acknowledge the different types of drug delivery systems and where they act.

1.1. AK prevalence

The ethnicity and geographical location are key factors that affect the risk of developing AK. Populations with fair skin are more susceptible to the carcinogenic effects of UV radiation, and for that reason, these populations have a higher risk to develop AK. The geographical location can translate the rate of UV radiation that a given population is exposed to and may even modify the prevalence rates (Reinehr & Bakos, 2019). As a result, the prevalence of AK is notably higher in Australia, followed by the United States and then Europe (Reinehr & Bakos, 2019). Whereas in the United States, the frequency in Caucasians over 30 varies from 11.5% to 26%, in Australia, it falls between 40% and 60% in this age group. In some European countries such as England, the prevalence in people over 40 years old is 15.4% in men and 5.9% in women, increasing with age showing a prevalence of 34.1% in men and 18.2% in women in patients over 70 years old. Countries like Spain and Austria also share the same pattern with the prevalence being higher in men, with the tendency to increase with age for both sexes (Reinehr & Bakos, 2019).

On other hand, AK in Asian countries is much lower when comparing to the rest of the globe. In South Korea the prevalence is 0.09% for people over 60 years old and in China there is a 0.52% prevalence of the disease, with a mean age of 69.8 ± 11.8 years (Reinehr & Bakos, 2019).

Finally, in South America more specifically in Brazil, AK represents the fourth most common dermatological diagnosis, and notably, the prevalence is higher in women (60.79%) than in men (30.9%) (Reinehr & Bakos, 2019). This difference may be ascribed to the lifestyle of Brazilian women, which favour outdoors activities, and thus prolonged sun exposure. These numbers further highlight the need to raise awareness on actinic keratosis in Brazil (Sociedade Brasileira de Dermatologia, 2006).

Figure 2 illustrates the incidence of non-melanoma skin cancer according to the continent in 2022.

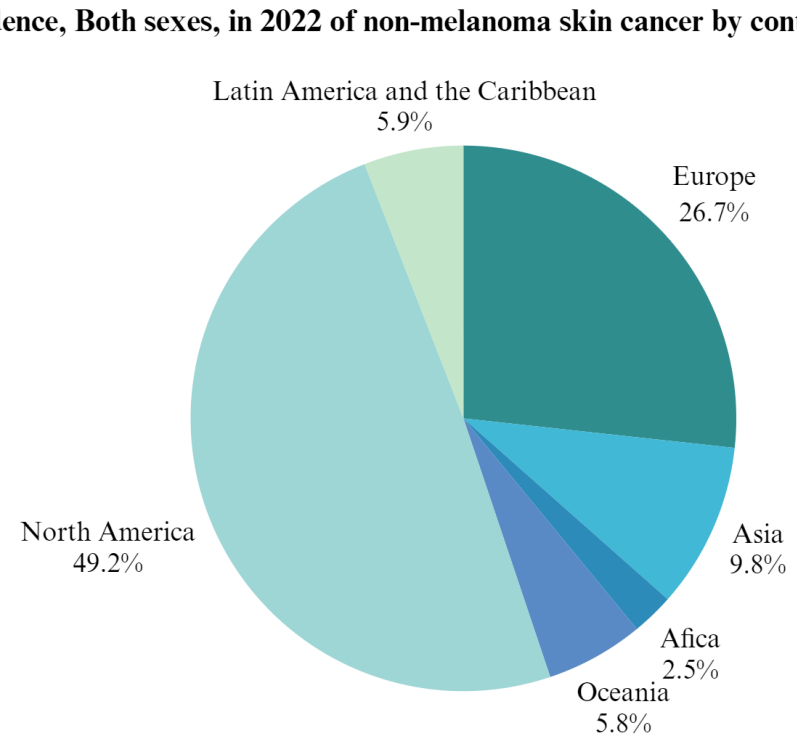

Figure 2 – Incidence of non-melanoma skin cancer in 2022 according to the Global Cancer Observatory, adapted from (International Agency for Research on Cancer, 2022a)

Figure 3 shows that there is a remarkably higher NMSC incidence and mortality in males compared to women. According to the Global Cancer Observatory, the incidence

of this cancer was 0.75 million cases diagnosed in men, which ultimately led to 0.04 million deaths. In 28 years, these numbers are expected to increase to 1.4 million men diagnosed with the disease and 0.09 million deaths.

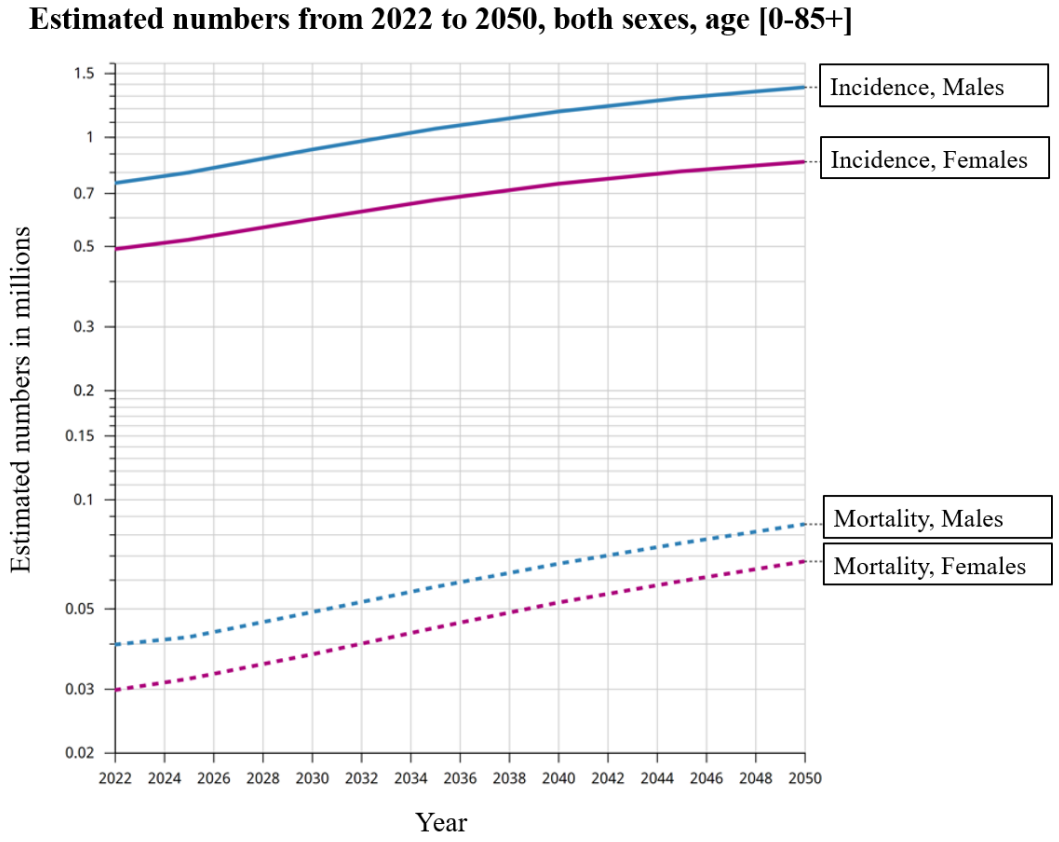

Figure 3 – Estimated evolution of non-melanoma skin cancer, according to the Global Cancer Observatory (International Agency for Research on Cancer, 2022b)

Even though these numbers report to NMSC, they are extremely important to understand the epidemiology of AK, as this condition is considered a precursor to squamous cell carcinoma, one of the main types of NMSC. By exploring the prevalence of NMSC, researchers can gain insights into how AK lesions develop and transform into more invasive cancers.

In Caucasian patients in ages of 20-29% the prevalence is <10%, however in patients aged 60-69 years the prevalence may rise to 80%.

There are special populations in which the appearance of AK lesions may be highly dangerous due to its high potential to progress into non-melanoma skin cancer. These include albinos and individuals carrying mutations in DNA repair genes, for example,

xeroderma pigmentosum, Rothmund-Thompson syndrome, Cockayne's syndrome and Bloom's syndrome. In these patients. the lesions may manifest sooner, more specifically in the first 10 years of life (Reinehr & Bakos, 2019).

Patients who have undergone organ transplants exhibit a notably higher risk of developing actinic keratosis (AK), with prevalence rates rising alongside the duration of immunosuppression, these patients have a 40–60% of developing AK after 20 years (German Guideline Program in Oncology (German Cancer Society, 2022). In fact, in predominantly white populations, skin cancer, more specifically, SCC is the most common malignancy observed after transplantation (Berg & Otley, 2002).

Figure 4 summarizes the main factors affecting the prevalence of AK.

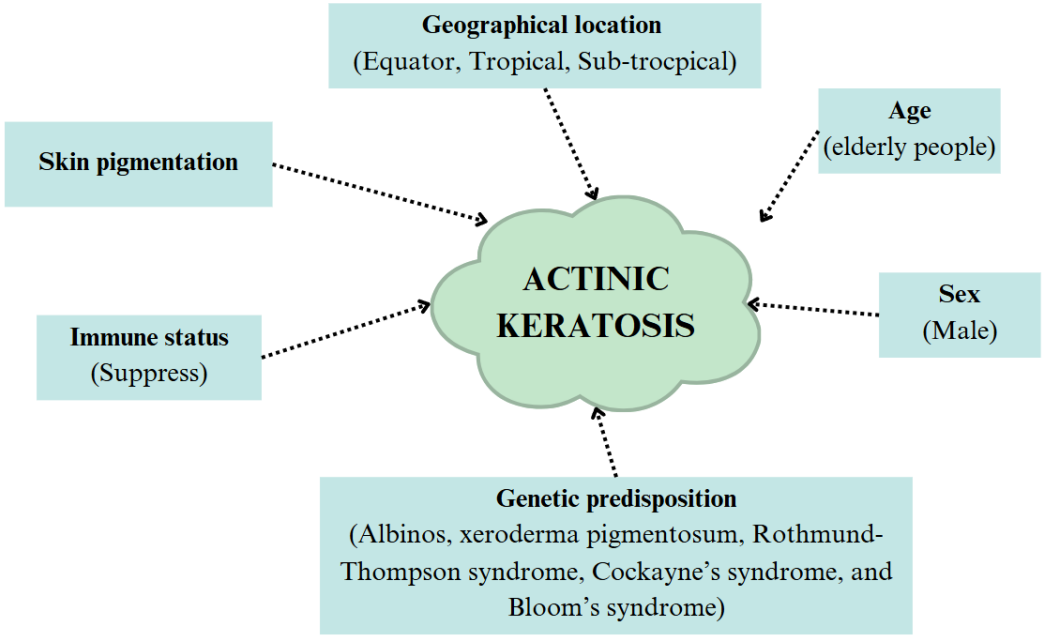

Figure 4 – Factors affecting AK prevalence. Adapted from (Sharma et al., 2019).

1.2. AK Etiology and pathophysiology

Ultraviolet (UV) radiation is the main etiological factor supporting AK development, due to its ability to penetrate the skin and directly damaging the DNA in epidermal keratinocytes, thereby reducing the skin's immunity (Costa et al., 2015).

Figure 5 shows the pathophysiological mechanisms involved in AK.

UV-A radiation (320-400 nm) enters the skin more deeply than UV-B. As a result, there is nucleic acids oxidative damage, being Thymine (T) — guanine (G) the signature mutation of this type of radiation. UV-A radiation is also responsible for membrane lipids and cell proteins impairment. These will ultimately be responsible for the formation off reactive oxygen species (ROS), that alter cell proliferation, due to their impact on transduction pathways, as well as cell-cell signalling (Costa et al., 2015). On the other hand, UV-B (290-320 nm) radiation is responsible for cytosine (C) - T and CC -TT mutations. Further, p53 (a tumour suppressor protein) inactivation by UV-B light, regards a crucial step in AKs development, since it is responsible for the appearance of genetically unstable keratinocytes (Costa et al., 2015). In fact, p53 mutations have been reported in 90% of human cutaneous SCCs. Additionally, UV light exposure increases the production of arachidonic acid and its metabolites, as well as other pro-inflammatory cytokines (Costa et al., 2015).

AK development involves mechanisms such as inflammation, oxidative stress, immunosuppression, impaired apoptosis, mutagenesis, cell growth dysregulation and proliferation, as well as tissue remodelling (Costa et al., 2015). These processes not only trigger AK lesion formation but also allow the disease to either regress or progress into SCC, leading to its classification as a premalignant condition. Due to this potential for malignancy, recent publications argue that AK should be reclassified as a "cancerous" condition (Costa et al., 2015).

Lately, cutaneous human papilloma virus (HPV) infection has also been related to AK development. Even though the mechanism is not totally understood, it is thought that the E6 protein, produced by the virus, appears to contribute to the reduction of Bak protein levels. Bak proteins are responsible for promoting apoptosis, especially in cells damaged by UV light. Taking this information into account, HPV increases the risk of AK by reducing the levels of Bak protein, allowing UV-damaged cells to survive and replicate (Costa et al., 2015).

AK and early SCC are very difficult to distinguish, since they share several cellular and histopathological features. Usually, both conditions are distinguished based on the relative depth of dysplastic cells within the skin. AK lesions are usually located in the basal layer, where keratinocytes are formed, whilst SCC lesions tend to extend beyond

the epidermis, through the basement membrane, and into the dermis. However, SCC and AK are indistinguishable at a cellular level (Costa et al., 2015).

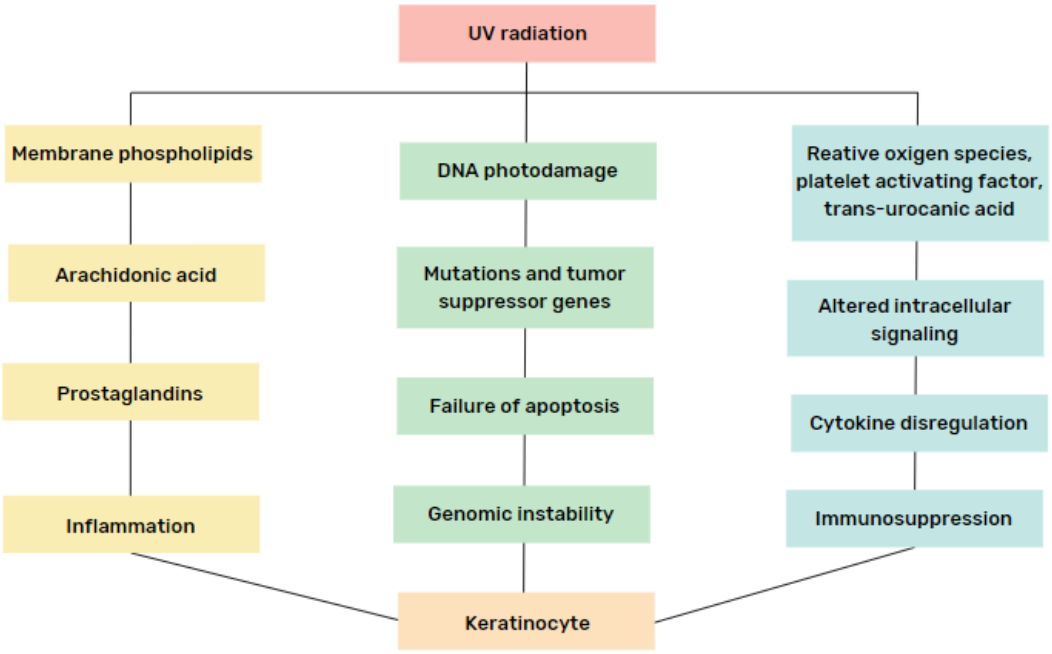

Figure 5 – Pathophysiological mechanisms involved in AK. Adapted from (Reinehr & Bakos, 2019).

1.3. AK Clinical Features

As previously mentioned, AK lesions regard erythematous, scaly plaques that occur on sun damaged skin, being a result of UV radiation exposure. Usually, the lesions manifest in sun-exposed areas (face, dorsum of the hands, shoulders, and in scalp in men with premature baldness (Rossi et al., 2007). Both single and multiple lesions are slow growing, small (1-3 mm) dry and flesh-coloured (Costa et al., 2015). The lesions may also present as erythematous papules to large plaques, with telangiectasias, often covered by yellow or brown adherent scales, and, except in their hypertrophic form, show little or no infiltration (Rossi et al., 2007). Occasionally, the lesions may show marked hyperkeratosis and may be similar to cutaneous horns(Rossi et al., 2007).

There are three main stages of AK, **Figure 6** however its diagnosis maybe subject to variable clinical interpretation (Casari et al., 2018). Briefly these are:

Stage I: Slightly palpable AKs (better felt than seen);

Stage II: Moderately thick AKs (easily felt and seen);

Stage III: Very dense, hyperkeratotic and more evident lesions. This stage resembles an early invasive SCC (Casari et al., 2018).

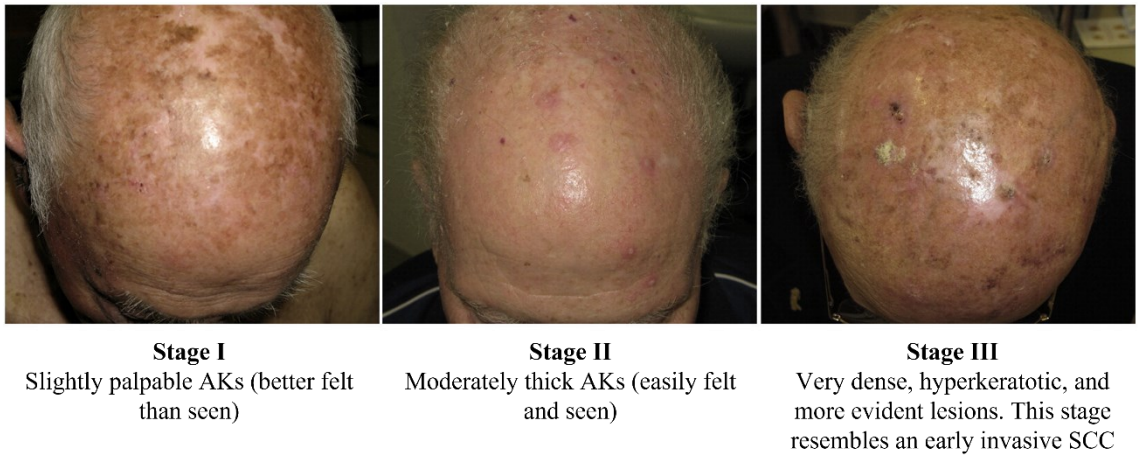

Figure 6 - Clinical grading of actinic keratosis. Adapted from (Zalaudek et al., 2014).

1.4. Objectives

AK is a prevalent disease in southern European countries because of the intense sun exposure, elderly population, together with the low phototype of the general population. Due to the potential of this condition to evolve into non-melanoma skin cancer, it is extremely important to educate the population on this disease, specifically in what concerns preventive measures, as well as on the available treatments.

Independently of their work area, the pharmacist should have a comprehensive insight on AK. In community pharmacies, the pharmacist has direct and frequent contact with the population, and because so the pharmacist is in a privileged position to guide and educate the population on AK risks. More specifically, to advise on the importance of avoiding prolonged sun exposure, which, has explained in the introduction chapter, is the main risk factor for AK development. The pharmacist is also in a privileged position to educate on the importance of using sunscreens on a daily basis, as well the importance of limiting sun exposure during the peak sun hours. Furthermore, even though the pharmacist is not responsible for AK diagnosis, he can play an essential role in referring the patient to a dermatologist for a formal clinical evaluation, if suspicious spots or lesions are identified. On the other hand, if the patient is diagnosed with AK, the pharmacist

should be able to guide and explain the treatment. On the other hand, the hospital pharmacist should also have an updated knowledge on this condition, as AK represents one of the most common reasons why patients visit the dermatologist. In this context, the hospital pharmacist should be aware of the current treatment guidelines, available treatments, as well as ongoing clinical studies. Finally, from a pharmaceutical industry/academia perspective, due to the high prevalence of this disease, it is important to continue to develop new drug delivery systems, able to increase the therapeutic potential of available drugs, but at the same time not compromising their safety profile.

AK treatment guidelines are divided into destructive therapies, also referred to as focal therapies, and field therapies. Destructive therapies regard lesion-directed destruction techniques, such as cryotherapy, curettage, phtotodynamic therapy and laser resurfacing, whilst field therapies are intended to eliminate lesions present in a field of cancerization, and usually comprehend topical drugs (Fleming et al., 2017).

Topical formulations exhibit good spreadability and bioadhesion characteristics due to their rheological profile. These physical attributes stimulate patient compliance to treatment, which is important as the patient has to apply the formulation directly to where the disease is visible. Furthermore, the topical route is non-invasive and has a lower side effect profile, due to its localized action (Miranda et al., 2024). As destructive therapies are invasive and display a higher predisposal to infections, pain, scarring, as well as skin dyspigmentation issues, topical drugs maybe of extreme utility for stimulating patient compliance to AK treatment.

Taking this background into account, firstly this monograph intends to review AK treatment guidelines. Then, due to the referred advantages concerning the dermal route, this work intends to present an overview on the topical AK treatments currently being tested in clinical trials, as well as to review promising topical drug delivery systems targeting this specific condition.

1.5. Methods

The study was conducted via bibliographical research which involved looking up information in several databases, including PubMed and Web of Science, using the terms actinic keratosis, drug delivery systems and drugs. The papers with publication dates ranging from 2018 to the present were considered. However, when relevant, older articles were also accounted for.

Ongoing and finished clinical trials for AK treatment were also considered. These were searched using the ClinicalTrials.gov, database. The clinical trials were identified by searching "actinic keratosis". This search yielded 480 clinical trials, where 158 regarded topical drugs. The remaining 322 studies regarded focal therapies or oral therapy. In the present work, solely the studies that regarded topical drugs were considered.

II. Development

2. Approved AK treatments

AK treatment can be divided into focal therapies and field therapies. The focal therapies target single lesions that can be identified, whilst the field therapies eliminate lesions present in a field of cancerization (Fleming et al., 2017).

AK treatment primarily consists of lesion-directed destruction (focal therapies) or/combined with field treatments, that regard topical drugs. The main aim of the treatment is to prevent the AK lesions progression into keratinocyte carcinomas (KC), such as squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). Furthermore, the treatment also seeks to improve the lesion appearance and to relieve the symptoms (Siegel et al., 2017).

In the following sections the most relevant pharmacotherapeutic regimens available for AK will be summarized.

2.1. Destructive therapies (Focal therapies)

2.1.1. Cryotherapy

Cryotherapy with liquid nitrogen, is the most common procedure to eliminate isolated AKs, in Canadian guidelines. However, UK guidelines suggest extensive cryosurgery (‘cryopeeling’) as a field treatment, this contrasts with European and Canadian guidelines that show low support for its extensive use (Fleming et al., 2017). The local adverse effects of this therapy regard in short term pain, edema, blistering, infection and pyogenic granuloma (rare). As predominant adverse effects related with long term exposure, the most common are nerve damage, as well as pigment changes (Siegel et al., 2017).

2.1.2. Curettage

Curettage is a surgical method that can be used as a diagnostic technique or as a treatment. It involves taking a small sample of tissue, which an the be used to perform a biopsy, or alternatively to simply remove a superficial skin lesion, such as a benign growth, warts, as well as some skin cancers (British Association of Dermatologists, 2024). Curettage is used for hypertrophic AKs to debride lesions before taking other

therapy, but according to the guidelines curettage is mainly used as a diagnostic tool (Fleming et al., 2017).

Local adverse effects include scars, infection, vital structure damage (rare). Systemic adverse effects may regard reactivation and local spread of herpes simplex virus (HSV) (Siegel et al., 2017).

2.1.3. PDT (Photodynamic therapy)

PDT is used to treat AKs lesions that are superficial and diffuse, or for lesions located at sites of poor healing. FDA approved PDT for lesion-directed treatment, however this technique is also used as field therapy, off-label (Siegel et al., 2017).

In Canadian, European and British guidelines, more sessions of PDT are recommended for thicker AKs, or AKs are pre-treated with curettage to remove hyperkeratotic tissue, before the application of the photosensitizing agents (Fleming et al., 2017).

There are two PDT photosensitizing agents: (i) aminolevulinic acid cream with blue light and (ii) methyl-aminolevulinate cream with red light (Costa et al., 2015). According to a study performed by (Moloney & Collins, 2007), the patients found the application of methyl-aminolevulinate less painful during and after light treatment, when compared to aminolevulinic acid, however both creams were equally effective in the reduction of AK lesions (Fleming et al., 2017).

PDT has a similar clearance rate to cryotherapy (69% PDT vs 75% cryotherapy), though cryotherapy is generally preferred for thicker lesions. However, PDT is rated as having a superior cosmetic outcome compared to cryotherapy. Nowadays, PDT concerns the first line treatment for patients with multiple AKs, according to the majority of the guidelines (Fleming et al., 2017).

Local adverse effects of this therapy include burning or stinging during light exposure, as well as pigmentary changes (Siegel et al., 2017).

2.1.4. Laser resurfacing

Laser resurfacing is a procedure that uses targeted laser energy to remove the outer, damaged layers of skin, where AK lesions form. This laser uses either carbon dioxide

(CO_2) or erbium: yttrium aluminium garnet (Er:YAG). CO_2 laser is usually preferred because it is normally less painful and allows for a faster wound healing. However, it should be referred that pain levels depended on the laser parameters and depth of treatment, and not on the laser modality itself (Fleming et al., 2017).

According to Canadian and European guidelines, laser resurfacing should be used for areas with clustered AKs lesions, suggesting multiple applications as needed. Additionally, the Canadian guidelines highlight laser resurfacing as an option for organ transplant patients (Fleming et al., 2017).

Another trial using ablative fractional laser resurfacing (AFXL), demonstrated enhanced methyl-aminolevulinate uptake and improved PDT efficacy, with an 88% lesion response rate for AFXL-PDT versus 59% observed with the conventional PDT. However, this technique is associated with pain and higher frequent pigmentary changes (Fleming et al., 2017).

Local adverse effects of this therapy include persistent erythema, dyspigmentation, infections, as well as scarring (Siegel et al., 2017).

Table 1 summarizes the focal therapies mentioned above used to treat AK.

Table 1 – Summary of the destructive therapies used for actinic keratosis treatment.

Therapy	Main features	Local Adverse Effects	Systemic Adverse Effects	Effectiveness	Guidelines	Ref.
Cryotherapy	Application of liquid nitrogen. Commonly used for isolated AKs, as well as for thicker lesions.	Short term: Pain, edema, blistering, infection, pyogenic granuloma (rare). Long term: Nerve damage, pigment changes. Cosmetic outcome rated lower than PDT	None	Similar clearance rate to PDT (75% for cryotherapy vs 69% for PDT).	Widely used in Canadian guidelines; UK guidelines suggest a broader application (cryopeeling).	(Fleming et al., 2017; Siegel et al., 2017)
Curettage	Used for hypertrophic AKs, primarily for diagnosis or to debride lesions before other treatments.	Scars, infection, rare vital structure damage.	HSV reactivation, local spread.	Effective for hyperkeratotic lesions	Primarily diagnostic tool	(British Association of Dermatologists, 2024; Fleming et al., 2017; Siegel et al., 2017)
PDT (Photodynamic Therapy)	Uses photosensitizing agents (aminolevulinic acid with blue light or methyl-aminolevulinate with red light) for superficial, diffuse	Burning/stinging during light exposure, pigment changes.	None	Response rates: 59.2%-82% (for 2 cycles); less painful with methyl-aminolevulinate;.	First-line treatment for multiple AKs. FDA-approved for lesion treatment, off-label for field therapy.	(Fleming et al., 2017; Siegel et al., 2017)

Therapy	Main features	Local Adverse Effects	Systemic Adverse Effects	Effectiveness	Guidelines	Ref.
	AKs or in sites of poor healing. Cosmetic outcome rated higher than cryotherapy PDT combined with laser is more effective.					
Laser Resurfacing	Uses CO_2 or Er Lasers. CO_2 allows for less pain and for a faster healing. Laser resurfacing combined with PDT is superior to either alone.	Persistent erythema, dyspigmentation, infections, scarring.	None	Effective when combined with PDT (88% response compared to 59% post-PDT).	Canadian/European guidelines recommend laser resurfacing for clustered AKs or for organ transplant patients.	(Fleming et al., 2017; Siegel et al., 2017)

2.2. Field Therapies

2.2.1. 5-FU cream

5-fluorouracil (5-FU) is an antineoplastic drug commonly used as a topical, field-directed treatment for AK. This drug irreversibly inactivates the thymidylate synthase through the direct interference with DNA synthesis. As a result, the high proliferative AK keratinocytes undergo apoptosis (Briatico et al., 2023). In AK, 5-FU is highly useful due to its erosive characteristics.

In Canadian guidelines, the use of 5% 5-FU cream twice daily for up to 4 weeks is recommended for AK treatment. On the other hand, in UK guidelines, 3 weeks of treatment are recommended instead. In European guidelines, the use of 0.5% 5-FU for once-daily application is recommended for a period ranging 1 to 4 weeks. However, 5% 5-FU is also described for once- or twice-daily application over 2 to 4 weeks (Fleming et al., 2017).

In Portugal, topical 5-FU is only commercialized in 2 different drug products: **Efflurak** which regards a 40 mg/g 5-FU cream, and **Actikerall**, a solution that combines 100 mg salicylic acid and 5 mg 5-FU (Infarmed, 2020, 2024).

A systematic review of 13 RCTs (n = 864) examining the efficacy of 5-FU, showed that the patients exhibited an 80% reduction in lesion count, and that about half of the patients displayed a complete lesion clearance. However, this review also highlighted that the quality of evidence was poor since there were limited studies comparing 5-FU with other topical drugs (Askew et al., 2009).

A different study conducted by Gupta and collaborators (2005), compared the efficacy of imiquimod 5% cream with 5-fluorouracil, by means of a cumulative meta-analysis, for the treatment of AK lesions in the scalp and face. The authors concluded that both drugs are effective and regard useful alternatives to destructive therapies, such as cryotherapy. However, imiquimod maybe a more effective treatment (Gupta et al., 2005)

Local adverse effects of topical 5-FU include erythema, inflammation, erosions, pain, pruritus, photosensitivity and burning. Systemic adverse effects regard headaches, insomnia, irritability, stomatitis, leucocytosis, thrombocytopenia, birth defects, herpes simplex reactivation, miscarriage, neutropenia, neurotoxicity, gastrointestinal toxicity. Systemic toxicity is mostly seen in patients with dihydropyrimidine dehydrogenase

deficiency (Siegel et al., 2017) and this toxicity can be intensified when the drug is applied into compromised skin e.g. cuts. In this case, the patients should be monitored for signs and symptoms of systemic toxicity. If any, the treatment should be interrupted (Infarmed, 2024). This therapeutic regimen should not be applied to patients with melasma or acne rosacea (Siegel et al., 2017).

2.2.2. Imiquimod cream

Topical imiquimod is used in AK treatment, due to its role as a toll-like receptor 7 (TLR-7) agonist. Imiquimod induces cytokines, in order to start an inflammatory skin reaction directed towards malignant or virus-infected cells, stimulating their apoptosis. Imiquimod has however no effect on normal skin (Kopera, 2020).

Imiquimod is used in two different dosages, 5% and 3,75%, in Canada and United Kingdom, respectively. Imiquimod 5% has a dosing cycle of 3 times a week over a 4-week treatment cycle, and the 3.75% formulation is used daily over two 2-week cycles separated by a 2-week rest (Fleming et al., 2017). On the other hand, European guidelines present three dosages of imiquimod: 2.5%, 3.75% and 5%. The 2.5% and 3.75% formulations are applied once daily in two 2-week cycles, with a 2-week break between cycles. The 5% dosage is applied 2 to 3 times per week over a period of 4 to 16 weeks (Fleming et al., 2017). In Portugal, according to the Infomed database, the marketed dosages are 3.75% and 5% cream.

A randomized trial was performed in 479 patients to assess the efficacy of imiquimod 2.5% and 3.75% compared to placebo. The results showed that imiquimod was more effective than the placebo, since complete and partial clearance rates for the placebo were 5.5% and 12.8%, for imiquimod 2.5% the clearance rates were 25.0% and 42.7%, and finally for imiquimod 3.75%, 34.0% and 53.7% clearance rates. Additionally, similar results were observed in 5 clinical trials studying 5% imiquimod, with complete clearance rates between 26.8% to 57.1% and partial clearance from 36.6% to 72.1%, over a treatment periods of 3 to 16 weeks. However, these studies also showed that applying imiquimod more than 3 times per week was poorly tolerated by the patients (Fleming et al., 2017).

Local adverse effects of this drug include erythema, whilst systemic adverse effects may present as upper respiratory tract infection, influenza-like symptoms or, predisposition to HSV (Siegel et al., 2017).

2.2.3. Ingenol mebutate gel

Ingenol mebutate is a macrocyclic diterpene ester that can be found in the sap of the *Euphorbia peplus* plant, used as a traditional treatment for common skin lesions, including malignant lesions (Lebwohl et al., 2012).

According to preclinical research, ingenol mebutate is a pleiotropic effector that triggers immunological responses and quick and direct cell death through particular activation of protein kinase C delta. These immune responses include neutrophil-mediated oxidative burst and tumor clearance (Lebwohl et al., 2012).

According to EMA, studies *in vivo* and *in vitro* have demonstrated a dual mechanism: 1) induction of cell death in the local lesion, and 2) promotion of an inflammatory response characterized by the local production of pro-inflammatory cytokines and chemokines, as well as infiltration of immunocompetent cells (European Medicines Agency, 2017)

This compound is used for many dermatological treatments, such as in AK lesions (Brooke Fidler, 2024).

EMA reviewed the use of Picato for AK, the assessment concluded that the risks of this product, including a potential link to skin cancer, outweigh its benefits. The decision was based on data from clinical studies and pharmacovigilance reports, which indicated a higher incidence of skin malignancies in patients treated with Picato compared to other therapies. Consequently, the EMA recommended the suspension of Picato's marketing authorization in the European Union (European Medicines Agency (EMA), 2020).

The 0.015% ingenol mebutate gel is recommended for use on the face or scalp, with a once-daily application over 3 days. For the trunk and extremities, a 0.05% gel formulation should be used instead, once daily over 2 days. Both therapies are referred in Canadian, UK and European guidelines (Fleming et al., 2017). In three studies, partial clearance rates ranged from 49.1% to 75.4%, with complete clearance between 42.2% and 71% over the 2- to 3-day treatment period (Fleming et al., 2017).

Lebwohl et al. (2004) conducted two phase III trials on imiquimod 5% cream for actinic keratosis, enrolling 436 patients. The complete clearance rate was 45.1% for the imiquimod group and 3.2% for the vehicle group.(Lebwohl et al., 2004). Thus, highlighting the efficacy profile of this API.

Siller et al. (2009) studied PEP005 (ingenol mebutate) gel in a phase IIa trial, involving 58 patients with AK. The results showed a complete clinical clearance of 71% of treated lesions ($P < 0.0001$ vs. vehicle gel) (Siller et al., 2009)

In a phase II experiment, Anderson et al. (2009) examined two different strengths of ingenol mebutate gel (0.025 and 0.05%) with a vehicle gel on 222 patients. Compared to the vehicle gel, which had a partial clearance rate of 21.7%, patients treated with ingenol mebutate gel had substantial higher clearance rates in both strengths. (Anderson et al., 2009).

The short treatment period is a key advantage of ingenol mebutate gel over the other topical drugs, especially for patients who struggle to comply with the longer therapeutic regimens necessary for imiquimod and 5-FU. Furthermore, no drug interactions have been reported thus far with ingenol mebutate. This topical drug product also showed high clearance rates after cryosurgery, making it a recommended option for single and multiple AK lesions (Fleming et al., 2017).

Local adverse effects of this dug include pain, itching, irritation, infection, dose-related erythema, flaking/scaling/ dryness and scabbing/crusting. Systemic adverse effects include: Headache, periorbital edema, nasopharyngitis, conjunctivitis, eye pain, herpes zoster and severe hypersensitivity (rare) (Siegel et al., 2017).

2.2.4. Other topicals

Other topical treatments of AK mentioned in the guidelines are diclofenac, salicylic acid, as well as emollients.

UK and European guidelines discuss the use of 3% diclofenac in a 2.5% hyaluronic gel, twice-daily, over 60 to 90 days for treating mild AK lesions. One randomised trial found that 47% of the actively treated patients, improved when compared with the placebo group. Currently, this formulation is available in Portugal (Solaraze, 3%

diclofenac gel). This NSAID drug can be a useful strategy for an early treatment of AK, before resorting to more invasive methods (Fleming et al., 2017).

UK guidelines also recommend the use of emollients for mild AKs, although no trials on its use have been conducted. This, however, is not addressed in other guidelines, probably due to its use as a placebo, as well as the lack of effect on the known AK pathophysiologic processes (Fleming et al., 2017).

The treatment with salicylic acid was recommended prior to 5-FU treatment by both UK and European guidelines. The application of salicylic acid should be conducted once daily for a period of 6 to 12 weeks, to allow the removal of overlying keratin. However, there is limited evidence on the efficacy of this procedure: In a small randomized study (n = 66), using a formulation with both 0.5% 5-FU and 10% salicylic acid, produced a greater histologic clearance, fewer clinical lesion counts, and a lower recurrence rate than two cryotherapy treatments spaced three weeks apart; However, drug-related adverse events were more severe, as in the latter group (Fleming et al., 2017).

The local adverse effects of topical diclofenac include dry skin, pruritus, and erythema. The only systemic adverse effect, which is extremely rare, regards hepatotoxicity (Siegel et al., 2017). **Table 2** summarizes the field therapies mentioned above used to treat AK.

Table 2 - Summary of the actinic keratosis field therapies.

Active Pharmaceutical Ingredient (API)	Dosage Form	Drug dose Regimen	Action Mechanism	Adverse Local Reactions	Adverse Systemic Reactions	Efficacy	Ref.
5-Fluorouracil (5-FU)	Cream	5% - twice daily for 2-4 weeks; 0.5% - once daily for 1-4 weeks Europe only	Pyrimidine analogue that inactivates thymidylate synthase, inhibiting DNA synthesis and causing apoptosis.	Erythema, inflammation, erosions, pain, pruritus, burning, photosensitivity.	Headache, insomnia, irritability, leucocytosis, birth defects, neurotoxicity, gastrointestinal toxicity, systemic toxicity in DPD deficiency patients.	80% lesion count reduction; 50% of patients achieve complete clearance. Poor quality evidence and less effective than imiquimod (52.2% complete response for 5-FU vs. 70.8% for imiquimod).	(Askew et al., 2009; Briatico et al., 2023; Fleming et al., 2017; Gupta et al., 2005; Infarmed, 2020, 2024; Siegel et al., 2017)

Active Pharmaceutical Ingredient (API)	Dosage Form	Drug dose Regimen	Action Mechanism	Adverse Local Reactions	Adverse Systemic Reactions	Efficacy	Ref.
Imiquimod	Cream	5% (3 times a week for 4 weeks); 3.75% (daily, 2 cycles of 2 weeks with a 2-week rest); 2.5% (once daily for 2 weeks with a 2-week rest, Europe only)	TLR-7 agonist that induces cytokine production, stimulating an inflammatory response directed at malignant/infected cells.	Erythema.	Upper respiratory tract infection, influenza-like symptoms, herpes simplex virus reactivation.	Complete clearance: 26.8%-57.1%, Partial clearance: 36.6%-72.1% depending on regimen. More effective than placebo and 5-FU.	(Fleming et al., 2017; Siegel et al., 2017)
Ingenol mebutate	Gel	0.015% (face/scalp, once daily for 3 days); 0.05% (trunk/extremities, once daily for 2 days)	Rapidly induces cell death in AK lesions.	Pain, itching, irritation, erythema, flaking, scabbing, crusting.	Headache, nasopharyngitis, periorbital edema, conjunctivitis, herpes zoster, hypersensitivity (rare).	Complete clearance: 42.2%-71%, Partial clearance: 49.1%-75.4%. Shorter treatment period makes it suitable for patients with adherence challenges.	(Anderson et al., 2009; Brooke Fidler, 2024; European Medicines Agency, 2017; European

Active Pharmaceutical Ingredient (API)	Dosage Form	Drug dose Regimen	Action Mechanism	Adverse Local Reactions	Adverse Systemic Reactions	Efficacy	Ref.
							Medicines Agency (EMA), 2020; Fleming et al., 2017; Lebwohl et al., 2004, 2012; Siegel et al., 2017; Siller et al., 2009)
Diclofenac	Gel (in hyaluronic gel)	3% (twice daily for 60-90 days)	NSAID that acts through anti-inflammatory effects, non-destructive, used for early-stage AKs.	Dry skin, pruritus, erythema.	Rare hepatotoxicity.	47% of patients rated as completely improved. Non-invasive option, suitable for early treatment.	(Fleming et al., 2017)

Active Pharmaceutical Ingredient (API)	Dosage Form	Drug dose Regimen	Action Mechanism	Adverse Local Reactions	Adverse Systemic Reactions	Efficacy	Ref.
						Not available in Canada.	
Salicylic acid	Cream	Used in combination with 5-FU, 0.5% (salicylic acid in 10% 5-FU, daily for 6-12 weeks)	Removes overlying keratin to enhance the efficacy of 5-FU.	Local irritation and more severe adverse events compared to cryotherapy.		Greater histologic clearance, fewer lesion counts, and lower recurrence rates compared to cryotherapy (in combination with 5-FU).	(Fleming et al., 2017; Siegel et al., 2017)

3. Clinical trials for AK

This chapter will cover clinical trials on actinic keratosis. As previously referred, the ClinicalTrials.gov database was used to identify all the clinical studies on AK. Currently, 480 clinical trials were identified. From this pool of results, 158 clinical studies regarded the evaluation of topical drugs, whilst the remaining ones focus on destructive therapies or oral drugs. These results are depicted in **Figure 7.**

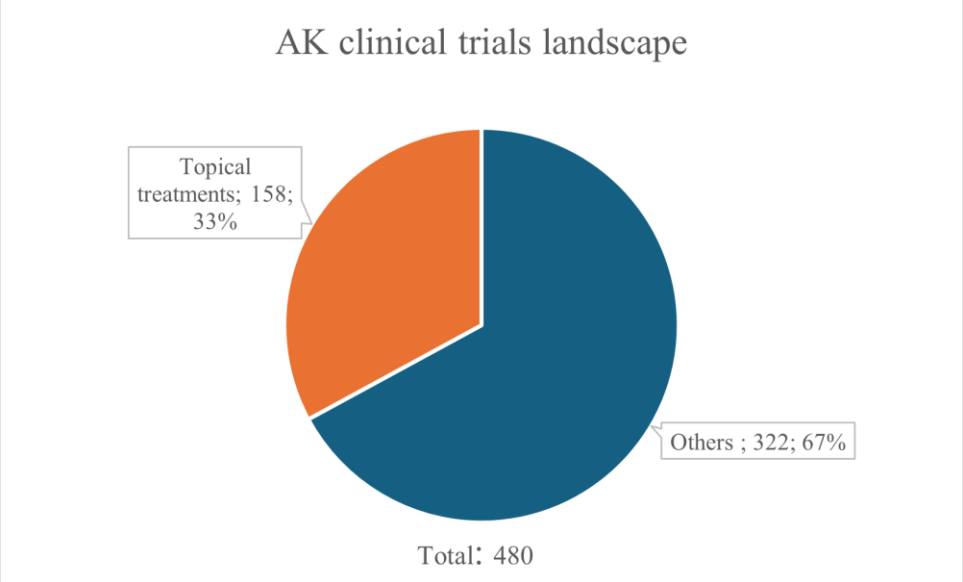

Figure 7 - Distribution of clinical trials about AK

Even though the majority of studies concerns the evolution of destructive therapies, the importance of the topical route in AK management is still relevant as illustrated by the ongoing 158 studies.

As previously referred in the introductory chapter, the topical delivery of drugs offers several advantages, when compared to other administration routes: (i) Direct access for localized delivery to the disease sit; (ii) The API does not undergo first-pass metabolism, or other variables related with the gastrointestinal tract; (iii) There are fewer side effects related with systemic toxicity and; (iv) This administration pathway is non-invasive, with ultimately favours patient compliance (Brown et al., 2024).

Figure 8 depicts the dosage forms currently being evaluated in the clinic. Please note that solely 144 studies were considered because in 14 studies the information provided by the clinicaltrials.gov database did not contemplate the dosage form tested.

Furthermore, there were also some studies that contemplated the evaluation of more than one API in distinctive dosage forms, therefore these were not accounted for.

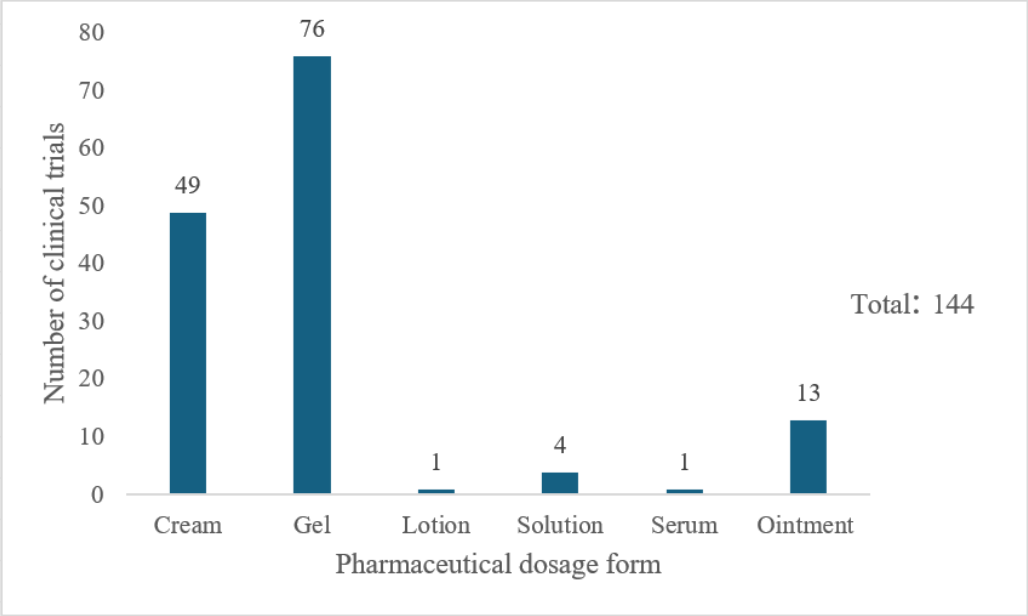

Figure 8 - Topical dosage forms currently being tested in AK management.

As illustrated in **Figure 8** , gel is the most used pharmaceutical dosage form, with 76 clinical studies. This dosage form regards a semi-solid system where the liquid phases are thickened with gelling agents such as carbomers or cellulose derivatives. Gels can be water-based or hydroalcoholic.

Afterwards, the most common type of dosage form regards creams, which are used in 49 clinical trials. Cream is semi-solid emulsion that can be water-in-oil (W/O) or oil-in-water (O/W) based.

The third most used dosage form regards ointments (13 studies). These are highly occlusive, oil-based formulations, usually anhydrous, that contain high concentrations of oils and waxes.

It should be highlighted that there is one study currently evaluating an uncoated nanoparticulate Paclitaxel Ointment to determine the safety, tolerability, and preliminary efficacy, compared to an ointment placebo, applied to AK lesions on the face. Even though this is solely one study, it may be indicative of the potential of nanotechnology in

topical drug delivery for AK management. This subject will be detailed in the next chapter.

The active pharmaceutical ingredients formulated in topical drugs currently being tested for AK treatment are summarized in **Figure 9.**

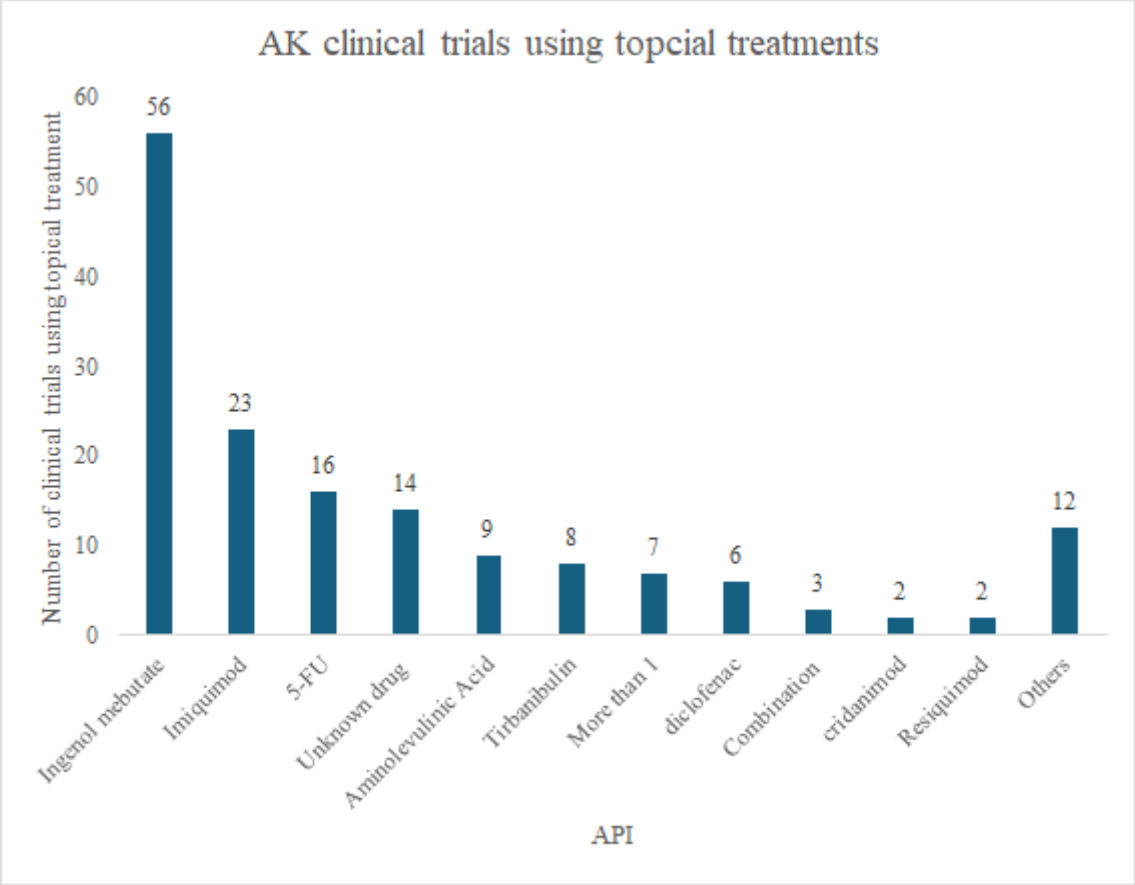

Figure 9 - API in Topical Drug Products used in clinical trials for AK

Ingenol mebutate is by far the most used API in topical products being tested for AK. The action mechanism of this drug still needs to be clarified, however studies both *in vivo* and *in vitro* have demonstrated a dual mechanism: 1) induction of cell death in the local lesion, and 2) promotion of an inflammatory response characterized by the local production of pro-inflammatory cytokines and chemokines, as well as infiltration of immunocompetent cells (European Medicines Agency, 2017).

After ingenol mebutate, imiquimod is the second most used API for AK management currently being tested in clinical trials. As previously referred, this drug can be classified

as an immune response modifier that acts as a toll-like receptor 7 (TLR-7) agonist, inducing cytokines to trigger an inflammatory skin reaction towards malignant or virus-infected cells, through the stimulation of their apoptosis.

5-FU is the third most common API in AK management. This drug is an analogue of uracil acting like an antimetabolite, after intracellular conversion into the active deoxynucleotide. Therefore, 5-FU interferes with DNA synthesis by blocking the conversion of deoxyuridilic acid into thymidylic acid through the cellular enzyme thymidylate synthase. Fluorouracil can also be incorporated into RNA, interfering with RNA synthesis.

Aminolevulinic acid (ALA) is the fifth most used API, with 9 clinical trials currently testing is effects in AK. This substance is metabolized into protoporphyrin IX (PpIX), a photoactive compound that accumulates intracellularly in the treated lesions. PpIX is activated by light illumination with an appropriate wavelength and energy. In the presence of oxygen, reactive oxygen species are formed. These damage the cellular components, leading to the destruction of the targeted cells.

In fourth place is Unknown drug, in this category, there are several clinical trials where the API is not said because they are patented.

Finally, tirbanibulin is currently being tested in 8 clinical studies. The action mechanism of this drug is that interrupts microtubules by binding directly to tubulin, which induces cell cycle blockage and apoptotic death of proliferating cells,and is associated with the interruption of Src tyrosine kinase signalling.

Some other active pharmaceutical ingredients that are being used are, for example, diclofenac, paclitaxel, resiquimod and cridanimod.

4. Promising new treatments for AK

As detailed in the previous chapters, there is a broad range of available treatments for AK, however, most of the guidelines privilege the surgical methods and destructive methods over topical drugs. This can be due to the fact that topical drug delivery can be less effective in the total removal of lesions and may also not display an adequate security profile when dealing with very potent API (Hmingthansanga et al., 2022). To overcome these problems, the use of nanotechnology can be helpful, since it enables the enhancement of the drugs pharmacokinetic profile, while maintaining their safety.

In this chapter, nanotechnology drug delivery systems for managing and treating AK will be the focus. These include liposomes, nanoparticles, ethosomes, microemulsions, as well as other drug delivery systems. Even though the aim of this monograph is to cover therapeutic systems for AK management, in this section papers that explored the topical nanotechnology delivery systems targeting SCC or BCC were likewise considered, due to their proximity with AK. According to the literature, the most common API in AK that are incorporated into nanosystems regard 5-FU, IMQ and ALA. Nevertheless, there are also several studies that point out to the applicability of doxorubicin and T4N5 within this scope.

4.1. Liposomes

Liposomes are colloidal spheres composed of cholesterol and phospholipids resulting in a hollow lipid bilayer, as depicted in **Figure 10.** These systems are highly organized structures, formed through self-assembly of amphiphilic phospholipids in an aqueous environment, with a morphology resembling cellular membranes. This unique structure allows the encapsulation of hydrophobic drugs (Desmet et al., 2017; Krishnan & Mitragotri, 2020).

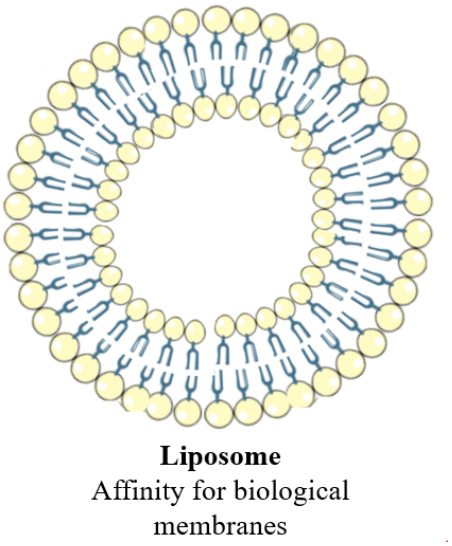

Figure 10 – Liposome structure, adapted from (Ewert de Oliveira et al., 2021).

Phospholipids are the main components of biological barriers and have a high affinity with the skin. These amphiphilic molecules provide liposomes the ability to encapsulate hydrophilic and lipophilic molecules. The first one in the inner aqueous phase, whilst the second one in the hydrophobic domains of the lipid bilayer (Desmet et al., 2017; Ewert de Oliveira et al., 2021). Liposomes can be divided into the following groups, according to their size and lamellarity:

i. Unilamellar vesicles (SUV, 20-100 nm).
ii. Large unilamellar vesicles (LUV, >100 nm).
iii. Giant unilamelar vesicles (GUV, >1000nm).
iv. Oligolamellar vesicles (OLV, 100-100 nm).
v. Multilamellar vesicles (MLV, >500 nm).

Lamellarity regards a parameter that describes how many lipid bilayers enclose the aqueous core of liposomes. Parameters such as drug release, entrapment capacity and storage ability, are highly influenced by lamellarity. The preferred size of liposomes for drug delivery ranges from 50 to 450 nm. Within the 50 – 100 nm range liposomes have the ability to circulate in the bloodstream and to penetrate the tissue, however up to 450 nm liposomes are able to carry more drug load (Desmet et al., 2017; Pattni et al., 2015).

When formulating a liposome formulation, it is strongly advised to use hydrogenated phospholipids, together with epidermal lipids like cholesterol and ceramides. These

structures should then be incorporated into a gel matrix, in order to increase the stability of the vesicles (Desmet et al., 2017). Due to their high loading capacity, liposomal formulations do not require the inclusion of high concentrations of drug. This feature makes them suitable for delivering extremely powerful API´s (Desmet et al., 2017).

The main therapeutic advantages of liposomes regards their ability to target API delivery to the action site, reduce the systemic absorption of the drug, and by doing so minimize the side effects (Raghu et al., 2020). Furthermore, liposomes also have the ability to improve the drug's pharmacokinetics profile and enhance its efficacy with reduced toxicity (Krishnan & Mitragotri, 2020).

Figure 11 depicts the pathways by which liposomes can permeate the skin:

I) A drug-free process in which molecules are released from carriers to permeate the skin independently or through direct drug exchange between the liposomal bilayer and the *stratum corneum*, or by a combination of both.

II) Enhancement of penetration by inducing structural changes in the *stratum corneum* due to liposome components.

III) Adhesion to the skin surface, with potential fusion or interaction with the *stratum corneum* lipid matrix.

IV) The trans-appendageal pathway (through sebaceous glands, sweat glands, and hair follicles), plays a minor role in transdermal liposomes delivery.

V) Intact vesicular penetration mechanism, where complete vesicles penetrate into or through the intact skin (Desmet et al., 2017).

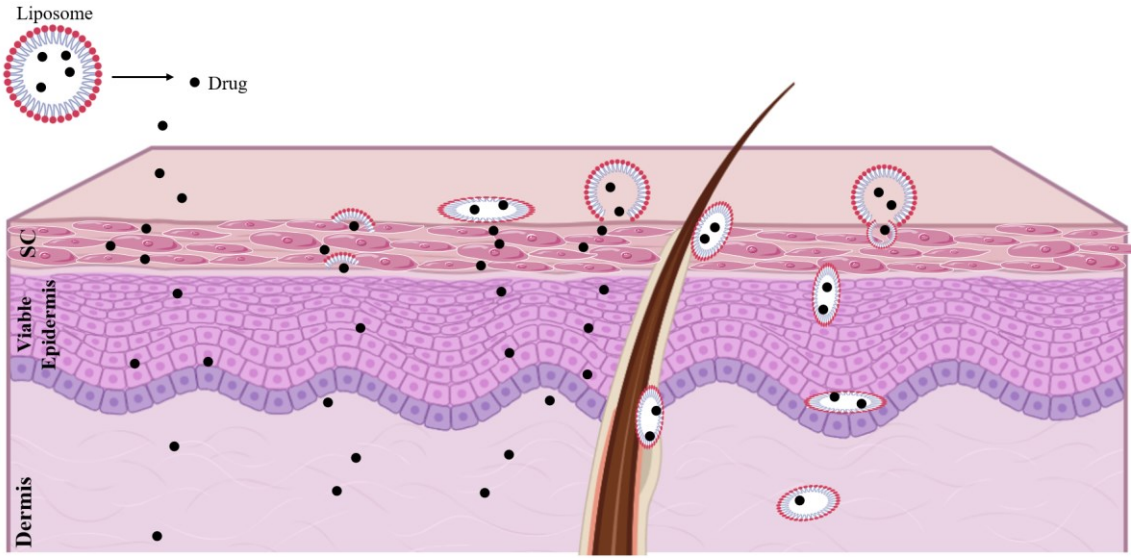

Figure 11 – Topical drug delivery pathways in liposomes. Adapted from (Desmet et al., 2017)

Glavas-Dodov et al. (2003) developed Phospholipon 90H liposomes and cholesterol for 5-FU encapsulation. Up to 25% of 5-FU was encapsulated by the authors, using an aqueous solution and a liposome-chitosan hydrogel as the drug carriers. The authors then evaluated the *in vitro* release profile of the drug (Glavas-Dodov et al., 2003). The extended-release profile was attributed to both the gel matrix and the liposome system. These results point out to the system's effectiveness in serving as a reservoir for a continuous drug release (Ewert de Oliveira et al., 2021).

Petrilli et al. (2017) developed immunoliposomes conjugated with cetuximab for the targeted administration of 5-FU to SCC. This drug regards a monoclonal antibody, which induces cell death through the interaction with the epidermal growth factor receptors (EGFR). These receptors are over-expressed in several types of cancer, such as SCC (Ewert de Oliveira et al., 2021).

Liposomes have also been studied for AK treatment. One example regards the T4N5 liposome lotion, which reduced AK lesions by 68% compared with placebo. This molecule acts by promoting DNA repair in sun-damaged skin (Cancer Network, 2001).

Despite these advantages, the pharmaceutical use of liposomes is still limited. The following constraints may be identified:

- **Physical**: High physical instability caused by vesicle leakage, alterations in size by aggregation or fusion, and/or ester hydrolysis and formation of oxidation products (Desmet et al., 2017).
- **Biological**: Liposomes tend to be rapidly eliminated from the systemic circulation, due to mononuclear phagocyte system (Nsairat et al., 2022)..
- **Manufacture:** Their production costs are higher than conventional dosage form. (Desmet et al., 2017; Rahman et al., 2019).

4.2. Nanoparticles

4.2.1. Solid lipid nanoparticles (SLN)

Solid lipid nanoparticles are composed of a solid lipid core coated with surfactant. Their size may range from less than 50 nm to 1000 nm, as described in **Figure 12.** Similarly to liposomes, emulsions and polymeric NP, SLN aim to provide a controlled drug release (Dianzani et al., 2021; Ewert de Oliveira et al., 2021; Krishnan & Mitragotri, 2020; Raghu et al., 2020).

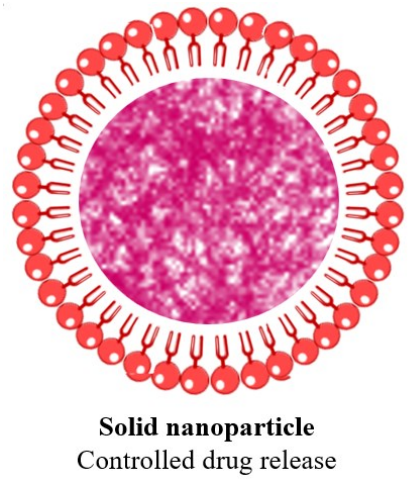

Figure 12 – Pictogram of a solid nanoparticle. Adapted from (Ewert de Oliveira et al., 2021).

SLN are composed of 0.1% to 30% (w/w) physiological solid lipid (i.e. solid at room and body temperature), which is dispersed in an aqueous medium or in a surfactant solution. The latter creates a surfactant shell over the particle surface that stabilizes the

dispersion. Lipid nanoparticles are useful for the transdermal and epidermal administration route due to the following reasons:

- Their composition is non-irritating and non-toxic to the skin, even when considering damaged skin
- The small size of the lipid enables a close interaction with the *stratum corneum*, thereby increasing the penetration, occlusion and accumulation of the API in the epidermis (Desmet et al., 2017; Ewert de Oliveira et al., 2021; Raghu et al., 2020).

SLNs can be used to incorporate highly insoluble drugs in its lipid matrix. When the drug is encapsulated in the lipid core, there is a prevention of its early release, as well as potential degradation. SLNs are also able to adhere to the skin, by forming a monolayer that creates an occlusive effect by increasing the water retention in the skin. This allows for an increased topical penetration. They are relatively easy to scale-up, and are cost-effective and reproducible (Krishnan & Mitragotri, 2020)

The production of SLNs does not require organic solvents. Instead, they resort to biodegradable and biocompatible solid lipids, emulsifiers and water via a high-pressure homogenization process. Typically, the used lipids regard triglycerides, glycerides, fatty acids and waxes, all raw materials that display limited toxicity (Desmet et al., 2017; Dianzani et al., 2021; Krishnan & Mitragotri, 2020).

There are three SLN manufacture methods: (i) solid solution model, in which hydrophobic drugs are dissolved within the lipid matrix; (ii) drug-enriched shell model, where the APIS are dispersed around a central lipid core and finally; (iii) drug-enriched core model, in which the drug is selectively located in the SLN core (Desmet et al., 2017).

SLNs have been extensively developed for cosmetic and skin applications. Indeed, SLNs have been used as carriers for several drugs relevant to the topical treatment of skin conditions, including clotrimazole, isotretinoin, triptolide, glucocorticoids and vitamin A (Mustfa et al., 2021).

Disadvantages associated with this system include poor drug loading due to a compact lipid matrix network; drug-lipid melt interaction; drug dissolution/dispersion rates in the lipid matrix and/or possible drug loss on account of polymorphic transition during storage (Desmet et al., 2017; Krishnan & Mitragotri, 2020).

In a study conducted by Khallaf et al. (2016), SLNs were developed using lecithin and Poloxamer 188, resulting in a 5-FU retention rate of up to 47%. SLNs containing 5-FU were introduced into a sodium carboxymethylcellulose gel, and their absorption was measured *in vitro* and compared with that of the free drug in the same gel matrix. The results demonstrated that the encapsulation process enhanced the penetration of the drug into lipophilic barriers. The hydrogel comprising 5-FU SLNs was then evaluated *in vivo* in mice with Ehrlich ascites carcinoma, twice a week. The study contemplated the evaluation of the developed system, placebo, as well as free 5-FU system. The histological results showed that, the SLN-5-FU displayed a better penetration and reduced the inflammation and haemorrhage in the tumour area, when compared with other formulations. However, it should be referred that the authors did not evaluate the systemic absorption of the drug during the treatment, nor did they mention whether there was a reduction in tumour mass during the evaluated period (Ewert de Oliveira et al., 2021).

4.2.2. Nanostructured lipid carriers (NLC)

Nanostructured Lipid Carriers (NLCs) are second-generation lipid nanoparticles designed to overcome the limitations of the first generation (SLNs). NLCs are in this context, “tailored” SLN, fabricated from a mix of solid and liquid lipids, preferably in a ratio of 70/30 up to 99.9/0.1, that do not possess the ideal crystalline structure. The structure of this system is illustrated in **Figure 13**. The lipid is either enclosed within the solid lipid matrix or localized at the surface of solid platelets and the surfactant layer. As a result, the liquid phase with a reduced water content provides a high drug loading and the solid lipid part imparts attributes for a controlled drug release (Desmet et al., 2017; Krishnan & Mitragotri, 2020).

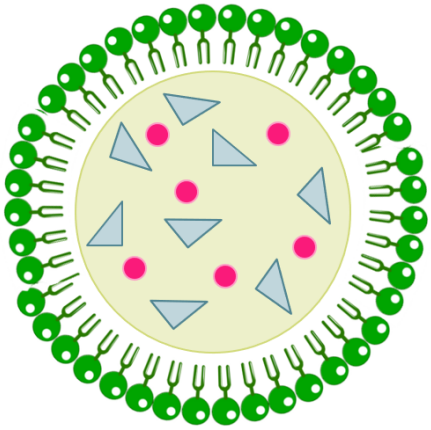

Figure 13 – Nanostructured lipid carrier structure. Adapted from (Subramaniam et al., 2020).

There are three types of NLC: amorphous (made by combining solid lipids with special lipids); multiple (where liquid lipids are added to the solid lipid to achieve higher solubility for lipophilic drugs); and imperfect (made from a mix of spatially different lipids creating an imperfect crystal matrix with area for drug molecules) (Desmet et al., 2017).

When compared to SLNs, NLCs have a higher drug loading capacity due to an increase in the distance between the fatty acid chains and the unstructured crystal. When made into their final dosage form, NLCs are simpler to produce. They are typically made using aqueous dispersion, nanoemulsion, or pressure homogenization procedures. They also have a wide surface area, which adds to the occlusion effect, enhances its penetration, and increases skin moisture, according to research on dermal delivery (Desmet et al., 2017; Krishnan & Mitragotri, 2020). NLC also improve the photo, oxidative, and hydrolytic stability of the encapsulated drugs. The dissimilar melting temperatures of liquid and solid lipids allows for a distinctive release profile. Firstly, there is an initial burst release, which is then followed by a gradual drug release (Desmet et al., 2017; Krishnan & Mitragotri, 2020).

For AK, topical treatment is ideal since it allows for a direct drug delivery to the lesions. NLCs provide sustained release, which enhances the therapeutic effect while reducing systemic toxicity. The small size and lipid composition of NLCs allows for a

deeper penetration into the skin, increasing the concentration of the therapeutic agent and a high drug load.

4.2.3. Polymeric micelles

Polymeric micelles are a type of nanostructure that arises from the combination of two or more hydrophobic polymer chains, known as amphiphilic block copolymers, that interact in aqueous solutions. These copolymers self-assemble into a micellar structure with a core-shell structure, where hydrophilic groups create a corona-like shell, which stabilizes the core by direct contact with the aqueous environment, while the hydrophobic groups form the core to minimize their exposure to water. The structure of this system is illustrated in **Figure 14** (Dianzani et al., 2021; Mustfa et al., 2021).

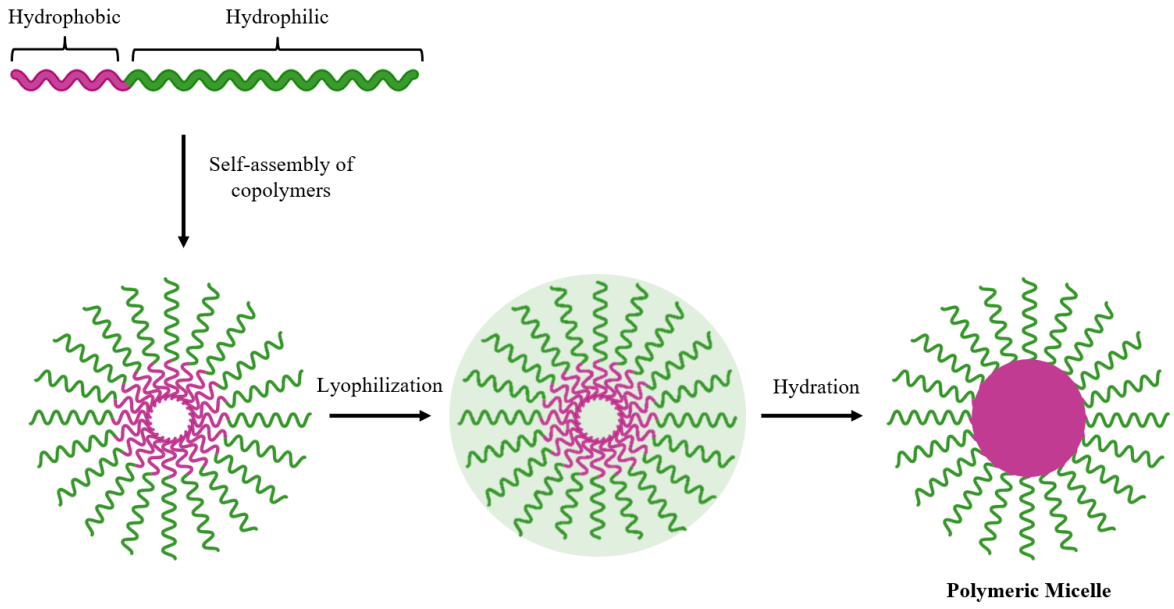

Figure 14 – Particle preparation of a polymeric micelle. Adapted from (Stern et al., 2017)

The standard size of polymeric micelles for pharmaceutical use ranges in size from 10 to 80 nm. Although being smaller, they have a shorter circulation time compared to liposomes, they exhibit a superior tumour uptake due to the enhanced permeability and retention (EPR) effect. Hydrophobic drugs are loaded into the core, while the hydrophilic shell ensures water solubility and core stability. This structure maximises the solubility

of hydrophobic drugs (Dianzani et al., 2021; Krishnan & Mitragotri, 2020; Mustfa et al., 2021).

The hydrophobic core can accommodate a high concentration of lipophilic drugs (5-25 wt%), while the hydrophilic portion of the system protects the drug, reducing systemic toxicity. Functionalizing the micelles with ligands further increases their specificity and efficacy. Additionally, this system displays a higher *in vivo* stability compared to liposomes and surfactant micelles. Due to their larger size, they can be used for co-delivery of drugs (Dianzani et al., 2021).

Polymeric micelles can be engineered for controlled drug release in response to external chemical and/or physical cues, such as changes in pH, temperature, ultrasound, or light, enhancing their precision in treatment, improving specificity and efficacy. Polymeric nanoparticles are known to penetrate the skin through hair follicles, enhancing drug delivery via the follicular pathway (Krishnan & Mitragotri, 2020).

Polymeric micelles are self-assembling nanocarriers used in drug delivery systems, including cancer therapies. Their unique structure and physicochemical properties have made them a promising candidate for treating actinic keratosis, because of their capability to enhance the penetration of drugs through the skin, increasing their effectiveness at the tumour site, allowing localized treatment, and reducing systemic exposure, minimizing side effects.

4.3. Niosomes

Niosomes regard surfactant vesicles, which are manufactured by hydrating non-ionic single-chain surfactant, together with the incorporation of cholesterol or other excipient as stabilizer (Desmet et al., 2017). The structure of this system is illustrated in **Figure 15**.

Similarly to liposomes, niosomes have an amphiphilic bilayer structure and form unilamellar or multilamellar vesicles. However, this system uses non-ionic surfactants, that do not suffer oxidation as the natural phospholipids used in liposomes. This makes niosomes more chemically stable, when compared towards liposomes, but they are less flexible (Desmet et al., 2017; Ewert de Oliveira et al., 2021).

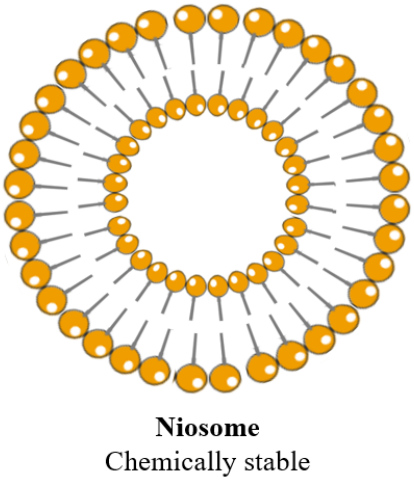

Figure 15 – Pictogram of a niosome. Adapted from (Ewert de Oliveira et al., 2021).

Niosomes can encapsulate polar and nonpolar drugs and are preferred over liposomes because of their high chemical stability, lower cost, as well as penetration enhancing properties (Desmet et al., 2017; Ewert de Oliveira et al., 2021).

Niosomes have other characteristics that make them be more advantageous compared to other technologies:

- Greater patient compliance, than oil based systems;
- Capacity to encapsulate a variety and large amounts of drugs;
- Biodegradable and biocompatible;
- Ability to control size, lamellarity and shape;
- Slow and controlled release, by acting as a depot;
- Capacity to be incorporated in a wide range of dosage forms, and thereby allow for different administration routes
- Ease of handling and storage (Desmet et al., 2017).

The disadvantage of this system relies on their stability profile, as the vesicles tend to suffer fusion, aggregation, leakage, and hydrolysis of the entrapped drugs. This occurrence leads to shelf malfunctions, with the formation of several derivatives such as proniosomes, aspasomes and niosomes (Desmet et al., 2017).

There are various parameters that explain the ability of niosomes to increase topical drug delivery: i) they diffuse as a whole; ii) smaller vesicles are formed in the skin; iii) interaction with the *stratum corneum* (aggregation, fusion or adhesion) causes a high concentration gradient the vesicle-*stratum corneum* surface which drives lipophilic drug

penetration; iv) they modify the *stratum corneum* structure loosening its intercellular lipid barrier; and v) the non-ionic surfactant itself enhances the drug permeation (Desmet et al., 2017).

One of these mechanisms regards the modification of the stratum corneum barrier, by enhancing its smoothness through the restoration of the lost lipids and reducing transepidermal water loss. However, this specific mechanism is highly dependent on the drug's physicochemical characteristics, as well as on the vesicle itself and the lipids that are employed to produce the niosomes.

Numerous studies have examined the role that charge, cholesterol, and surfactants like Span 40, Span 60, and Brij 72 play have in improving niosome skin penetration and retention (Krishnan & Mitragotri, 2020).

Paolino et al. (2008) developed a niosome delivery system for topical application of 5-FU using a novel ball-type surfactant. These surfactants are amphiphilic molecules with a central carbonyl chain and two ether-based rings at each end. The niosomes, formulated with the ball surfactant, Span® 80, and cholesterol in a 2:5:2 ratio, achieved a 45% encapsulation efficiency for 5-FU. The created niosomes exhibited an eight-fold higher capability for skin penetration than the free medication, according to the *in vitro* studies results. Confocal laser scanning microscopy results revealed that the ball-niosomes effectively underwent endocytosis, with this mechanism being responsible for the intracellular levels of 5-FU (Paolino et al., 2008).

This enhanced delivery significantly improved the anticancer activity of 5-FU in both melanoma and non-melanoma skin cancer cell lines, compared to free 5-FU (Ewert de Oliveira et al., 2021).

The application of niosomes in the treatment of actinic keratosis is descried in the literature using 5-FU (Priyadarshini K., 2024). Research findings indicated that niosomes notably boosted 5-FU's percutaneous penetration, resulting in an increased anticancer activity. The human *stratum corneum* and epidermal layers were much more susceptible to drug penetration, as a result of the niosomal system. Additionally, 5-FU's vesiculization enhanced its cytotoxic impact and improved topical distribution, when compared to the non-vesiculized dosage forms. This demonstrates how niosomes may be used as a delivery mechanism to improve the effectiveness of anticancer drugs in dermatological applications (Priyadarshini K., 2024).

4.4. Ethosomes

Ethosomes are non-invasive nanovesicle carriers composed of phospholipids, cholesterol, water, and 10–40% ethanol. They are soft, flexible and stable vesicles. The structure of this system is illustrated in **Figure 16**.

Ethanol is a polar solvent capable of directly interacting with the polar head of phospholipids in the *stratum corneum*, resulting in the reduction of its melting point and enhancing permeability of drugs to be delivered in the deep skin layers and/or to the systemic circulation (Ewert de Oliveira et al., 2021). Furthermore, ethanol helps in improving the interaction of the ethosomes with the skin lipids, being responsible for a better drug delivery, when compared to liposome (Desmet et al., 2017; Ewert de Oliveira et al., 2021; Krishnan & Mitragotri, 2020).

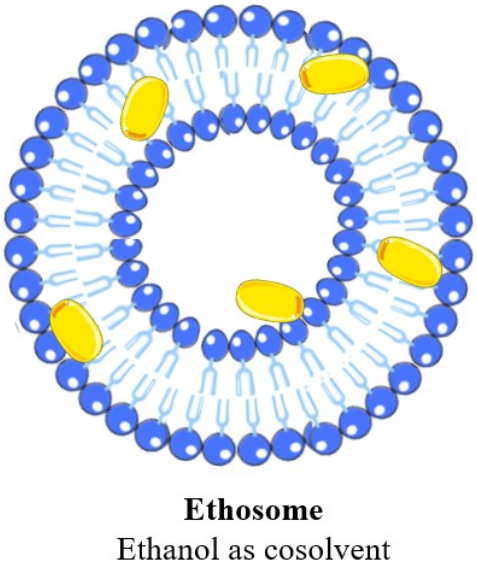

Figure 16 – Ethosome structure. Yellow elements represent alcohol as a solvent. Adapted from (Ewert de Oliveira et al., 2021)

Because of their high concentration of ethanol, ethosomes are more fluid than liposomes, enabling them to squeeze through narrow spaces between skin cells. In fact, skin permeability is increased by ethanol making the cell membrane lipids more fluid and reducing the density of the lipid multilayer in cell membranes. Because of this, ethosomes are able to enter the deep layers of the skin easily, fusing with skin lipids to release the drug (Desmet et al., 2017). Due to this reason, ethosomes are promising drug delivery systems in topical conditions, such as AK.

This system displays a low-risk toxicological profile, enhanced patient compliance and comfort and improved drug efficacy. Furthermore, production costs are not high. In other hand, it should be considered that the high concentration of ethanol can cause skin irritation or dermatitis (Desmet et al., 2017).

In a study made by Fang et al., ethosomes evaluated the permeation of aminolevulinic acid (ALA) encapsulated in ethosomes with liposomes. Both treatments also contemplated photodynamic therapy. The results showed that the skin permeation was higher in the ethosome formulation (Krishnan & Mitragotri, 2020).

In another study, Puri and Jain (2012) created an ethogel, an ethosome-based topical gel formulation for the topical administration of 5-FU (5%). The authors used 10-40% of ethanol and 1-5% of phosphatidylcholine to form the vesicle, and as a gelling agent, Carbomer 980 was used. The formulation efficiency was then compared with a commercial 5-FU cream. *In vitro* studies demonstrated that the developed ethogel increased 5.9- to 9.4- fold the drug deposition. The developed system had also a better permeation efficiency, higher anticancer activity and less side effects, *in vitro* (Ewert de Oliveira et al., 2021).

4.5. Microemulsions

Microemulsions are optically transparent, thermodynamically stable, and spontaneously formed dispersions of two or more immiscible liquids (water and oil), where one liquid forms spherical droplets in the other phase (Ewert de Oliveira et al., 2021). A surfactant/cosurfactant or emulsifier mixture has to be added to stabilize the emulsion, by reducing the interfacial tension between the different phases and by generating a physical repulsion between them. This reaction forms a transparent, isotropic and thermodynamically stable liquid(Desmet et al., 2017). The structure of this system is depicted in **Figure 17** (Desmet et al., 2017; Ewert de Oliveira et al., 2021).

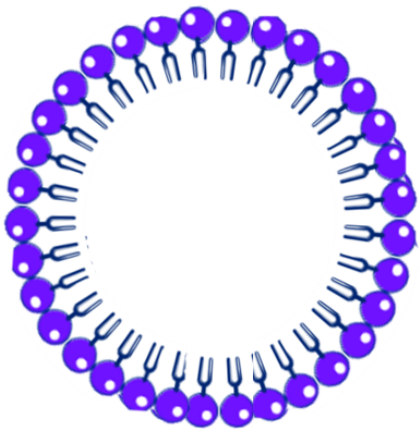

Figure 17 – Microemulsion structure. Adapted from (Ewert de Oliveira et al., 2021)

To achieve drug solubility, microemulsions boost the concentration gradient towards the skin, which leads to improved absorption of hydrophilic and lipophilic APIs. The effectiveness of microemulsions in (trans)dermal drug administration is further influenced by the surfactant's role as a penetration enhancer and the drug's internal mobility inside the vehicle. Drug-surfactant interactions and/or the drug's partitioning between the oil and water phases, regulates the drug's release from the emulsion (Desmet et al., 2017).

Microemulsions have the following advantages: large surface area, small droplet size, low production costs, high scale-up potential and easy manufacture. Moreover, microemulsions can alter drug pharmacokinetics and improve drug loading, as well as penetration across biological membranes (Desmet et al., 2017; Ewert de Oliveira et al., 2021). Nevertheless, this system also displays some disadvantages: Premature drug release or leakage, phase inversion, high (co-)surfactant concentration and associated toxicity, and frequently a difficult complex and time-consuming pharmaceutical development ((Desmet et al., 2017).

Goindi et al. (2014) developed a topical 5-FU created ionic water-in-oil microemulsions based on 1-Butyl-3-methylimidazolium bromide (BMIMBr), Tween® 80/Span® 20, and isopropyl myristate. Several controls, including a 0.2% water-in-oil microemulsion and a 5% commercial formulation, were likewise tested. The produced microemulsions exhibited a superior skin penetration and retention *in vitro*. Furthermore,

in an *in vivo* evaluation, all the microemulsions were able to minimize tumour damage in an animal model, something that the traditional formulation did not show (Ewert de Oliveira et al., 2021).

Microemulsions can be studied as a way to improve the delivery of active compounds for the treatment of AK, providing advantages in drug solubility and others. Also, microemulsions have the potential to achieve high drug penetration through the *stratum corneum*, where they can more effectively target the abnormal keratinocytes responsible for AK lesions.

4.6. Nanoemulsions

Nanoemulsions (NEs) are nanoscale dispersions of oil in water (o/w) or water in oil (w/o), stabilized by an interfacial film of surfactant molecules, as illustrated in **Figure 18**. This type of mechanism is ideal for topical drug delivery with reduced skin irritation, because of the small particle size ranging between 20 - 200 nm and a low quantity of surfactant. The production can be made by high-energy (e.g., high-pressure homogenization) or low-energy (based on physicochemical properties of components) emulsification techniques (Krishnan & Mitragotri, 2020).

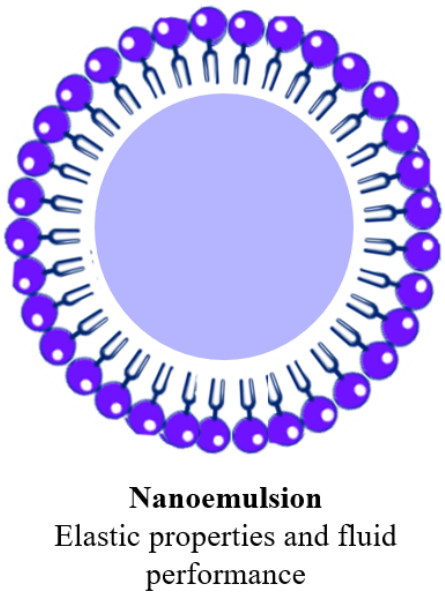

Figure 18 – Representation of a nanoemulsion. Adapted from(Zhang et al., 2022).

There are three types of NEs categorized by the composition of the dispersed phase and continuous phase:

i) Oil-in-water, where oil droplets are dispersed in the continuous aqueous phase;

ii) Water in-oil, where the water droplets are dispersed in the continuous oil phase;

iii) Bi-continuous NEs, where microdomains of oil and water are interspersed within the system (Krishnan & Mitragotri, 2020).

NEs have a high loading capacity and can solubilize and transport hydrophilic and lipophilic drugs together. Due to its vast surface area, NEs may form a tight occlusive membrane when in contact with the *stratum corneum*. This eases drug permeation. The addition of oil and surfactants, such as oleic acid or eucalyptol, may alter the lipid composition of the *stratum corneum*, thereby contributing to the increase of drug permeation (Krishnan & Mitragotri, 2020).

Ameluz® topical gel (Biofrontera Pharma GmbH, Leverkusen, Germany) is used to treat actinic keratosis and basal cell carcinoma. It contains aminolevulinic acid (logP 1.5) in a nanoemulsion made of soybean phosphatidylcholine, water, polysorbate, propylene glycol, and isopropyl alcohol (Nastiti et al., 2017).

4.7. Dendrimers

Dendrimers are unimolecular, monodispersed, synthetic polymers that are less than 15 nm in size. They have layered architectures made up of a central core, an internal symmetrical region made up of repeating units, and different terminal groups that determine the characteristic structures of the dendrimers in three dimensions. The repeating units in these structures have functional groups that grow exponentially (Dianzani et al., 2021; Krishnan & Mitragotri, 2020).

The structure of this system is illustrated in **Figure 19.**

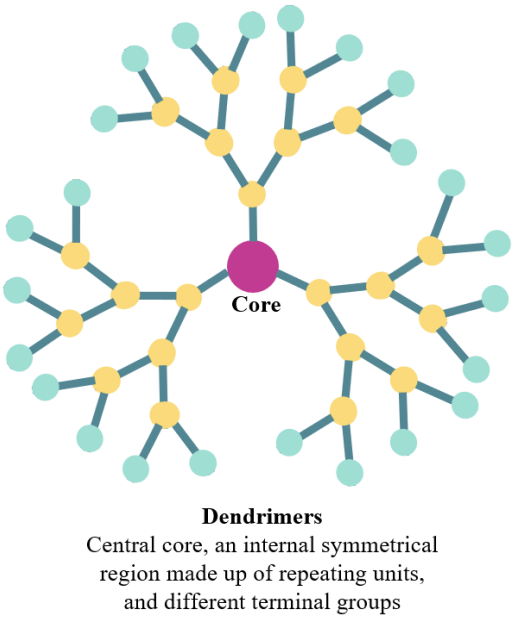

Figure 19 – Dendrimers structure. Adapted from (Zhang et al., 2022).

Their core-shell architecture, well-defined size and molecular weight, monodispersity, multivalency, number of available internal cavities, the high degree of branching, the high number of surface functional groups, and concentration, allows dendrimers to penetrate the skin. Dendrimers can be used to deliver hydrophobic and hydrophilic drugs, nucleic acids, as well as imaging agents (Dianzani et al., 2021; Krishnan & Mitragotri, 2020).

Dendrimer-targeted ligands have been described in several literature reports as a tool for the tumour specific target and mass reduction. Together with folate and tumor-associated antigens, they consist of oligosaccharides, polysaccharides, oligopeptides, and polyunsaturated fatty acids. But it's still challenging to have drugs delivered via dendrimers, with a controlled release profile (Dianzani et al., 2021).

Dendrimers are ideal for drug delivery applications because of their highly branched macromolecules with a well-defined structure. They also provide a promising vehicle for delivering APIs due to their unique properties, including precise control over size, and the ability to target specific tissues. The small size and surface functionality of these particles make them able can penetrate the skin more efficiently than larger particles.

4.8. Exosomes

Exosomes are originated from intraluminal vesicles (ILV) of multivesicular late endosomal compartments (MVB), the fusion of endosomes with the cell membrane release the ILVs into the extracellular environment and the exosomes travel to cells in biological fluids. Receptor-mediated endocytosis is the mechanism by which the entrance in the recipient cells takes place. This is followed by opening, degradation, or re-internalization into a target cell's MVB. The exosome material is released directly upon internalization by fusion with the secondary cell's plasma membrane. Exosomes have a lipid and protein composition which is very important for their intrinsic targeting capacity and essential for cell docking and fusion, like phosphatidylserine, an identified biological docking site for signalling molecules, is often expressed. Exosomes are present in body fluids and have biological components such as mRNA and microRNA species, they are endogenous nanocarriers as a result (Desmet et al., 2017).

A schematic image of the mechanisms of exosomes can be seen in **Figure 20**.

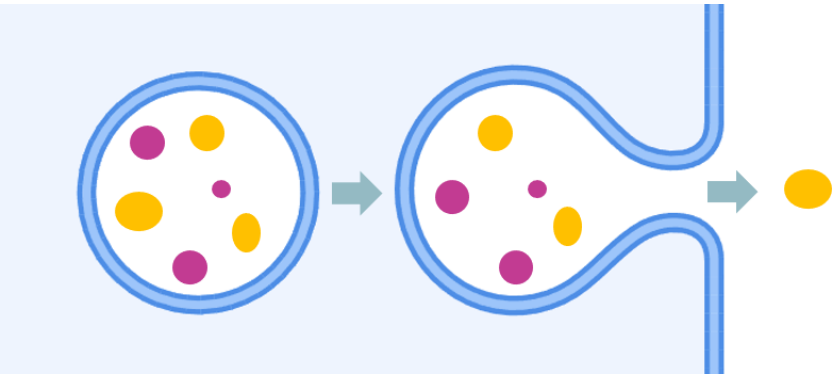

Figure 20 – Exosomes mechanism. Adapted from (Desmet et al., 2017).

Exosomes expect to be used to deliver therapeutic drugs or RNA molecules because of their biogenesis. As of now, there have been no documented clinical studies, commercial products, or research publications on the use of exosomes for (trans)dermal medication delivery in the treatment of skin problems. The current deficiency is a result of unsolved problems with exosome endogenous content, ideal dose and distribution, and improved isolation techniques. It will be easier to translate these problems into clinical practice if they are explained. Utilizing synthetic exosome mimetics, which include creating liposomes with just the essential components of natural exosomes, is another beneficial option (Desmet et al., 2017).

Exosomes play a crucial role in cell-to-cell communication and because of their natural origin and ability to transfer bioactive molecules across biological barriers gaining attention as potential adjuvant in treating AK. They can transport therapeutic agents into AK lesions, having the capacity to fuse with target cells since they are biocompatible, efficiently delivering API.

Another possibility for the use of exosomes is since they can naturally carry RNA, they have the potential to perform gene therapy. A possible mechanism is the delivery of RNA to stop genes involved in the progression of AK to SCC, becoming a more personalized treatment option and reducing the risk of disease progression.

4.9. Nanotubes

Nanotubes are a carbon structure, a type of fullerenes, and are made of coaxial graphite sheets rolled into a cylinder shape with a size range of <100 nm and can be either a single one graphite sheet or a multiwalled nanotube (Dianzani et al., 2021).

The structure of this system is illustrated in **Figure 21**. In this figure it is possible to observe several concentric graphite sheets, which provide to the system excellent physical, photochemical, and electrochemical properties (Dianzani et al., 2021).

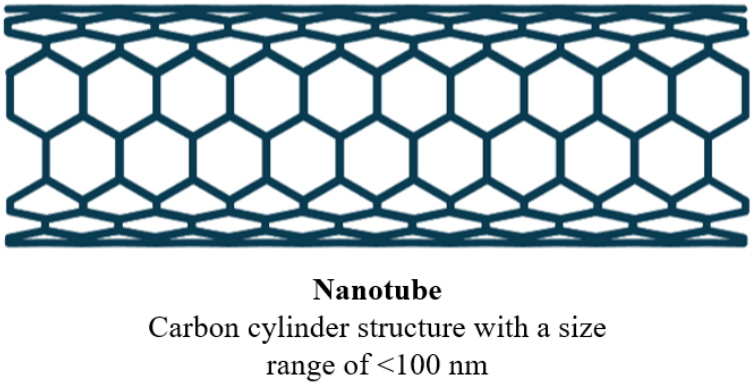

Figure 21 – Pictogram of a nanotube. Adapted from (Negri et al., 2020)

This type of technology due to its metallic or semiconductor behaviour is often used as biosensors, however it can also be used as drug carriers and tissue repair scaffolds (Dianzani et al., 2021).

Due to their large surface area, nanotubes can be seen as a possible strategy to treat AK, due to their higher drug loading capacity and because of their unique shape which allows them to permeate the *stratum corneum* more easily, than larger particles. Another specificity of nanotubes is that they can be functionalized to interact with skin lipids, facilitating a deeper drug permeation and retention in the epidermis (Karimi et al., 2015)

4.10. Nanospheres

The structure of this system is illustrated in **Figure 22**.

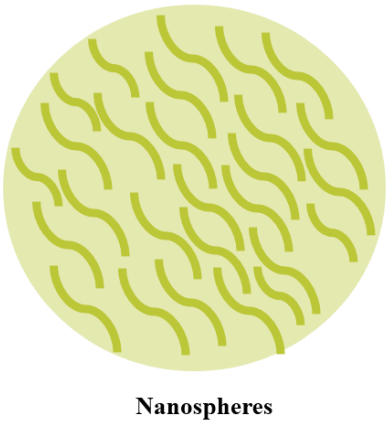

Figure 22 – Nanosphere structure. Adapted from (Bahamonde-Norambuena et al., 2015)

Polymeric nanospheres are insoluble colloidal particles, either on nano- or microscale. These systems are characterized by a polymeric core and typically have a side range of 10 to 1000 nm. They are primarily engineered as pH-sensitive drug delivery systems, tailored for oral administration due to its stability in the highly acidic conditions of the stomach (Dianzani et al., 2021). Nevertheless, nanospheres can carry various drugs used in AK treatment, including chemotherapeutic agents and anti-inflammatories,. Furthermore, the surface of nanospheres can be functionalized with ligands to enhance delivery to specific cells or tissues.

4.11. Microneedles

Since the microscopic needles only pierce the SC and do not reach the underlying nerves, microneedle technology is considered to be minimally invasive. This allows for a painless and efficient drug delivery. With the shortcomings of conventional topical treatments, microneedles have become a ground-breaking technique for transdermal and topical drug administration (Kim et al., 2023).

It is challenging to deliver drugs to non-melanoma skin cancers (NMSCs) since the barrier's thickness varies considerably within individual lesions. The highest depth that microneedles made for this purpose could reach was 2-3 mm. Three main categories may be used to classify different types of microneedles: solid, hollow and dissolving (Yamada & Prow, 2020)

Generally, solid microneedles are fabricated form stainless steel, polymers, ceramics or other materials that allow them to pass through the epidermal barrier. Another type of is hollow microneedles they are like a small version of syringe needles for the extraction or delivery of fluids made of metal or silicon. Finally, dissolving microneedles can be made of sugars, polymers and drug crystals, this type of technology depend on wetting the skin to release the drug through dissolution (Yamada & Prow, 2020).

Controlled depth delivery can be possible by adjusting the microneedle's length and the application dynamics, which can both aid to broadly manage the penetration depth. There are a lot of restrictions with this technology. Because of the devices' size and delivery method, only very little amounts may be applied topically. As a result, fewer active compounds are suitable for delivery by microneedles (Yamada & Prow, 2020).

Although dry-coated formulations provide advantages like increased penetration and durability, they also come with drawbacks including higher costs for drug reformulation, obtaining regulatory permission for formulation modifications, and changing the kinetics of drug dissolution in the skin and dried actives' activity. The amount of active delivered can vary, contrasting with needles and syringes, and can be increased when the delivery sites are not defined or when the skin properties vary, such as lesion delivery (Yamada & Prow, 2020).

Also, this type of technology is set to a specific area and cannot be simply expanded without the use of multiple devices and is needed to use high-speed applications or some

levels of pressure, increasing penetration depth variability. The manufacturing of microneedles can be expensive and challenging (Yamada & Prow, 2020).

One study developed stainless steel microneedles containing 5-Aminolevulinic acid (5-ALA) for photodynamic therapy. The system showed a delivery efficiency of around 90%(Jain et al., 2016). The microneedles penetrated the skin considerably deeper (~480 μm) than their topical cream equivalent (~150 μm). Based on an *in vivo* animal investigation, the 5-ALA-coated microneedles dramatically inhibited the development of subcutaneous tumors by around 57%. On the other hand, much like the untreated control group, the topical cream containing 5-ALA (5 mg) was unable to inhibit the tumor volume, resulting in tumor development (Kim et al., 2023).

According to a recent study, polyvinylpyrrolidone-co-vinyl acetate polymer microneedles loaded with IMQ were developed. In the pig skin model, the microneedle patch showed a penetration depth of 426 ± 72 μm. The microneedle achieved a similar degree of IMQ intradermal administration even though its IMQ load was six times lower than the clinically relevant dosage of Aldara®, which is frequently used for BCC therapy, according to the *ex vivo* permeation trials. Moreover, skin cross-sections subjected to time-of-flight secondary ion mass spectrometry analysis demonstrated that, after being delivered by microneedle, IMQ was localized inside the skin, while skin treated with Aldara® mostly displayed the medication within the SC (Kim et al., 2023).

Table 3 is a summary of the treatments mentioned above.

Table 3 - Microneedle patches for the treatment of non-melanoma skin cancer. Adapted from (Kim et al., 2023)

Therapeutic Agent	Materials used for microneedle	Skin Cancer Model	Study description
5-Aminolevulinic acid (5-ALA)	Stainless steel	A20 tumour-bearing Balb/cA nude mice	Stainless steel microneedles coated with 5-ALA demonstrated substantially deeper skin penetration (~480 μm) in comparison to its topical cream equivalent (~150 μm). The subcutaneous tumour development was considerably suppressed by the microneedles by around 57%. On the other hand, the 5-ALA (5 mg) topical cream was unable to reduce the tumor's volume.
IMQ	Polyvinylpyrrolidone and vinyl acetate Polyethylene glycol 400	-	Microneedles loaded with IMQ Using a polyvinylpyrrolidone-co-vinyl acetate polymer had a penetration depth of 426 ± 72 μm. The microneedle achieved an equal amount of IMQ intradermal administration even though its IMQ load was six times lower than the therapeutic dosage of Aldara®.

4.12. Polymeric, Drug-in-Adhesive, and Matrix-Type Patches

Reservoir, matrix-type, and drug-in-adhesive (DIA) patches are types of polymeric patches that utilize polymers as structures and have countless properties. The structure of this system is illustrated in **Figure 23.**

Reservoir patches have the drug between a backing layer and a rate-controlling membrane, matrix-type and DIA patches have the drug inside the polymeric matrix or adhesive layer, this last two types of patches can be adjusted and cut to fit various areas, unlike reservoir patches, making them more adaptable for treating the varying forms of skin cancer (Kim et al., 2023).

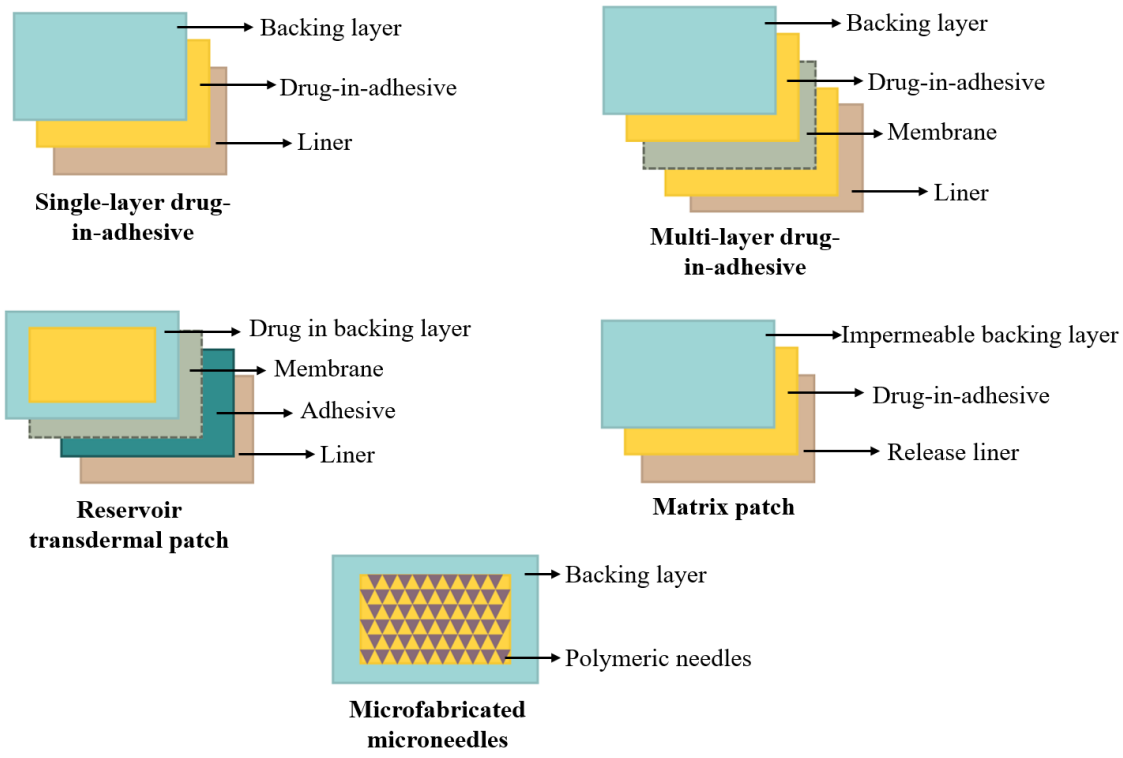

Figure 23 – Types of polymeric patches. Adapted from (Kim et al., 2023).

One study developed polymeric imiquimod patches at concentrations of 4.75, 9.50, and 12.50 mg/cm². These patches exhibited strong bioadhesion (1.76 N/cm²) when applied to porcine skin, releasing a higher amount of imiquimod across a Cuprophan® dialysis membrane compared to the commercial cream Aldara® over six hours, indicating improved drug delivery potential (Kim et al., 2023).

Another study developed polymeric films containing gold nanorods (GnRs) for local hyperthermia applications. The GnRs with thiolated polyethylene glycol were used to improve biocompatibility and were combined into a crosslinked polymeric film made of a copolymer of methyl vinyl ether and maleic acid. These films are favorable candidates for non-invasive hyperthermia treatments against NMSC, because on porcine skin demonstrates no polymer residue and is heated the skin to over 40°C (Kim et al., 2023).

Additionally, DIA patches containing 5-fluorouracil (5-FU) were developed using a cationic copolymer (Eudragit® E) as the adhesive matrix. Patches with 40% triethyl citrate, dibutyl sebacate, or triacetin as plasticiser achieved adhesive properties like several marketed patches, controlling 5-FU release, emphasizing their potential for skin cancer treatment. A combination of 5-FU and IMQ was study in a DIA patch, resulting

in a faster release of 5-FU compared to IMQ, which could be valuable for initial topical application (Kim et al., 2023).

In a more recent study, IMQ nanostructured lipid carriers (NLCs) were developed in a matrix-type patch of hydroxypropyl methylcellulose (HPMC) and propylene glycol, the results show a better IMQ deposition in the skin compared to a commercial cream. These patches have advantages because increase drug delivery and patient adherence and the possibility of using environmentally friendly practices in NLC production (Kim et al., 2023). **Table 4** summarises all treatments mentioned above.

Table 4 – Patches for potential non-melanoma skin cancer treatment. Adapted from (Kim et al., 2023)

Patch Type	Therapeutic Agent	Nanosystem	Study description
Polymeric patches	Imiquimod	-	Patches with imiquimod of 4.75, 9.5 and 12.5 mg/cm² released a significantly higher amount of drug compared to Cuprophan® and Aldara®
Polymeric patches	Gold	Gold nanorods (GnRs)	Polymeric films containing gold GnRs for local hyperthermia effectively heated the skin to over 40 °C → Promising candidates for non-invasive hyperthermia treatments against NMSC
DIA	5-FU	-	Patches with 40% triethyl citrate, dibutyl sebacate, or triacetin as a plasticiser achieved adhesive properties, similar to several marketed patches. Controlled 5-FU release
DIA	5-FU and imiquimod	-	Faster release rate for 5-FU, compared to IMQ
Matrix	Imiquimod	NLCs	IMQ-NLC patches significantly enhanced IMQ deposition in deeper skin layers, when compared to a commercial cream

4.13. Hydrogels

Hydrogels are made from hydrophilic polymers and show a crosslinked three-dimensional system that allows them to absorb water. They can be developed from natural materials, such as chitosan, hyaluronic acid, and alginate, or from synthetic materials like

polyethylene glycol, polyvinyl alcohol, sodium polyacrylate, and their copolymers (Kim et al., 2023). In some cases, hydrogels are hybrid systems that combine both natural and synthetic components. These materials are highly versatile as drug delivery platforms due to their adjustable physical properties, controllable degradation rates, and capacity to encapsulate a variety of drugs (Kim et al., 2023).

In the context of NMSC, doxorubicin-loaded hydrogels were developed using natural compounds such as dextran, chitosan, gelatine, and xanthan. These systems were bonded with acrylamide and N,N′-methylenebis (acrylamide) for topical use (Kim et al., 2023). All formulations exhibited swelling and bioadhesion when tested in biological fluids and membranes (Kim et al., 2023). Moreover, doxorubicin was released over a period of at least 50 hours, effectively inhibiting the A431 epidermal cell line, a type of a human cell line derived from epidermoid carcinoma, *in vitro* by disrupting cell division and inducing apoptosis (Kim et al., 2023).

Table 5 is a summary of all the new treatments mentioned above for possible use in the treatment of AK.

Table 5 – Promising new treatments for actinic keratosis.

Technological system	Main Features (Size, Structure)	Drug Release Kinetics	Advantages	Disadvantage	Available for AK	Carried Drug	Ref.
Liposome	Colloidal spheres made of cholesterol and phospholipids (20 nm- 500 nm)	Premature release or targeted release	Easy synthesis, easy uptake by cells	Chemical/physical instability, low drug load, expensive	-	5-FU, T4N5	(Cancer Network, 2001; Desmet et al., 2017; Ewert de Oliveira et al., 2021; Glavas-Dodov et al., 2003; Krishnan & Mitragotri, 2020; Nsairat et al., 2022; Pattni et al., 2015; Raghu et al., 2020; Rahman et al., 2019)
SLN	Solid lipid core with surfactant, (50nm-1000nm)	Burst followed by controlled release	Physical stability, lower dosing frequency	Drug expulsion, limited drug load, drug-lipid interactions	-	5-FU	(Desmet et al., 2017; Dianzani et al., 2021; Ewert de Oliveira et al., 2021; Krishnan & Mitragotri,

Technological system	Main Features (Size, Structure)	Drug Release Kinetics	Advantages	Disadvantage	Available for AK	Carried Drug	Ref.
							2020; Mustfa et al., 2021; Raghu et al., 2020)
NLC	SLN with a mix of solid and liquid lipids (50nm-1000nm)	Burst followed by sustained release	High drug load, physical stability, economical		-	-	(Desmet et al., 2017; Krishnan & Mitragotri, 2020; Subramaniam et al., 2020)
Polymeric Micelles	Nanosized core-shell structures formed by self-assembly of amphiphilic block copolymers (10 to 80 nm)	Controlled release	High drug loading, good solubility for hydrophobic drugs	Limited stability under physiological conditions	-	-	(Dianzani et al., 2021; Krishnan & Mitragotri, 2020; Mustfa et al., 2021; Stern et al., 2017)
Niosomes	Non-ionic surfactant vesicles (10 – 100 nm)	Slow and controlled release	Chemical stability, enhanced penetration, flexibility	Physical instability	-	5-FU	(Desmet et al., 2017; Ewert de Oliveira et al., 2021; Krishnan & Mitragotri, 2020;

Technological system	Main Features (Size, Structure)	Drug Release Kinetics	Advantages	Disadvantage	Available for AK	Carried Drug	Ref.
							Paolino et al., 2008; Priyadarshini K., 2024)
Ethosomes	Soft vesicles composed of phospholipids and high ethanol content (30 nm – few µm)	Slow sustained drug delivery	High drug permeation, enhanced solubility, physical stability	Skin irritation, poor yield, expensive	-	5-FU and ALA	(Desmet et al., 2017; Ewert de Oliveira et al., 2021; Krishnan & Mitragotri, 2020)
Microemulsions	Thermodynamically stable, isotropic mixtures (5 – 100 nm)	Controlled release	Ease of formation, economical	Premature leakage, stability influenced by environment	-	5-FU	(Desmet et al., 2017; Ewert de Oliveira et al., 2021)
Nanoemulsions	Fine oil-in-water dispersions with droplets (20 - 200 nm)	Controlled release	High surface area, good bioavailability	Stability issues, high surfactant content	Yes	ALA	(Krishnan & Mitragotri, 2020; Nastiti et al., 2017; Zhang et al., 2022)
Dendrimers	Highly branched, monodisperse macromolecules (<15 nm)	Controlled release	Precise control of size and function	Expensive to produce, toxicity concerns	-	Immunotherapy and Radioimmunotherapy	(Dianzani et al., 2021; Krishnan & Mitragotri, 2020)

Technological system	Main Features (Size, Structure)	Drug Release Kinetics	Advantages	Disadvantage	Available for AK	Carried Drug	Ref.
Exosomes	Natural vesicles derived from cells (30 – 100 nm)	Sustained drug release	Endogenous carrier, immunologically inert	Complex production, various unresolved issues	-	-	(Desmet et al., 2017)
Nanotubes	Cylindrical nanostructures made of carbon (<100 nm)	Sustained release	High drug loading, unique mechanical properties	Toxicity concerns, complex fabrication	-	-	(Dianzani et al., 2021; Karimi et al., 2015; Negri et al., 2020)
Nanospheres	Solid, colloidal particles (10 – 80 nm)	Controlled release	High drug encapsulation efficiency, controlled release	Limited by drug properties, potential toxicity	-	-	(Dianzani et al., 2021)
Microneedles	Arrays of microscopic needles	Controlled release	Minimally invasive, painless administration	Limited to transdermal applications	-	5-ALA, Imiquimod	(Kim et al., 2023; Yamada & Prow, 2020)
Matrix-type patches	Drug embedded in polymeric matrix	Controlled release	Continuous, controlled release over time	Limited by skin permeability	Yes	5-FU, Imiquimod	(Kim et al., 2023)
Hydrogels	Water-swollen, crosslinked polymer networks	Controlled release	High water content, good biocompatibility	Mechanical weakness, low drug loading	-	Doxorubicin	(Kim et al., 2023)

III. Conclusions and Future Perspectives

Pharmacists, due to their proximity with the population, can significantly contribute to the prevention of AK by advising on the importance of using daily sunscreen, as well as the need to avoid peak UV exposure, especially in elderly people as well as in people with low skin phototypes. Furthermore, in patients with diagnosed AK, community pharmacists should be able to guide and explain the treatment.

The current guidelines for treating AK focus on a combination of destructive therapies and field therapies. Focal therapies such as cryotherapy, curettage, PDT and laser resurfacing offer a targeted lesion destruction. However, these treatments often contemplate the removal of some healthy tissue around the lesions. Even though they are broadly referred in the guidelines, they are invasive, can cause scars, dyspigmentation, pain besides local discomfort.

Field therapies on the other hand, target the eradication of both clinically visible and subclinical AKs within the treatment area, and often regard topical drug delivery. The most common API´s used in AK management are: 5-fluorouracil (5-FU), imiquimod, ingenol mebutate, diclofenac and salicylic acid. There are several advantages concerning topical drug delivery such as its non-invasiveness, the direct access for localized delivery to the disease site, the absence of first-pass metabolism, or other variables related with the gastrointestinal tract; fewer systemic toxicity side effects. All these characteristics ultimately favour patient compliance to treatments. However, overcoming the skin barrier is a major challenge in topical drug delivery, which conditions the efficacy of these delivery systems.

Due to the increasingly high prevalence of AK, there is the need to develop new topical drugs with a superior therapeutic efficacy, but that are able to maintain its safety profile. For this reason, there are 158 clinical trials registered in the clinicaltrial.gov database investigating new topical drug products intended for AK treatment. The most common dosage forms regard gel formulations, followed by creams and then ointments. On the other hand, the most common API´s being tested for AK treatment is ingenol mebutate, followed by imiquimod and 5-FU. It should also be pointed out that there is one clinical trial evaluating the safety, tolerability and preliminary efficacy of an uncoated nanoparticulate paclitaxel ointment formulation in AK lesions in the face. Even though this is solely one study, it may be indicative of the potential of nanotechnology in topical

drug delivery for AK management. In fact, there is a wide range of new drug delivery systems that seek to overcome the limitations associated with the conventional topical formulations. In this monograph the following systems were briefly described due to their potential in AK management: liposomes, nanoparticles (SLN and NLC), polymeric micelles niosomes, ethosomes, microemulsions, nanoemulsions, microemulsions, dendrimers, exosomes, nanotubes, polymeric micelles and nanospheres, microneedles, Polymeric, Drug-in-Adhesive, and Matrix-Type Patches, and hydrogels. These systems are designed to improve drug stability, enhance skin penetration, provide controlled release and increase the concentration of the active ingredients in the target site.

In hospital and industrial settings, pharmacists contribute to AK treatment by staying updated on evolving clinical research and supporting the development of innovative, patient-friendly drug delivery systems that offer a more effective treatment option. Their position emphasizes how important pharmacists are on raising public health awareness and improving the standard of care for AK patients, bridging current treatment standards with emerging therapeutic innovations.

Figure 24 summarizes all treatments herein described. Ranging from the treatments referred in the guidelines, to the treatments undergoing clinical evaluation, and finally to the new drug delivery systems currently being evaluated in academia / industry.

Figure 24 – Graphical abstract: Actinic keratosis treatment – From current guidelines to promising drug delivery systems

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