UNIVERSIDADE D
COIMBRA

Beatriz da Silva Gomes

Relatórios de Estágio orientados pela Dra. Alexandra Miranda e pelo Dr. João Maia e Monografia intitulada "Nanocarriers-based topical treatments for skin cancer: focus on cutaneous melanoma" orientada pela Professora Doutora Filipa Mascarenhas Melo referentes à Unidade Curricular "Estágio", apresentados à Faculdade de Farmácia da Universidade de Coimbra para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Fevereiro 2023

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Fevereiro 2023

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Coimbra, 31 de janeiro de 2023.

Beatriz da Silva Gomes

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PARTE I

Relatório de Estágio em Indústria Farmacêutica

Departamento de Assuntos Regulamentares

Laboratórios Basi - Indústria Farmacêutica S.A.

Sob orientação da Dra. Alexandra Miranda

Lista de Abreviaturas

AIM - Autorizações de Introdução no Mercado

AR - Assuntos Regulamentares

CPP - *Certificate of Pharmaceutical Product*

CTD - Documento Técnico Comum

FFUC - Faculdade de Farmácia da Universidade de Coimbra

FI - Folheto informativo

IF - Indústria Farmacêutica

MICF - Mestrado Integrado em Ciências Farmacêuticas

ORIMED - *Online Regulatory Information MEDicines*

QIS - *Quality information summary*

QOS - *Quality Overall Summary*

QRD - *Quality Review of Documents*

RCM - Resumo das Características do Medicamento

SWOT - *Strenghts, Weakness, Opportunities and Threats*

1. Introdução

"O Farmacêutico é um profissional de saúde com competências para executar todas as tarefas que respeitam ao medicamento e outras tecnologias de saúde"[1], desempenhando um papel ativo nas seguintes áreas profissionais: Investigação científica, Assuntos Regulamentares, Distribuição Farmacêutica, Ensino Farmacêutico, Análises Clínicas e Genética Humana, Indústria Farmacêutica (IF), Distribuição, Farmácia Comunitária e Farmácia Hospitalar.[2] Contudo, com o vasto conhecimento teórico e prático adquirido ao longo do curso de Ciências Farmacêuticas, o Farmacêutico pode também enveredar por áreas como Análises Não Clínicas (Toxicológicas, Hidrológicas e Bromatológicas), Marketing Farmacêutico e em âmbito de outros produtos de saúde (por exemplo, cosméticos, suplementos alimentares e dispositivos médicos). Além do estágio curricular em Farmácia Comunitária ou Farmácia Hospitalar, os estudantes do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC) podem realizar outro estágio curricular numa das áreas profissionais do setor farmacêutico.

Podendo aproveitar esta oportunidade, decidi concluir o meu percurso académico com um estágio em IF, nomeadamente nos Laboratórios Basi – Indústria Farmacêutica S.A., em Mortágua. Esta decisão foi fruto do interesse que as seguintes unidades curriculares suscitaram em mim "Tecnologia Farmacêutica (I, II e III)", "Assuntos Regulamentares do Medicamento" e "Gestão de Processos Regulamentares". Deste modo, realizei um estágio curricular no departamento de Assuntos Regulamentares, sob orientação da Dra. Alexandra Miranda, durante 3 meses perfazendo um total de 420 horas.

O presente relatório de estágio apresenta-se por meio de uma análise *SWOT* (do inglês *Strenghts, Weakness, Opportunities and Threats*), através do qual é feita uma exposição e consequentemente análise crítica e subjetiva da minha experiência nos Laboratórios Basi.

2. Laboratórios Basi - Indústria Farmacêutica S.A.

Os Laboratórios Basi são uma empresa farmacêutica, fundada em 1956 em Coimbra, de capitais privados exclusivamente nacionais, com foco no desenvolvimento, fabrico e comercialização de diversos produtos farmacêuticos. Em 2007, os Laboratórios Basi passaram sinergicamente a integrar o Grupo Farmacêutico *The Future of Health Care* - FHC, proporcionando assim o crescimento da empresa além-fronteiras, tendo atualmente relações comerciais com mais de 60 países.[3]

Desde que os Laboratórios Basi se sediaram em Mortágua, as suas instalações têm vindo a sofrer alterações e subsequente expansão, havendo atualmente duas unidades fabris: uma dedicada ao desenvolvimento e fabrico de formas farmacêuticas líquidas e semisólidas e outra, mais recente, dedicada ao fabrico de formulações parentéricas, de grande e pequeno volume.[3] A criação destas duas unidades possibilitou o crescimento de um portefólio que conta com mais de 240 produtos farmacêuticos de 17 áreas terapêuticas diferentes, sob diversas formas farmacêuticas.[4]

2.1. Assuntos Regulamentares

A função do departamento de Assuntos Regulamentares (AR) é assegurar a conformidade com os requisitos legais e regulamentares exigidos pelas autoridades de saúde, no que diz respeito aos processos de desenvolvimento, registo, acesso ao mercado, informação e apoio aos profissionais de saúde, bem como na monitorização da utilização dos medicamentos.[2]

Nos Laboratórios Basi, o departamento de AR é constituído por uma equipa multidisciplinar, estando dividida pelas seguintes secções: Registos, Renovações, Alterações de Autorizações de Introdução no Mercado (AIM), Departamento Médico (*Medical Affairs*), Transferências de Fabrico (*Manufacture Transfers*), Acesso ao Mercado e Gestão de Produto.

3. Análise *SWOT*

Forças
Strenghts

- Integração na empresa;
- Competências pessoais;
- Conhecimentos adquiridos num percurso académico diferenciado.

Fraquezas
Weaknesses

- Sequenciação de tarefas.

Oportunidades
Opportunities

- Interligação com outros; departamentos da IF;
- Variabilidade de tarefas.

Ameaças
Threats

- Formações internas;
- Complexidade do sistema documental;
- Duração de estágio;
- Estágio não creditado.

3.1. Forças (*Strenghts*)

3.1.1. Integração na empresa

Gostaria de salientar a preocupação dos Laboratórios Basi relativamente às condições de trabalho dos seus colaboradores, pois, para além de facultarem equipamentos necessários para o desempenho das suas funções, possuem excelentes instalações de trabalho e de lazer, estando incluído um refeitório.

No primeiro dia de estágio, após uma apresentação institucional por parte dos recursos humanos, os estagiários foram apresentados e alocados às equipas dos respetivos departamentos, no meu caso o departamento de AR. Nesse momento fui apresentada à minha orientadora de estágio, a Dra. Alexandra Miranda, que me deu a conhecer a todos os colaboradores do departamento, bem como à diretora do departamento de Investigação, Desenvolvimento e Inovação, a Dra. Catarina Cardoso. A amabilidade, simpatia, espírito de entreajuda, partilha de conhecimentos e disponibilidade da equipa facilitou a minha integração e consequentemente a execução de um estágio inigualável.

Destaco também a integração dos estagiários no recente projeto mensal, *Lab Meeting*, no qual são feitas exposições do trabalho desenvolvido por colaboradores do departamento de Investigação, Desenvolvimento e Inovação. Este projeto permite a transmissão do conhecimento sobre o que tem vindo a ser feito neste departamento da empresa e proporciona um espaço de cooperação e união entre colaboradores de diferentes setores com um objetivo comum, a melhoria contínua.

3.1.2. Competências pessoais

Toda a documentação inerente à atividade dos AR encontram-se na língua universal, o inglês, à exceção de documentos nacionais que são obrigatórios estarem apresentados na língua-mãe. Para a preparação da documentação são requeridas ferramentas informáticas, como o *Microsoft Word* e o *Microsoft Excel*. Deste modo, através deste estágio pude fortalecer competências digitais, linguísticas e profissionais, onde se enquadra o trabalho em equipa, pensamento crítico e proatividade.

3.1.3. Conhecimentos adquiridos num percurso académico diferenciado

Os conhecimentos adquiridos ao longo do MICF abrangem áreas distintas da área farmacêutica. No entanto, o curso lecionado na FFUC disponibiliza unidades curriculares opcionais que proporcionam formações distintas aos seus estudantes.[5]

No meu percurso académico optei pela realização da unidade curricular "Gestão de Processos Regulamentares", lecionada pela Dra. Catarina Cardoso que, tal como mencionado anteriormente, é também Diretora do departamento de Investigação, Desenvolvimento e Inovação nos Laboratórios Basi. A realização desta unidade curricular, por intermédio de casos práticos, deu-me a possibilidade de conhecer a legislação regulamentar do medicamento na União Europeia, contactar com Autoridades Competentes com o intuito de obter mais informações quanto a pedidos de *Certificates of Pharmaceutical Product* (CPPs) e ainda identificar e corrigir erros nos Folhetos informativos (FIs) e Resumos das Características do Medicamento (RCMs), complementando e consolidando conhecimentos adquiridos em unidades curriculares obrigatórias direcionadas para esta área profissional, mais concretamente "Assuntos Regulamentares do Medicamento". Estas competências favoreceram uma rápida aprendizagem e destreza no desempenho de grande parte das tarefas por mim realizadas no decorrer do estágio.

Por outro lado, a realização de formações extracurriculares, nomeadamente um curso de *Microsoft Excel,* facilitou a realização de tarefas que requereram domínio no seu uso.

3.2. Fraquezas (*Weaknesses*)

3.2.1. Sequenciação de tarefas

O departamento de AR recebe vários pedidos de elementos por parte das Autoridades Competentes e/ou clientes, pelo que tem de estabelecer prioridades para a execução das respostas, consoante os prazos a cumprir. O plano de estágio, definido pela minha orientadora, tinha como principal objetivo a execução de tarefas que estivessem envolvidas nas diferentes fases regulamentares do ciclo de vida de um medicamento, permitindo que eu contactasse com cada subsecção do departamento de AR.

Em consequência do número elevado de solicitações inesperadas, o volume de trabalho deste departamento muitas das vezes não pode ser programado. Como tal, a ordem das tarefas que executei não seguiu uma ordem cronológica dos eventos regulamentares (desde um registo até à renovação e/ou alterações à AIM), dependendo sempre do que em cada momento era mais prioritário e/ou moroso. Por este motivo senti dificuldades na compreensão do propósito de algumas tarefas. De qualquer modo, a equipa apresentou sempre disponibilidade para esclarecer as minhas dúvidas e facilitar a localização da tarefa nos eventos regulamentares.

3.3. Oportunidades (*Opportunities*)

3.3.1. Interligação com outros departamentos da IF

O departamento de AR encontra-se situado no *Open Space* da empresa, uma área de trabalho comum a outros departamentos. O trabalho efetuado em AR é interligado e é dependente da ação de outros departamentos, como por exemplo Controlo de Qualidade, Garantia da Qualidade, Gestão de Produto, *Marketing* e *Market Access.* Como tal, a proximidade física de diferentes departamentos permite otimizar a execução dessas tarefas.

Apesar de não ter contactado pessoalmente com elementos de outros departamentos na execução das minhas tarefas, o posicionamento do departamento ao qual fiquei alocada permitiu-me conhecer as atividades realizadas noutros departamentos e quais eram fulcrais para o trabalho desenvolvido no departamento de AR.

3.3.2. Variabilidade de tarefas

O departamento de AR desempenha atividades regulamentares tanto pré-AIM como pós-AIM. Como tal, é responsável pela preparação e gestão de toda a documentação necessária para a submissão e aprovação de novas AIMs de medicamentos, bem como pelas renovações de AIM e alterações aos termos das AIMs. Como tal, as funções que desempenhei ao longo do estágio passaram maioritariamente pelo contacto com documentação regulamentar relacionada com as diversas fases do ciclo do medicamento (pré- e pós-AIM), tendo sido, por isso, um estágio enriquecido com tarefas bastante diversificadas.

Mais especificamente, neste estágio tive a oportunidade de:

- Preencher formulários tanto para novos pedidos de AIM como para alterações de AIM;
- Redigir secções de qualidade do Documento Técnico Comum (CTD), tais como *Quality information summary* (QIS) e *Quality Overall Summary* (QOS);
- Efetuar uma renovação de AIM na íntegra;
- Acompanhar a submissão de um dossier completo (CTD);
- Contactar com plataformas de diferentes Autoridades Competentes do medicamento da União Europeia, com o intuito de pesquisar RCMs e Relatórios Públicos de Avaliação de medicamentos;
- Elaborar uma secção não clínica do CTD, nomeadamente a parte toxicológica do *Non-Clinical Overview*;
- Elaborar um RCM e um FI de um medicamento;

- Elaborar um material promocional de um medicamento, cumprindo todos os requisitos legais exigidos.

Ao longo do estágio colaborei ainda no processo de implementação da gestão documental regulamentar, tendo auxiliado na migração e organização de toda a informação relacionada com os medicamentos do portefólio Basi (nomeadamente a inserção de nomes, formas farmacêuticas, apresentações, estados das AIMs, preços e comparticipações, etc.) para uma plataforma de base de dados, *Online Regulatory Information MEDicines* (ORIMED).

A realização deste enorme leque de tarefas possibilitou-me adquirir muitos conhecimentos relativamente aos trabalhos desempenhados e constatar a complexidade, exigência e disciplina do setor regulamentar do medicamento.

3.4. Ameaças (*Threats*)

3.4.1. Formações internas

Os Laboratórios Basi recebem os estagiários com um plano de integração que é primeiramente geral e transversal a todos os departamentos da empresa. Este plano inclui uma Formação de Integração, uma formação de Ambiente e Segurança no Trabalho e uma formação de Farmacovigilância. Posteriormente, abrange ainda formações específicas de acordo com as funções que o estagiário ou colaborador irá desempenhar no departamento ao qual ficou alocado. Uma vez que eu estagiei no departamento de AR, contactando assim com as duas plataformas do sistema documental (*Q-Pulse* e ORIMED), recebi formação sobre o funcionamento das mesmas. Estas formações foram uma mais-valia para a realização do meu estágio. Contudo, acredito que teria sido frutífero se houvesse também formações de carácter regulamentar.

Ainda assim, a ausência de formações regulamentares no plano de integração foi contornada pela disponibilização de ferramentas na primeira semana de estágio. Forneceram-se, para leitura, diferentes procedimentos internos, *guidelines* e legislação farmacêutica, que me permitiram adquirir conhecimentos quanto à estrutura do CTD de um dossier de AIM, bem como aos procedimentos de registos, renovações e alterações de AIM. Mais direcionado para o Departamento Médico (*Medical Affairs*), também me foi possível adquirir conhecimentos relativamente à atividade de *Medical Writing,* ao uso de modelos de referência (*Quality Review of Documents,* QRD) na elaboração da informação do produto (RCM, FI e rotulagem) e à atividade da revisão regulamentar da publicidade de medicamentos e de outros produtos de saúde. Após a leitura dos diferentes documentos, foram-me facultados vários exemplares e esclarecidas todas as dúvidas que foram surgindo.

Esta abordagem inicial permitiu-me conhecer logo as diferentes tarefas que são desempenhadas dentro do departamento de AR, facilitando assim a realização das mesmas durante o meu estágio. No entanto, acredito que a existência de formações dinâmicas e práticas ao longo do meu estágio facilitaria ainda mais o processo de aprendizagem, dado que o trabalho nos AR é muito complexo e a relação entre a legislação e processos, na prática, não é muitas vezes evidente.

3.4.2. Complexidade do sistema documental

O sistema documental dos Basi, no qual se armazenam e arquivam todos os documentos internos da empresa, é bastante extenso e complexo, representado por duas plataformas: *Q-Pulse* e ORIMED, atualmente em processo de atualização. Tal complexidade constituiu um obstáculo na execução das minhas tarefas, principalmente no início do estágio, visto que necessitava de aceder frequentemente às plataformas para procurar e reunir informações dos produtos do portefólio, e nem sempre foi fácil, por falta de domínio das mesmas.

Acredito que esta adversidade fosse ultrapassada se o estágio tivesse durado mais tempo e se o processo de transição da informação dos produtos para o ORIMED já estivesse concluído, tal como mencionado anteriormente. Contudo, graças ao apoio constante da equipa do departamento de AR, esta dificuldade foi sendo ultrapassada e superada ao longo do tempo.

3.4.3. Duração de estágio

O estágio curricular no departamento de AR dos Laboratórios Basi teve a duração de, aproximadamente, 3 meses, sendo, portanto, considerado um estágio de curta duração.

No meu ponto de vista, a duração do estágio demonstrou ser insuficiente por dois motivos: o primeiro prende-se com a dificuldade que senti em ambientar-me e contextualizar-me rapidamente com os processos da empresa e a área regulamentar propriamente dita, impedindo-me de executar as tarefas de forma autónoma e meticulosa em grande parte do estágio; o segundo prende-se com o facto do tempo entre a execução das minhas tarefas e a concretização dos projetos ter sido, por vezes, superior à duração do meu estágio, pelo que não tive a oportunidade de acompanhar os processos na íntegra.

3.4.4. Estágio não creditado

A unidade "Estágio Curricular" obedece à Diretiva 2013/55/EU, de 20/11, que refere as alterações efetuadas à Diretiva 2005/36/CE relativamente ao reconhecimento das qualificações profissionais. Segundo esta Diretiva, o título de formação de farmacêutico só é obtido ao fim

de, pelo menos 5 anos, sendo que a última etapa, referente ao estágio decreta a realização de um estágio de 6 meses em farmácia aberta ao público ou num hospital.[5,6]

Como se constata, não existe menção a outras áreas do medicamento, pelo que, caso o aluno pretenda realizar estágio curricular numa Indústria Farmacêutica, tem imperativamente de efetuar, no mínimo, 810 horas de estágio em Farmácia Comunitária, à semelhança dos alunos que apenas pretendem realizar estágio curricular nesta última área.

Contrariamente a outras instituições de ensino, a FFUC tem parcerias com algumas entidades empregadoras, o que permite e facilita o acesso a um segundo estágio, independentemente de este ser (como o caso de Farmácia Hospitalar) ou não creditado.

A não creditação é um fator decisivo na escolha do segundo estágio, visto que acarreta um maior esforço por parte do aluno. Todavia, na minha opinião, a realização de outro tipo de estágio é indubitavelmente uma mais-valia para mercado de trabalho futuro. Em suma, pelos motivos mencionados anteriormente, decidi realizar um segundo estágio não creditado em AR numa Indústria Farmacêutica.

4. Considerações Finais

A IF é um setor do ramo farmacêutico que emprega, para além de farmacêuticos, profissionais de outras áreas. O âmbito da sua atividade abrange investigação, desenvolvimento, fabrico, controlo e comercialização de medicamentos e outros produtos de saúde. Dentro de uma IF, o departamento de AR assegura que todas as etapas do ciclo de vida do medicamento cumprem as exigências regulamentares das Autoridades Competentes. Por este motivo, AR é um departamento essencial, polivalente e interligado aos outros departamentos da IF.

A possibilidade de realizar outro estágio curricular, à exceção de Farmácia Hospitalar, não é transversal a todos os cursos de Ciências Farmacêuticas do país, deste modo, destaco o facto da FFUC providenciar esta oportunidade aos estudantes. Assim sendo, realizei um estágio complementar à minha formação na área regulamentar do medicamento, onde constatei que o estágio curricular que optei por realizar ultrapassou as minhas espectativas e permitiu-me evoluir tanto a nível pessoal como profissional, na certeza que será um fator diferenciador no mercado de trabalho.

Dirijo os meus sinceros agradecimentos à Dra. Alexandra Miranda e a toda a equipa do departamento de AR dos Laboratórios Basi, pelos ensinamentos, rigor e valores transmitidos, que culminaram num estágio curricular único e exímio.

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PARTE II

Relatório de Estágio em Farmácia Comunitária

Farmácia Machado Suc. – Coimbra

Sob orientação do Dr. João Maia

Lista de Abreviaturas

FC - Farmácia Comunitária

FFUC - Faculdade de Farmácia da Universidade de Coimbra

FM - Farmácia Machado

***Glintt* -** *Global Intelligent Technologies*

INFARMED - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

MICF - Mestrado Integrado em Ciências Farmacêuticas

MNSRM - Medicamentos Não Sujeitos a Receita Médica

MSRM - Medicamentos Sujeitos a Receita Médica

SARS-CoV-2 - *Severe acute respiratory syndrome coronavírus*

SI - Sistema informático

SNS - Serviço Nacional de Saúde

***SWOT* -** *Strenghts, Weakness, Opportunities and Threats*

1. Introdução

A Farmácia Comunitária (FC) constitui a estrutura de saúde de primeira linha do Serviço Nacional de Saúde (SNS) pela sua proximidade à comunidade.[1] Dada a panóplia de complicações em saúde, o Farmacêutico Comunitário deve seguir o conceito de Desenvolvimento Profissional Contínuo, incluído na filosofia *Kaizen*, que se pode definir pela atualização e desenvolvimento de conhecimentos, competências e aptidões permanentemente ao longo da sua vida ativa.[2]

As funções de um Farmacêutico Comunitário vão muito além da cedência de medicamentos, nomeadamente a promoção da literacia em saúde e estilos de vida saudáveis, a adesão à terapêutica, a monitorização de parâmetros bioquímicos e fisiológicos, a administração de medicamentos injetáveis, entre outros.

De acordo com o n.º2 do artigo 44º da Diretiva 2013/55/EU 2013 do Parlamento Europeu e do Conselho da União Europeia, é reconhecido o estatuto de Farmacêutico ao fim de cinco anos de formação teórica e de uma formação prática de seis meses em farmácia aberta ao público.[3] Em concordância com esta diretiva, o último semestre do Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC) culmina na execução de um estágio de carácter obrigatório em FC.[4] Por conseguinte, realizei o estágio curricular na Farmácia Machado (FM), sob orientação do Dr. João Maia, durante 4 meses perfazendo um total de 670h.

O presente relatório de estágio apresenta-se por meio de uma análise *SWOT* (do inglês *Strenghts, Weakness, Opportunities and Threats*) culminando na exposição de cinco casos práticos e respetiva análise crítica e subjetiva, que se localizam em Anexo.

2. Farmácia Machado

A FM encontra-se situada na Rua Bernardo Albuquerque, perto da Cruz de Celas e apresenta um horário alargado, estando aberto das 8h às 21h durante a semana e das 9h às 13h ao sábado. Para além deste horário, conforme o mapa de turnos de serviço das farmácias elaborado pela Administração Regional de Saúde, a FM realiza serviço permanente, isto é, durante 24h está disponível para atendimento ao público em casos de urgência.[5]

Fundada em 1917, a FM muda-se em janeiro de 2018 para um espaço vizinho maior, modernizando, expandindo e melhorando a sua imagem. Este novo espaço apresenta uma área de atendimento ao utente com quatro balcões, duas gondolas, prateleiras para exposição de produtos de venda livre e um gabinete. O *back-office* é uma área ampla de arrumação de

Medicamentos Sujeitos a Receita Médica (MSRM) e de outros produtos, contando ainda com um espaço informático e um laboratório para a preparação de manipulados.

A FM insere-se no grupo de Farmácias Portuguesas da Associação Nacional de Farmácias e utiliza o sistema informático (SI) *Sifarma* 2000®, criado pela *Global Intelligent Technologies* (*Glintt*). Este SI é de uso exclusivo a farmácias, assegurando atividades referentes à faturação, gestão de encomendas, dados estatísticos, informações científicas de suporte à dispensa e muito mais. A FM conta ainda com três armazenistas: a Empifarma – Produtos Farmacêuticos, S.A.; a Plural – Cooperativa Farmacêutica e a *Alliance Healthcare*, a fim de adquirir a medicação ou outros produtos farmacêuticos solicitados pelos seus utentes.

3. Análise *SWOT*

Forças
Strenghts

- Integração na equipa;
- Conteúdos programáticos do MICF;
- Competências linguísticas.

Fraquezas
Weaknesses

- Insegurança nos atendimentos;
- Aconselhamento de produtos cosméticos.

Oportunidades
Opportunities

- Localização e Utentes;
- Procedimentos internos;
- Formações;
- Acordo com a Liga Portuguesa contra o Cancro;
- Grupo de Farmácias Portuguesas.

Ameaças
Threats

- Sistema Informático.

3.1. Forças (*Strenghts*)

3.1.1. Integração na equipa

A equipa é constituída por quatro farmacêuticos, Dr. João Maia, Diretor Técnico, Dra. Rita Garrett, Dra. Mariana Lopes e Dr. João Teixeira e por um técnico de farmácia, o Sr. Eduardo Cruz. Denota-se pelo ambiente de entreajuda, simpatia, profissionalismo, dinamismo e respeito que, aliado à juvenilidade da equipa, providenciou uma célere integração na mesma. Destaco ainda a disponibilidade apresentada por todos os colaboradores da FM na explicação dos procedimentos internos, no esclarecimento prontamente de questões, na partilha de

conhecimentos, vivências e valores, bem como no voto de confiança depositado em mim, que contribuíram para um processo de aprendizagem contínuo, enriquecimento e valorização pessoal.

3.1.2. Conteúdos programáticos do MICF

FC é a saída profissional farmacêutica que requere um conhecimento abrangente de várias áreas da saúde, como a Farmacologia, Fitoterapia, Nutrição, Dermocosmética entre outras, sobretudo para a Indicação Farmacêutica. Esta compreende o ato profissional farmacêutico de tomada de decisão sobre a necessidade da toma de Medicamentos Não Sujeitos a Receita Médica (MNSRM) com base na avaliação clínica de um problema de saúde e no historial de patologias do utente.[6] Neste sentido destaco a parte prática do plano de estudos multidisciplinar de MICF da FFUC, em particular os casos clínicos escrupulosamente redigidos pelos docentes, pelo facto de incitarem o raciocínio crítico dos estudantes, a partilha e a discussão de opiniões fundamentadas com o objetivo de solucioná-los. Por ter realizado estes exercícios em diversas unidades curriculares do MICF denotei uma maior capacidade de decisão nos atendimentos que realizei ao longo estágio.

3.1.3. Competências Linguísticas

A localização da farmácia, como será posteriormente mencionado, apresenta proximidade a unidades de saúde, institutos de ensino e zonas habitacionais, por conseguinte alguns utentes da FM são estudantes estrangeiros ou turistas. O número destes utentes aumenta significativamente na altura do verão, que curiosamente coincidiu com o período do meu estágio. Assim, através de vários atendimentos a este grupo de utentes pude colocar em prática a língua universal em contexto profissional, especialmente na identificação de sintomas e na transmissão de informação referente à patologia ou aos medicamentos, nomeadamente a sua indicação, posologia, modo de administração, efeitos adversos, entre outros. O domínio da língua inglesa é imprescindível na grande maioria das áreas profissionais dado os fenómenos decorrentes da globalização. No caso dos profissionais de saúde que trabalhem diretamente com o público, como é o caso do farmacêutico comunitário, esta competência revela-se de extrema importância. Como tal destaco a oportunidade que tive de colocar em prática este idioma no final do meu percurso académico e em contexto profissional.

3.2. Fraquezas (*Weaknesses*)

3.2.1. Insegurança nos atendimentos

FC é a saída profissional que emprega grande parte dos farmacêuticos, por conseguinte os alunos de MICF deveriam ter contacto com esta área durante o curso e não apenas no seu término.[7] Por este motivo, a utilização do SI, o conhecimento de procedimentos internos e a associação dos nomes comerciais aos fármacos constituíram uma profunda lacuna durante os meus primeiros atendimentos ao público, originando alguma incerteza e insegurança na resposta, suscitando desconfiança e dúvida perante o membro estagiário por parte dos utentes. Graças à amabilidade, compreensão e voto de confiança dos utentes fidelizados e dos membros integrantes da equipa da FM estas lacunas foram colmatadas no decorrer do estágio.

3.2.2. Aconselhamento de produtos cosméticos

A "Dermofarmácia e Cosmética" é uma unidade curricular do 4º ano do MICF da FFUC que se denota pelo elevado grau de exigência em virtude dos seus extensos conteúdos programáticos teóricos e práticos. A existência de resolução de casos clínicos como objeto de avaliação constituiu uma mais-valia na indicação farmacêutica de produtos cosméticos. Durante o aconselhamento dermocosmético consoante as necessidades que o utente procurava ou as patologias da pele sabia qual a forma farmacêutica ou quais os constituintes que haveria de procurar nos produtos cosméticos, porém, dado o elevado número de constituintes dos produtos, do elevado número de marcas e de produtos no mercado o processo de escolha era dificultado. Com o objetivo de ultrapassar esta lacuna, assisti regularmente a atendimentos desta área realizados pelos membros da equipa da FM e também recorri às ferramentas de suporte informativo dos produtos cosméticos vendidos na FM. Todavia, teria sido mais benéfico se tivesse tido formações na área da cosmética por parte das marcas durante o estágio.

3.3. Oportunidades (*Opportunities*)

3.3.1. Localização e Utentes

A localização de uma FC é um fator determinante do seu sucesso. A FM encontra-se localizada numa área central de Coimbra com várias unidades de saúde nas redondezas como o Centro Hospitalar e Universitário de Coimbra que inclui, nesta localidade, os Hospitais da Universidade de Coimbra, o Hospital Pediátrico e a Maternidade Bissaya Barreto; o Intituto Português de Oncologia de Coimbra Francisco Gentil; a Unidade de Saúde Familiar da Cruz de Celas e os consultórios médicos privados. Para além disto, esta área da cidade tem muitas

instituições de ensino, público e privado, do ensino básico, secundário e superior, entre os quais a Escola EB1 dos Olivais, EB 2,3 Martim de Freitas, Escola Secundária José Falcão, Instituto Superior Miguel Torga, Escola Superior de Enfermagem de Coimbra e as Faculdades de Farmácia, Medicina e Economia da Universidade de Coimbra. Dada a proximidade a estas entidades, os utentes da FM eram estudantes (portugueses ou estrangeiros), moradores das redondezas ou utentes das unidades de saúde mencionadas abrangendo, assim, diversas faixas etárias, desigualdades financeiras e literárias. Graças à excelente localização da FM deparei-me com diferentes situações clínicas e pude melhorar as minhas competências interpessoais com os utentes, adequando a linguagem ao tipo de utente garantindo que a informação transmitida era facilmente compreendida.

Os utentes fidelizados são uma mais-valia para a farmácia, pelo acesso ao seu historial de compras, personalizando os atendimentos e facilitando o acompanhamento terapêutico. O processo de fidelização resulta da manutenção da relação de confiança com o utente por mérito do trabalho austero e contínuo do farmacêutico. Relativamente a este grupo de utentes, pude compreender em primeira pessoa o papel determinante do farmacêutico neste processo, já que eu, enquanto membro temporário da FM, também tive um papel ativo no mesmo.

3.3.2. Procedimentos internos

Numa primeira fase do estágio, foi me dado a conhecer os procedimentos internos relativamente ao ciclo do medicamento e outros produtos farmacêuticos no interior da FM, sendo que a maioria é realizada no SI *Sifarma* 2000®. A tarefa primordial é a receção das encomendas que consiste na leitura dos produtos das encomendas seguida da confirmação do seu estado inviolado, prazo de validade e o preço, inserindo os dados necessários no SI. No final deste processo confirma-se que o número de unidades e o valor final da fatura apresentados neste SI coincidem com a nota de encomenda, procedendo-se de seguida à arrumação dos produtos nos sítios respetivos. No caso de um produto se encontrar violado, danificado, fora do prazo de validade ou não coincidir com aquele que o utente pretendia procede-se à sua devolução, também através do SI, com criação de uma nota de crédito. Esta etapa permite registar todas as informações de todos os produtos da FM no SI permitindo saber o *stock* da FC em tempo real, recolher dados estatísticos de vendas, ter conhecimento dos produtos com validade mais curta, entre outras funcionalidades. Dos procedimentos internos da FM fazem ainda parte o *VALORMED*, os medicamentos estupefacientes e psicotrópicos e as receitas manuais que passo a citar.

O *VALORMED* é uma entidade portuguesa fundada recentemente por várias empresas do setor farmacêutico responsável pela gestão de resíduos de produtos farmacêuticos. As farmácias comunitárias constituem a base desta cadeia como sendo os locais de recolha dos resíduos, para esse fim existem contentores de cartão.[8] O farmacêutico, ao receber os resíduos por parte dos utentes, tem a responsabilidade de assegurar que apenas os resíduos autorizados são colocados no contentor e que este é enviado para um centro de triagem através de um dos seus distribuidores quando atingir a sua capacidade máxima.

Os estupefacientes e psicotrópicos atuam ao nível do sistema nervoso central produzindo efeitos temporários sobre a perceção, humor, comportamento e consciência, evidenciando inúmeras potencialidades no ramo da medicina, como por exemplo em doenças psiquiátricas ou como terapêutica adjuvante em oncologia. Por apresentarem efeitos temporários, tolerância e dependência representam um dos fármacos mais suscetíveis a tráfico ilícito no mundo. Como tal, necessitam de um controlo redobrado por parte das autoridades competentes, no caso de Portugal este controlo é realizado pela Autoridade Nacional do Medicamento e Produtos de Saúde, I.P. (INFARMED). Neste sentido o farmacêutico é obrigado a recolher dados pessoais do utente e do adquirente que avia a receita, introduzindo-os no SI. No final do atendimento é recolhido um comprovativo das informações relativas à dispensa, procedendo-se ao seu arquivamento.[9,10]

As receitas manuais têm vindo a cair em desuso por causa da sua elevada suscetibilidade a erros e em consequência da evolução tecnológica, porém existem ainda quatro exceções para a prescrição manual, entre as quais a falência informática. O farmacêutico aquando do aparecimento deste tipo de receitas deve assegurar-se que os MSRM cedidos estão corretos em termos de nome, dosagem e número de unidades conferindo que a receita está válida, viabilizando, deste modo, o financiamento da comparticipação desses MSRM pelo estado.[9] Esta comparticipação advém de uma rede nacional de prestação de cuidados de saúde criada pelo estado para todos os utentes do SNS.[11]

Em simultâneo com o trabalho de *back-office* inicial fui assistindo aos atendimentos realizados pela equipa da FM, durante os quais pude colaborar através do levantamento de produtos. Este trabalho permitiu-me consolidar conhecimentos adquiridos na faculdade, familiarizar-me com os produtos da FM e associar fármacos aos nomes comerciais e respetivas embalagens. Gradualmente fui realizando atendimentos com supervisão, obtendo capacidades para os realizar autonomamente.

No decorrer do estágio tive ainda oportunidade de presenciar duas situações insólitas. Uma das situações foi a não conformidade da forma farmacêutica de um MSRM, neste caso a equipa

da FM contactou o seu delegado comercial e aguardou a devolução do dinheiro ou a troca do produto. A outra situação foi o comunicado do INFARMED decretando a suspensão imediata da comercialização e a retirada do mercado nacional dos alimentos ou suplementos contendo monacolina K de arroz vermelho fermentado para alteração da sua rotulagem.[12]

A aquisição do conhecimento destes procedimentos foi indubitavelmente indispensável para a execução do meu estágio, tal como será para a vida profissional, dado que compreendem as bases práticas de um farmacêutico comunitário.

3.3.3. Formações

O farmacêutico deve procurar atualizar os seus conhecimentos em busca de melhoria contínua, filosofia *Kaizen*. Neste sentido a Ordem dos Farmacêuticos demanda que os seus membros efetivos alcancem no mínimo 15 créditos a cada 5 anos, esta creditação pode ser obtida por meio de diversas atividades, entre as quais a execução de formações.[13] A equipa da FM, demonstra interesse nas formações facultativas, visto que é através destas que obtém ou atualizam os seus conhecimentos nas mais variadas áreas da saúde, aprimorando os posteriores atendimentos ao público. A excelente integração de um novo membro na equipa é também demonstrada aqui, uma vez que assim que tinham conhecimento das formações convidaram e incentivaram o membro estagiário a realizá-las.

Durante a pandemia do *severe acute respiratory syndrome coronavírus* (SARS-CoV-2), as formações pararam de ser feitas presencialmente e tiveram de se adaptar ao ensino à distância. Em consequência da maioria das formações não ser transponível para esse método de ensino observou-se uma queda abrupta no seu número, de tal modo que durante o meu estágio apenas tive oportunidade de realizar uma formação da Gameiros Material Clínico, intitulada por "Doença Venosa Crónica e a Terapia Compressiva" na Plural. Através desta formação tive oportunidade de adquirir conhecimentos desta área, sobretudo no que diz respeito às meias de compressão. Recebi ainda duas formações internas, uma da marca *Easyslim* na qual pude conhecer os diferentes planos dietéticos e os produtos que detêm, e uma da marca *Exeltis* referente ao anel vaginal *Ornibel*®.

As formações colmatam lacunas do curso pelo que considero vantajoso ter tido estas oportunidades neste estágio curricular.

3.3.4. Acordo com Liga Portuguesa contra o Cancro

A Liga Portuguesa contra o Cancro é uma instituição de apoio ao doente oncológico e familiares, apoiando financeiramente os doentes mais carenciados relativamente às suas

despesas, entre as quais a sua medicação.[14] A FM celebra um acordo com esta instituição garantindo medicação a custo zero para o utente. De forma a efetivar este acordo o utente precisa de trazer juntamente com a receita uma ficha de referenciação intransmissível, assinada pelo assistente social da associação, com indicação do nome do utente e da(s) receita(s) médica(s) que a instituição apoia financeiramente. No final do atendimento o farmacêutico pede ao utente para assinar a fatura que irá ser arquivada juntamente com a fotocópia da receita e ficha de referenciação. Todos os documentos arquivados são mensalmente enviados à entidade por forma a que a FM receba o valor monetário correspondente à comparticipação que efetuou.

Em virtude deste acordo saliento a oportunidade de contactar proximamente com esta realidade e de conhecer as principais terapêuticas adjuvantes desta patologia, mais concretamente analgésicos estupefacientes, antieméticos ou profilaxia da candidíase oral.

3.3.5. Grupo de Farmácias Portuguesas

A FM integra um grupo de farmácias, denominado por farmácias portuguesas, que apresenta vantagens para os seus utentes. Através da adesão ao "Cartão Saúda", um programa de fidelização na qual os utentes desfrutam de inúmeras vantagens e benefícios em farmácias comunitárias associadas e outras empresas parceiras. Em relação às farmácias comunitárias, o utente adquire pontos na compra de produtos das farmácias que podem ser convertidos em dinheiro deduzível noutra compra ou em produtos do catálogo de pontos elaborado pelo grupo.

Este programa por outro lado também é vantajoso para as farmácias associadas, visto que é um agente potencializador para a fidelidade do utente à farmácia. Por meio deste estágio pude incentivar os utentes da FM a aderir ao programa, aprender a efetivar a sua adesão na plataforma, a associar o cartão à sua ficha de utente e a rebater pontos quer em dinheiro quer em produtos. Esta competência será sem sombra de dúvida importante no futuro profissional visto que uma grande percentagem das farmácias comunitárias é aderente a este programa.

3.4. Ameaças (*Threats*)

3.4.1. Sistema Informático

O SI é a ferramenta basilar do trabalho de uma FC. Neste sentido a FM adotou o SI *Sifarma 2000* ®, como mencionado anteriormente, para o auxílio da grande maioria das tarefas dos seus membros trabalhadores. Este SI agiliza os atendimentos ao público, permitindo o acesso ao historial de um utente com ficha na FM e fornecendo também informações de suporte

científico, processa a faturação dos produtos da FM, apresenta dados estatísticos e financeiros, gere encomendas, notas de devolução e stock, entre outras ferramentas.

A *Glintt*, empresa de consultoria que desenvolveu este SI procedeu ao lançamento de uma versão moderna e aprimorada em 2020. Esta versão, contrariamente à anterior, divide-se em três módulos, um para o atendimento, outro para as encomendas e ainda um clínico, tornando o SI consideravelmente mais rápido, e também apresenta ferramentas novas, permite alocar produtos a diferentes fichas de utentes num só atendimento. Esta ferramenta irá mitigar as fichas conjuntas de diferentes utentes, simplificar a leitura do historial da ficha do utente e, desta forma, aprimorar o acompanhamento terapêutico. Infelizmente, este SI apresenta algumas lacunas que são colmatadas com o SI antigo, designadamente o processo de entrada de encomendas e os atalhos através das teclas.

Por esta razão, e talvez por comodidade, a FM ainda utiliza o SI mais antigo na maioria dos procedimentos, salvo exceção de dois procedimentos que foram recentemente introduzidos devido ao aparecimento da pandemia do vírus SARS-CoV-2 em 2020. Estes dois procedimentos são relativos à emissão do resultado do teste rápido de antigénio para esse vírus, segundo a Circular Informativa Conjunta n.º 006/CD/100.20.200 de 16 de dezembro de 2020, e à dispensa de medicamentos hospitalares na FM, segundo o Despacho n.º 4270-C/2020, de 7 de abril.[15,16]

A implementação do novo SI tem sido uma prática comum na maioria das FCs por conseguinte teria sido benéfico o contacto rotineiro com este SI. De qualquer modo, destaco o esforço da equipa na apresentação minuciosamente do novo SI e dos novos procedimentos do mesmo.

4. Considerações Finais

A FC é a saída profissional vulgarmente associada à atividade farmacêutica, pela sua proximidade à comunidade e por empregar grande parte dos farmacêuticos. O farmacêutico comunitário é o agente de saúde pública mais acessível à comunidade, pelo que deve promover o aumento da literacia em saúde, a adoção de estilos de vida saudáveis e a adesão à terapêutica contribuindo para a redução do agravamento das patologias ou do recurso a unidades de saúde médica. Apresenta ainda outras funções como a indicação farmacêutica, a administração de injetáveis, a avaliação de parâmetros bioquímicos e fisiológicos, entre outros. Face à responsabilidade perante a sociedade, os estudantes de Ciências Farmacêuticas finalizam os seus estudos com a execução obrigatória de um estágio curricular nesta saída profissional.

Considero a realização de estágios uma porta de entrada para o mercado de trabalho pelo conhecimento profissional que se adquire e pela responsabilidade que se assume mesmo apenas por breves momentos. O local de estágio tem um papel indiscutivelmente importante na aquisição destas competências. Por essa razão, considero que a equipa e as parcerias da FM contribuíram fortemente para a minha formação profissional e crescimento pessoal que serão sem sombra de dúvida uma mais-valia num futuro próximo.

Resta-me apenas agradecer uma vez mais a todos os elementos da equipa da FM pelo estágio que me proporcionaram.

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Anexos

Caso Clínico 1 – Eritema da fralda com infeção fúngica por *Candida albicans*

Utente idoso do sexo masculino contactou por via telefónica a farmácia a fim de obter esclarecimentos sobre o/s produto/s farmacêutico/s mais adequado/s para a situação clínica da sua companheira. Iniciou a chamada por uma contextualização da situação clínica, referindo que a sua companheira se encontrava acamada e que, portanto, utilizava diariamente fralda. Sendo que a higienização da zona e a muda da fralda estava ao encargo de uma terceira pessoa. De seguida informou que a companheira aparentava ter eritema da fralda e que atualmente era aplicado uma mistura da pomada *Halibut*® e do creme *Bepanthene*®. Posto isto, e tendo em conta que não havia sinais de melhoria, procurou saber se esse tratamento era o mais indicado para a situação.

Comecei a minha intervenção pela avaliação do grau de gravidade do eritema da fralda através de questões relativas aos sinais e sintomas, mais especificamente se apresentava lesões ou secreções na zona e se experienciava prurido ou dor. Relativamente a estas questões o utente respondeu que a companheira não apresentava esses sinais, porém apresentava esses sintomas. De seguida questionei qual o tipo de creme *Bephantene*® que estava a aplicar, visto que esta marca apresenta diferentes formulações consoante diferentes indicações, sendo que para o eritema da fralda apenas o *Bephantene Baby*® seria indicado. A esta última questão o utente respondeu que se tratava de *Bepanthene* Creme®.

Tendo por base estas informações, concluí que se tratava de um eritema da fralda com infeção fúngica por *Candida albicans*, como tal aconselhei a suspensão imediata do *Bepanthene* Creme® e a introdução de uma pomada reparadora e antifúngica, *Isdin Baby Naturals* Pomada Reparadora *AF*® por 7 dias. Sendo que esta seria alternadamente aplicada com a pomada *Halibut*®. Neste caso a introdução de um antifúngico era imprescindível.

Com o propósito de explicar a minha decisão, primeiro deve saber-se o principal constituinte dos produtos, relativamente ao *Halibut*® é o óxido de zinco, um composto absorvente, antisséptico, cicatrizante e protetor da pele, no caso do *Bepanthene* Creme® é o dexpantenol, um agente condicionador e emoliente. Assim sendo, a associação de ambos é indicada para a cicatrização e regeneração da pele no caso de cortes superficiais, queimaduras ligeiras ou secura da pele, e nunca para o eritema da fralda já que gera um ambiente propício para o desenvolvimento de microrganismos, como se observou neste caso. A decisão de manter a pomada *Halibut*® foi sobretudo pelas suas propriedades absorventes e antissépticas que mitigam as condições necessárias para o crescimento de fungos. Paralelamente à

terapêutica farmacológica recomendei a adoção de medidas não farmacológicas especialmente efetuar uma correta higienização e limpar suavemente a pele lesada, aumentar a frequência da muda da fralda, após a limpeza deixar a pele respirar por breves instantes.

Após este atendimento por telefone o utente deslocou-se à FM para comprar a pomada antifúngica.

Caso Clínico 2 – Cessação abrupta da terapêutica antidepressiva

Utente de 41 anos do sexo masculino demonstrando inquietação aproxima-se do balcão de atendimento e menciona que precisa de um medicamento para tonturas e náuseas.

Os únicos MNSRM disponíveis na FM para esta sintomatologia contém na sua composição o dimenidrinato, um antagonista dos recetores H1 de histamina, contudo apenas são indicados para vómitos, náuseas, tonturas, vertigens ou indisposição provenientes do movimento (viagem), de distúrbios do labirinto ou da doença de Ménière.[17,18] Posto isto, uma vez que nada indicava se tratar de uma destas situações seria incongruente ceder um destes MNSRM.

Em todo o caso, a fim de avaliar a gravidade da situação, procurei saber a duração dos sintomas, ao que o utente me responde que foi desde que parou subitamente de tomar o antidepressivo, sem indicação médica. No seguimento desta resposta, elucidei o utente sobre a terapêutica antidepressiva, salientando que a mesma apresenta um lento início de ação, ou seja, os seus efeitos terapêuticos são visíveis apenas algum tempo após o início do tratamento (semanas a meses), e que a sua suspensão deve ser gradual e segundo indicação médica, com o intuito de mitigar a gravidade dos sintomas de descontinuação.[19] Neste sentido, disse ao utente que deveria consultar um médico para reiniciar a terapêutica ou proceder à substituição do antidepressivo. Informei ainda que os sintomas que apresentava eram autolimitados.

Após estas advertências o utente mencionou que se sente agitado e tem muitas insónias, outros dois sintomas de descontinuação. Para estes sintomas aconselhei a toma de 1 comprimido de *Valdispert*® 125 mg, um MNSRM à base de *Valeriana officinalis*, até 3 vezes ao dia.

Caso Clínico 3 – Dermatite atópica

Num dia quente de verão, uma utente de 28 anos do sexo feminino aproximou-se do balcão de atendimento apresentando pele seca com lesões edematosas e eritematosas dolorosas na face, no pescoço, no peito e nas mãos com a intenção de obter um aconselhamento farmacêutico e porventura tratamento. Ao deparar-me com a situação aterradora questionei se era comum ter este tipo de lesões e se já tinha sido vista ou era acompanhada por um

médico dermatologista. Relativamente a estas questões a utente informou pensa ter tido dermatite atópica durante a infância, mas que até então não tinha tido mais nenhuma exacerbação da doença. Portanto não aplicava produtos específicos nem era acompanhada por um dermatologista. Posto isto era necessário intervir imediatamente.

A dermatite atópica é uma patologia da pele que se caracteriza por estados inflamatórios recorrentes do contacto da pele com um composto alergénico ou da colonização de *Staphylococcus aureus* em consequência das alterações na barreira cutânea. Por este motivo nesta patologia é crucial a aplicação diária de produtos hidratantes e emolientes que restauram e preservam a função barreira da pele reduzindo a recorrência das fases agudas desta patologia. Assim, aconselhei a aplicação dos produtos pela seguinte ordem: óleo lavante *La Roche Posay Lipikar AP+*® 400 mL para lavagem facial e corporal, sérum e creme fluido da linha *Toleriane* da marca *La Roche Posay* para a hidratação profunda da pele, respetivamente *La Roche Posay Toleriane Serum Ultra Derm*® 20 ml e *La Roche Posay Toleriane Dermallergo*® 40 mL. Optei por esta linha de produtos, porque para além de ter na sua composição compostos emolientes não contém compostos irritantes para a pele.

Após a aplicação destes produtos segue-se a proteção solar, como tal questionei se utilizava proteção solar diariamente e uma vez mais a utente referiu que não utilizava. Mencionei que a proteção solar é imprescindível, sobretudo numa pele lesada, dado que os raios solares provocam dano celular potencializando o aparecimento de cancro da pele. Neste caso, uma vez que a pele estava lesionada e seria também alérgica aconselhei um protetor solar mineral em creme, *Lrposay Anthelios Mineral One SPF50+*® 30 ml, para aplicar na zona do rosto, pescoço e peito. Recomendei ainda a toma de 10 mg de cetirizina, um antihistamínico, uma vez por dia à noite para o alívio da dor e de prurido.

Como medidas não farmacológicas ainda referi que deveria de usar água tépida nos banhos, reduzir a frequência e o tempo de duração, limpar a pele sem esfregar a toalha e utilizar roupas leves e, de preferência, de algodão.

Por fim, aconselhei a utente a consultar um médico dermatologista caso não visse melhorias nos 3 dias seguintes ao começo da aplicação destes produtos e posteriormente, se voltassem a aparecer sintomas similares, a procurar um profissional de dermatologia afim de obter um tratamento mais indicado.

Caso Clínico 4 – Dor de barriga

Utente de 23 anos do sexo masculino aproxima-se do balcão de atendimento informando que a sua companheira apresentava dores de barriga e náuseas. Visto que as dores de barriga podem estar associadas a inúmeras patologias convém perceber a localização, a intensidade e a evolução da dor e se existem outros sintomas associados. Por conseguinte questionei se os sintomas tinham aparecido na sequência de uma refeição ou da introdução de um novo medicamento e qual a duração dos mesmos; se a dor era localizada, se sim, em que região da barriga; se estava associado a dismenorreia, ou seja, dores menstruais; se tinha febre, vómitos ou diarreia. O utente esclareceu que a dor era difusa, que não tinha febre e que desconfiava ser consequência do *stress* causado pela época de exames.

Assim sendo, a situação clínica pareceu ser mais simples do que receava, portanto aconselhei para as dores abdominais a toma de 1 comprimido de *Buscopam Compositum N*® (10 mg brometo de butilescopolamina e 500 mg de paracetamol) de 8h em 8h horas durante 3 dias consecutivos, podendo espaçar as doses consoante os sintomas. Se, entretanto, aparecesse febre ou a intensidade da dor aumentasse deveria consultar o médico, bem como, se ao fim dos 3 dias não visse melhorias.

Associado a esta terapêutica aconselhei, para o alívio de sintomas ligeiros de *stress* a toma de 1 comprimido de *Valdispert Stress*®*,* um MNSRM à base de plantas (200 mg de extrato de *Valeriana officinalis* e 68 mg de extrato de *Humulus lupulus L.*) até 3 vezes ao dia.

Caso Clínico 5 – Obstipação

Utente de 40 anos do sexo feminino aproxima-se do balcão e informa que tem diverticulose do intestino grosso e não tem conseguido defecar há 6 dias, mesmo após a toma de contínua *Laevolac*®, medicamento contendo 10 mg de lactulose, ou da aplicação de glicerina retal em supositórios. Ambos os medicamentos são laxantes osmóticos, isto é, aumentam a retenção de água nos intestinos amolecendo as fezes facilitando a evacuação, sendo que no caso dos supositórios apresentam também ação irritante local. A patologia desta utente facilmente pode ocasionar diverticulite, caracterizada pela inflamação (associada ou não a infeção) dos divertículos, que são bolsas que se desenvolvem na parede enfraquecida do intestino grosso, produzindo dores abdominais. Esta inflamação pode aparecer em sequência do aprisionamento das fezes no local, dado que pode lesar os divertículos e potencializar a proliferação bacteriana.

Posto isto, comecei por perguntar qual a frequência da purgação, se costuma tomar laxantes e se tinha dores. A utente informou que costumava purgar de 2 em 2 dias, que tomava frequentemente *Laevolac*® e que não sentia dores, apenas mal-estar abdominal e sensação de

barriga inchada. O laxante que a utente indica tomar com frequência apresenta como efeitos adversos os sintomas que a mesma apresenta, melhor dizendo, dores abdominais e o aumento de gases. Além disto provoca desidratação se for usado recorrentemente o que poderá ser uma das razões pelas quais tem tido dificuldade em defecar.

Por conseguinte mencionei que os laxantes apenas devem ser utilizados em casos de urgência e que, dada a sua condição patológica, deveria adotar medidas não farmacológicas, entre as quais o aumento da ingestão de líquidos e de fibras, a reeducação do intestino e a prática regular de exercício físico.

Deste modo, e tendo em conta a necessidade urgente de defecar da utente dada a sua patologia, aconselhei a toma de 1 comprimido de *Advancis Easylax Forte*® à noite sem associação de mais nenhum laxante. Este laxante de contacto à base de plantas (sene, cáscara-sagrada o ruibarbo) insere-se no grupo de laxantes mais eficazes, causando cólicas abdominais mais marcantes. Portanto alertei a utente sobre este efeito secundário e informei que procederia à sua suspensão assim que conseguisse defecar não ultrapassando 7 dias de toma consecutivos. Aconselhei também a utente a consultar o médico a fim de saber qual o laxante mais indicado para a sua patologia em situações de urgência.

PARTE III

Monografia

Nanocarriers-based topical treatments for skin cancer: focus on cutaneous melanoma

Sob orientação da Professora Doutora Filipa Mascarenhas Melo

Abbreviations

2D - two-dimensional

3D - three-dimensional

5-FU - 5-fluorouracil

ADMET - absorption, distribution, metabolism, excretion, and toxicity

AgNP - Silver nanoparticle

AK - Actinic keratosis

ALA - 5-aminolevulinic acid

anti-PD1 - Anti-programmed cell death protein 1

APT - Aptamer

AuNP - Gold nanoparticle

BCC - Basal cell carcinoma

CHOL - Cholesterol

CQA - Critical quality attribute

cSCC - Cutaneous squamous cell carcinoma

CUR - Curcumin

CV - Cell viability

DE - Dermis

EGCG - (-)-epigallocatechin-3-gallate

EMA - European Medicines Agency

ET - Ethosome

FDA - Food and Drug Administration

FTIR - Fourier transform infrared spectroscopy

GCO - Global cancer observatory

HSPC - Hydrogenated soybean phosphatidylcholine

IMQ - Imiquimod

INC - Inorganic-based nanocarrier

IT - Imunoterapeutic

K-TRP-2 - Tyrosine-related protein-2

LNC - Lipid-based nanocarrier

LNP - Lipid nanoparticle

LP - Liposome

LPHNP - Lipid-polymer hybrid nanoparticle

MAL - Methylaminolevulinate

MSC - Melanoma skin cancer

MSNP - Mesoporous silica nanoparticle

NC - Nanocarrier

NE - Nanoemulsion

NIR - Near-infrared

NLC - Nanostructured lipid carrier

NMSC - Non-melanoma skin cancer

O/W - Oil-in-water

PDT - Photodynamic therapy

PEG - Polyethylene glycol

PEV - Pegylated liposome

PL - Phospholipid

PLA - Polylactic acid

PLGA - Polylactic glycolic acid

PNC - Polymeric-based nanocarrier

PNM - Polymeric nanomicelle

PNP - Polymeric nanoparticle

PS - Photosensitizer

PTT - Photothermal therapy

PVP - Polyvinylpyrrolidone

QbD - Quality by Design

ROS - Reactive oxygen species

RT - Radiation therapy

SC - *Stratum corneum*

siRNA - Small interfering ribonucleic acid

SkC - Skin Cancer

SLN - Solid lipid nanoparticle

TEWL - Transepidermal water loss

UV - Ultraviolet

VE - Viable epidermis

VEDE - Viable epidermis and dermis

W/O - Water-in-oil

W/O/W - Water-in-oil-in-water

Abstract

Skin cancer is a heterogeneous, sudden, and silent disease that affects the skin, causing symptoms and visible skin lesions only in advanced stages. It can be divided into two main groups, melanoma and nonmelanoma skin cancers. Traditional therapies for skin cancer include surgical excision or other invasive therapies combined with chemotherapy or radiotherapy, which have numerous systemic side effects and high recurrence rates. To overcome this drawback, researchers are looking for other ways to deliver drugs with fewer side effects, as in the case of topical treatment, whose main challenge, however, is the impenetrable upper layer of the skin, the *stratum corneum*. Therefore, nanocarriers appear here as an important nanotechnology tool to improve drug penetration into the skin, increase targeted drug delivery to the tumor, significantly reduce side effects, and improve clinical outcomes. On this basis, there is a growing interest in the research and development of nanocarriers, as evidenced by the increasing number and diversity. In this review, scientific articles on the use of nanocarriers for the topical treatment of skin cancer were collected and classified into three general groups according to the nature of their main composition, namely lipids, polymers, and inorganic substances. Despite the promising results of the presented nanocarriers and considering that some are already available on the market, there is an urgent need for investment in the development of manufacturing methods, as well as better toxicological and regulatory evaluation, since the conventional methods that are currently used make production and regulation much more time-consuming and expensive than for conventional products.

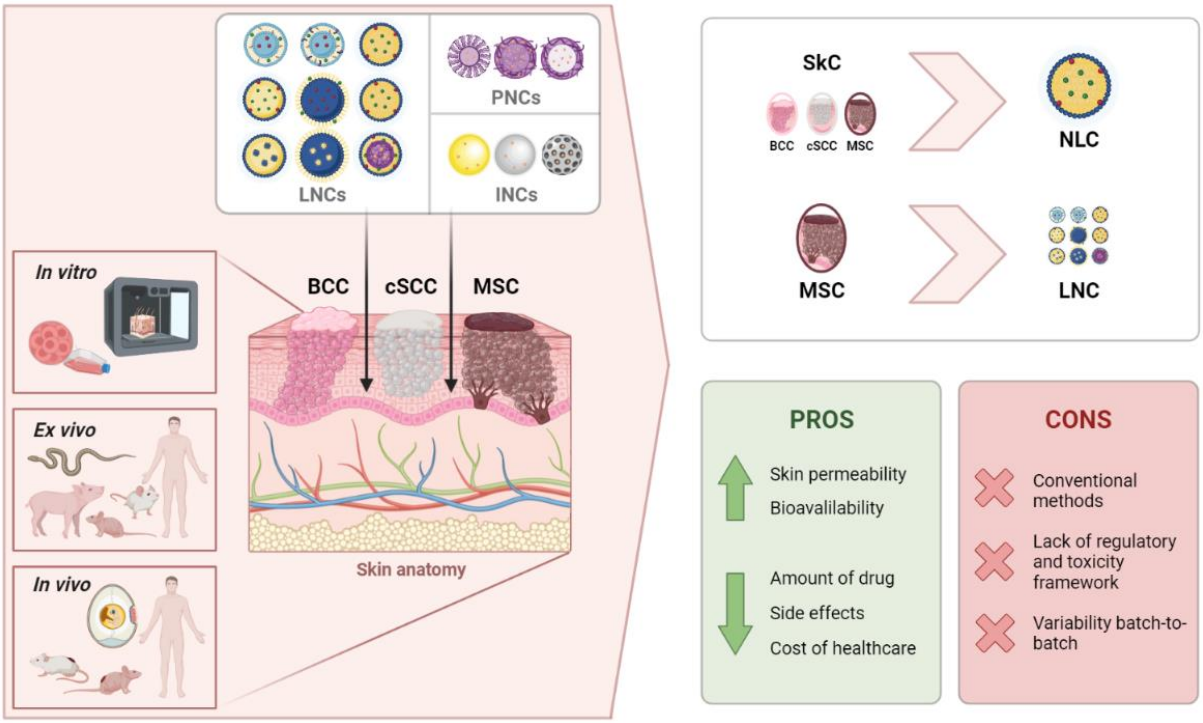
Keywords: Skin cancer, Melanoma, Nanocarrier, Topical administration, Nanotoxicity, Scale-up, Regulatory issues.

Resumo

O cancro cutâneo é uma doença heterogénea, súbita e silenciosa que afeta a pele, causando sintomas e lesões cutâneas visíveis apenas em fases avançadas. Pode ser dividido em dois grupos principais, melanoma e cancros de pele não-melanoma. As terapias tradicionais para o cancro cutâneo incluem a excisão ou outras terapias invasivas associadas a quimioterapia ou radioterapia, que têm numerosos efeitos secundários sistémicos e altas taxas de recidiva. Para ultrapassar estes inconvenientes, procura-se outras formas de administrar medicamentos com menos efeitos secundários, como o tratamento tópico, cujo principal desafio, no entanto, é a camada superior impenetrável da pele, o *stratum corneum*. Por conseguinte, os *nanocarriers* aparecem aqui como uma importante ferramenta nanotecnológica para melhorar a penetração dos fármacos na pele, aumentar a condução dos fármacos ao tumor, reduzir significativamente os efeitos secundários e melhorar os resultados clínicos. Nesta sentido, observa-se um crescente interesse na investigação e desenvolvimento de *nanocarriers*, como evidenciado pelo crescente número e diversidade dos mesmos. Nesta revisão sistemática, artigos científicos sobre a utilização de *nanocarriers* para o tratamento tópico do cancro de pele foram recolhidos e classificados em três grupos gerais de acordo com a natureza da sua composição principal, nomeadamente lípidos, polímeros e substâncias inorgânicas. Apesar dos resultados promissores dos *nanocarriers* e da existência de alguns no mercado, existe uma necessidade urgente de investimento no desenvolvimento de métodos de fabrico, bem como numa melhor avaliação toxicológica e regulamentar, uma vez que os métodos convencionais atualmente utilizados tornam a produção e regulamentação muito mais demorada e dispendiosa do que para os produtos convencionais.

Palavras-chave: Cancro cutâneo, Melanoma, Nanossistema, Administração tópica, Nanotoxicidade, Transposição de escala , Assuntos regulamentares.

Graphical abstract



1. Introduction

Cells are the fundamental units of life, their sets result in tissues, which in turn, culminate in organs consequently forming systems that shape a living being. As an open thermodynamic system with self-regulating capabilities, they are constantly ingesting matter and generating energy, that is central to all activities of the cell, such as metabolism, movement, proliferation, differentiation, and apoptosis, that fulfills the needs of the organism. In case of disorder on the regulatory mechanisms, depending on the severity, either the cell dye or uses compensatory mechanisms.[1,2] In the latter case although the cell is compatible with life, it will never be the same, therefore it can turn into a tumor cell, i.e., a cell whose elementary regulatory mechanisms such as survival, proliferation, and differentiation are abnormal. Since it is a complex process, it is not possible to name a single cause for the appearance of tumor cells. There are agents, called carcinogens, that have been identified in several studies as enhancers of the tumor process, such as radiation, and chemical or biological compounds, but cellular or host susceptibility is also a very important factor in this process.[1] In the case of Skin Cancer (SkC), environmental factors include exposure to ultraviolet (UV) radiation, ionizing radiation and chemical compounds, as in the case of arsenic; host factors include genetic alterations or predispositions, such as fair-skinned and light-eyed people, and autoimmune diseases like Human Immunodeficiency Virus or Human Papilloma Virus.[1,3–7] As with any cancer, reducing exposure to controllable risk factors can reduce the risk of disease.[6] Unlike normal cells, tumor cells can: i) constantly proliferate and create cultures of high cell density thanks to the abnormal production of growth factors, that stimulate cell division; ii) form new blood vessels (angiogenesis), also due to secreted growth factors; iii) invade adjacent normal tissues and form metastasis because of their low expression of surface adhesion molecules, results in low cell adhesion and morphological changes (most of them are rounder than normal cells), and because they secrete proteases that digest the extracellular matrix.[1] The mass formed by these cells is called a tumor. It can be classified according to the severity and the type of tumor cells. As for the severity, they can be benign or malignant. The benign tumor does not invade the surrounding normal tissues or distant parts of the body, it remains confined to the original site and therefore can be removed by surgery. In contrast, a malignant tumor, also known as cancer, can spread to other systems through the bloodstream and cause metastasis, making it more difficult to treat. Depending on the cells from each tumor arises, they can be divided into broad groups in the order of their prevalence: carcinomas (epithelial cells), leukemias (hematopoietic cells), lymphomas (cells of the immune system) and sarcomas (connective tissue - muscle, bone, cartilage and fibrous tissue) within which the tumor can be classified

according to the tissue of origin and by extension being stage 0, I and II for localized tumor, III for regional and IV for distant metastases.[1,8] SkC belongs to the group of carcinomas, which account for 90% of human cancers.[1]

Skin is the biggest organ of the human body and consists of three main layers: viable epidermis (VE), dermis (DE) and hypodermis. The uppermost layer of the skin, the VE, is a stratified squamous epithelium with several sublayers that are distinguished by the differentiation of keratinocytes, which occur in this order: basal layer, spinous layer, granular layer and *stratum corneum* (SC).[9] In the SC, the outermost sublayer of the VE, keratinocytes are called corneocytes because they have lost the nucleus and changed shape.[10] The inner sublayer bordering the DE is called the basal layer, where melanocytes are located and mitosis of the keratinocytes begins. As mentioned above, SkC is a carcinoma that originates in epithelial cells of the skin such as corneocytes, keratinocytes, melanocytes, Langerhans cells, Merkel cells, and others localized in the VE.[9] There are two main types of SkC, melanoma skin cancer (MSC) and non-melanoma skin cancer (NMSC).[6] The charts on the Global Cancer Observatory (GCO) website (available in Annex I and II) show that the incidence of SkC is increasing over the years with NMSCs being the most predominant while MSC, although less common, is the most lethal because is highly prone to metastasis[4,11,12]

NMSCs, also called keratinocyte carcinomas because they arise from keratinocyte cells, are common in light-eyed and fair-skinned people with chronic sun exposure, but may also occur in association with other diseases such as xeroderma pigmentosum or genetic predisposition. NMSCs include two types of cancer, basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC). Based on the literature BCC is the most common type.[10–16] BCC induced by UV radiation, the most common cause, leads to activation of Hh or mutations in the TP53 tumor protein gene of keratinocyte progenitor cells, stem cells, localized in the basal layer of the VE and in hair follicles, resulting in mutagenesis.[17,18] BCC can be divided into several subtypes: nodular, superficial, infiltrative, fibroepithelial, infundibulocystic, micronodular, basosquamous, and morphoeic, with varying appearance and aggressiveness, the last three subtypes being the most severe.[19] Diagnosis is made by dermoscopy, and depending on the subtype, telangiectasias, bluish-grey lumps, multiple erosions or ulcerations may be observed. Superficial and basosquamous subtypes can be confused with cSCC, so immunohistochemical markers can also be used.[18] As for cSCC, it arises from pre-existing skin damage on the squamous cells, cells with spiny projections, located above the basal layer, on the spinous layer.[20,21] It occurs mainly as a result of genetic mutations caused by exposure to UV radiation, such as the TP53 tumor protein gene, RAS, the cyclin-dependent kinase inhibitor 2A -

CDKN2A, NOTCH1 and NOTCH2, which are responsible for the production of proteins involved in cell division or external cell signaling. The TP53, RAS, and CDKN2A genes are thought to play a role in the early stages of the disease, as in the case of Bowen's disease also known as Actinic keratosis (AK), which are considered stage 0 or *in situ* cSCC. Several types of cSCC differ in location in the body, appearance and metastatic risk. The lowest risk cSCCs are keratoacanthoma and verrucous carcinomas, whose appearance presents small glomerular and stripped vessels, and the highest risk/invasive cSCC, which includes acantholyti and adenosquamous carcinomas, where serpiginous vessels can be seen at diagnosis by dermoscopy.[22–26] Regarding conventional treatment, surgical excision is the first choice for both NMSCs. For low-risk BCC, especially facial tumors, cryotherapy or laser ablation can be performed as an alternative to surgery. As an additional therapy for both cancers, topical treatment such as 5% 5-fluorouracil (5-FU) or 5% imiquimod (IMQ) creams or photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) or methyl 5-amino-4-oxopentanoate as a photosensitizer (PS) can be used. In more severe cases, radiation therapy (RT) or chemotherapy may be used, the latter being the last option for patients who have not responded to previous therapies or whose disease has progressed. There are also systemic therapies for severe cases too, such as Hh inhibitors (sonidegib and vismodegib) for BCC and anti-programmed cell death protein 1 (anti-PD1) (cemiplimab) and epidermal growth receptor factor inhibitor (cetuximab) for cSCC. Except for anti-PD1, all of these therapies have significant side effects that often cause patients to discontinue therapy. There is a high risk of recurrence when these therapies are used alone, because they usually have difficulty reaching the deeper parts of the tumor, resulting in low efficacy. Therefore, a combination is desirable.[18,23,27,28]

Melanoma is a type of cancer that originates from melanocytes, the dendritic cells responsible for melanogenesis, the production and distribution of melanin.[29–31] Since melanocytes are found in many tissues, melanoma is named for the location of the melanocytes that cause cancer, such as cutaneous melanoma (MSC), mucosal melanoma, and uveal melanoma.[31,32] MSC is the most common and well-studied melanoma, which is why the term melanoma usually refers to this type. MSC as the name implies, develops in the skin, more specifically at the proximal dermo-epidermal junction of the VE, where melanocytes are located. The high mortality rate of MSC results from the fact that it is a highly proliferative and metastatic disease that rapidly spreads systemically.[33] It can be further divided into superficial melanoma (most common), lentigo melanoma, desmoplastic melanoma, spitz melanoma and acral melanoma. They differed in terms of epidemiology, clinic and

histopathology, genotypes (genetic alterations on the BRAF gene, NRAS gene, NF1 gene, etc.), type of the skin (whether it is glabrous or not), localization of the skin and origin (whether it arises from chronic sun exposure, recognizable by the transition of cytosine to thymine bases).[30–33] Regarding conventional treatment, the gold standard is also surgical excision. If this isn't possible, RT can be used, which occurs mainly in lentigo malignant melanoma. For this type of cancer, topical IMQ is often used in addition to RT. As adjuvant therapy for stages above III, interferon-alpha, as an immunotherapeutic (IT), was the first choice thanks to its high therapeutic effect, but due to its considerable toxicity, it has been replaced by other systemic therapies such as anti-PD-1 (nivolumab or pembrolizumab), BRAF inhibitor (vemurafenib), or BRAF/MEK inhibitors (dabrafenib/trametinib), the latter two being specifically suitable for patients with BRAF-mutated MSC.[32–34] For tumors larger than 1.5 mm and at a high risk of progressing to metastases, IT is used with nivolumab or pembrolizumab or a combination of dabrafenib and trametinib (for BRAF-mutated MSC). For metastases that cannot be submitted to surgery, destructive therapies, such as cryotherapy, laser therapy or electrochemotherapy, or systemic therapies, such as IT (intralesional or topical) with talimogen, laherparepvec or interleukin 2 can be used. In addition to these therapies, chemotherapeutic agents such as paclitaxel are essential and should be used when IT or targeted therapies cannot be used.[33,34] Figure 1 (see Annex III) is an illustration of the skin structure with the three SkC types, showing in which skin layer the respective SkC type thrives.

These conventional systemic cancer therapies (injectable or oral) have various toxic side effects because they are poorly targeted and therefore require large amounts of drugs to achieve the desired therapeutic effect.[13,35] To overcome this systemic toxicity, the skin is an alternative drug delivery route, namely transdermal administration, which offers great advantages such as non-invasive, no systemic side effects, skin targeting, avoidance of the hepatic first-pass effect, can be self-administration, and higher compliance with a much lower effective dose of the drug.[36–40] Although it is already used in clinical practice, drug delivery is a real challenge because it must penetrate the skin barrier, more specifically the SC. This sublayer contains corneocytes surrounded by lipid material such as triglycerides, fatty acids, cholesterol (CHOL) and ceramides, forming a lipid barrier to the permeability of organisms or xenobiotics.[6,9] Here are several possible strategies to increase drug penetration into the skin, ranging from the use of nanotechnology, such as reducing the size of drugs or incorporating them into a delivery system, to structural changes to the skin.[41]

Nanotechnology is a technological branch interdisciplinary responsible for the development of matter at the nanoscale for several applications in science (biology, chemistry, physics and medicine) and engineering, which are becoming increasingly prominent in research and in the market.[42,43] Nanomedicine is one of the most researched fields in academy and industries, concerned with the production and application of nanoscale molecules and structures in the field of health, particularly in the diagnosis and treatment of diseases, where promising results have been obtained.[44–46] Nanomedicines comprise nanodiagnostics, nanopharmaceuticals, nanotheranostics and nanobiomaterials.[47] Focusing only on the therapeutic side, nanopharmaceuticals can be divided into two main forms, nanoscale pharmaceutical compounds and nanocarriers (NCs) to overcome the limitations of conventional pharmaceuticals, such as their low solubility, permeability, stability and poor bioavailability.[45,46,48] The first group is essentially the downsizing of pharmaceutical compounds by simple manufacturing methods, with the resulting formulation offering them advantages in terms of solubility, permeability and bioavailability. The second group refers to pharmaceutical compound delivery systems capable of holding and transporting pharmaceutical compounds to a specific target, it has even more advantages than the previous group, as it not only reduces the size of the compounds but also ensures protection against degradation, which increases the stability of the pharmaceutical compound and allows controlled release directed it to a specific tissue or cell, which consequently minimizes the effective dose required and the toxic effects.[36,49] The use of this technological approach in the topical application offers advantages over the conventional application, as even lower concentrations of the pharmaceutical compound penetrate deeper into the skin and increase its bioavailability while causing less skin irritation.[37,50] In addition, it can also integrate other types of compounds, such as biotherapeutics or large molecules.[51] The NCs have a variety of types, in terms of size, morphology, composition, charge surface, and their ability to conjugate with certain target agents, among others.[52] Depending on their complexity, they may be more difficult to manufacture, and here lies the main limitation of them, because their final physicochemical properties have an even greater impact on their performance, their production is much more complex and slight changes in the process can affect their biologic performance, efficacy and safety.[51,52]

This review brings together several experimental studies using NCs for the topical treatment of the three types of SkC: BCC, cSCC, and MSC. The prominence of the MSC among the three types is because this type is more severe and therefore more studies were found. One of the main goals of this review is to understand which NCs-based topical

formulations are most appropriate for each type of SkC. Concerns related to nanotoxicity, scale-up, and regulatory issues that nanomedicines face to reach the market are also addressed.

2. Nanocarriers

Nanocarriers (NCs) are nanotechnology-based delivery systems that can entrap one or more drugs by dispersion, adsorption, conjugation, or encapsulation.[45,49] The NCs can be divided into three main groups according to their main composition Lipid-, Polymer-, or Inorganic-based NCs consisting mainly of lipids, polymers, and inorganic substances, respectively. The studies analyzed were divided into the three groups mentioned above: the first group includes liposomes (LPs), ethosomes (ETs), nanoemulsions (NEs), lipid nanoparticles (LNPs) which include solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and lipid-polymer hybrid nanoparticles (LPHNPs); the second group includes polymeric nanomicelles (PNMs) and polymeric nanoparticles (PNPs) comprising polymeric nanocapsules and polymeric nanospheres; and the third group includes the gold nanoparticles (AuNP), silver nanoparticles (AgNPs) and mesoporous silica nanoparticles (MSNPs). Detailed information from the studies, such as the type of NC used, its composition, the SkC applied and the results, can be found in the Table 1 and 2, shown in Annex IV and V, respectively. In each study found, the benefits and limitations of the nanocarrier in question are described and it is mentioned whether it is specific to a particular type of SkC.

2.1. Lipid-based nanocarriers

Lipid-based nanocarriers (LNCs) group has been the most studied because it is the most promising for drug delivery due to its high biocompatibility and high scalability, offering a variety of options for the delivery of hydrophobic drugs and, if the structure has an aqueous domain, also hydrophilic drugs.[53,54] Hydrophobic drugs are very sensitive to environmental properties such as enzymatic reactions, pH and temperature changes, and other factors that may occur in the presence of pathology. The microenvironment of cancer, for example, is complex and characterized by low oxygen availability (hypoxia), low pH (acidosis), and expression of proteases (enzymes), all of which contribute to the instability of these drugs.[50] The use of LNCs reduces drug instability, controls their release, increases their absorption, and improves their targeting, resulting in greater efficacy with fewer side effects.[54] Figure 2 (see Annex VI) shows all LNCs discussed in this section in illustrated images.

2.1.1. Liposomes

Liposomes (LPs) were the first LNCs to appear in 1956. They are inserted into the vesicular systems of LNCs, which means that they have a phospholipid bilayer surrounding the aqueous compartment. The lipid portion of LPs contains natural or synthetic phospholipids (PLs) and CHOL, which provide fluidity and elasticity to the vesicle.[54–56] There is a wide range of PLs that can be selected depending on the desired properties of the LPs such as the size of the vesicles, surface properties, or combining ability with other ingredients.[54] LPs are an amphiphilic system that resembles the cell membrane and is, therefore, more biocompatible and has low immunogenicity. They can also transport both lipophilic and hydrophilic drugs, allowing the transport of a wide range of drugs. On the other hand, the PLs in the composition of LP make the system more susceptible to degradation processes, which generates greater difficulty in controlling the drug release rate compared to other NCs. In addition, if a natural PL is chosen, LP may cause allergic reactions.[10,35,54,55,57] To overcome these limitations, some studies have proposed modifications to conventional liposomes.

In two studies by Ahmed *et al*., two drugs, celecoxib and doxorubicin hydrochloride, were encapsulated in an LP for the treatment of SkC. The optimized formulation of LP contained CHOL, hydrogenated soybean phosphatidylcholine (HSPC) and ammonium sulfate, the latter two of which appear to increase the stability of LP. In the first study, solutions and liposomal preparations of doxorubicin hydrochloride isolated or associated with celecoxib were tested. LP, in contrast to the corresponding solutions, showed sustained release without an initial burst release, which seems to have improved therapeutic efficacy by allowing its absorption through endocytosis. According to the cell studies performed, after the application of different formulations, it was concluded that liposomal formulations are better absorbed by the cancer cells and are more cytotoxic than corresponding solutions, leading to significant inhibition of the proliferation of the tested cells. [55] Cadinoiu *et al*. developed LPs for the treatment of BCC with the drug 5-FU and an active targeting conjugated to the surface, namely an aptamer (APT), which is better than antibodies because it is smaller, more stable, easy to synthesize and manipulate, and has higher targeting recognition. In this case, they used a specific APT, the AS1411-APT, a hydrophilic compound consisting of a specific 26-mer DNA sequence that binds to the nucleolin protein found only in the plasma membranes of cancer cells.[58] In the *in vitro* tests, unlike all other formulations containing the drug (5-FU free, LP and LP gel) the Blank LP did not prove to be irritating to the skin; in terms of cytotoxic effect free 5-FU, showed the highest cytotoxic effect, with the highest number of dead and apoptotic cells and a lower number of preapoptotic cells, while the LPs formulations with 5-FU also decreased cell viability

but with an increase of apoptotic and preaptotic cells. It is also important to highlight that this formulation is safe and biocompatible, which is of particular importance in this type of SkC, as contact with the formulation with blood is inevitable.[59] El-Kayal *et al.* encapsulated (-)-epigallocatechin-3-gallate (EGCG) in penetration enhancer-containing vesicles, i.e. pegylated liposomes (PEV), and it showed good stability, biocompatibility, and increased protection of the drug from photodegradation. In this study, PEV and the other NCs, particularly ET, LP containing ethanol (results of them can be seen in the respective section) and transethosomes, LP containing an edge activator, Tween 80, in addition to ethanol, showed higher intradermal deposition capacity compared with the solution. Although PEV has a lower capacity because is larger and does not contain ethanol in its structure, this NC proved to be more beneficial in the other assays. *In vivo*, PEV showed greater tumor reduction compared to EGCG solution. At the histological level, PEV showed normal skin thickness and architecture with the presence of large hollow cells, indicating the presence of epidermal hyperplasia (a sign of the onset of carcinogenesis). Although the EGCG solution also showed no abnormality in skin structure, the number of these cells was higher and hyperactivity in cell division was observed in the epidermal layer, which could lead to cancer recurrence. In addition, blank PEV showed similar results to the positive control group, indicating that it does not have an inhibitory effect, suggesting safety.[15] The study by Magdy *et al.* follows a repurposing strategy, similar to the study by Barone *et al.* mentioned in the section of NLCs. Although metformin is indicated for diabetes, in this study it was used for cancer treatment. The results of this study were very promising as LP showed a high degree of permeation of almost 70% and had a better cytotoxic effect compared to the drug solution.[60]

Biocompatibility and safety are the main advantages of LP, as shown in the case of the study by Cadinoiu *et al*. that LP showed less than 5% hemolysis at all concentrations and overall time periods and did not cause irritation effects, or in the case of El-Kayal *et al*. that the blank LP had no inhibitory effect. Compared with the free drug, the LPs in these studies showed greater therapeutic efficacy against cancer cells, even the LP developed by Ahmed *et al*. without additional compounds. As shown, the choice of formulation composition is of great importance for properties such as stability using HSPC, a more resistant LP, or targeting by adding APT, further improving overall its performance.[15,55,59,60]

2.1.2. Ethosomes

Ethosomes (ETs) are deemed ethanolic LPs, so they are also a vesicular system with a PL bilayer with CHOL surrounding the aqueous compartment which also can entrap lipophilic and hydrophilic drugs, but additionally contains ethanol in its composition.[38,54,61] Ethanol is an

enhancer that increases the deformability of the vesicle and interacts with the lipid molecules of the lipid bilayer of SC via the alcoholic chains lowering their transition temperatures, resulting in temporary disorganization and liquefaction, improving permeation into the skin.[38,54,62,63] Moreover, due to the negative surface charge of ethanol, they can reduce the size of the vesicles.[64] Despite the advantage of this last aspect, it is also a limitation, since it can cause skin irritation.[7] Because of their properties, ETs have been shown to be well-suited for transporting drugs through the skin, as seen in the studies cited below.

As the article by El-Kayal *et al*., discussed in the previous section, the developed ET showed a threefold higher intradermal deposition compared to LP, which can be explained by the presence of ethanol in the composition; a similar percentage of photodegradation of the drug as the LP, which corresponded to a half of the value degradation from the solution of the drug; however, in terms of stability ET showed significant changes in all parameters, so it was discarded for subsequent assays.[15] Cristiano *et al*. developed an ET containing sulforaphane for SkC treatment. This ET proved stability, showing no signs of aggregation, based on both zeta potential and polydispersity index values, and no signs of creaming and sedimentation, evidenced by the values of back scattering obtained through the Turbiscan Lab® Expert. The permeation and anticancer activity results show that ET penetrates skin four times more and is more cytotoxic than the drug solution at all concentrations and for a longer period of time, which is probably due to the fusion with the cell layer leading to the release of the drug into the cytoplasm.[38]

Three studies with ETs have been conducted specifically for the treatment of MSC. The first study by Lin *et al*. included two active compounds, berberine chloride and evodiamine. ETs have shown better results than the two drugs' solutions, such as achieving greater cytotoxicity to cancer cells, thanks to their ability to penetrate the cell by transmembrane transport, or reaching the basal layer, thanks to their ability to squeeze, allowing passage through the intercellular spaces. Remarkably, in the distribution experiment on human skin, the solution was not even able to deposit the dye in the SC; the dye used was a drug surrogate to observe its localization on the skin. Given the cytotoxic effect and the ability to reach melanocytes in the basal layer, it is reasonable to assume the efficacy of ET for the topical treatment of MSC. [64] In the second study by Peram *et al*., curcumin (CUR) was used free, in solution, and in LP in addition to ET, the latter being the more favorable for all studies. Figure 3A (see Annex VII) shows the actual appearance of the microscopic image of the ET. The comparison with LP was made only in the *ex vivo* assays, i.e., permeation and deposition assays. According to the results, unlike the other formulations, ETs showed a higher cumulative

amount and permeation flux of drug through the skin, and in terms of penetration ability the ETs had a homogeneous distribution in the viable epidermis and dermis (VEDE). In contrast, the solution and liposome were restricted to the uppermost layers of the skin, the SC, while only a lesser extent was detected in the deeper layers of the LP, as shown in Figure 3B. These results can be explained by the smaller size and greater elasticity of ETs. As for the cellular studies, ETs, blank ETs and free CUR were tested on healthy and on cancer cells, and none of the formulations proved to be toxic to healthy cells. However, as can be seen in Figures 3C and 3E, ETs proved to be much more cytotoxic than free CUR in cancer cells due to their sustained release and higher cellular uptake, as demonstrated by the use and visualization of cell internalization of a dye. Given these properties of ETs, it also showed a stronger antiproliferative effect, resulting in a lower number of live cells and a higher number of apoptotic cells than free CUR, shown in Figures 3D and 3E, respectively. In conclusion, these ETs have proven their effectiveness and safety in use. Another advantage is that it can be stored for months at low temperatures without changing its properties.[63] In the third study, Ismail *et al*. developed optimized ETs containing brucine that met the desired standards according to the Quality by Design (QbD) approach and was later incorporated into a gel for application on the skin. The three formulations - brucine suspension, ET and ET gel - were compared in terms of release and skin permeation. In the first assay, ET gel showed the highest sustained release, followed by ET, as the viscosity of the formulations directly affects this parameter. In the second assay, ET without gel was the formulation with the highest permeation, twice the brucine suspension, due to its low viscosity, which is advantageous in this assay. For the cytotoxicity assay, ET gel had the higher cytotoxicity, again due to the sustained release. This reveals that ET gel can be used for the cutaneous treatment of MSC as it is not only effective but also safe. The latter can be confirmed as the blank ET did not show cytotoxicity and since no skin irritation is expected due to the pH value.[61]

Nayak *et al*. developed a green ET for the treatment of NMSC containing two chitosan-coated nanoparticles, one with silver nitrate and one with sericin, with chemotherapeutic effect by increasing the activity of reactive oxygen species (ROS) in cancer cells, while normal cells remain intact, unlike the drug cisplatin. These results are confirmed by the *in vitro* assays performed and by the observation of membrane blebs in cancer cells during the apoptotic process. In cytotoxicity and DNA damage assay, ET, as mentioned above, showed cell specificity with the same percentage of cell viability (CV) of cancer cells as cisplatin, but with higher efficiency in damaging the mitochondrial membrane. In terms of safety, ET has proved its hemocompatibility and non-immunogenicity in the kidney and liver if it reaches the

bloodstream. Overall, the study demonstrates the advantages of using ETs in topical treatment and concludes that they are not only more economical but also more environmentally friendly.[65]

The greater efficacy and skin penetration of ET compared to the non-encapsulated drug and even compared to LP can be demonstrated due to its higher deformability. Regarding stability, two of the six studies found that the developed ETs were stable, while El-Kayal *et al.* found the opposite.[15,38,61,63–65] As mentioned early, the type of PL used in the LNC formulation has an impact on this parameter. Phosphatidylcholine, one of the most abundant PL in biological cell membranes, was used in both LP and ET, and due to its lower transition temperature, it can be concluded that it is more susceptible to degradation and thus reduces the stability of NC.[66,67] However, since ET was more unstable than LP, another property could affect this parameter.[15] If the other study that included phosphatidylcholine in the ET had conducted a stability study, some conclusions could be drawn in this regard.[65] Regardless, ET has been shown to be safe, with good hemocompatibility and non-immunogenicity. Unfortunately, none of the studies tested skin irritation except one study that concluded that ET is probably not irritating based on pH alone.[15,38,61,63–65]

2.1.3. Nanoemulsions

Nanoemulsions (NEs) are nanoscale emulsions with a droplet size of less than 500 nm that is visually transparent or slightly cloudy.[16,68–70] NEs consist of the mixture of two immiscible liquids, oil and water, in the obligatory presence of an emulsifier. The emulsifier should be added until the interfacial forces are overcome, which allows the formation of very small droplets and prevents aggregation and coagulation phenomena, giving kinetic stability to the formulation. When selecting an emulsifier, care must be taken to ensure that it has low toxicity and that its effects on rheological properties are known. Usually, a surfactant or co-surfactant is chosen as the emulsifier, but it can also be a peptide, protein, PL, or polysaccharide.[68] NEs are easily manufactured using low-energy or high-energy processes suitable for scale-up, and also require fewer emulsifiers than microemulsions, which reduces associated skin irritation.[3,16,68,70] The most used NEs are single phase, oil-in-water (O/W) and water-in-oil (W/O), but there are also biphasic, oil-in-water-in-oil and water-in-oil-in-water (W/O/W). In comparison, two-phase NEs provide better protection against drug degradation but are more susceptible to coalescence phenomena.[71,72] NEs can incorporate lipophilic and hydrophilic drugs, are biocompatible, and can easily bind target molecules to their surface, which increases their specificity for cells, have more advantageous properties for topical application such as low viscosity and good rheological properties without creamy or flocculation phenomena, and

also minimize transepidermal water loss (TEWL), which proves their superiority over the application of conventional emulsions.[54,68,70]

Asasutjarit *et al*. prepared an andrographolide-containing O/W NE by microfluidization, a high-energy emulsification technique, which can be scaled up to an industrial level while maintaining the optimized conditions in terms of pressure and number of homogenization cycles determined at the laboratory scale. In *in vitro* assays, NE proved to be more cytotoxic to cancer cells and safer for healthy cells compared to the solution. Thus, NE had a higher selectivity index, justified by its controlled release and the route of cellular uptake that prevents drug efflux, namely endocytosis. Regarding the cytotoxic effect of these formulations on cells, it was found that the number of necrotic cells was higher in the case of the solution, as it results from more violent cytotoxic processes, in contrast to AG-NE, which acts by inducing apoptotic processes. In all experiments, blank NE had no effects, proving its safety. In addition to the therapeutic component, NE also showed efficacy in preventing SkC by inhibiting tyrosinase cell activity, which seems to be an advantage since this formulation can also be used to prevent recurrences.[3] In contrast, Morton *et al*. conducted a phase III clinical trial comparing two formulations already on the market with different PS, Ameluz®, the O/W NE gel with ALA, and Metvix® or Metvixia®, a cream with methylaminolevulinate (MAL), for PDT treatment of non-aggressive BCC, particularly superficial and nodular BCC. Both formulations are already approved for the treatment of AK, with the cream MAL additionally approved for Bowen's disease and non-aggressive BCC, with the latter indication recently approved also for NE gel. This study was conducted to demonstrate the non-inferiority of NE gel to MAL cream, which was successful. Although efficacy, clearance, and recurrence rates varied according to BCC type, the number of lesions and lesion zones overall the results showed that NE gel had the greatest efficacy and the lowest recurrence rate, especially in patients with nodular BCC only, possibly due to higher skin penetration. Treatment-related adverse events for both formulations were application site skin reactions consistent with PDT treatment, such as edema, pruritus, erythema, and pain, therefore it is not possible to say anything about the toxicity of NE.[73]

Cordeiro *et al*. developed a biphasic NE W/O/W type without required organic solvents for the treatment of MSC containing two drugs with different solubilities, sodium diethyldithiocarbamate and 4-nitrochalcone, to improve their bioavailability, stability and permeability. As shown by the cellular assays performed, NE showed rapid internalization by cancer cells as a result of their interaction with cell membrane phospholipids, resulting in cytotoxic effects that were clearly superior to the free drugs in a wider range of

concentrations, while the free drugs had an inhibitory effect only at the highest concentration tested. Another advantage of this NE is that its preparation, a double emulsion together with the melt dispersion method, doesn't require organic solvents.[14]

5-FU is an anticancer drug already on the market for topical use in the form of creams and ointments (e.g., Efudex®, Carac®, or Flonida®) to treat certain skin conditions, such as AK and NMSC. 5-FU is an extremely water-soluble drug. Therefore, it has very poor skin permeability in these conventional formulations, leading to safety concerns related to skin irritation, redness, soreness, crusting, swelling, or bleeding. To improve permeability and safety, several nanotechnology studies have been conducted.[74–76] An example is the study by Ahmad *et al*. in which an optimized W/O NE was developed that exhibited good physicochemical stability and sustained release. In the cytotoxic assay, the drug solution showed no quantifiable inhibitory effect, in contrast to NE, which showed a significant cytotoxic effect in which about 62% of the cells died. Interestingly, different skin models were used for the permeation assay, namely rat, cow and goat skin. The results showed that the permeation of rat skin was higher than the other skins, and NE permeated significantly more compared to the drug solution. According to the literature, cow and goat skin are very similar to human skin and have the anatomical morphology and structure of VEDE. Although these skins lack some dermal structures than human skin, the correlation is better than laboratory animal skin (mice or rats) because these animals have different structures, such as a thin VE and a straight interface between the VEDE. Although animal skin is often weaker than human skin, performing this assay on different animal species may be more predictive of human skin behavior than using only one animal or *in vitro* assays. The authors believe that this NE does not irritate the skin because the choice of surfactants has a great influence on skin irritation, therefore they chose nonionic surfactants because they are safe and were used in small amounts. [76] In an *in vivo* study by Wakabayashi *et al*., a cancer vaccine for the treatment and prevention of MSC was developed based on W/O NE, which contained a peptide antigen, tyrosine-related protein-2 (K-TRP-2). Permeation of the peptide was tested and NE was abundant in SC, especially in the intercellular spaces, acting as a reservoir that allowed K-TRP-2 to reach a greater depth in the skin by continuous release, in contrast to the solution that presented K-TRP-2 only at the skin surface. In terms of antitumor efficacy, NE showed an immunological effect when applied topically, as infiltration of T lymphocytes and migration of Langerhans cells in the lymph node was observed, as well as a significant reduction in tumor growth compared to the non-treated group and a better survival rate and reduction in the number of nodules than when the solution injected subcutaneously. In addition, NE, although not tested, appears to be safe for topical

use, since the ingredients of the formulation are commonly used in pharmaceutical products and cosmetics. [77]

These studies show that NE is more effective than the active compound in free form or in solution in terms of both permeability and cytotoxicity. In addition, we also see its practical application in the clinic with promising results compared to creams on the market. Although none of the studies examined skin application safety, some efforts are evident to ensure some safety by selecting scalable manufacturing methods and minimizing the use of toxic excipients, such as organic solvents.[3,14,73,76,77]

2.1.4. Lipid nanoparticles

Lipid nanoparticles (LNPs) are colloidal systems with a size between 150 and 300 nm, whose lipid core is in the solid or semisolid state surrounded by one or two emulsifiers. These NCs can also include lipophilic and hydrophilic drugs.[78–80] Currently, there are two main types of LNPs: the solid lipid nanoparticles (SLNs) and the nanostructured lipid carriers (NLCs), and another as the newest and possible next generation, the lipid-polymer hybrid nanoparticles (LPHNPs). All types contain a lipid phase, the difference between them is the variation of the core, which in SLNs is completely solid, in NLCs is a mixture of liquid and solid lipids, and finally LPHNPs contain polymeric nanoparticles encased in a lipid layer.[78] Unlike conventional creams or ointments that contain microparticles, the topical application of LNPs has a significant occlusive effect due to their small size, which brings greater resistance to allergic reactions or skin fragility. This also lowers TEWL and reduces corneocyte compaction, allowing the drug to penetrate deeper into the skin.[80] In addition, their fluidity and elasticity provide good rheological properties making them easy to apply. LNPs are more suitable for the encapsulation of lipophilic drugs because lipophobic drugs are adsorbed on the surface and a burst effect can occur. In general, they scale up easily, have a high encapsulation rate, are stable and most of their components are non-toxic and biodegradable.[4,78] LPHNPs have only been around for a short time, so it may be justified that only one study was found in this review.

2.1.4.1. Solid lipid nanoparticles

SLNs are a type of LNP whose core consists of biodegradable lipids in a solid state. In this state, the drugs disperse uniformly, which allows controlled release and protection from degradation. Their preparation is free of organic solvents and is suitable for scale-up, e.g., by high-pressure homogenization or solvent evaporation.[54,81] Despite these advantages, during storage solid lipids may crystallize and change their polymorphism, which may cause the drug

to leak and decrease its charge, increase particle size, and produce aggregation phenomena.[80] Palliyage *et al*. developed an SLN containing nonionic surfactants, including CUR and resveratrol, two polyphenols that are nontoxic. These two aspects could provide more safety for topical application for the treatment of MSC. Normally, this type of NC is stable over long periods of time, but Compritol®, the only solid lipid in the formulation, is prone to polymorphic phenomena, so this could be the cause of the reduction in stability of the formulation, which reaches stability of 2 weeks at 4°C. The permeation assay was performed on the skin of a snake, which is similar to humans in terms of lipid content, and proved that resveratrol reaches the DE. Unfortunately, the release from the SLN depends on the affinity to the main lipid, and since CUR has a higher affinity, it took longer to be released, so it did not reach the same depth. This assay was not performed for other formulations, unlike the others, as in the impedance method assay, which allows accurate evaluation of cellular changes according to the resistance value (parameter directly proportional to it), indicating cell proliferation when it reaches higher values and cell death when it reaches lower values. In this assay, a similar decrease in resistance was observed for both SLN and polyphenol solutions, compared to untreated cancer cells and blank SLN. These results were similar to the results obtained in the confluence assay demonstrating its cytotoxicity, although here we observed barely perceptible better values for SLN compared with polyphenols solution, which can be justified by the reduction in the size, the increased stability, solubility and cellular uptake of the drug.[82] It should be noted that, based on this review selection criteria, only this study could be found for this NC, possibly because it is gradually being replaced by other LNPs. Nevertheless, this study mentioned the problem of polymorphism and there were no significant improvements compared with the drug solution.[82]

2.1.4.2. Nanostructured lipid carriers

NLCs were developed to overcome some limitations of SLNs. In their core, lipids are not only solid but also in a liquid state, which increases stability by overcoming the shortcomings of recrystallization of solid lipids and drug losses during storage.[49,54,57,78,80] They can be divided into three types: imperfect, amorphous and multiple lipids, depending on the composition and method of preparation.[78] Compared to SLN, a larger amount of drug can be encapsulated, and controlled release can be modulated by the composition of the lipid matrix; however, the surfactants used in their preparation can be irritating.[54,78]

Imran *et al*. developed an optimized NLC gel containing two drugs, quercetin and resveratrol. Previously, an LP containing these drugs was developed, but it had several limitations, such as high degradation and low drug loading so, in this study, they decided to

choose this NC to overcome them. Thus, NLC and free drugs were incorporated into the gel and compared in all studies. NLC showed higher permeability in both models, which can be justified by its size that increases surface area and solubility, and also by the disruption of the lipids of the SC, caused by its lipid composition and surfactants, as demonstrated by Fourier transform infrared spectroscopy (FTIR). This increased permeability allowed the NLC gel to extensively penetrate the skin, allowing the drug to reach the VEDE layers, where mainly cancer cells were located, in contrast to the drug gel, which was mainly located in the SC. At the cellular level, NLC showed greater cytotoxicity and more anti-metastatic effect, which is evident from its lower percentage of wound healing, as NLC reduced cell migration into the wound.[57]

As previously stated, 5-FU is a drug with several limitations, so the use of NCs is a solution to overcome them. On this basis, two studies were performed for the topical treatment of SkC with NLCs. The first study by Amasya *et al.* included the drug into LNPs, namely SLNs and NLCs, that were prepared by the high-pressure homogenization method, which is simple, scalable and does not require organic solvents. In this study, a QbD approach was used to select the formulation closest to the defined parameters being selected, and an NLC-type formulation was selected for further assays. In all assays, this NLC was compared with the free drug, and in the release assay also with the commercial cream. The NLC showed some selectivity for healthy cells and proved to be more cytotoxic to cancer cells than the free drug, although contrary to expectations, some of this toxicity was due to the presence of surfactants in the formulation, which was confirmed by the toxicity observed with the blank NLC. In the release assay, NLC and the free drug were incorporated into a hydrophilic gel to facilitate application to the skin. The cream was the fastest, while the NLC-gel was the slowest, which could be due to the different lipophilicity of the matrix, gel and cream. This result proves the benefit of NLC-gel for others, as it leads to an increase in therapeutic effect and a decrease in adverse effects of the drug contained in the formulation. In addition, an approximately eightfold increase in occlusion factor was observed with the NLC gel compared to the gel without NLC, increasing TEWL, which is reflected in the widening tight junctions of corneocytes, allowing deeper penetration of the drug. This was demonstrated in the permeation assay, although the smaller size of NLC may also contribute to this result.[83] In the second study by Iqubal *et al.*, a different manufacturing process was used to produce an NLC that contained resveratrol in addition to the 5-FU, and a different design for optimization, the central composite rotatable design. Then the NLC was placed in a gel and compared with a gel containing both free drugs in all assays. The release, permeation, and cytotoxicity results were similar and with the same

possible causes as in the previous study, but no cell selectivity or safety was tested for this NLC.[5]

In a 2018 study, Iqbal *et al*. developed a formulation of NLC with silymarin that was optimized by the same design as the previous study and incorporated into a gel. Permeation and potential skin irritation were evaluated, and the results showed that the permeation of this NLC was three times higher than that of the drug gel, which was due to the nanosize and structural changes of SC caused by the composition, as demonstrated by FTIR. In the skin irritation assay, NLC did not show irritation to the skin. In a second study conducted in 2019, which focused on evaluating the efficacy of this NLC for the treatment and prevention of SkC, its cytotoxicity to cancer cells was demonstrated. Thanks to its greater penetration and deposition, it showed better results than the drug gel in the other assays, such as a higher increase in antioxidant enzymes (glutathione, superoxide dismutase and catalase) and an anti-inflammatory effect, through the decrease in the number of proinflammatory cytokines, a reduction in edema and a more significant reduction in the burden and the number of tumors.[84,85] Barone *et al.* conducted a study following a repurposing strategy in which simvastatin, a drug indicated for dyslipidemia, was added to an NLC, and then incorporated into a film for topical treatment of MSC because it also has an antitumor effect by blocking the cell cycle. In this study, various permeability enhancers were added to the film and their effects were evaluated in the assays. However, since this is not our aim, the results presented in Annex V refer only to the film without them. In the permeation assay where only NLC was included in the films, good permeation results were obtained and good adhesion to the skin was also observed, meaning that no residue remained when the film was removed. In the tolerance assay, none of the NLC and NLC in films with or without permeation enhancers showed irritation to human skin, based on the erythema index and TEWL values, which were equal to or lower than those of the negative control. In the *in vitro* assay, NLC showed cell selectivity and greater cytotoxicity to cancer cells than the drug alone in the most drug-sensitive MSC cells, Colo-38 cells.[4]

Through these studies, we found that NLCs can overcome some limitations of LPs and SLNs. They showed favorable results in terms of permeation and cytotoxicity. Although their higher permeation is due to the nanosize and disruption of SC, NLCs did not cause skin irritation or edema.[4,5,57,83–85]

2.1.4.3. Lipid-polymer hybrid nanoparticles

Abdi *et al.* developed an LPHNP containing bevacizumab, an anti-angiogenesis agent, for topical therapy of MSC. LPHNP was chosen to overcome the limitations of lipid and polymer NCs by combining both into a single NC, resulting in an NC with higher permeation capacity, drug loading and better drug protection. The permeation of LPHNP was more than 30-fold higher than that of the free drug. At the cytotoxic level, LPHNP also showed higher cytotoxicity than the free drug, and the composition of LPHNP did not affect this result and even showed safety. The same was observed *in vitro* and *in vivo* tubulogenesis and angiogenesis assays, where their inhibition was higher than that of the free drug and significantly higher than in the untreated groups. This can be justified by the rapid release of the drug in large quantities from the NC. Moreover, the drug remains unchanged until released.[86] Although only one LPHNP study has been found in the context of this review, its potential seems evident and could be a strong bet for the near future.

2.2. Polymeric-based nanocarriers

The adverse effects of polymeric-based nanocarriers (PNCs) are not yet well known, so biodegradable and biocompatible polymers should be developed to be more easily approved for clinical practice. These can be of natural or synthetic origin, with the latter being preferred because their chemical composition is known, they have greater similarity from batch to batch, and they have low immunogenicity. Some examples of polymers are polylactic glycolic acid (PLGA), polylactic acid (PLA), polyethylene glycol (PEG), polyaniline, polypyrrole, polydopamine and polyvinylpyrrolidone (PVP).[87] PLGA is most used worldwide because the products resulting from its hydrolysis are biocompatible. The great advantage of the PNC is that it can change the structure to increase compatibility with the drug. There has been an increase in the number of polymeric materials used for drug delivery. However, they are unstable in preclinical studies *in vivo*, so more efforts need to be made so that they can be used in clinical practice. For this reason, it is of utmost importance to choose a method to prepare a PNC stable. The physical encapsulation method has been shown to be effective but is extremely expensive.[88] Figure 4 (see Annex VIII) shows all PNCs discussed in this section in illustrated images.

2.2.1. Polymeric nanomicelles

Polymeric nanomicelles (PNMs) have a corona structure with a size of less than 200 nm, consisting of amphiphilic copolymers, i.e., in their structure, they have hydrophobic and hydrophilic monomers.[89–91] Copolymers can be of di-block, tri-block or ionic type, their

hydrophilic part may consist of PEG, PVP, polytrimethylene carbonate or polyacryloylmorpholine and the hydrophobic part of polyesters such as PLGA or PLA. These copolymers have a very low critical micelle concentration, which proves stability, and can self-form by using low molecular weight surfactants such as amphiphiles. [90,91] Pharmacokinetic properties are determined by the shell, so it can be modified to increase targeting and thus efficacy. Both the shell and the core are capable of entrapping drugs depending on their polarity and they can be placed in an intermediate position. Compared to other NCs, they are smaller, easy to produce and scale-up (even compared to polymeric nanoparticles), and have high efficiency in cell internalization, but low stability *in vivo*, as this type of polymers can interact with biological membranes and change their activities, it is already known that capability to inhibit efflux pumps. [90,91]

Lapteva *et al.* incorporated 0,05% of IMQ into the copolymer methoxy-poly(ethylene glycol)-)-hexyl-substituted lactide PNM. IMQ leads to the stimulation of immunity against cancer cells by binding to Toll-like receptor 7 of antigen-presenting target cells present in the VE, so the goal is to target this layer. The commercial cream, named Aldara®, containing 5% IMQ, had high drug concentrations in all layers, but the drug delivery efficiency was much better with the PNM-gel. The PNM-gel showed good distribution in the VE and upper DE as the commercial cream, but the latter one showed high transdermal permeation, resulting in undesirable deposition of the drug in the lower DE, allowing it to enter the bloodstream. On the other hand, the drug gel showed no transdermal permeation ability, so it only deposited in the SC. Through these results, it is already possible to prove the target capacity of PNM-gel compared with the other formulations, even at IMQ concentration 100 times lower than the commercial cream. In addition, the PNM-gel was shown to reach 12 times half of the maximum effective concentration, making it potentially effective for the topical treatment of SkC.[92] Bano *et al.* incorporated lycopene into a PNM containing the thermosensitive and biocompatible copolymer PNIPAAM-PEG formed from the combination of N-isopropylacrylamide, N-vinyl-2-pyrrolidone and poly (ethylene glycol) monoacrylate. The PNM protects the drug from oxidation and allows sustained release, making the formulation more beneficial than free lycopene in terms of antioxidant (demonstrated in the radical scavenging assay) and cytotoxicity activity. In the *in vivo* assays, even at a lower concentration than free lycopene, PNM showed a stronger chemopreventive effect by decreasing compounds associated with tumorigenesis and the inflammatory process and increasing compounds that cause cell apoptosis and reduce edema, as well as reducing the number and burden of tumors. This can be justified by its ability to deliver the drug to the target site.[93] Wang *et al.*

incorporated a small interfering ribonucleic acid (siRNA) to act on survivin, a protein that inhibits cell apoptosis, into a cationic PNM, poly (β-aminoester), for the treatment of MSC. This siRNA is a hydrophilic and large negatively charged molecule that has been bioengineered and has very low cellular uptake, which in turn leads to a low cytotoxic effect on cancer cells, as shown in this study, and also showed low permeation in the skin, which means that no antitumor effect was observed *in vivo*. In contrast, in the case of PNM, despite some pH-dependent parameters such as size, degradation, and release of siRNA, promising results were obtained in all assays compared with free siRNA. PNM increased cellular uptake with the immediate intracellular release of siRNA due to the pH variation, which significantly increased cytotoxicity and showed a higher percentage of cell apoptosis compared with free siRNA and blank PNM. As for permeation, PNM reached 210 μm, showing that it reaches the DE thanks to its amphipathic properties that allow it to use the transcellular and intercellular pathways, unlike free siRNA. In the *in vivo* assay, PNM increased animal weight and decreased tumor size 10-fold, with apoptotic and necrotic regions, resulting in a 75% decrease in survivin levels. In this assay, blank PNM showed some apoptotic and necrotic effects, but like siRNA, no reduction in survivin levels. Although cationic polymers are associated with high toxicity, the polymers comprising this PNM degrade into nontoxic products. The safety and biocompatibility were demonstrated in the last assay, which showed that the PNM with and without siRNA showed no toxicity in different organs.[94]

The PNM preserve the drug during transport, but its peculiarity of being composed of polymers with different lipophilicities allows it to reach a greater range in the skin. As seen in the above studies, the PNM reached the DE, unlike the free drug, which only reached the upper layers. Compared to the commercial product, it was more efficient and safer because it has a lower concentration of the drug in its composition, but still showed a high concentration of the drug in all layers of the skin.[92–94] Despite some advantages, more studies are needed to conclude about its viability for SkC approaches.

2.2.2. Polymeric nanoparticles

Polymeric nanoparticles (PNPs) are particles with a size between 100 and 300 nm, which can be divided into two main groups: nanocapsules and nanospheres. Nanocapsules, a reservoir system, represent a vesicular structure in which the solid polymeric shell surrounds a lipidic or non-lipidic liquid core containing the drugs. The release of the drug depends on the rate of biodegradation of the polymer.[95–97] In nanospheres, a matrix system, the entire solid spherical structure is made of polymers, and the drugs may be entrapped inside or on the surface of the structure. The release of the drug depends on the diffusion of the drug

through the polymer matrix.[95–97] The production of PNP is a spontaneous and autonomous process, its control and the choice of polymer determine the behavior of the resulting PNP. PNPs are easy to design and produce with a wide range of structural options, are biocompatible, and can be targeted to specific cells by coupling design monomers or polymers to the surface of the PNPs.[96,97]

Ferreira *et al*. conducted two studies on a poly (ε-caprolactone)-based PNP containing diphenyl diselenide for the treatment of MSC. In the older study, these PNP were developed, characterized physicochemically, tested the drug stability in PNP and an *in vitro* assay was performed. PNP showed high drug stability due to its encapsulation and protection from photodegradation, as its nanoscale size scatters radiation. In both studies, PNP showed cell selectivity concerning cell assays, as it exhibited high cytotoxicity to all cancer cells tested and low cytotoxicity to healthy cells, unlike the free drug, which exhibited high cytotoxicity to both cells. Note that blank-PNP did not exhibit cytotoxicity. In the most recent study, both formulations were incorporated into a gel for application to human skin and the results were compared. It was found that the drug was more localized in the superficial layers, perhaps due to its lipophilicity, and only in the DE layer did PNP have a higher drug content than the free drug. This suggests that the nanoscale size or larger surface area increases the residence time and provides the drug for a longer period of time.[39,98] Casarini *et al*. developed a PNP containing the flavonoid phloretin for topical therapy of MSC. The polymer used was the same as in the previous study, a biodegradable, biocompatible and bioadhesive polymer, properties that give this NC an even greater advantage. In the cell assays, blank-PNP and PNP were found to be safe and cell-selective, respectively, as they did not significantly reduce the viability of healthy cells. In cancer cells, PNP showed greater efficacy than the free drug in reducing cell proliferation through senescence, as shown by nuclear analysis. As for skin assays, the PNP showed stronger adhesion to the skin (less washability), which in turn resulted in a higher drug concentration than the free drug in all layers, except for DE, where this difference was not statistically significant. However, this was not only due to the adhesion factor but also to the nanosize and its reservoir effect, which led to deeper penetration into the skin appendages.[99] Md *et al*. developed an optimized PNP based on PLGA copolymers containing α-mangostin, which was then incorporated into a gel. PLGA is a biodegradable polymer whose compounds formed during degradation are nontoxic. This formulation were then compared to the gel containing the free drug in all assays performed. Both gels had a pH of 6.72, so skin toxicity is not expected. In the permeation assay, the PNP gel showed a higher amount of drug in all skin layers, including the DE, and also proved a higher penetration than the gel containing the free

drug, thanks to its size, higher drug stability, control of drug release from the formulation and good compatibility with the skin. In addition, this nanoformulation also proved to be more beneficial in terms of cytotoxicity to cancer cells and antioxidant activity, which was still, albeit slightly, higher than that of the vitamin E analog -Trolox.[100] Rata *et al*. developed poly (N-vinylpyrrolidone-*alt*-itaconic anhydride) copolymer-based PNP that was functionalized with APT and incorporated 5-FU for topical treatment of BCC. The permeation assay was conducted in the *ex vivo* model and the results showed that PNP had higher permeability than the free drug. In cell assays, both PNP and the free drug decreased cell viability and increased dead, apoptotic and preapoptotic cells. The safety of PNP was demonstrated both in the hemolysis assay, as the value obtained was well below 1%, and in the potential skin irritation assay, as the percentage of CV was above 50%. In the *in vivo* study, the PNP proved to be more beneficial as it did not cause changes to the skin and also activated the immune system, unlike the free drug which caused local toxicity by showing cellular infiltration in the dermis and edema in addition to changes in the epidermis. Once again, the safety and efficacy of the use of the NC were confirmed, in addition to the immunological advantage of coupling APT to the NC surface.[101] The choice of polymer is an important step because it can influence the toxicity of PNCs. In these studies, the selected polymers were biodegradable, biocompatible, or appeared to be non-toxic. The resulting PNPs proved that encapsulation of drugs protects them from degradation, allows more drugs to reach the site of action and serves as a drug reservoir. Since PNPs are nanoscale, they also provide better adhesion to the skin, better permeation, and longer residence time. Finally, the association with immunostimulants appears to further enhance the efficacy of this NC by activating the immune system to combat tumor growth in a complementary manner without associated toxicological effects.[39,98–101] Unfortunately, the studies described above on the use of these NCs do not distinguish or provide information that allows inferences about the type of PNPs used, preventing a more assertive conclusion about the specific use of nanocapsules and nanospheres.

2.3. Inorganic-based nanocarriers

Inorganic-based nanocarriers (INC) are characterized by high stability, low degradation rate, adjustable size, easy fabrication, excellent physicochemical and optical properties, and surface functionalization by adding functional groups or ligands that make them more biocompatible.[102–104] INCs contain in their composition inorganic compounds, like silica, graphene or metals like silver, gold, copper, titanium, and zinc, among others.[102,105] Metallic nanoparticles can be used for therapeutic and diagnostic purposes, but their biodegradation and elimination need further studies. These include gold nanoparticles and metal oxides such

as iron oxide, manganese dioxide, calcium carbonate, and others.[106] INCs can be used for both diagnosis (sensing or imaging) and treatment, even simultaneously as theranostics. The area of most interest and commonly used for the application of INCs is PDT. PDT is a cancer therapy that involves irradiating PS with visible light, with near-infrared radiation (NIR) in the presence of molecular oxygen for the generation of ROS.[102,107] Photothermal therapy (PTT) like PDT is a noninvasive and localized phototherapy that uses light radiation, particularly in the NIR range, to stimulate PS to kill cancer cells. PTT was the first therapy to appear and, in this case, the excitation of PS leads to the generation of heat. Both heat and ROS lead to cancer cell death at the periphery of PS.[104,107] Nevertheless, their use in clinical practice has been rather limited because they accumulate in large amounts in the organs of the reticuloendothelial system causing toxicity there. In the last decade, this limitation has been overcome by the development of biodegradable or easily eliminable INCs.[106] Figure 5 (see Annex IX) shows all INCs discussed in this section in illustrated images.

2.3.1. Gold nanoparticles

Gold nanoparticles (AuNPs) have antifungal and antibacterial properties, are wound healing, and provide flexibility to the skin, which is why they are usually used in cosmetic anti-aging formulations.[103] The presence of gold, a high atomic number element, allows AuNPs to be used in medicine as radiosensitizers or contrast agents in medical imaging. In addition, AuNPs have optical properties, namely the plasmon resonance effect, which means that their electrons present on the surface are excited upon exposure to radiation photons, resulting in the generation of heat and ROS. As such, they can be used in PDT with NIR radiation because the absorption wavelength of gold is in this range, which provides more safety for healthy cells in the periphery.[102] Among all INCs, AuNP is considered the most advantageous because it has low toxicity, is easy to synthesize and its physicochemical properties such as size and shape can be modified and easily functionalized, preventing aggregation phenomena, increasing its stability, and improving its target.[102–104]

Safwat *et al.* developed and optimized an AuNP surrounded by cetyltrimethylammonium bromide (CTAB), a toxic cationic surfactant that imparts a positive charge to the AuNP, increases its stability, establishes ionic bonds with 5-FU, increases the drug loading capacity and improves its interaction with the skin. The charge of the drug depends on the pH of the environment, which affects ionic bonding and thus release from the AuNP. The release is faster in acidic environments and delayed when the AuNP is contained in a cream or gel. At a pH of 11.5, it showed excellent stability for four months at both 4°C and room temperature, as shown in Figure 6A (see Annex X). In the permeation assay, the gel and cream AuNPs were

compared with their respective free drug forms, which also contained CTAB. Figure 6B shows that the AuNP formulations exhibited higher permeation due to their size, charge, and high lipophilicity caused by ionic bonding. In tumor models *in vivo*, it was again observed that AuNP formulations showed a more rapid effect in reducing tumor size compared to the free drug, i.e., they were more effective in inhibiting tumor growth, as shown by the reduction in tumor weight and the reduction in histopathological features, such as the cancer cells infiltration in the VEDE, shown in Figure 6C and 6D, respectively. In addition, positive effects on animal weight were observed in contrast to the free drug.[108]

2.3.2. Silver nanoparticles

Silver nanoparticles (AgNPs) have antimicrobial activity, are ease to synthesize and functionalize, and also have optical properties with high efficiency in converting light to heat, so they can be used on PTT and have the same medical applications as AuNPs.[104,105,109,110] Since these NCs tend to aggregate, a coating with hydrophilic molecules is required to give them stability.[104] AgNPs are already on the market to increase the stability of cosmetic formulations and also for the treatment of burns and wounds as they stimulate cells involved in the skin regeneration process.[103,109] AgNPs have anticancer activity because their silver ions alter cell homeostasis and lead to the formation of ROS in mitochondria, even under hypoxia. Their major limitation is their toxicity to healthy cells and their accumulation outside the target area, especially in the spleen and liver, although their effects are somewhat less severe than those of conventional drugs only because of their nanosized. Therefore, their use requires local application in cancer tissues or their functionalization to limit their toxic effects.[109,110]

Amatya *et al*. developed an AgNP for PTT, which was coated with bovine serum albumin as a hydrophilic molecule to prevent aggregation that is biodegradable, biocompatible and nontoxic. In this context, this study demonstrated the ability to generate heat proportional to silver concentration or radiation power. AgNPs were found to be significantly more cytotoxic than silver cations, and no cytotoxicity was observed in cells exposed to radiation only or to AgNPs only. Cytotoxicity was observed mainly when AgNPs were exposed to radiation that could lead to the generation of 45°C or more since PTT is only effective above this temperature. In the *in vivo* tests, the increase in skin temperature was observed only at the site where the gel was applied where mild burn marks were also observed. Here, the efficacy of AgNPs was tested at 40°C and 50°C, and only at the latter temperature was there a reduction in cancer cells (demonstrated in histological analysis) and volume, with the tumor virtually ablated with only one application. In this study, it was not possible to determine cell selectivity or off-target toxicity of AgNPs, but when they were administered systemically to

determine their toxicity, the animal was dead after 24 hours. Therefore, caution should be exercised with successive administrations as accumulation may occur leading to adverse toxic effects.[110]

2.3.3. Silica nanoparticles

Silica is a silicon oxide that is a semiconductor, a nontoxic material, translucent to radiation, and its degradation generates silicic acid, a biocompatible product that is excreted in the urine.[102] Amorphous silica is the more degradable form of silica there is present on silica nanoparticles. These nanoparticles are biocompatible, biodegradable and inert to the photophysical and photochemical processes of PDT or bioimaging so they can be used for these proposals.[102,106,111,112] They can be divided into two main groups: nonporous and porous silica, with MSNP belonging to the last group and being the most common form of silica nanoparticles.[112] MSNP corresponds to polymerized silica in honeycomb form, which has hollow channels where the drug (regardless of its lipophilicity) is normally bound. The advantage of MSNP is their high encapsulation capacity, good stability, inertness, easy functionalization and changeable structure and channels, the latter in terms of morphology, diameter and number. Because of their complex matrix, their degradation is more complicated and not yet fully known. [102,112,113] Nevertheless, MSNPs are of toxicological concern because they have silanol groups on the surface that can interact with the cell membrane and lead to death; therefore, long-term toxicity studies are needed.[112]

Ghazaeian *et al.* developed a silica nanocomplex with porous membranes (SNPM) containing curcumin, which is not only an anticancer drug but can also be used as PS for PDT, as demonstrated in this study by its ability to generate ROS, for the treatment of MSC. The efficacy of this NC was demonstrated only in the presence of radiation with selectivity for cancer cells. Here, neither the blank MSNP nor the radiation used had any effect on the outcome. MSNP caused apoptosis of cancer cells and reduced their ability to form colonies. Its cytotoxic effect was shown to be superior to that of free PS even at a much lower concentration. In addition, MSNP has been shown to be hemocompatible, indicating some safety.[114] Clemente *et al*. performed two studies on an amine-functionalized MSNP containing a PS, verteporfin, to be used for PDT of MSC. The *in vitro* assays demonstrated the ability of this MSNP to produce ROS and its efficacy in inhibiting cancer cells upon irradiation. Also, in this last assay, it was found that both MSNP without irradiation and blank MSNP, irradiated or not, showed a decrease in CV. This must be because they are easily taken up by cells and hardly metabolized. In the *in vivo* assays, the anti-tumor growth effect was tested only upon irradiation, with the same results as in the *in vitro* study of the two MSNPs, where glycerol,

the vehicle, had no effect. Here, the inhibitory effect on angiogenesis, lymphoangiogenesis and the number of lung metastasis was also studied because, as mentioned earlier, MSC is more likely to metastasize than other cancers because they can grow and develop new blood and lymphatic vessels to reach other parts of the body. Through these assays, the higher inhibitory effect of MSNP was demonstrated in contrast to blank MSNP and the vehicle. In the case of lymphoangiogenesis and lung metastasis blank MSNP also had some inhibitory effect through the aforementioned cytotoxic effect.[115,116]

Based on these few studies, it is not possible to draw definitive conclusions about the efficacy and toxicity of each type of NC, but overall the results are very encouraging, especially about efficacy against cancer cells. And in the studies in which they were compared with the free active compound, they proved to be better. Regarding toxicity, it should be noted that in some studies care was taken to coat the NC with a more biodegradable compound to reduce potential toxicity.[108,110,114–116]

3. Nanotoxicity

Nanomedicine is the area of nanotechnology with the greatest interest, especially in oncology, an increasingly prevalent disease with high morbidity and lethality, accounting for 35% of the nanoscale pharmaceutical market.[43,45,46,52,67] Despite the above-mentioned advantages of these products, market access is often hampered by the occurrence of adverse effects in the first phase of clinical trials, which is thus the phase with the highest failure rate of about 90%, implying a high financial loss.[43,45,46,52,67] This is mainly due to the lack of targeted investment in the toxicological aspect of nanomedicine, known as nanotoxicology.[42,43,45,46,52] Toxicity is the result of factors related to the formulation, route of administration, and frequency of exposure and its evaluation is called toxicity assessment.[42,117] Currently, there are no specific regulations for nanotoxicity assessment of nanopharmaceuticals, so it is performed using conventional analytical methods of toxicity assessment, which include *in vivo*, *in vitro,* and more recently *in silico* studies, i.e., computerized studies whose main objective is to predict the harmful effects of the developed pharmaceutical on living beings and the environment.[42,43,117–119]

In vivo studies are the gold standard of toxicity assessment because they evaluate pharmacodynamic behavior in terms of absorption, distribution, metabolism, excretion, and toxicity (ADMET) and tissue or physiological changes; these studies include assessment of apoptosis, inflammation, and structural tissue changes, but are financially expensive and results depend on the type of species studied.[43,46] Currently, all research studies conducted on animals

are governed by an ethical principle, the 3Rs principle, which includes the three principles of Reduction, Refinement and Replacement and aims to reduce the number of animals used, refine experimental protocols to reduce animal distress, and prioritize alternative studies such as *ex vivo*, *in vitro*, or *in silico* studies.[43,117,119] *In vitro* studies evaluate the behavior of the developed pharmaceutical in a cell culture that can be set up in two-dimensional (2D) with stem cells or in three-dimensional (3D) with tissue engineering, the latter being more advantageous because it overcomes the saturation processes that occur in the top-a-top system (2D) and it is more similar to *in vivo* studies. They include the following studies - cell uptake, hemolysis, oxidative stress, immune response, chemoattractant, and cytotoxicity. In general, *in vitro* studies are commonly performed because they are the cheapest, easiest, and fastest. However, the results depend on the cell culture protocol and do not allow the determination of toxicokinetic parameters, so the results obtained are not considered a complete substitute for *in vivo* studies.[43,117,120] For this reason, other studies have been developed, such as *in silico* studies, which include several computational studies that analyze the pharmaceutical and predict its efficacy and toxicity, such as quantitative structure-activity relationships, read-across, structural alerts, and others, some of which can predict the ADMET profile, they can also be applied in the development phase of the pharmaceutical, unlike the other studies. *In silico* studies are financially accessible and can be performed quickly, but because they have only recently been applied, there is still uncertainty about their applicability and reliability.[43,46,117–119,121]

Some of the toxicological studies were performed in the experimental studies mentioned above, with the exception of *in silico* studies. As an *in vitro* toxicological assay, red blood cells were used as a 2D model in the Cadinoiu *et al.*, Nayak *et al.*, Rata *et al.* and Ghazaeian *et al.* studies. This model and SkinEthic™ reconstructed human epidermis tissues as a 3D model were used in the studies of Cadinoiu *et al.* and Rata *et al.* studies.[58,59,65,101,114] In this last study, although not related to toxicological tests, this 3D skin model did not show similar results in the permeation test to the *ex vivo* model, the skin of a chicken, showing that the transfer to animal models is not 100% reliable and that in these situations, as the authors explain, other models must be used to ensure that the results obtained are viable.[101] In the *in vivo* toxicological assays, Imran *et al.*, Iqbal *et al.*, Rata *et al.*, and Amatya *et al.* used mice as a model, and Morton *et al.* and Barone *et al.* used humans to evaluate the adverse effects on the skin such as erythema, edema, burning and pain.[4,57,73,84,85,101,110] Considering the studies by NC type, some results were reported. For LNC, the study by Cadinoiu *et al.* evaluated the hemocompatibility of LP using the 2D *in vitro* assay by quantifying the hemolysis, which was less than 5%, thus it

qualifies as hemocompatible. In the 3D *in vitro* assay, both the free drug and LP caused skin irritation because tissue viability was less than 50%, although this value was even lower for the free drug, whereas the incorporation of LP in a gel resulted in an irritation-free nanoformulation.[59] The study by Nayak *et al*. also developed the same 2D *in vitro* toxicity assay and concluded that ETs were hemocompatible.[65] Morton *et al*. analyzed the frequency of the severity of local adverse effects (pain, edema, and erythema) of NE (O/W) and, in general, the severity was mild to moderate, with only 6% of the 143 patients having more severe adverse effects.[73] The three studies with NLCs analyzed the degree of erythema, and all NLCs were rated 0 on a scale of 0 to 5, meaning that they are not irritating to the skin. In addition to this assay, Barone *et al*. also assessed TEWL and no change in the value was obtained supporting the result of the first assay.[4,57,84,85] The study by Rata *et al*. is the only study with PNC found with the toxicologic assay in which three different assays were performed, two *in vitro*, 2D and 3D, and one *in vivo*. Both results of the *in vitro* assays were similar to those of the study of Cadinoiu *et al*. In the *in vivo* assay, the skin lesions after PNP application were evaluated, and the changes present did not cause major alterations in the skin, unlike the free drug, which showed a very thin and dense epidermis and increased cellular infiltration in the dermis.[101] As for the INCs, the toxicologic assay of Amatya *et al*. study, AgNP administered intravenously resulted in the death of the animal in less than 24 hours. Despite the observed systemic toxicity, the topical application doesn't appear to be toxic, although some burn marks and loss of cancer cells have been noted.[110] In the study by Ghazaeian *et al*. the hemocompatibility of MSNP was demonstrated based on the hemoglobin spectrum, more precisely in the Soret band.[114]

To have the developed nanopharmaceuticals approved by regulatory authorities, the results obtained in the toxicological assessment must be consistent with the standard values established for bulk materials. However, this leads to unreliable toxicity profiles as the physicochemical properties of nanopharmaceuticals differ significantly from those of bulk materials, not only in terms of size but also in terms of surface coating, ability to form a protein corona, or aggregation state, leading to different pharmacokinetic and pharmacodynamic aspects.[52,122] For this reason, and because nanocarriers are considered novel substances, regulatory authorities require a more detailed and comprehensive assessment in the preclinical and clinical phases, based on some reflection papers, guidelines or reports published by American Society for Testing and Materials (AS^{TM} International), International Organization for Standardization, Food and Drug Administration (FDA) and European Medicines Agency (EMA), in addition to traditional toxicological studies, to gather information on their

physicochemical properties and their interactions with living organisms.[43,45,46,120] Finally, a benefit-risk assessment of the nanopharmaceuticals is conducted to decide on its marketing approval by regulatory authorities such as the FDA or the EMA, more specifically, in the latter the Nanomedicines Expert Group is designated for this task.[42,45,52,120] Given the high complexity and variability of nanopharmaceuticals, there's still no standardized and internationally accepted regulatory guideline for nanotoxicity assessment, which makes the process time-consuming and doesn't ensure reliability in terms of product efficacy and safety.[42,52]

4. Future challenges: scale-up and regulatory issues

The concept of nanotechnology was developed by physicist Richard Feynman in 1959, and it was not until 36 years later that the first nanopharmaceutical product was approved, Doxil®, a liposome containing doxorubicin, was approved by the FDA. [43,45,48,52] Since then, the FDA and EMA have approved more than 80 nanomedicines and interestingly twice this number is currently in clinical trials.[45–47,120] In the nanomedicine market, the therapeutic aspect, i.e., nanopharmaceuticals, have a burden of 75%, 35% of which is in the field of oncology.[47,52] The following trade names can be identified in this group: Doxil®, Daunoxome® (daunorubicin), Onivyde® (irinotecan), Marqibo® (vincristine sulfate), and Vyxeos® (cytarabine and daunorubicin) as LNC; Genexol-PM®, Apealea® (both with paclitaxel), Eligard® (leuprolide acetate) as PNC; and Hensify® (hafnium oxide) as INC, showing that most of the NC approved are lipid and polymer based. In addition, biotechnological agents are gradually being incorporated. Successful examples of LNCs type containing antibodies are Adcetris® (brentuximab vedotin) and Kadcyla® (trastuzumab emtansine), and those containing different types of ribonucleic acids are Onpattro™ (siRNA), which was the first to be approved, and more recently vaccines against the SARS-CoV-2 virus (messenger ribonucleic acid). [43,45,46,52,67] Moreover, these last examples show that this field is promising in overcoming the remarkable limitations of bioengineered compounds, namely their superior size, low penetration capacity, and high susceptibility to degradation, which only allow them to be administered by local and systemic routes.[52,67,123,124] Despite this visible growth in nanomedicine's market share due to the benefits, there are still a very high number of nanoformulations that fail during development, so those on the market represent only a portion of what would be possible if they overcame the challenges they face, such as regulatory hurdles, safety, and scale-up.[45,67]

The regulatory framework for pharmaceutical products includes legal and scientific guidelines and standard instruments that apply to each stage of the pharmaceutical product cycle, from development (research, manufacturing, preclinical and clinical phases, and

marketing authorization) to marketing, always ensuring the quality, efficacy and safety of these products.[45,67,125] Regarding the regulation of nanopharmaceuticals, there are still many limitations resulting from their high complexity and heterogeneity, starting with the lack of consensus among regulators on the definition and categorization of nanopharmaceuticals. Despite all efforts, it has not been possible to develop a commonly agreed guideline, which may also be related to the lower knowledge of the impact of their variable constituents and physicochemical properties on their biological and toxicological effects.[43,46,120] This in turn leads to a lack of standard methods, which consequently is the main reason for the high failure rate of nanopharmaceuticals development in scale-up and phase 1 clinical trials. In addition to this, they require about 15% more funding than conventional drugs and the duration of the patent term has not been extended, but remains the same as for conventional drugs, i.e. between 10 and 20 years.[46,52]

The production of nanopharmaceuticals in the laboratory is challenging because it is done using conventional methods that are not adapted to the production of nanoscale systems.[46,120] At this stage, careful selection and evaluation of excipients based on their biological and toxicological effects are of utmost importance, as the penetration and residence time of these formulations is higher than that of conventional drugs, increasing the exposure time and consequently the toxicological effects.[67] The scale-up process is a critical step for the success of nanopharmaceuticals, because only if it is successful can it move into the preclinical phase of development.[47,52,67] Therefore, during small-scale production, efforts should be made to identify the critical quality attributes (CQAs) of the nanoformulations, i.e., the attributes of the product that are measured and monitored to ensure that it remains within acceptable limits, such as size, stability and residues produced, to understand the impact of small variations in process parameters on the CQAs. In addition, robust, feasible, and industrially reproducible production methods should be used to reduce the risk of scale-up failure.[45,52,67] Despite these laboratory-scale efforts, large-scale production still has some drawbacks, such as the fact that many steps are required due to the complexity of the nanopharmaceuticals, resulting in higher costs and a greater likelihood of batch-to-batch variability, which can affect CQAs and thus influence the performance and biological toxicity of the nanopharmaceutical.[45,46]

This safety assessment of nanopharmaceuticals is important for the protection of patients and workers who handle them, as they may come into contact with them by inhalation or via the topical route, but also for the protection of the environment, which is less studied and has a prominent role.[46,52] Therefore, some strategies can be used to reduce the toxicity of NCs,

such as reducing or eliminating the use of organic solvents and attempting to develop biodegradable, biocompatible, and nonimmunogenic NCs.[43,45,46,48]

Despite the challenges faced by nanopharmaceuticals, summarized in Figure 7 (see Annex XI), the impact of nanomedicine on the healthcare system is undoubtedly significant, and it is expected that, in line with the strong growth of the last decades, these challenges will be overcome shortly through the development of more robust production techniques and the necessary regulation, which will improve product quality, shorten manufacturing time, reduce costs and failure rate give more confidence to all stakeholders, consumers, manufactures and policymakers.[45,52]

5. Concluding remarks and futures prospects

SkC is one of the most common cancers, so new therapies should be sought. MSC is the most worrisome of themes because it has the highest mortality rate, which is why it attracts the most investment and most studies have been performed. Regarding current cancer therapy, systemic therapies are known to cause serious side effects due to low therapeutic efficacy and high remission rates, as the tumor mass is poorly penetrable and highly resistant to these therapies. For this reason, topical therapies have been developed, but they also have their limitations, such as low penetration into the skin, which means that the drug remains in contact with the skin surface for a long time, leading to skin toxicity. Nanotechnology is a technological and multidisciplinary tool that has gained interest to overcome these limitations.

In this review, experimental studies on the use of NCs in the topical treatment of SkC were compiled and, in general, they showed more favorable therapeutic results than the free drug However, there are still some toxicological concerns related to their use and, for this reason, a more conscientious selection of ingredients of formulations, functionalization or coatings of NCs was observed. A clear preference for LNCs is observed mainly because of their biocompatibility, but this brings the lower ability to retain the drug as a limitation, which is why new NCs are emerging. The main drawback with PNCs and INCs is the lack of knowledge about their toxicity, so the use of the above-mentioned tools to improve biocompatibility has been strongly observed in these groups. In this regard, PNCs have an advantage over INCs because the polymers can be easily manipulated to increase their biocompatibility. On the other hand, NCs have the highest stability and also optical properties, which is advantageous for oncology therapeutics. Regarding the types of SkC, the vast majority of studies were found for SkC as a whole, so in this case, the developed NCs can be used for all types of SkC, since the active compounds used are not specific for a certain type of cancer cells. They have shown

that the NCs have very beneficial results in terms of skin permeation, selective cytotoxicity, and tumor shrinkage *in vivo* and, in some studies, they also proved to be superior to the free drug. We also highlight the NLC studies, not only because they were performed in greater numbers, but also because they evaluated other less common parameters such as wound healing and occlusion factor, and in two studies of them they also showed no skin irritation. The second largest group of studies found was for MSC treatment, and their main objective was the depth of permeation to ensure that NCs reach the basal layer of the VE. In terms of cellular and *in vivo* anticancer efficacy, the same results were obtained as for the SkC group. However, only one nanotoxicity study for NLC was found, in which no signs of toxicity were observed. Since the INCs or PNCs do not provide for toxicological evaluation, so the safety of these groups cannot be guaranteed, one of the LNCs seems to be the most appropriate NC group for this type of cancer. However, more studies are needed to make inferences about the most appropriate LNC for MSC, since not all studies have evaluated the same parameters. For NMSC, especially BCC and cSCC, although positive results were obtained in the evaluation of efficacy and toxicity, very few studies were found. No studies with topical application were found for cSCC. Based on the uncertain results, it is essential to conduct further studies on NCs for the topical treatment of different types of SkC to determine which NCs are most appropriate for each type of cancer to potentiate therapeutics and make them more personalized for each type of SkC.

Despite the advantages presented so far in terms of efficacy and safety of the use of NCs, there are still many obstacles that limit their use in clinical practice. Since physicochemical properties have a major impact on the biological effects of NCs, including toxicological ones, more specific manufacturing and toxicological methods are needed to ensure that NCs remain within acceptable limits, the batch-to-batch similarity is ensured, and non-toxicity is also evaluated in the long term. In addition, the attention of competent authorities should be drawn to their regulatory issues, in particular the development of specific guidelines and the need to extend the patent duration, given the higher investment associated with nanomedicines compared to conventional drugs.

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Annexes

Annex I

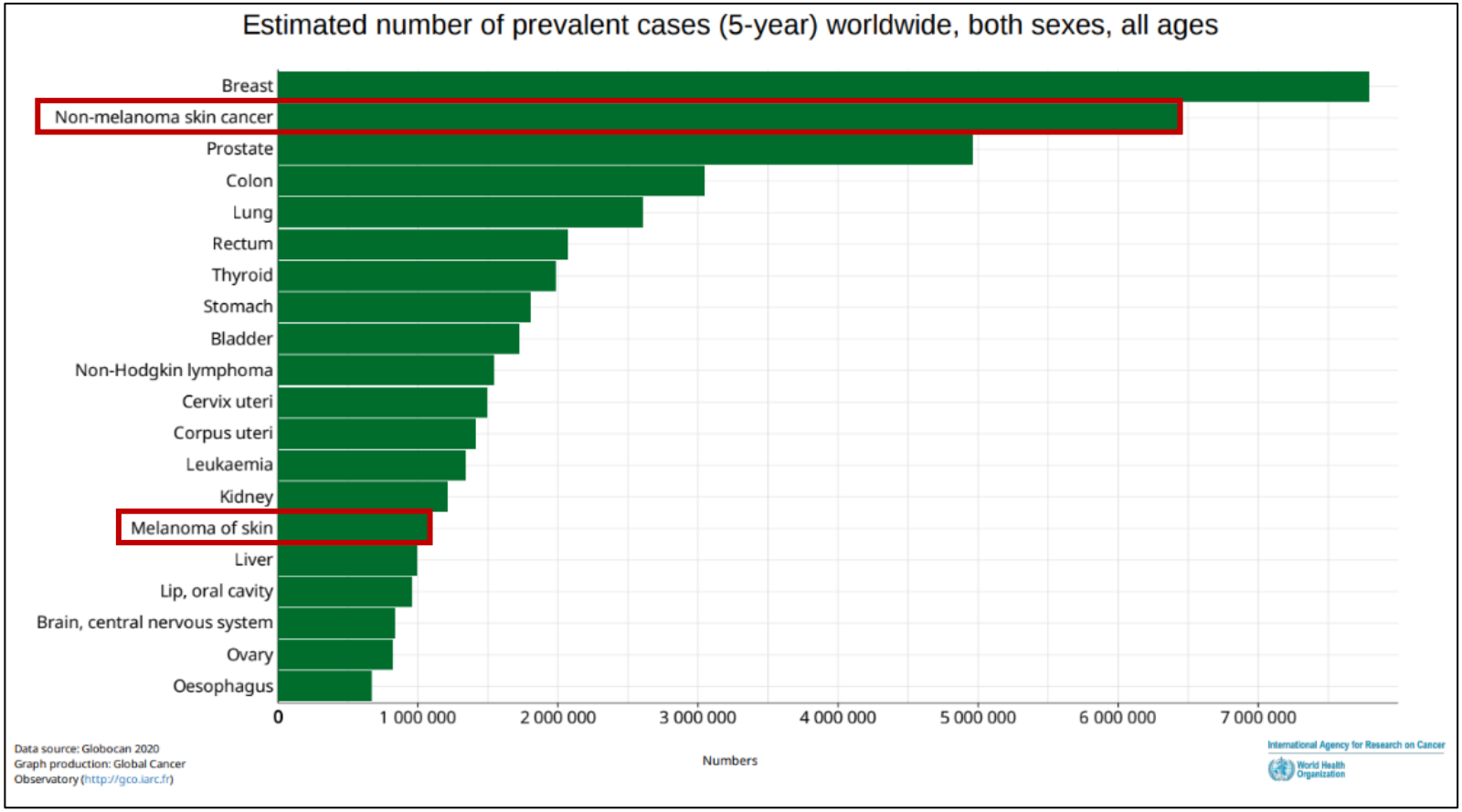

Chart 1 – Estimated number of prevalent cases (5-year) worldwide, both sexes, all ages by World Health Organization (WHO). Open access in Global Cancer Observatory (GCO) website.[12]

Annex II

Estimated number of cases worldwide, both sexes, all ages

Cancer	Incidence	Prevalence	Mortality
Breast	2 261 419	7 790 717	684 996
Lung	2 206 771	2 604 791	1 796 144
Prostate	1 414 259	4 956 901	375 304
Non-melanoma skin cancer	1 198 073	6 458 885	63 731
Colon	1 148 515	3 045 225	576 858
Stomach	1 089 103	1 805 968	768 793
Liver	905 677	994 539	830 180
Rectum	732 210	2 066 732	339 022
Cervix uteri	604 127	1 495 211	341 831
Oesophagus	604 100	666 388	544 076
Thyroid	586 202	1 984 927	43 646
Bladder	573 278	1 720 625	212 536
Non-Hodgkin lymphoma	544 352	1 544 488	259 793
Pancreas	495 773	379 958	466 003
Leukaemia	474 519	1 340 506	311 594
Kidney	431 288	1 207 547	179 368
Corpus uteri	417 367	1 415 213	97 370
Lip, oral cavity	377 713	959 248	177 757
Melanoma of skin	324 635	1 092 818	57 043
Ovary	313 959	823 315	207 252

Data source: Globocan 2020
Graph production: Global Cancer Observatory (http://gco.iarc.fr)

International Agency for Research on Cancer
World Health Organization

Chart 2 – Estimated number of incidence, prevalence (5 years) and mortality cases worldwide in 2020, both sexes and all ages by World Health Organization (WHO). Open access in Global Cancer Observatory (GCO) website.[12]

Annex III

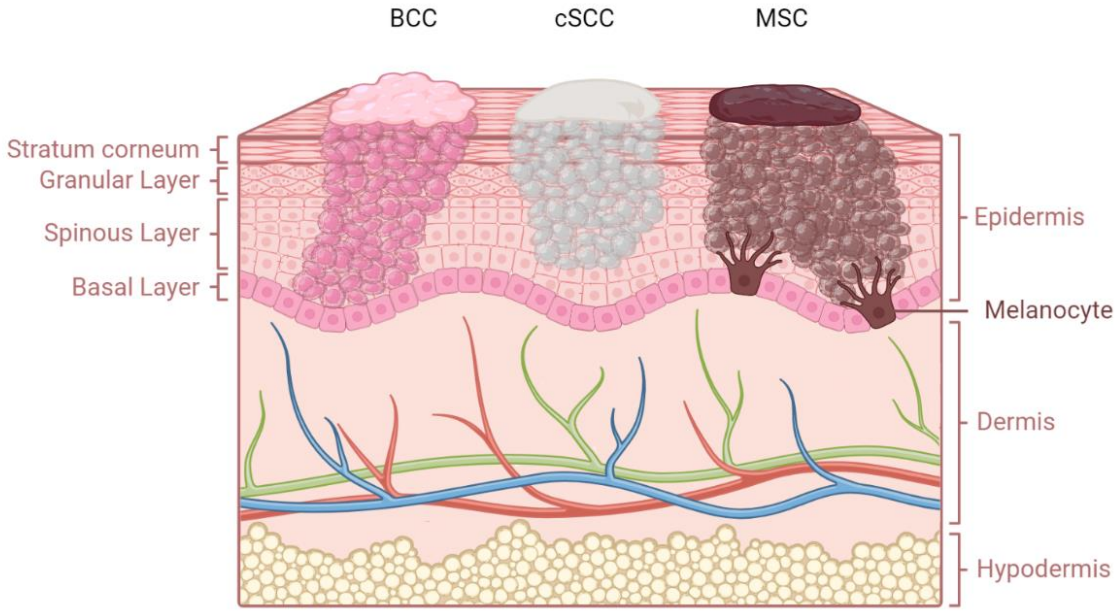

Figure 1 – Illustrative representation of the three types of skin cancer: basal cell carcinoma (BCC), cutaneous squamous cell carcinoma (cSCC) and melanoma skin cancer (MSC).

Annex IV

Table I – Physicochemical properties of nanocarriers-based formulations for topical treatment of skin cancer, separated by skin cancer types.

SkC type	NCs	AC	Preparation method	Starting materials	Composition gel/cream	PS (nm)	ZP (mV)	PDI	EE (%)	*In vitro* released study		Stability (shelf-time)	Ref.
										Outcome	Kinetik		
SkC	LP	DOX CEL	Thin film hydration and pH gradient methods	HSPC CHOL CHCl3 AMS	NA	164,83	-5,21	0,352	98,37 99,69	Sustained with no initial burst release	NA	Physical stability (2 months at 4°C): = PS and PDI, ≈ EE	55
SkC	LP	EGCG	Thin film hydration method	PC PEG-400 Water	NA	403,0	-7,58	0,504	83,45	NA	NA	↑↑Photostablitity *1 Physical stability (3 months at 4-8°C): ↑PS, PDI; ↓ %EE	15
SkC	ET	SFN	Mechanical dispersion	PL90 EtOH Water	NA	227	-26	0,01	87,5	Sustained and steadily release (67% after 24h) with initial burst release (first 5h)	NA	Kinetic profile: No significant changes	38
SkC	NE (O/W)	AG	Microfluidization technique	CNO SO JO TW 80 LEC EtOH PG Paraben Water	NR	176,6	-11,78	0,332	94,8	Constant release rate (0,66 µg/min)	Zero-order	Physical stability (12 weeks at ambient conditions): no significant changes	3
SkC	NE (W/O)	5-FU	Aqueous micro titration with ultra-sonication method	CTO TC PEG-400 Water	NR	66,97	-21,63	0,216	NA	Sustained released with burst release (first hour 30,55% and	Higuchi model	Physical stability (3 months at 4°C): no significant changes	76

SkC type	NCs	AC	Preparation method	Starting materials	Composition gel/cream	PS (nm)	ZP (mV)	PDI	EE (%)	*In vitro* released study		Stability (shelf-time)	Ref.
										Outcome	Kinetik		
										65,48% after 25h)			
SkC	NLC	QCT RSV	Melt emulsification and ultra-sonication method	Labrafil® (M2125CS and M2130CS) CrRH40 Water	Carbopol® 934	191	-10	0,33	>89,05	NA	NA	NA	57
SkC	NLC	5-FU	High-pressure homogenization method	TPM TSR LEC TC Water TW80	Carbopol® 940 PG TEA	205,8	-30,2	0,279	48,17	Sustained released with burst release (57,67% after 6h)*3	NA	NA	83
SkC	NLC	5-FU RSV	Emulsiosonicatio n method (modified)	Labrasol® Emulcire™ TW 80 Water	Carbopol® 934 TEA	178,97	-13,31	0,29	70,12 97,03	Sustained released with burst release *3	Non-Fickian Higuchi kinetic *3	Physical stability (3 months at 4°C): no significant changes	5
SkC	NLC	SM	Solvent diffusion and ultrasonication method	Geleol® Sefsol-218® EtOH CrRH40 BS Water	Carbopol® 934 TEA	126,1	-28,8	0,33	85	NA	NA	NA	84,85
SkC	PNM	IMQ	Solvent evaporation method (modified)	mPEG-hexPLA ACE AcOH NaOAc	CMC	27	NA	0,112	99,4	NA	NA	Physical stability (3 months at 4°C): ~EE%	92
SkC	PNM	LY	Free radical polymerization (modified)	NIPAAM VP PEG-A Water DMSO	NA	65	NA	0,594	> 85	Sustained release at pH 7,4 (≈ 50% after 25h)	NA	NA	93

SkC type	NCs	AC	Preparation method	Starting materials	Composition gel/cream	PS (nm)	ZP (mV)	PDI	EE (%)	*In vitro* released study		Stability (shelf-time)	Ref.
										Outcome	Kinetik		
SkC	PNP	MNG	Emulsion–diffusion–evaporation technique	PLGA DCM PVA	Carbopol® 934 Water PG MP PP TEA	168,06	-25,3	0,201	84,26	Sustained released with burst release (45,34% and 87,07% after 4h and 24h, respectively)	Higuchi model	Physical stability (30 days at 5°C, 30°C and 40°C): no significant changes	100
SkC	AuNP	5-FU	NS	HAuCl4 CTAB NaBH4	Pluronic F127	16,02	44,52	0,18	70	Sustained released pH-dependent (↓ pH leads to ↑ drug release rate)	Higuchi model, except at pH 8 or included in gel (Zero-order model)	Physical stability (pH 11.5, 4 months at 4°C and room temp.): ↑PS	108
SkC	AgNP	Ag	Single-step reduction Ag+	AgNO3 BSA Water NaBH4	GEL Water GLY	105	-28,2	0,22	NA	NA	NA	Physical stability (5 days at 4°C): ≈ PS	110
MSC	LP	MTF	Lipid hydration method or the modified injection method	Pluronic® F127 CHOL	NA	1.405	NA	NA	41.7	NA	NA	NA	60
MSC	ET	BBR EVO	Single step injection technique (modified)	LEC CHOL EtOH PG Water	NA	171	NA	0,12	92,7 >99,7	Sustained release (~100% after 12h)	NA	NA	64
MSC	ET	CUR	Classical cold method (modified)	PL90 EtOH PBS	NA	247	-9,13	0,19	92,24	Sustained release	NA	Physical stability (120 days): - at 4°C: no significant changes	63

SkC type	NCs	AC	Preparation method	Starting materials	Composition gel/cream	PS (nm)	ZP (mV)	PDI	EE (%)	*In vitro* released study		Stability (shelf-time)	Ref.
										Outcome	Kinetik		
												- at 25°C: ↑↑PS ↓↓%EE	
MSC	ET	BRU	Thin film hydration method	LEC CHOL PL EtOH Water	HPMC Water	145,6	-23,3	0,259	72,9	Sustained release (50,87% after 6h)	NA	NA	61
MSC	NE (W/O/W)	DETC 4NC	Double emulsion with melt dispersion method	WB LEC C8 Acid TW 80 Water	NA	213	-50	0,3	86,2 98,7	NA	NA	NA	14
MSC	NE (W/O)	K-TRP-2	NS	L-195 IPM CHX Water R-848	NA	68,6	NA	<0,37	99,5	Sustained release (72% after 48h)	NA	Physical stability (2 weeks at 40°C): ~EE%	77
MSC	SLN	CUR RSV	High shear homogenization method	Compritol®888 P188 TW80 Water	NR	180,17	<-30	<0,25	92 62,82	Sustained release (51% RES and 10% CUR after 120h)	NS - difference kinetics beetween the drugs	Physical stability (21 days): -at 4°C: ↑↑ PS by day 14 -at 25°C: ↑↑ PS by day 4	82
MSC	NLC	SV	Hot high-pressure homogenization technique	Precirol®ATO 5 SQ AcOH TW 80 CS	SRB PEG 400 HPMC EtOH	108	17	0,226	99,86	Sustained release (98% after 48h)*3	NA	NA	4

SkC type	NCs	AC	Preparation method	Starting materials	Composition gel/cream	PS (nm)	ZP (mV)	PDI	EE (%)	*In vitro* released study		Stability (shelf-time)	Ref.
										Outcome	Kinetik		
MSC	LPHNP	BVZ	Ion exchange method	CS AcOH TP Water LEC CHOL CHCl3	NR	283,31	-15	0,24	37,03	Sustained release (62% and 74% after 24h and 48h, respectively)	First order	NA	86
MSC	PNM	siRNA - survivin	NS	ACTGSTQHQ CG peptide HA CHCl3 PBAE Water	NA	1500 at pH 7.4 300 at pH 6.8 200 at pH 5.8	> 0	0,27	NA	Sustained release at pH 7.4 (17% after 24h) and 6.8 (25% after 24h) but not at pH 5.8 (80% after 3h)	NA	Stable 24h at pH 7.4 ↑ degradation with ↓ pH	94
MSC	PNP	(PhSe)2	Interfacial deposition of preformed polymer methodology	PCL Span ™ 80 MCT ACE TW 80	XG Water	240	-10,9	0,15	98	NA	NA	Physical stability (30 days at 25°C): no significant changes and no degradation products detected Physical stability (30 days at 25°C): ↑PS and PDI , ~EE% *3	39,98
MSC	PNP	PHL	Nanoprecipitation	CPO Span 60 PCL ACE EtOH TW 80 Water	NR	202,5 - 252	-8,78	0,1	> 99	Controlled released with burst release (20% and 54% after 4h and 20h, respectively)	First order	Physical stability (14 day): no significant changes in PS and EE% No drug crystal formed	99

SkC type	NCs	AC	Preparation method	Starting materials	Composition gel/cream	PS (nm)	ZP (mV)	PDI	EE (%)	*In vitro* released study		Stability (shelf-time)	Ref.
										Outcome	Kinetik		
MSC	SNPM	CUR	NS	SiO2 PEG-400 Water	NA	36-40	NA	NA	5	NA	NA	NA	114
MSC	MSNP	VER	Co-condensation method	TEOS CTAB Water NaOH TOL APTES	GLY	160–180	NA	NA	NA	NA	NA	NA	115, 116
NMSC	ET	AgNP SER	Spontaneously (CS NPs)	CS AA STPP	NA	261,3	-37,1	Highly singular entity	62,15 62,15	Sustained with initial burst release (55% SER and 56-59% AgNPs after 12h)	NA	NA	65
			Classical cold method	LEC PC EtOH Water									
BCC	LP	5-FU	Film hydration method and sequential extrusion	PC CHOL DSPE-PEG-MAL AS1411-APT	ALG NaCl HA CaCl2 GLY Or Pluronic® F108 Water GLY	182	-12,9	0,16	8,3	No equilibrium phase is reached	Korsmeyer Peppas model	NA	59
BCC	NE (O/W)	ALA	NS	MCT SPC PG IPA DSP MSP TW 80	XG	NA	NA	NA	NA	NA	NA	NA	73, 126, 127

SkC type	NCs	AC	Preparation method	Starting materials	Composition gel/cream	PS (nm)	ZP (mV)	PDI	EE (%)	*In vitro* released study		Stability (shelf-time)	Ref.
										Outcome	Kinetik		
				Water SB									
BCC	PNP	5-FU	Interfacial condensation method	PNVPAI copolymer DMSO ACE Span 80 CS AS1411-APT TW 80	HA ALG Water CaCl2 GLY	133	-19,2	0,33	22,5	NA	Peppas–Sahlin equation	NA	101

(PhSe)2: diphenyl diselenide; 4NC: 4-nitrochalcone; 5-FU: 5-fluorouracil; AA: ascorbic acid; AC: active compound; ACE: acetone; AcOH: acetic acid; AG: andrographolide; Ag: silver; AgNO3: silver nitrate; AgNP: silver nanoparticle; ALA: 5-aminolaevulinic acid; ALG: alginic acid; AMS: ammonium sulfate; APT: aptamer; APTES: aminopropyltriethoxysilane alkoxide; Au: gold; AuNP: gold nanoparticle; BBR: berberine chloride; BCC: basal cell carcinoma; BRU: brucine; BS: bile Salt; BSA: bovine serum albumin; BVZ: bevacizumab; C8 Acid: caprylic acid; CaCl2: calcium chloride; CEL: elecoxib; CHCl3: chloroform; CHOL: cholesterol; CHX: cyclohexane; CMC: carboxymethyl cellulose; CNO: coconut oil; CPO: copaiba oil; CrRH40: cremophor RH40; CS: chitosan; CTAB: cetyltrimethylammonium bromide; CTO: castor oil; CUR: curcumin; DCM: dichloromethane; DETC: sodium diethyldithiocarbamate; DMSO: dimethyl sulfoxide; DOX: doxorubicin hydrochloride; DSP: disodium phosphate dihydrate; DSPE-PEG-MAL: N-[(3-Maleimide-1-oxopropyl)aminopropyl polyethyleneglycol-carbamyl] distearoylphosphatidyl-ethanolamine; EE: encapsulation efficacy; EGCG: (-) -epigallocatechin-3-gallate; EGFR protein: Recombinant human epidermal growth factor; ET: ethosome; EtOH: Ethanol; EVO: evodiamine; GEL: Gelatin; GLY: Glycerol; HA: Hyaluronic acid; HAuCl4: Gold chloride; HPMC: hydroxy propyl methyl cellulose; HSPC: hydrogenated soybean phosphatidylcholine; IMQ: imiquimod; IPA: isopropyl alcohol; IPM: Isopropyl myristate; JO: Jojoba oil; K-TRP-2: Melanoma antigen peptide KKKGSVYDFFVWL; L-195: sucrose laurate; LEC: Lecithin; LP: liposome; LPHNP: Lipid-polymer hybrid nanoparticles; LY: lycopene; MCT: medium chain triglyceride; MCT: Medium Chain Triglycerides; MNG: α-Mangostin; MP: methyl paraben; mPEG-hexPLA: methoxy-poly(ethylene glycol)-)-hexyl-substituted lactide; MSC: melanoma skin cancer; MSNP: Mesoporous silica nanoparticles; MSP: sodium dihydrogen phosphate dihydrate (Monosodium phosphate); MTF: Metformin; NA: not available; NaBH4: Sodium borohydride; NaOAc: Sodium acetate; NaOH: sodium hydroxide; NC: Nanocarrier; NE: Nanoemulsion; NIPAAM: N-isopropylacrylamide; NLC: nanostructured lipid carrier; NMSC: non melanoma skin carcinoma; NP: nanoparticle; NR: not relevant; NS: not specified; O/W: Oil in water; OA: Oleic acid; P: Poloxamer; PBAE : Poly β-aminoester; PBS: Phosphate buffer saline; PC: Phosphatidylcholine; PCL: Poly(ε-caprolactone); PDI: polydispersity index; PEG: polyethylene glycol; PEG-A: polyethyleneglycol monoacrylate; PG: propylene glycol; pH: potential of hydrogen; PHL: Phoretin; PL: phospholipid; PL90: phospholipon 90G; PL90: phospholipon 90G; PLGA: Poly (D, L-lactide-co-glycolide) ; PNM: Polymeric nanomicelles; PNP: Polymeric nanoparticles; PNVPAI: Poly(N-vinylpyrrolidone-alt-itaconic anhydride) ; PP: propyl paraben; PS: particle size; PVA: Polyvinyl alcohol; QCT: quercetin; R-848: resiquimod; RA: Rosmarinic acid; RSV: resveratrol; SB: Sodium benzoate; SER: sericin; SFN: sulforaphane; SiO2: Silicon dioxide (artigo Ghazaeian); siRNA: small interfering ribonucleic acid; SkC: skin cancer; SM: Silymarin; SNPM: silica nanocomplex with porous membranes; SO: Sesame oil; SPC: soybean phosphatidylcholine; SQ: Squalene; SRB: Sorbitol; STPP: Sodium tripolyphosphate; SV: Simvastatin; TC: Transcutol; TEA: Triethanolamine; temp.: temperature; TEOS: Tetraethylorthosilicate; TOL: Toluene; TP: Triphosphate; TPM: Tripalmitin; TSR: Tristearin; TW: Tween; VER: verteporfin; VP: N-Vinyl-2-pyrrolidone; W/O: Water in oil; W/O/W: Water in oil in water; WB: White beeswax; XG: Xanthan gum; ZP: zeta potential.

Annex V

Table 2 – *In vitro, in vivo, and nanotoxicity* results of nanocarriers-based formulations for topical treatment of skin cancer, separated by skin cancer type.

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
SkC	LP	DOXCEL	Cellular viability assay (MTT assay) - MR	*In vitro* (B16 and MGC80-3 cells)	↓↓ IC50 of B16 cells ↓IC50 of MGC80-3 cells	NA	NA	NA	NA	NA	NA	55
SkC	LP	EGCG	Cellular viability assay (MTT assay) - MR	*In vitro* (A431 cells)	↓↓ IC50 *2	*In vitro*	%CV > 85%	Balb/C nude mice induced with DMBA in liquid paraffin	↓ TS *2 ↑ TGIR *2	NA	NA	15
			Permeation assay (FDC) - UPLC	*Ex vivo* (abdominal rat skin)	↑ intradermal deposition, mostly in VE	*In vivo*	≈					
SkC	ET	SFN	Permeation assay (Dynamic skin permeation systems) - HPLC	*Ex vivo* (fresh abdominal human skin)	↑↑ permeation *2	NA	NA	NA	NA	NA	NA	38
			Cellular viability assay (MTT assay) - ELISA MR	*In vitro* (SKMEL-28 cells)	↓↓ %CV (↓*2)							
SkC	NE (O/W)	AG	Permeation assay (FDC) - HPLC	*Ex vivo* (newborn pigs skin)	0,3 µg/cm2.h flux	*In vitro*	No cytotoxicity for all cells at all concentratio	NR	NR	NA	NA	3

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
			Cellular viability assay (MTT assay) - MR	*In vitro* (HFF-1, A375 and A431 cells)	%CV<70% of HFF-1 cells ≥ 100µg/mL (↑↑%CV *2) ↑↑ SI for A375 and A431 *2		ns tested No inhibition TA					
			Apoptotic assay - BD FACS Verse™ software	*In vitro* (A375 and A431 cells)	Apoptosis induction (≠ *2, both apoptosis and necrosis)							
			Intracellular Inhibition TA - MR	*In vitro* (A375 cells)	Inhibition TA (↓*2,6.1)							
SkC	NE (W/O)	5-FU	Permeation assay (FDC) - UHPLC - MS and FM	*Ex vivo* (ear pinna skin of CGR)	↑↑permeation *2 ↑↑ deposition *2	*In vitro*	No cytotoxicity	NA	NA	NA	NA	76
			Cellular viability assay (WST-1 assay) - ELISA reader	*In vitro* (SKMEL-5 cells)	↓%CV (≠*2)							
SkC	NLC	QCT RSV	Permeation assay (FDC) - spectrophotometer, FTIR, DSC and CLSM	*In vitro* (Strat-M™ membrane)	↑ permeation (coefficient and flux) *4,3 ↑↑ penetration depth *4,3	NA	NA	NA	NA	Skin irritation assay (Swiss albino mice skin)	No irritation after 6 days (erythema or edema) *4	57

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
			Cytotoxicity study (MTT assay) - MR	*Ex vivo* (rat skin)	↓ IC50 *4,3							
			Wound healing assay - PCM	*In vitro* (A431 cell)	↓↓% wound healing *4 (↓*3)							
SkC	NLC	5-FU	Cytotoxicity assay (MTT assay) - MR	*In vitro* (A431 cell, HACAT cell)	%CV < 70% for both cells ↓↓%CV (↓*2) %CV A431 cells < %CV HACAT cells	*In vitro*	%CV 63,6% for A431 cells	NA	NA	NA	NA	83
			Occlusion assay – NS	*In vitro* (glass beaker plus paper filter)	↑↑occlusion factor *4 (↑↑*3)							
			Permeation assay (FDC) - HPLC	*Ex vivo* (Sprague-Dawley rat skin)	↑↑ cumulative amount of drug *4 (↑↑*3)							
SkC	NLC	5-FU RSV	Permeation assay (FDC) - HPLC, DSC, FTIR and CLSM	*In vitro* (Strat-M™ membrane) *Ex vivo* (Wistar rat skin)	↑↑ cumulative amount of drug *4 (↑↑*3)	NA	NA	NA	NA	NA	NA	5
			Cytotoxicity assay (MTT assay) - MR	*In vitro* (A431 cell)	↓↓IC50 *4 (↓↓*3)							
SkC	NLC	SM	Cytotoxicity assay (MTT assay) - MR	*In vitro* (B16 cells)	%CV < 70%	NA	NA	Albino Swiss mice induced with UVB	↓biochemical markers of oxidative stress *4 (↓*3) ↓ cutaneous	Albino Swiss mice	No skin irritation *4	84,85

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
			Permeation assay (FDC) – SP, DSC, FTIR and CLSM	*In vitro* (Strat-M™ membrane) *Ex vivo* (Wistar rat skin)	↑↑ permeation *4,3 ↑ penetration depth *4,3 (VEDE ≠*3)				edema *4 (↓*3) ↓↓ TB (↓↓*3) ↓ NT *4 (~*3) ↑ anti-inflammatory activity *4 (↑*3)			
SkC	PNM	IMQ	Skin delivery assay (FDC) - UHPLC-MS	*Ex vivo* (porcine ear skin and human skin)	↑permeation and deposition (~ *6.2) ↑ delivery efficiency (↑↑*6.2) ↑ penetration depth (~ *6.2)	NA	NA	NA	NA	NA	NA	92
SkC	PNM	LY	DPPH Assay - SP	*In vitro* (DPPH solution)	↓↓ IC50 (↓↓*2)	NA	NA	Swiss albino mice induced with 12-O-tetradecanoylphorbol-13-acetate	↑ anti oxidative enzymes (↑*2) ↑ Bax protein ↓ Bcl-2 protein ↓↓ COX-2 enzyme and edema ↓↓ NT and TB	NA	NA	93
			Anticancer activity (MTT assay) - FC	*In vitro* (B16 cells)	↓↓ %CV (↓*2) ↑↑ % apoptotic cells (↑↑*2)							
SkC	PNP	MNG	Permeation (FDC) - HPLC, SP, CM	*Ex vivo* (rat skin)	↑↑ cumulative amount of drug and flux *4 (↑ in SC and↑↑ in VEDE* 3)	*In vitro*	%CV>70%	NA	NA	NA	NA	100
			Cell Viability assay - MR	*In vitro* (B16F10 cells)	↓↓ IC50 *4 (↓↓*3)							
			DPPH assay - SP	*In vitro* (DPPH solution)	↓↓ IC50 *4(↓↓*3 and ↓*6.3)							

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
SkC	AuNP	5-FU	Skin permeation study (FDC) - SP	*Ex vivo* (C57BL/6 mice dorsal skin)	↑↑ %drug permeated (↑*2, with CTAB)	NA	NA	C57BL/6 mice induced with A431 cells	↑↑ inhibition of the TG *4 (↑↑*3) ↓↓ TW *4 (↓↓*3) ↑ BW *4 (↑*3) ↓↓ skin pathology features *4 (↓↓*3)	NA	NA	108
SkC	AgNP	Ag	Photothermal activity -E5IR camera Cytotoxicity (WST-1 assay) - NS	*In vitro* (B16F10 cells)	↑↑ temp. after NIR (0,5W -> 45°C) ↓↓ %CV (↓↓*2)	NA	NA	Athymic nude mice induced with B16F10 cells	↓↓ TS and cancer cells (only with surface temp. at 50°C) *4	Athymic nude mice induced with B16F10 cells	Slightly reddish burn marks	110
MSC	LP	MTF	Cellular viability assay (MTT assay) - NS	*In vitro* (B16 cells)	↓↓ IC50 (↓↓*2) 67% permeation	NA	NA	NA	NA	NA	NA	60
MSC	ET	BBR EVO	Permeation and distribution assays (FDC) - HPLC and CM	*Ex vivo* (human cadaver skin)	All skin extension even in the basal layer (≠*2)	*In vitro*	The highest EtOH content did not lead to B16 cells death	NA	NA	NA	NA	64
			Cellular viability assay (MTT assay) - MR	*In vitro* (B16 cells)	↓↓ %CV (↓*2)							
MSC	ET	CUR	Permeation and deposition assay (FDC) - HPLC and FM	*Ex vivo* (Wister albino rat abdominal skin)	↑↑ permeation *2,7 Uniformity throughout SC and VEDE (≠*2,7 - SC)	*In vitro*	%CV ≈ 90%	NA	NA	NA	NA	63

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
			Cellular viability assay (MTT assay) - MR	*In vitro* (A375 and L-929 cells)	↓↓ IC50 and %CV of A375 cells (↓*2) %CV≈90% of L-929 cells (~*2)							
			Antiproliferation assay - Open CFU software	*In vitro* (A375 cells)	↑↑ %inhibition of colony formation (↑*2)							
			Apoptotic assay - FC		↑↑ apoptotic cells (↑↑*2) ↓↓ live cells (↓↓*2) ~ necrosis (↓↓ *2)							
MSC	ET	BRU	Permeation assay (FDC) - SP	*Ex vivo* (Wister albino rat abdominal skin)	↑↑ permeation *2	*In vitro*	%CV>95%	NA	NA	Characterization of ET	pH ≈ 6	61
			Celular viability assay (MTT assay) - MR	*In vitro* (A375 cells)	↓↓ IC50 *4,3							
MSC	NE (W/O/W)	DETC 4NC	Cellular viability assay (MTT assay) - SP	*In vitro* (B16F10 cells)	↓↓ IC50 *2	NA	NA	NA	NA	NA	NA	14
MSC	NE (W/O)	K-TRP-2	Permeation assay (FDC and Skin pieces)- PL-SP	*Ex vivo* (C57BL/6N mice back and ear skin)	↑↑permeation *2 SC and VEDE (≠*2, only SC)	NA	NA	C57BL/6N mice induced with B16F10 cells	↑ survival rate (↑*2) ↓↓number of nodules (↓*2) Immunological effect	NA	NA	77

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
MSC	SLN	CUR RSV	Permeation (FDC) -UV-SP	*Ex vivo* (Shed Snake Skin)	↑↑ penetration depth	*In vitro*	No resistance signal No cytotoxicity	NA	NA	NA	NA	82
			ECIS assay - electrophoresis	*In vitro* (B16F10 cells)	↓↓ resistance (≈*2)							
			Growth Inhibition Assay - IncuCyte cell analysis system and MR	*In vitro* (SKMEL-28 cells)	↓↓ confluence (≈*2) ↓↓ IC50 (≈*2)							
MSC	NLC	SV	Permeation assay (FDC) - NS	*Ex vivo* (newborn pigs skin)	↑↑ permeation of *4	NA	NA	NA	NA	Human	Erythema Index < 0 *4 TEWL ~ negative control	4
			Cytotoxicity assay (MTT assay) - MR	*In vitro* (HACAT, Cos-7, Colo-38 and SKMEL-28 cells)	%CV > 70% of healthy cells *4 (~*3) ↓%CV of melanoma cells *4 (↓*3 for Colo-38 cells)							
MSC	LPHNP	BVZ	Dermal absorption assay (FDC) - UV-SP	*Ex vivo* (Balb/c mice skin)	↑↑ cumulative amount of drug (↑↑*2)	*In vitro*	%CV > 70% Number of tubular vascular branches ≈ untreated cells	Chicken chorioallantoic membrane	↑↑ angiogenesis inhibition (↑*2)	NA	NA	86
			Cell proliferation assay (MTT solution) - ELISA reader	*In vitro* (HBMEC cells)	↓↓ %CV (↓↓*2)							

SkC type	NCs	AC	*In vitro*/*Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
			Tubulogenesis assay - NS		↑↑ suppressor effect (↑↑*2)							
MSC	PNM	siRNA - survivin	Cellular uptake assay - FC and FM	*In vitro* (B16F10 cells)	↑↑ cellular uptake (↑↑*2)	*In vitro*	%CV = 83% 3% apoptosis (≈ *2)	C57BL/6 nude mice induced with B16F10 cells	↓↓ TS (≠*2, ~ control) ↑↑ necrosis and apoptosis regions (↑↑ *2) ↓↓ % survivin (↓↓ *2)	NA	NA	94
			Cell viability assay (MTT assay) - MR		↑↑ apoptosis (↑↑*2)	*In vivo*	Slightly necrosis and apoptosis No damage in the various organs					
			Skin permeation assay (FDC) - CLSM	*Ex vivo* (C57BL/6 nude mice skin)	↑↑ permeation (≠*2)							
MSC	PNP	(PhSe)2	Cytotoxicity assay (MTT assay) -SP and inverted microscope	*In vitro* (A375, SKMEL-103, HACAT, B16F10, SKMEL-28 cells)	↓↓ IC50 for A375 cells (~*2) ↓ IC50 for SKMEL-103 cells (↓*2) ↑↑ IC50 for HACAT than melanoma cells (≠*2)	*In vitro*	%CV > 70%	NA	NA	NA	NA	39,98
			Skin permeation assay (FDC) - HPLC	*Ex vivo* (human skin)	↑↑ cumulative amount of drug in SC and VE *4 (↓*3) ↑ cumulative amount of drug in DE *4 (↑*3)							

SkC type	NCs	AC	*In vitro/Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
MSC	PNP	PHL	Cytotoxicity assay (MTT assay) – SP and FC	*In vitro* (MRC5, HACAT, SKMEL-28 cells)	%CV>70% for MRC5 and HACAT cells (~*2) ↓ proliferation rate (↓*2) ↑ nuclear enlargement of SKMEL-28 cells	*In vitro*	%CV>70% for MRC5 and HACAT cells	NA	NA	NA	NA	99
			Washability and permeation assay (FDC) - HPLC	*Ex vivo* (porcine ear skin)	↓↓ washability (↓↓ *2) ↑↑ penetration in SC and VE (↑↑*2)							
MSC	SNPM	CUR	Photocytotoxicity - (MTT assay) - ELISA reader	*In vitro* (A375 and HDF cells	For A375 cells: %CV > 90% (dark) ↓↓ %CV (↓*2) (light) ↓ colony forming Cell selectivity (no cytotoxicity to HDF cells) (~ *2)	*In vitro*	No citotoxicity, melanoma cells with intact nuclei	NA	NA	Human blood - spectrophotometer	No changes in Soret band, globin or Q band	114
			Apoptotic assay - FM		Early and apoptotic cells (dark) apoptotic and necrotic (light)							
MSC	MSNP	VER	Cytotoxicity (MTT assay) - MR	*In vitro* (B16F10 cells)	↓↓ %CV (↓ in light)	*In vitro*	↓ %CV (light)	C57BL6/J mice induced with B16F10 cells	↓↓ TG and TW *4 ↓↓ angiogenesis *4 ↓↓ lymphangiogenesis and micrometastasis	NA	NA	115, 116
						In vivo	↓ TG and TW *4 ↑ angiogenesis *4 ↓ lymphangiog					

SkC type	NCs	AC	*In vitro/Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
							enesis and micrometastasis					
NMSC	ET	AgNPSER	Cellular viability assay (MTT assay) - MR	*In vitro* (A431 and HACAT cells)	↓ %CV of A431 cells (≈*6.4) Cell selectivity (≠*6.4)	NA	NA	NA	NA	Human blood (RBC)	<5% hemolysis with "button-like structure"	65
			DNA damage assay - electrophoresis		DNA bands for A431 cells Intact DNA for HACAT cells					Immune response assay (Balb/c mice)	Negligible levels of IgG and IgD	
			Mitochondrial membrane and ROS assays - FM	*In vitro* (A431 cells)	↑ damaged mitochondrial membrane (↓*6) ↑ internal ROS							
BCC	LP	5-FU	Transdermal diffusion assay (FDC) - SP	*In vitro* (Strat-M® membrane)	↓ permeation rate *2	NA	NA	NA	NA	SkinEthic™ reconstructed human epidermis tissues	↑ irritant effect (≈*2) non-irritant (Blank NC)	59
			Cellular viability assay (MTT assay) - SP	*In vitro* (TE 354.T cell)	↓ %CV *3 (but, ↑ *2)					RBC	<5% hemolysis	

SkC type	NCs	AC	*In vitro/Ex vivo* studies			Outcome Blank NC		*In vivo* studies		Nanotoxicity assessment		Ref.
			Test method	Model	Outcome *1	Model	Outcome *1	Model	Outcome*1	Model	Result	
			Apoptosis assay - FC		↓ dead cells *3 (but, ↓↓ *2) ↑ preapoptotic *3 (but, ↑ *2) ↑ apoptotic cells *3 (but, ↓↓ *2)							
BCC	NE (O/W)	ALA	NA	NA	NA	NA	NA	Human diagnosed with BCC	↑↑ clearance rates *4 (↑*6.5)	Human diagnosed with BCC	≤6% report adverse event (pain, pruritus, erythema and oedema)	73, 126, 127
								Human diagnosed with nonaggressive BCC only	↑↑clearance rate *4 (↑↑*6.5) ↓↓recurrence rates *4 (↓↓*6.5)			
BCC	PNP	5-FU	Permeation assay (FDC) - SP	*In vitro* (Stat-M® artificial membrane) and *Ex vivo* (chicken skin membrane)	↑↑ permeation *4 (↑*3)	*In vitro*	%CV > 91,35%	Albino mice	↑ activated Langerhans cells (≠ *3)	Human blood (RBC) - spectrophotometer	<0,55% haemolysis (= *4,5)	101
			Cytotoxicity assay (MTT assay) - Biochrom EZ Read	*In vitro* (TE 354.T cells)	↓↓ %CV (~ *3)		% tissue viability > 50% (≈ *4 and *5			SkinEthic™ Reconstructed Human Epidermis tissues - microplate spectrophotometer	% tissue viability < 50% (↑*2)	
			Apoptosis assay - FC		↑ preapoptotic, apoptotic and dead cell *4 (≈ *3)					Albino mice	No skin alteration (≠ *3)	

(PhSe)2: diphenyl diselenide; *1: compared with positive control; *2: compared with free active compound; *3: compared with free active compound in gel; *4 : NC in gel, cream or film; *5: plain gel;*6: compared with commercial product or other compound; *6.1: Kojic acid solution; *6.2: Aldara; *6.3: Trolox; *6.4: Cisplatin; *6.5: MAL cream; *7: compared with LP; ↓/↑: ≤ 2-fold or low variation; ↓↓/↑↑: ≥ 2-fold or significant/substantial variation; 4NC: 4-nitrochalcone; 5-FU: 5-fluorouracil; A375 | SKMEL-28 | SKMEL-5 cells: malignant human melanoma cell line; A431: human epidermoid carcinoma cell line; AC: active compound; AG: andrographolide; Ag: silver; AgNP: silver nanoparticle; ALA: 5-aminolaevulinic acid; Au: gold; AuNP: gold nanoparticle; B16 | B16F10 cells: murine melanoma cell line; Bax: Bcl-2-associated X; BBR: berberine chloride; BCC: basal cell carcinoma; Bcl-2: B-cell lymphoma 2; BRU: brucine; BVZ: bevacizumab; BW: body weight; CEL: celecoxib; CGR: cow, goat and Swiss albino rat; CLSM: confocal laser scanning microscopy; CM: confocal microscopy; Colo-38: melanoma cell line; Cos-7 | MRC5 | HDF | HFF-1 cells: human fibroblasts cell line; COX-2: Ciclo-oxigenase-2; CTAB: cetyltrimethylammonium bromide; CUR: curcumin; CV: cell viability; DETC: sodium diethyldithiocarbamate; DMBA: 7,12-dimethylbenz[a]-anthouracene; DNA: deoxyribonucleic acid; DOX: doxorubicin hydrochloride; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DSC: differential scanning calorimetry; EGCG: (-) -epigallocatechin-3-gallate; ELISA: enzyme-linked immunosorbent assay; ET: ethosome; EtOH: ethanol; EVO: evodiamine; FC: Flow cytometry; FDC : franz diffusion cell; FM: fluorescence microscopy; FTIR: fourier-transform infrared spectroscopy; HACAT: human keratinocyte cell line; HBMEC : human bone marrow microvascular endothelial cell line; HPLC: high performance liquid chromatography; IC50: half maximal inhibitory concentration; IgD: immunoglobulin D; IgG: immunoglobulin G; IMQ: imiquimod; K-TRP-2: melanoma antigen peptide KKKGSVYDFFVWL; L-929: mouse fibroblast cell line; LP: liposome; LPHNP: lipid-polymer hybrid nanoparticles; LY: lycopene; MGC80-3: human gastric carcinoma cell line; MNG: α-mangostin; MR: microplate reader; MSC: melanoma skin cancer; MSNP: mesoporous silica nanoparticles; MTF : metformin; NA: not available; NC: nanocarrier; NE: nanoemulsion; NIR: near-infrared radiation; NLC: nanostructured lipid carrier; NMSC: non melanoma skin carcinoma; NP: nanoparticle; NR: not relevant; NS: not specified; NT: number of tumors; O/W: oil in water; PCM: phase-contrast microscope; pH: potential of hydrogen; PHL: phoretin; PL: photoluminescence; PNM: polymeric nanomicelles; PNP: polymeric nanoparticles; QCT: quercetin; RBC: red blood cell; ROS: reactive oxygen species; RSV: resveratrol; SC: stratum corneum; SER: sericin; SFN: sulforaphane; SI: selectivity index; siRNA: small interfering ribonucleic acid; SkC: skin cancer; SKMEL-103: resistant human malignant melanoma cell line; SM: Silymarin; SNPM: silica nanocomplex with porous membranes; SP: spectrophotometrically; SV: simvastatin; TA: tyrosinase activity; TB: tumor burden; TE 354.T: human BCC cell line; temp.: temperature; TEWL: transepidermal water loss; TG: tumor growth; TGIR: tumor growth inhibition rate; TS: tumor size; TW: tumor weight; UHPLC-MS: ultra-high performance liquid chromatography-tandem mass spectrometry; UPLC: Ultra-performance liquid chromatography; UV: ultraviolet radiation; VE: viable epidermis; VEDE: viable epidermis and dermis; VEDE: viable epidermis and dermis; VER: verteporfin; W/O: water in oil; W/O/W: water in oil in water; WST-1: [2-(4-iodophenyl)-3-(4- nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium].

Annex VI

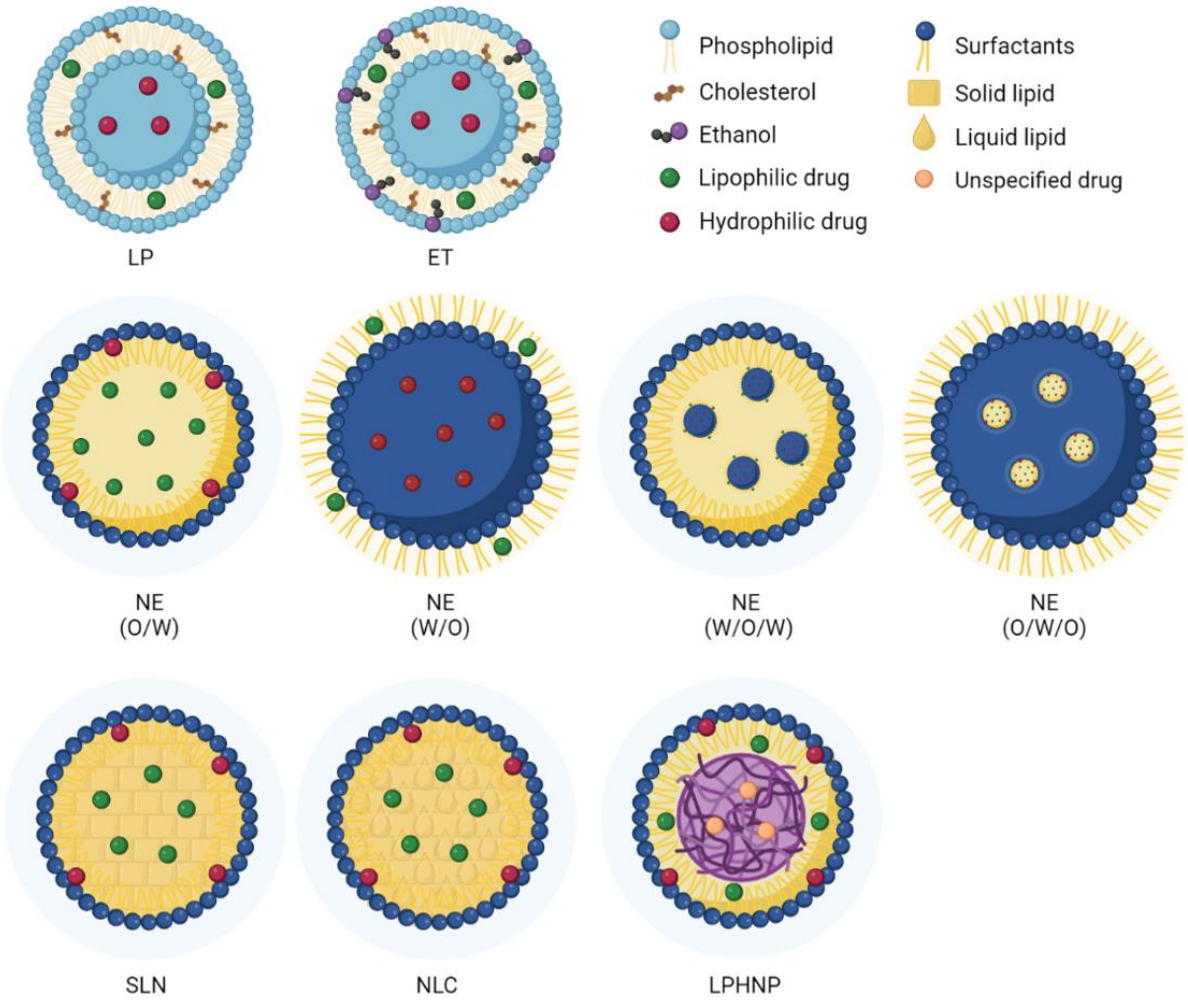

Figure 2 – Illustrative representation of the lipid-based nanocarriers: liposome (LP), ethosome (ET), nanoemulsions (NE different types: O/W, W/O, W/O/W, O/W/O), nanostructured lipid carrier (NLC), solid lipid nanoparticle (SLN) and lipid-polymer hybrid nanoparticle (LPHNP).

Annex VII

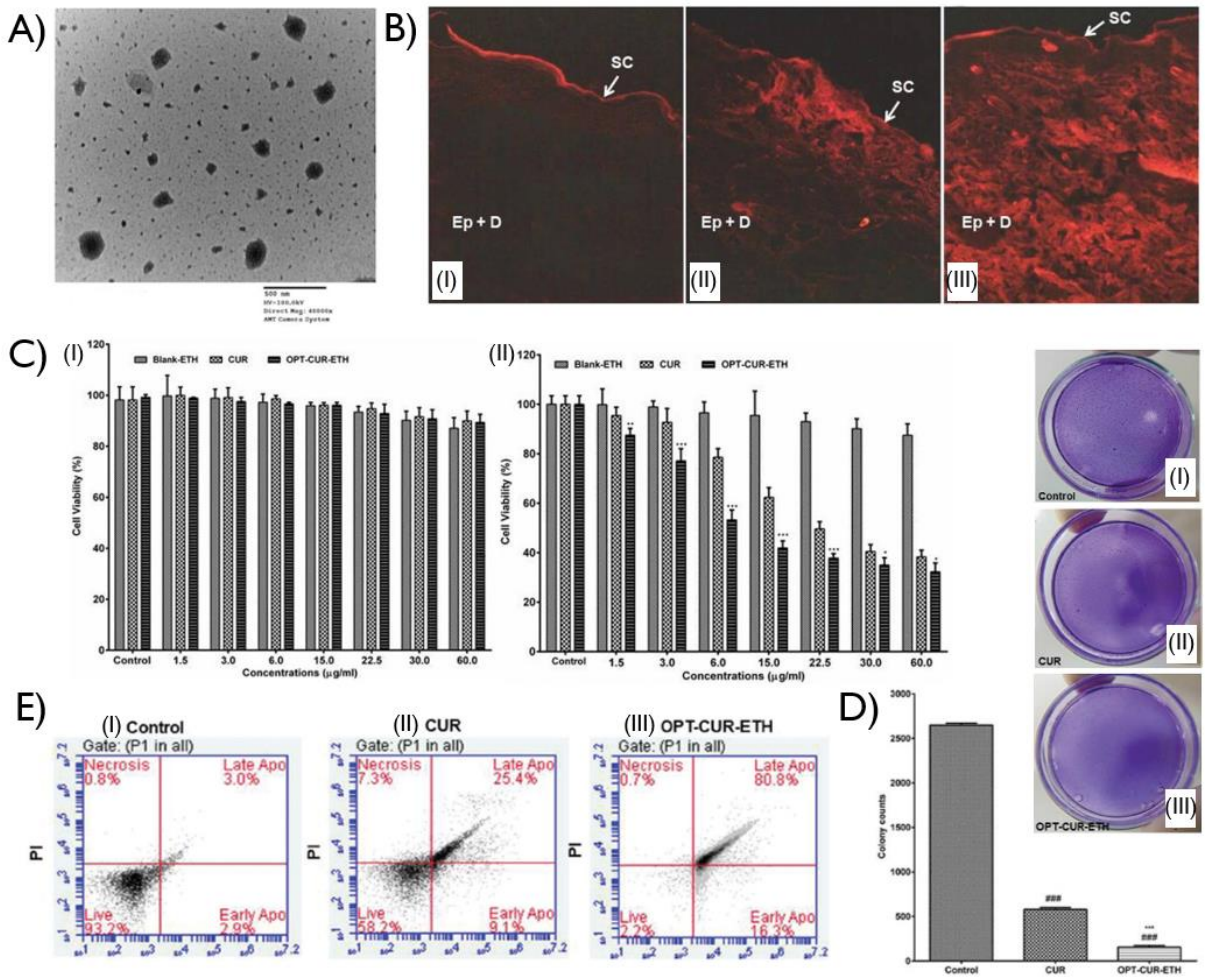

Figure 3 – A) Transmission electron micrograph of the developed ethosome; B) Fluorescence microscopy visualization of skin permeation of fluorophore solution (I), fluorophore contained in the liposome (II), and fluorophore contained in the ethosome (III); C) cytotoxicity effect of the formulations blank ethosome, free curcumin, and optimized ethosome with curcumin-on L-929 cells (I) and on A375 cells (II); D) Clonogenic potential of untreated A375 cells (I) treated with free curcumin (II) and with curcumin contained in the ethosome; E) Flow cytometric analysis of untreated A375 cells (I) treated with free curcumin (II) and with curcumin contained in the ethosome (III). Adapted from the study of Peram *et al.*[63]

Annex VIII

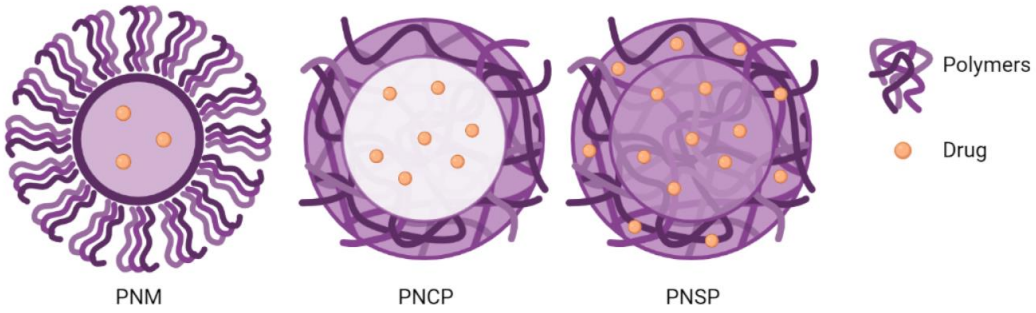

Figure 4 – Illustrative representation of the polymeric-based nanocarriers: polymeric nanomicelle (PNM), polymeric nanocapsule (PNCP) and polymeric nanocapsule (PNSP).

Annex IX

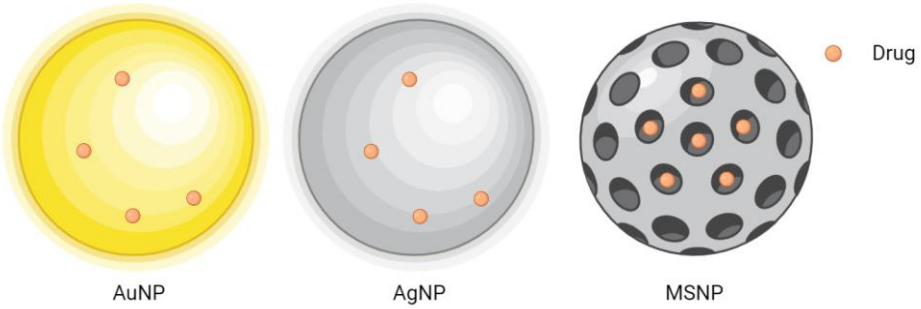

Figure 5 – Illustrative representation of the inorganic-based nanocarriers: gold nanoparticle (AuNP), silver nanoparticle (AgNP) and mesoporous silica nanoparticle (MSN).

Annex X

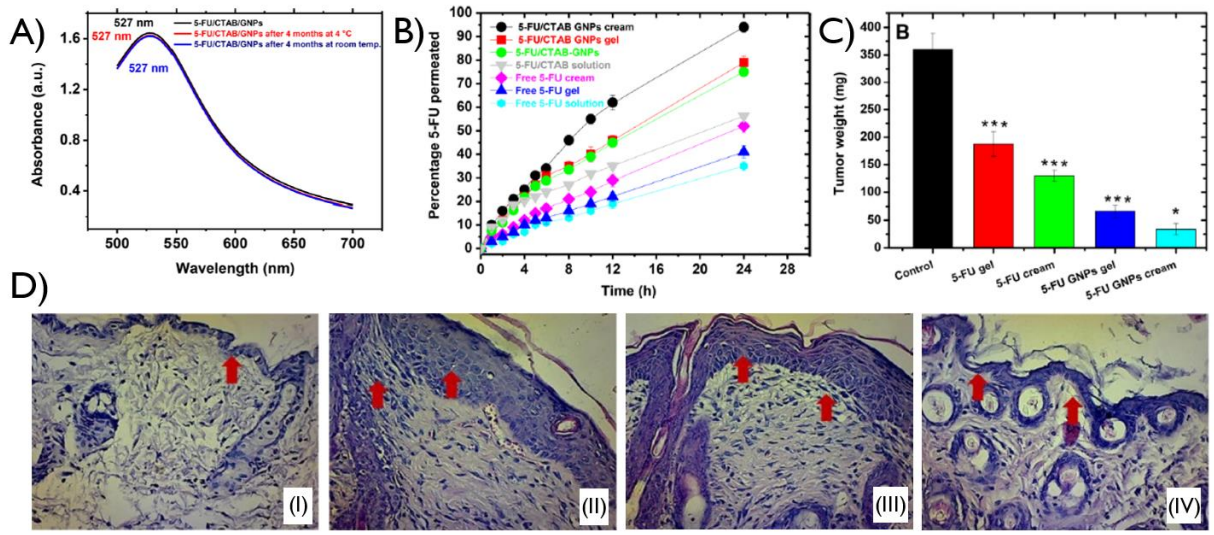

Figure 6 – A) UV-visible spectrum of 5-fluorouracil used to evaluate the long-term stability of gold nanoparticles; B) Permeability of the different formulations studied - gold nanoparticles and free 5-fluorouracil in solution, gel or cream - through the skin of mice; C) Tumor size at the end of the study in untreated mice and after application of gold nanoparticles and free 5-fluorouracil formulations; D) Histological representation of healthy mice skin (I), malignant mice skin without treatment (II), after treatment with free 5-fluorouracil gel (III) and gold nanoparticle gel (IV) (arrows point to epidermis). Adapted from the study of Safwat *et al.*[108]

Annex XI

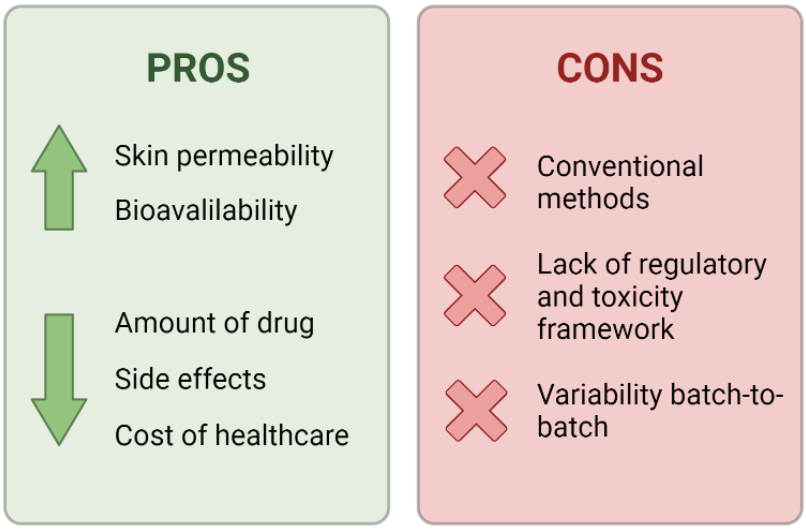

Figure 7 – Main pros and cons of nanomedicine.