UNIVERSIDADE D
COIMBRA

Ana Rita Sampaio Moreira

Relatório de Estágio e Monografia intitulada "Anti-PD-1Immunotherapy in Advanced Metastatic Melanoma" referentes à Unidade Curricular "Estágio", sob a orientação, da Dra. Ângela Ramos e do Professor Doutor André Pereira apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

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Julho 2019

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Coimbra, 11 de julho de 2019.

Ana Rita Sampaio Moreira

Agradecimentos

À minha mãe e ao meu irmão, pelo apoio incondicional e por invariavelmente acreditarem em mim quando já nem eu mesma acredito.

Aos meus avós, desde sempre a minha força motriz, aos quais dedico o fruto final deste trabalho.

Ao Seara – porque também é família.

À Lara – por todos os conselhos pessoais e académicos, os seus preferidos.

À Sara – por ser tão parecida comigo que nunca precisamos de palavras.

À Irene, à Chica e ao Pedro – por todas as confidências e memórias construídas.

À Eunice, à Teresa ao André e à Maria – porque Roma nos uniu para sempre.

Ao Marco – por ser o meu técnico informático.

À Dra Ângela Ramos, orientadora do meu estágio na Farmácia Confiança, o meu obrigada pela forma como me recebeu e ensinou sempre com a sua boa-disposição.

Ao Dr. Felice Musicco, o grande impulsionador desta monografia, o meu mais sincero obrigada pelo acolhimento em Roma e por me impulsionar a ir mais além. Estar-lhe-ei sempre grata.

Ao Professor Doutor André Pereira, o meu muito obrigada pela disponibilidade e ajuda constante e crucial para levar avante esta monografia.

À Faculdade de Farmácia e a Coimbra, que jamais esquecerei!

Índice

Parte I

Relatório de Estágio em Farmácia Comunitária

Abreviaturas

CHTS – Centro Hospitalar do Tâmega e Sousa

DC – Dermofarmácia e Cosmética

DCI – Denominação Comum Internacional

DM – Dispositivos Médicos

EC – Estágio Curricular

FC – Farmácia Confiança

FFUC – Faculdade de Farmácia da Universidade de Coimbra

HMP – Hospital da Misericórdia de Paredes

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamento Não Sujeito a Receita Médica

MSRM – Medicamento Sujeito a Receita Médica

MUV – Medicamento de Uso Veterinário

NH – Nutrição Humana

PNV – Plano Nacional de Vacinação

PUV – Preparações de Uso Veterinário

SWOT – *Strengths, Weaknesses, Opportunities and Threats*

I. Introdução

O farmacêutico é um profissional de saúde com um vasto e variado leque de saídas no mercado de trabalho. Na farmácia comunitária, os farmacêuticos são o primeiro profissional de saúde em contacto com o doente, estabelecendo com este uma relação de proximidade muito antes do aconselhamento médico. O farmacêutico não é um mero dispensador de medicamentos. Apesar de ser o especialista do medicamento, o seu papel transcende essa função – constitui um agente de saúde pública, um prestador de serviços e cuidador, fulcral no aconselhamento do doente e no apoio da população e da comunidade em que a farmácia se encontra inserida, zelando sempre pelo bem-estar do doente e do cidadão em geral [1].

Após nove semestres de formação curricular, com vista a adquirir competências teóricas e científicas no Mestrado Integrado em Ciências Farmacêuticas (MICF) da Faculdade de Farmácia da Universidade de Coimbra (FFUC), chega a etapa final do Estágio Curricular (EC), visando auxiliar o estudante a pôr em prática os conhecimentos adquiridos previamente. O EC permite assegurar a aptidão do estagiário, crescendo profissionalmente e desenvolvendo as competências necessárias para exercer a profissão farmacêutica e se tornar num profissional exímio. No EC promove-se ainda o contacto do estudante com o utente e instituições relacionadas com a atividade farmacêutica, sendo abordadas as problemáticas do uso racional do medicamento e promoção para a saúde.

O meu EC teve início a 24 de setembro de 2018 e findou a 31 de dezembro do mesmo ano, na Farmácia Confiança (FC), em Paredes, Porto, sob orientação da Dra. Ângela Ramos, farmacêutica adjunta responsável pelo meu estágio. O plano de estágio consistiu primeiramente na realização de tarefas de *backoffice*, incluindo receção de encomendas, gestão de reservas e gestão de devoluções, seguido de organização de lineares, preparação de manipulados, análise de parâmetros antropométricos e bioquímicos e atendimento ao balcão.

O presente relatório diz respeito ao estágio por mim realizado na FC, examinando a relação entre o plano curricular do MICF e a aplicabilidade do mesmo no contexto profissional, sendo apresentado na forma de Análise SWOT – *Strengths, Weaknesses, Opportunities and Threats*. Nesta, através da descrição de um conjunto de fatores internos – forças e fraquezas e fatores externos – oportunidades e ameaças, farei uma análise global do meu EC que se traduz nos pontos fortes e fracos do mesmo, nas oportunidades que considero ter tido e nas ameaças que fui sentindo ao longo de todo o percurso [2].

2. Análise SWOT

2.1. *Strenghts* (Forças)

2.1.1. Localização, população abrangida e horário de funcionamento alargado

A Farmácia Confiança (FC) encontra-se num local privilegiado da cidade, centralizado e residencial, com ótimos acessos, próximo do Centro Hospitalar do Tâmega e Sousa (CHTS), Hospital da Misericórdia de Paredes (HMP) e de outras clínicas privadas. É, de facto, uma farmácia que assegura os serviços farmacêuticos a um agregado populacional elevado, possuindo, para além de utentes provenientes do meio hospitalar ou das clínicas mencionadas, um vasto número de utentes habituais e fidelizados há já bastantes anos, visto que a FC é uma das mais antigas do concelho. Desta forma, a assistência e serviços farmacêuticos são prestados a um grande número de cidadãos oriundos de várias regiões do concelho de Paredes e arredores. Para além da localização estratégica, a FC apresenta um horário de funcionamento alargado, encontrando-se aberta de segunda-feira a sábado das 8:30h às 23:00h e aos domingos e feriados entre as 9:00h e as 23:00h, sem interrupção para almoço ou jantar. Possui ainda um parque de estacionamento privativo, o que a torna de mais fácil acesso e comodidade para os utentes.

A localização e o horário de funcionamento alargado refletem-se na heterogeneidade dos utentes frequentadores da FC, desde idosos a jovens, utentes de passagem ou utentes fidelizados e cidadãos de diferentes contextos socioeconómicos e com diferentes quadros clínicos. Destaco ainda o facto de ter estagiado aos sábados, pois permitiu-me ter a perceção da mudança no movimento e do tipo de utentes ao longo do dia comparativamente com os dias de semana. Considero estes factos uma grande vantagem na medida em que tive de me adaptar rapidamente às diferentes necessidades e contextos que iam surgindo, adotando diferentes abordagens e atendimentos consoante os casos. Desenvolvi, assim, capacidades a nível da dispensa e aconselhamento farmacêutico, permitindo-me uma maior perceção e preparação para os diferentes casos que foram surgindo.

2.1.2. Diversidade de produtos e *stocks*

A FC apresenta uma grande dimensão e, como tal, uma grande variedade de produtos e *stocks*. Para além dos habituais medicamentos sujeitos e receita médica (MSRM) e medicamentos não sujeitos a receita médica (MNSRM), a farmácia apresenta ainda uma vasta gama de produtos no que diz respeito às áreas de dermocosmética, fitoterapia, suplementação

alimentar, nutrição desportiva, puericultura, ortopedia e medicamentos de uso veterinário (MUV). Inclusivamente, a farmácia dispõe de dois andares, dos quais um é exclusivamente dedicado a puericultura e ortopedia. Também os colaboradores da farmácia se preocuparam em transmitir-me vários ensinamentos acerca das várias categorias de produtos, no que diz respeito às suas características, vantagens e desvantagens de uns em detrimento de outros (com base no utente e na patologia em questão) e funcionamento dos mesmos. Estes conhecimentos também contribuíram para o enriquecimento da minha formação nas várias áreas que deverão ser dominadas por um farmacêutico.

2.1.3. Diversidade de serviços prestados

A FC dispõe de um conjunto de serviços variados tais como a determinação de parâmetros bioquímicos, fisiológicos e antropométricos, – colesterol total, colesterol HDL, glicémia, pressão arterial e cálculo do índice de massa corporal – administração de injetáveis e de vacinas não incluídas no Programa Nacional de Vacinação (PNV), consultas de nutrição e diagnósticos de pele e capilares. A determinação dos parâmetros enumerados facilita a adesão à terapêutica ao mesmo tempo que assegura a própria comodidade e bem-estar do doente, não sendo necessário deslocar-se a outro local para usufruir de um serviço que poderia ter sido prestado na farmácia. Além disso, o doente sente-se mais motivado a tomar a sua medicação se, através dos testes, vir melhorias significativas; por outro lado, os resultados permitem acompanhar a evolução do doente, podendo auxiliar os profissionais de saúde – o farmacêutico pode ajudar numa melhor adesão caso os resultados não cumpram o esperado, tentando perceber o motivo para tal, enquanto o médico pode acompanhar e ter conhecimento da evolução do doente, podendo alterar a dose, intervalo da toma, o fármaco, ou o que considere necessário caso os resultados não estejam dentro dos limites de valores esperados.

Durante o estágio, realizei várias vezes estes testes, o que considero ser extremamente positivo, não só pela aprendizagem na sua execução, mas também pela comunicação que fui capaz de estabelecer com vários doentes em múltiplas situações, longe da azáfama do balcão, aconselhando-os e esclarecendo dúvidas sobre as suas patologias, sensibilizando-os ao mesmo tempo para a importância de medidas não farmacológicas como alimentação, exercício físico e adoção de hábitos de vida mais saudáveis.

2.1.4. Preparação de Medicamentos Manipulados

As especialidades farmacêuticas devem apenas ser descondicionadas com a finalidade de serem utilizadas em medicamentos manipulados em casos excecionais, tais como: se não for possível encontrar no mercado nenhum medicamento com igual dosagem e forma farmacêutica, em caso de aplicação cutânea, preparações de uso pediátrico e ainda em medicação destinada a doentes cujas condições de administração ou farmacocinética se encontrem alteradas.

Aquando da preparação de um medicamento manipulado, o farmacêutico, responsável pela sua preparação e dispensação, deve assegurar-se da qualidade e segurança do mesmo, de acordo com as Boas Práticas de Farmácia. Um manipulado pode ser uma fórmula magistral, caso o medicamento seja executado segundo a descrição da receita médica ou um preparado oficial, executado segundo os procedimentos da Farmacopeia ou do Formulário Galénico Português [3].

A FC prepara medicamentos manipulados regularmente, possuindo instalações, matérias-primas e equipamentos adequados. Durante o meu estágio, participei na preparação de medicamentos manipulados, como a **Suspensão Oral de Trimetoprim**, um antibiótico utilizado normalmente para tratar infeções da bexiga, dos rins ou do ouvido (cistites, otites) provocadas por bactérias Gram negativas [4]; o manipulado do **Creme Quadriderme®**, uma associação de betametasona (corticosteróide), clotrimazol (antifúngico) e gentamicina (antibacteriano), descontinuado pela indústria farmacêutica e utilizado para o tratamento de afeções cutâneas, nomeadamente eczema [5] e a **Solução de Minoxidil a 5%**, uma solução cutânea hidroalcoólica indicada no tratamento da alopécia androgenética e areata (peladas) [6]. A preparação de medicamentos manipulados foi um ponto forte do meu estágio pois permitiu-me pôr em prática conhecimentos teóricos e laboratoriais obtidos na unidade curricular de Farmácia Galénica.

2.1.5. Importância do Robot

A receção, conferência e processamento de encomendas é realizada quase exclusivamente no segundo piso da farmácia, onde está localizado o Robot, modelo Rowa, ligado ao Sifarma 2000®. O Robot permite o armazenamento de uma maior quantidade de medicamentos, cuja entrada é dada manualmente e pelo mesmo processo quer exista ou não Robot na Farmácia. Os medicamentos são dispostos dentro do robot segundo o princípio

"*first in, first out"*, o que significa que os medicamentos saem de acordo com o prazo de validade, saindo primeiro do robot para venda aqueles com prazo de validade mais curto.

O Robot tem várias vantagens, nomeadamente rentabilizar o espaço da farmácia, já que a maior parte dos MSRM são ali armazenados; controlar os prazos de validade e o *stock* real dos medicamentos e aumentar a eficiência no próprio ato da dispensa ao público, fornecendo os produtos pedidos de forma automática, minimizando os erros de troca de medicação ou dosagens e tornando o processo de atendimento muito mais rápido e facilitado. É também importante numa farmácia com elevada afluência de clientes, já que desta forma se diminui o tempo de espera entre atendimentos.

Desta forma, minimiza-se o tempo que o farmacêutico despenderia na procura do medicamento, aumentando o tempo que está ao balcão, podendo fazer um aconselhamento mais completo. Para além disso, a existência de Robot foi também importante para o meu estágio na medida em que aquando da introdução dos medicamentos no Robot na zona de receção de encomendas, me pude familiarizar com as embalagens e associar as substâncias ativas às respetivas marcas comerciais.

2.1.6. Realização de rastreios e ações educativas em escolas

Para além das atividades mais comumente desempenhadas por farmacêuticos comunitários, a FC preocupa-se também em avaliar e educar a população para a saúde. Assim, durante o meu estágio realizei várias ações deste tipo, o que considero claramente um ponto forte. Primeiramente, fiquei responsável pela organização de um Rastreio Cardiovascular, realizado no dia 29 de setembro de 2018, dia Mundial do Coração. Neste, avaliei vários parâmetros como o peso, determinação do perímetro abdominal, medição da pressão arterial, medição da glicemia e do colesterol. Esta foi uma ótima forma de ter um contacto diferente e mais prolongado com os doentes, num ambiente extra-farmácia, já que na farmácia o tempo de atendimento é mais limitado e, muitas vezes, não é possível realizar um aconselhamento tão personalizado a cada paciente. Para além deste facto, foi ótimo para mim executar todos estes testes, pois para além da notável melhoria na sua execução, permitiu-me relembrar mais uma vez os valores de referência e aconselhar os doentes, pondo em prática os conhecimentos obtidos em Farmacoterapia.

Realizei ainda duas ações educativas em escolas sobre "Higiene Oral" e "Lanches Saudáveis", o que foi muito positivo na minha aprendizagem, visto que preparei toda a apresentação, enriquecendo os meus conhecimentos nos temas e saindo da minha zona de

conforto, já que a apresentação foi feita para crianças, demonstrando versatilidade enquanto futura farmacêutica e agente de saúde pública.

2.2. *Weaknesses* (Fraquezas)

2.2.1. Plano de Estágio

O ponto fraco do meu estágio foi, indubitavelmente, a ausência de um plano de estágio fixo e definido. O farmacêutico exerce uma grande variedade de funções em Farmácia Comunitária, portanto considero que teria sido importante e mais proveitoso para mim a existência de um plano de estágio bem delineado, de forma a não me sentir "perdida" como algumas vezes aconteceu. Sinto que, devido a uma má gestão do meu estágio, não passei por algumas áreas como a organização e conferência do receituário e gestão do *stock*. Mais importante ainda, passei pouco tempo ao balcão comparativamente com o tempo de *backoffice.*

O elevado fluxo de utentes da FC torna difícil a planificação das tarefas a atribuir aos estagiários, o que provocou em mim uma certa desmotivação e algumas vezes um sentimento de não realização.

2.2.2. Programa curricular do MICF

Apesar de o programa curricular do MICF abranger várias áreas e ser focado nas diversas vertentes que podem fazer parte do exercício profissional de um farmacêutico, considero que apresenta algumas falhas no sentido de ser um pouco estático, devendo evoluir e modernizar-se no sentido de assegurar todas as competências aos futuros farmacêuticos para que possam enfrentar com mais confiança os desafios da profissão, nomeadamente a nível do aconselhamento farmacêutico.

Penso que unidades curriculares como Dermofarmácia e Cosmética (DC), Preparações de Uso Veterinário (PUV), Nutrição Humana (NH) apresentam algumas falhas. Senti alguma dificuldade a aconselhar utentes em relação a afeções cutâneas e capilares, já que na área curricular de DC o aconselhamento é muito pouco abordado, não estando o conteúdo programático adaptado à realidade diária de uma farmácia comunitária. Relativamente a PUV, o conteúdo programático centra-se em antiparasitários, sendo as patologias mais comuns pouco abordadas, o que suscitou também dificuldades no aconselhamento.

Falando agora de NH, penso que o conteúdo lecionado está desatualizado, havendo inúmeras situações e avanços na área da Nutrição que não são abordados. Na minha opinião, seria importante que o conteúdo programático desta unidade curricular fosse atualizado, já que enquanto futuros farmacêuticos poderíamos auxiliar de melhor forma os pacientes nesta temática da alimentação, sabendo-se da importância das medidas não farmacológicas no sucesso da terapêutica.

Em relação a outra unidade curricular, Dispositivos Médicos (DM), penso que não deveria ser uma unidade curricular opcional, visto ser uma área tão presente e em expansão em Farmácia Comunitária. Visto que não escolhi esta opcional durante o meu percurso, no estágio tive dificuldade, por exemplo, com a área de ortopedia, tão presente na farmácia onde estagiei, tendo de recorrer à restante equipa para que me auxiliassem no aconselhamento nesta área.

2.2.3. Nome comercial

Na maior parte das situações que decorrem ao balcão, os medicamentos são pedidos por nome comercial e não pelo nome do princípio ativo. Inicialmente foi uma dificuldade associar o nome comercial ao princípio ativo inerente. Durante o MICF os conhecimentos são-nos transmitidos com base no princípio ativo, respetivo mecanismo de ação e patologia inerente, havendo uma falta de informação relativamente aos nomes comerciais dos medicamentos.

Nos MSRM, esta barreira é ultrapassada devido à prescrição feita por Denominação Comum Internacional (DCI), estando nestes casos o trabalho facilitado. No entanto, quando se tratam de MNSRM, a dificuldade aumenta, dado que na maior parte das situações estes são requisitados por nome comercial. Foi crucial estudar bem a panóplia de produtos existentes na FC de modo a evitar uma descredibilização por parte dos utentes.

Uma solução para este problema poderia passar por ser lecionado nas respetivas unidades curriculares ou em simulações práticas o nome comercial associado ao princípio ativo correspondente, diminuindo a insegurança inicial neste tema por parte do estagiário.

2.2.4. Imagem do estagiário por parte da população

Durante o meu estágio, deparei-me várias vezes com a falta de confiança demonstrada pelos utentes quando eram atendidos por mim ou por outro dos meus colegas estagiários,

exigindo algumas vezes que um farmacêutico os atendesse. Esta situação criou em mim um sentimento de alguma impotência, já que durante o estágio o estagiário encontra-se num percurso de aprendizagem, a qual é dificultada caso não realize o máximo de atendimentos e se depare com as mais variadas situações.

Os utentes fidelizados apresentam um sentimento de desconfiança perante a nova figura que é o estagiário, pensando muitas vezes que não somos capazes e que não vamos prestar um atendimento e aconselhamento ao mesmo nível que outro farmacêutico pelo qual costumam ser atendidos. Os utentes de passagem associam também, não raras vezes, um estagiário a falta de conhecimento e experiência.

Na minha opinião, seria importante educar a população para o facto de ser determinante para um estagiário realizar o maior número de atendimentos e aconselhamentos, pois só desta forma adquirirá prática e poderá tornar-se no melhor profissional de saúde possível.

2.3. *Opportunities* (Oportunidades)

2.3.1. Acesso a formações externas e internas com delegados de informação médica

É de extrema importância para qualquer profissional de saúde manter-se atualizado sobre novos produtos e quaisquer inovações na sua área. As formações são uma mais-valia para o farmacêutico e, na minha opinião, algo que devemos aproveitar para realizar enquanto estagiários, já que muitas vezes existe falta de compatibilidade entre o horário de trabalho do farmacêutico e a hora das formações, impossibilitando a comparência nestas últimas.

Devido à dimensão da farmácia e quantidade de produtos, *stocks* e marcas, as próprias marcas e delegados de informação médica faziam várias visitas à FC para dar formações internas de vários produtos, principalmente das novidades e produtos mais recentes, o que contribuiu também muito positivamente para a minha formação e maior conhecimento de vários produtos. Durante o meu estágio na FC tive a oportunidade de assistir a várias formações internas, focadas essencialmente na área de dermocosmética e suplementação alimentar. As formações tinham normalmente uma duração curta, referindo o conceito da marca e destacando as vantagens, indicações, aconselhamento e dicas/técnicas de *cross-selling* a serem realizadas aquando da venda de alguns produtos. Estas formações são muito importantes pois auxiliam bastante no aconselhamento do utente ao balcão, para além do seu

caráter informativo. Atualmente há uma panóplia enorme de produtos na farmácia, principalmente na área de dermocosmética, daí a importância destas formações na transmissão das características de cada produto de uma forma individualizada. Destaco as formações da SkinCeuticals®, Nuxe®, Vichy®, MartiDerm®, Lierac® e Avene®.

Tive também oportunidade de participar em várias formações fora do ambiente físico da farmácia, das quais destaco a da Nutribén®, já que tratava especificamente da nova gama da marca – "Nutribén Innova", o único leite de fórmula do mercado com bifidobactérias *BPL1*, usualmente presentes no trato gastrointestinal de bebes alimentados com leite materno. Tem assim várias vantagens relativamente a outros leites, como na questão da saciedade, anti-inflamatório e único da marca que não possui óleo de palma na sua composição. As formações a nível da puericultura foram de grande importância para mim na medida em que não dominava de todo o assunto, preenchendo desta forma a lacuna do MICF em relação a este tema.

2.3.2. Programa VALORMED

A VALORMED é uma sociedade sem fins lucrativos cuja função é a gestão de resíduos de medicamentos fora de uso e respetivas embalagens, resultante da colaboração entre a indústria farmacêutica, distribuidores grossistas e farmácias comunitárias. Os contentores de recolha são entregues aos distribuidores, sendo transportados para um centro de triagem e tratados de acordo com o tipo de resíduo [7].

Vários utentes foram entregando os seus medicamentos sem uso, fora de validade ou vazios para colocar no contentor da VALORMED. Senti que os utentes da FC estavam muito consciencializados dos benefícios ambientais deste ato, fazendo-o com grande frequência e livremente, devido ao excelente trabalho de sensibilização feito pela equipa da FC ao longo do tempo. A minha função também passou por contactar e promover este sistema junto dos utentes que se encontravam menos sensibilizados para o tema, explicando o seu significado e importância.

A participação ativa e empenhada da FC na informação e sensibilização dos utentes traduziu-se na taxa de resíduos recolhida no ano de 2017, tendo sido a Farmácia em Portugal com maior taxa de recolha de resíduos de medicamentos naquele ano.

2.3.3. Consultas de nutrição

A FC trabalhava com uma equipa de nutricionistas da *Uriach Theralab*, cujo regime alimentar proposto tinha o nome de "Dieta do pH". Durante o mesmo estágio, o laboratório reformulou o conceito da dieta, a qual passou a designar-se "Prato". Incluía quatro regimes alimentares diversos, pensados e adaptados às necessidades individuais – "Prato e Bikini" adaptado ao excesso de peso, gordura localizada e celulite; "Prato e Saúde" adaptado a doentes com patologias crónicas; "Prato e Biberão" pensado para grávidas e "Prato e Vida" adaptado a regimes alimentares vegetarianos e vegans.

O conhecimento desta nova dieta e a presença de uma nutricionista semanalmente na farmácia foram uma boa oportunidade para melhorar os meus conhecimentos na área da nutrição e alimentação, assim como dar dicas que poderão ser benéficas para os doentes medicados conjuntamente com a terapêutica farmacológica.

2.4. *Threats* (Ameaças)

2.4.1. Medicamentos Esgotados

Os medicamentos esgotados foram um assunto recorrente em todo o meu estágio. Muitas vezes tive de lidar com a revolta e desagrado dos doentes por não lhes conseguir ceder um medicamento que tomavam cronicamente.

A realidade dos medicamentos esgotados é cada vez mais frequente graças à exportação paralela que permite que qualquer país da União Europeia possa exportar medicamentos para outro país membro. Como Portugal apresenta dos preços mais baixos da Europa, exporta uma grande parte dos medicamentos, havendo rutura de stock dentro do próprio país [8].

Recordo, por exemplo, o caso do Pritor® 80mg, contendo a substância ativa Telmisartan, indicado para o tratamento da hipertensão e redução de eventos cardiovasculares [9]. Este medicamento tem um caráter crónico, e a constante rutura de stock causava ansiedade e descontentamento nos doentes e nos profissionais. Constituiu uma ameaça ao meu estágio porque muitas vezes não consegui satisfazer as necessidades do doente durante o atendimento.

2.4.2. Parafarmácias

As parafarmácias e outros locais de venda de MNSRM e dispositivos médicos são, de facto, uma ameaça devido aos preços inferiores que conseguem praticar e à banalização do conceito de medicamento. Estes locais, muitas vezes inseridos em grandes superfícies, apresentam preços competitivos devido ao grande volume de compras que efetuam comparativamente com uma farmácia. Muitas vezes nestes estabelecimentos os MNSRM são vendidos por pessoas sem qualquer formação na área, o que representa um risco para a saúde pública.

Através do seu atendimento, o farmacêutico desempenha um papel preponderante em demonstrar que a profissão farmacêutica é imprescindível e insubstituível, evidenciando o seu papel único a nível do aconselhamento do utente.

2.4.3. Medicamentos Genéricos

Desde a alteração da prescrição de medicamentos para a prescrição por DCI, o utente tem o direito de optar por um medicamento com a mesma DCI, forma farmacêutica, dosagem e tamanho de embalagem similares ao prescrito, devendo ser questionado pelo farmacêutico sobre a sua preferência e informado sobre os que têm o preço mais baixo disponível no mercado [10].

Considero os medicamentos genéricos uma ameaça ao meu estágio, já que muitas vezes os utentes se recusaram a comprá-los, duvidando destes medicamentos ao nível da qualidade e eficácia. Aconteceram também alguns casos em que os utentes afirmavam não querer comprar genéricos, acabando eu por constatar que sempre estiveram a tomar genéricos, acreditando que tomavam o medicamento de marca. Posto isto, existe uma ideia pré-concebida sobre os medicamentos genéricos e até alguma ignorância neste tema, o que constituiu muitas vezes uma ameaça no meu atendimento.

Os farmacêuticos devem também tentar desmistificar este tema junto da população e contribuir para uma maior literacia em relação aos medicamentos genéricos.

3. Considerações Finais

Findados ambos os estágios, tanto em Farmácia Comunitária como em Farmácia Hospitalar (feito ao abrigo do programa Erasmus) compreendo agora, mais do que nunca, a importância da profissão farmacêutica.

O estágio curricular é o momento de pôr em prática todos os conhecimentos técnico-científicos adquiridos e de aprender tantos mais, com uma equipa de profissionais incansável e que me transmitiu a sua experiência, auxiliando-me diariamente. Aprendi a trabalhar em equipa, a ser mais autónoma, empática e paciente. Através do contacto com a equipa e com os utentes, cresci enquanto farmacêutica, mas também enquanto pessoa.

Assisti de perto à importância da figura do farmacêutico comunitário para muitos utentes, através da confiança tantas vezes depositada, dos conselhos pedidos, das conversas e dos agradecimentos. Vi também o outro lado, o da desvalorização da profissão, da banalização do conceito de medicamento e da não cooperação entre profissionais de saúde.

Mais do que nunca, o farmacêutico deve apostar na sua formação contínua, através da do estudo e atualização científica constantes, proporcionando o melhor aconselhamento possível aos cidadãos e à sociedade, desmistificando a ideia de que o seu papel se resume a dispensar medicamentos.

Juntamente com o estágio em Farmácia Comunitária, o estágio em Farmácia Hospitalar na área de Farmacovigilância realizado em Roma permitiu-me percecionar o caráter polivalente do farmacêutico, através da diversidade de funções que pode desempenhar dependendo da área onde se encontra, destacando mais uma vez a importância da formação constante.

Considero ambos os estágios como etapas essenciais do meu percurso e formação enquanto futura farmacêutica, proporcionando-me tantas aprendizagens, que sempre levarei comigo independentemente da área profissional onde me encontre.

Anexos

Caso Clínico 1

Uma senhora com cerca de 30 anos dirige-se à farmácia com queixas de infeção urinária, afirmando apresentar ardor e dor ao urinar, micções frequentes e em pequena quantidade e uma necessidade urgente de urinar que estava a prejudicá-la no seu trabalho. Estes sintomas duram há 1 dia.

Após questionada, a utente revela ter infeções urinárias recorrentemente e que, inclusivamente, tinha acabado há pouco tempo a toma de um antibiótico receitado pelo médico. Expressa ainda a vontade de não querer tomar novamente antibiótico, perguntando se não existe alguma medida menos invasiva e mais natural. Questionei ainda a senhora sobre a existência de alguma patologia como diabetes, já que a infeção poderia dever-se aos elevados níveis de glicémia. A resposta foi negativa, assim como quando questionei se estava a tomar alguma medicação – como por exemplo imunossupressores, que poderiam aumentar a suscetibilidade à infeção.

Aconselhei o suplemento alimentar Systelle®, contendo 400mg de extrato seco de uva-ursina, antissético natural cujas folhas ricas em arbutina ajudam a limpar o trato urinário, sendo conhecidas as suas propriedades antibacterianas e diuréticas. Assim, alivia os sintomas de infeções leves e recorrentes no trato urinário inferior, tais como sensação de ardor ao urinar e a elevada frequência das micções. Deve tomar-se dois comprimidos três vezes por dia, durante sete dias, depois das refeições. O principal mecanismo de ação da arbutina consiste na hidrolisação pela beta-glucosidase presente na bactéria, libertando hidroquinona, cuja ação antissética e de destabilização das membranas destroem a parede bacteriana [11].

Informei a doente da existência de imunoterapia oral para este tipo de casos, constituindo uma medida profilática eficaz para pessoas que desenvolvem infeções urinárias recorrentemente. Expliquei que consiste numa vacina para administração oral (em comprimidos) com o nome de Uro-Vaxon® (lisado bacteriano de *Escherichia coli*) [12] e sugeri que propusesse ao médico na próxima consulta, pois poderia ser uma boa opção para diminuir as recidivas.

No entanto, alertei a doente para o facto de o suplemento alimentar que acabara de comprar ser apenas eficaz em casos de infeções urinárias não instaladas, devendo consultar o médico novamente caso a sintomatologia permanecesse ou agravasse.

Caso Clínico II

Uma senhora com cerca de 50 anos apresentou-se na farmácia com queixas de formigueiro, dor, sensação de peso nos membros inferiores e algum inchaço, sobretudo no final do dia. Queixou-se ainda que passa muitas horas de pé no trabalho diariamente. Depois de ter examinado as pernas da utente no gabinete, concluí que apresentava algumas veias varicosas em estado inicial, tratando-se de uma paciente com potencial Doença Venosa Crónica em estado inicial. Após questionada, a utente afirmou não fazer nenhuma medicação habitual, com exceção da pílula contracetiva.

Aconselhei-lhe um medicamento não sujeito a receita médica venoativo com diosmina na sua composição, já que esta possui uma ação a nível da macro e microcirculação, aumentando o tónus venoso, diminuindo a permeabilidade capilar e atuando sobre a parede e válvulas venosas, protegendo as células da hipoxia e prevenindo o refluxo venoso. Promove também uma melhoria do fluxo linfático e têm uma ação anti-inflamatória. Algumas destas formulações contêm também hesperidina na sua composição. Daflon® e Flabien® são duas das alternativas disponíveis no mercado. Juntamente com as medidas farmacológicas, aconselhei também o uso de meias de compressão elástica que previnem a formação do edema, diminuindo o calibre venoso e aumentando a velocidade do fluxo, reduzindo o refluxo na posição vertical [13].

Alertei também para algumas medidas não farmacológicas a adotar, tais como procurar sentar-se nas pausas do trabalho com as pernas esticadas, deixar de fumar, fazer exercício físico e preferir desportos como ginástica, natação, ciclismo ou dança, já que estimulam a contração muscular e o retorno venoso. Caminhadas também são uma boa opção. Aconselhei a evitar expor as pernas ao sol durante um longo período de tempo ou estar em lugares muito quentes como sauna ou banhos quentes, porque o calor provoca dilatação e estase venosa; evitar o uso de roupa apertada que dificulta a circulação e o uso de sapatos altos ou muito rasos. Durante o sono o sistema venoso não é estimulado, devendo elevar os pés 10 a 15cm na cama depois do trabalho ou antes de se deitar. A contraceção oral também pode piorar a doença uma vez que os estrogénios aumentam a permeabilidade venosa e a progesterona promove a dilatação [14].

Aconselhei a doente a dirigir-se ao médico para confirmar o diagnóstico de Doença Venosa Crónica, através da história clínica e do exame físico a ser realizado (Doppler).

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Parte II

Monografia

Anti-PD-1 Immunotherapy in Advanced Metastatic Melanoma

Resumo

A imunoterapia com inibidores do *checkpoint* imunitário, como os fármacos anti-PD-1, é uma área em crescente desenvolvimento devido à sua eficácia terapêutica e às vantagens no tratamento do melanoma metastático avançado. De facto, a imunoterapia tem sido alvo de vários estudos recentes em diversos tipos de cancro, nomeadamente no melanoma, uma ameaça crescente a nível mundial. A contribuir para a incidência crescente deste cancro estão as alterações climáticas, em particular o aquecimento global do século passado, que veio aumentar a tendência para passar mais tempo ao ar livre e, consequentemente, a exposição à luz solar e radiação ultravioleta. De entre os fatores de risco mais relevantes para o melanoma, o aumento da radiação ultravioleta devido à destruição da camada do ozono constitui um dos principais responsáveis pelo número crescente de novos casos. Os agentes anti-PD-1, tais como o Nivolumab e o Pembrolizumab, permitem um tratamento mais eficaz, aumentando a duração das respostas à terapia e prolongando a sobrevida do paciente. No entanto, estudos recentes de segurança e tolerabilidade afirmaram que, embora estes fármacos apresentem menos efeitos adversos e toxicidade, podem ser responsáveis por eventos adversos específicos mediados por autoimunidade. No geral, a imunoterapia com agentes anti-PD-1 representa uma área altamente promissora no tratamento de alguns tipos de cancro, tal como o melanoma.

Palavras-chave: Cancro, Eventos adversos mediados por autoimunidade, Imunoterapia anti-PD-1, Inibidores do *checkpoint* imunitário, Melanoma metastático avançado, Nivolumab, Pembrolizumab, Toxicidade.

Abstract

Immunotherapy with immune checkpoint inhibitors, such as anti-PD-1 drugs, is an area in increasing development for its efficacy and advantages in the treatment of advanced metastatic melanoma. In fact, immunotherapy has been the target of several and recent studies in different types of cancer, namely in melanoma, a globally growing threat. Contributing to the increasing incidence of this cancer is climate change, particularly global warming of the past century, which has increased the tendency to spend more time outdoors and, consequently, the exposure to sunlight and ultraviolet radiation. Among the most relevant risk factors for melanoma, the increase in ultraviolet radiation due to ozone layer depletion is one of the main factors responsible for the incidence of new cases. Anti-PD-1 agents like Nivolumab and Pembrolizumab allow a more effective treatment, increasing the duration of the responses to the therapy and prolonging the survival of the patient. However, recent studies about safety and tolerability have stated that, although these drugs present less adverse effects and toxicity, those may lead to specific autoimmune-mediated adverse events. Overall, immunotherapy with anti-PD-1 agents represents a highly promising area in the treatment of some types of cancer such as melanoma.

Keywords: Advanced metastatic melanoma, Anti-PD-1 immunotherapy, Autoimmune-mediated adverse events, Cancer, Immune checkpoint inhibitors, Nivolumab, Pembrolizumab, Toxicity.

Abbreviations

ADL – Activities of Daily Living

AE – Adverse Event

AJCC – American Joint Committee on Cancer

APC – Antigen Presenting Cells

BANS – Back, Arm, Neck and Scalp

CD – Cluster of Differentiation

CNS – Central Nervous System

CPDs – Cyclobutane Pyrimidine Dimers

CR – Complete Response

CTLA-4 – Cytotoxic T Lymphocyte Antigen-4

DDFS – Distant Disease-Free Survival

DFS – Disease-Free Survival

FDA – Food and Drug Administration

GITR – Glucocorticoid-Induced Tumor Necrosis Factor Receptor-Related Protein

HR – Hazard Ratio

IDO – Indoleamine 2,3-Dioxygenase

IFN-α – Interferon Alpha

IFN-γ – Interferon Gama

IgG4 – Immunoglobulin G4

IL-2 – Interleukin-2

IR – Infrared

irAE – Immune-related Adverse Event

ITM – In-transit Metastasis

IV – Intravenous

LAG-3 – Lymphocyte Activation Gene-3

LDH – Lactate Dehydrogenase

LNs – Lymph Nodes

MAPK – Mitogen Activated Protein Kinase

MC1R – Melanocortin Receptor 1

MDN – Melanocytic Dysplastic Nevi

MHC – Major Histocompatibility Complex

MM – Malignant Melanoma

MSH – Melanocyte Stimulating Hormone

NCI – National Cancer Institute

NK – Natural Killer

ORR – Overall Response Rate

OS – Overall Survival

OX-40 – Tumor Necrosis Factor Receptor-4

PD-1 – Programmed Cell Death Protein-1

PD-L1 – Programmed Cell Death Protein Ligand 1

PD-L2 – Programmed Cell Death Protein Ligand 2

PFS – Progression-Free Survival

PI3K – Phosphoinositol-3-Kinase

PR – Partial Response

RECIST – Response Evaluation Criteria in Solid Tumours

RGP – Radial Growth Phase

ROS – Reactive Oxygen Species

TCR – T-cell Receptor

TH – T-Lymphocytes Helper

TIM-3 – T-cell Immunoglobulin and Mucin domain-3

TNFR – Tumor Necrosis Factor Receptor

T-VEC – Talimogene Laherparepvec

UV – Ultraviolet

UVR – Ultraviolet Radiation

VGP – Vertical Growth Phase

I. Introduction

Melanoma is an aggressive form of skin cancer, caused by the transformation of melanocytes (pigment-producing cells) into malignant cells. Melanoma is responsible for most of the deaths related to skin cancer [1]. The incident rates for melanoma are increasing: in 2018 in Europe, it was the fifth and eighth cancer with more estimated new cases among females and males, respectively, posing a major health threat worldwide [2].

The most relevant risk factors for melanoma are ultraviolet (UV) exposure, number of melanocytic nevi, family history and genetic susceptibility, being considered a multi-factorial disease based on the environmental exposure-genetic susceptibility interaction. The destruction of the ozone layer and the consequent increase in the amount of radiation that reaches the earth's surface are associated with a high risk of melanoma. Melanocytic nevi, benign agglomerations of pre-existing melanocytes or nevus cells (congenital or acquired) are responsible for a small percentage of melanoma cases when compared to UV radiation [3,4]. Two principal areas have been investigated: ultraviolet radiation (UVR) and genetic abnormalities. It is known that UVR is implicated in the most part of melanoma cases, approximately 86%, while the genetic predisposition, although exists, constitutes a small percentage of the cases. Malignant melanoma (MM) is associated with repeated and sporadic UV exposure and occurs more frequently in people who have had multiple sunburns as children or adolescents [5–7]. Being intermittently and repeatedly exposed to sunlight from childhood is epidemiologically considered the primary cause of benign and malignant skin tumours, including MM [8,9]. Also, the incidence of MM changes according to the skin phenotype, being more frequent among fair-skinned population in Northern Europe comparing to darker-skinned individuals in Southern Europe. Higher levels of melanin mean increased photoprotection, explaining a lower percentage of MM in Southern Europe in countries such as Portugal, Spain and Italy [10]. Additionally, its incidence increases with age [11].

Five-year survival rates – the percentage of people who are alive five years after they were diagnosed or started treatment [12], depend on the stage of the disease at the time of diagnosis. The best way to reduce mortality rates is the early detection [13].

The most part of melanomas are normally located in the skin, but also can be found in the eyes, ears, gastrointestinal tract, leptomeninges and oral and genital mucous membranes [14]. In the Figure 1 are represented the layers of the skin and types of skin cells, including melanocytes, the most important cells in melanoma [15].

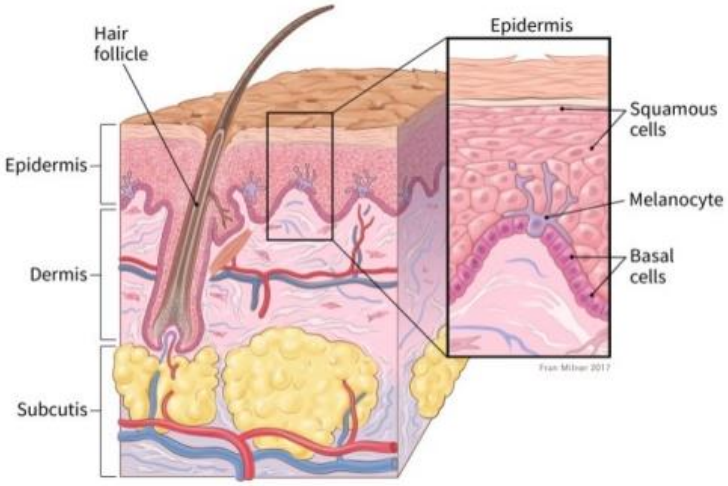

Figure 1 – Layers of the skin and types of skin cells [15]

2. Ultraviolet radiation

2.1. Ultraviolet radiation and risk of melanoma

Solar light is composed by a wide range of wavelengths from infrared (IR) to ultraviolet range. Radiation in the UV range (100-400 nm) is the most potent risk factor for skin cancers due to its genotoxic effect. The UV range can be divided into three different spectral regions, according to their biological effects – the band 100-280 nm is designated as UVC, 280-315 nm as UVB and 315-400 nm as UVA. Each spectrum has a characteristic limit of efficiency in penetrating the epidermal and dermal layers of the skin [3].

Normally, UVC rays would not be a problem in skin cancer because they are mostly blocked by the ozone layer before they could reach the earth´s surface. With the ozone depletion, this scenery has changed and now this UV range can also constitute a risk factor. If this short-wave radiation penetrates the skin, even if it can only penetrate until a maximum depth of 60–80 μm, it can damage DNA molecules and cause mutations, because this type of radiation is energetic and mutagenic [8,9].

UVB radiation is the main responsible for most of the cutaneous damage, including non-melanoma and melanoma skin cancers, being the most cytotoxic and mutagenic type of UV radiation [8]. Although UVA is the most part of the solar UV radiation (90–95%), UVB exhibits higher energy which is more potent in inducing damages in DNA [16]. Also, DNA is a chromophore for UVB radiation and not for UVA. A chromophore is a substance that absorbs light in the molecule [17], which means in this case that DNA bases absorb directly incident photons on the wavelength of UVB. It can penetrate the skin to a depth of approximately 160–180 μm. It can cross the whole epidermis layer and penetrate the dermis

inducing adverse biologic effects, directly or indirectly – oxidative stress, DNA damage, premature aging of the skin and multiple effects on the immune system. UVB can act as a tumor initiator, tumor promoter and co-carcinogen [5,7]. Among the most known and most frequent DNA damage related to UVR is the formation of cyclobutane pyrimidine dimers (CPDs). The 6-4 pyrimidine dimers cause mutation in the epidermal cells which can lead to the development of cancer cells. UVB is responsible to upregulate gene expression through intracellular signal transduction pathways, which may contribute to developing skin cancer at the tumor promotion stage. In addition, UVB is proved to suppress immune reaction and to induce tolerance to antigens [8].

The longest UV wavelengths (UVA) can penetrate deeper into the epidermis and dermis of the skin, until a depth of approximately 1000 μm. Because of their lower energy, it was thought that their effects on skin diseases were minimum. Now, it is known that UVA radiation is involved in many processes, including carcinogenesis, due to penetrate until deeper skin layers and produce reactive oxygen species (ROS), which cause secondary damage to DNA and induce skin cancer [5,16].

Both UVA and UVB radiations produce DNA damage directly and indirectly through oxidative stress. Skin diseases induced by UVR are mainly caused by excessive induction of inflammation, oxidative stress and DNA damage [9]. Although UVR from the sun is responsible for the most part of the skin diseases, some cases are due to UVR from lamps and tanning beds [5,18].

The skin is the first line of defense against external insults and adverse biological events. It possesses several mechanisms to defend from the aggressions like specialized cells and cellular structures. Despite this fact, the excess of exposure to UVR destroys the cutaneous defense system, leading to the development of some skin disorders like MM [5,13].

3. Cancer immunology

Cancer is an inflammatory disease. Neoplastic cells have the capacity to evade the immune system and create the best microenvironment for their own proliferation. The immune system consists basically of two different cell types in different maturation phases and can be subdivided in innate immune system and adaptive immune system. The innate immune system is our first line of defence trough abnormalities but has no immunological memory, meaning it does not involve specific recognition of immunogenic proteins called antigens. The adaptive immune system consists in a specific response, meaning it identifies an aggressor or

antigen and maintains immunological memory in case of a new exposure to the same agent. In this case, the response is given to a tumor-associated antigen. The innate and the adaptive systems are both involved in fighting cancer, playing an important role in regulating the growth of cancer, stimulating or inhibiting it, depending on the inflammatory environment they create [19].

Depending on the type of cells released by both systems, they can inhibit or stimulate cancer growth. Immune system cells are involved in tissue repair, however the factors secreted by these cells can enhance rather than inhibit tumor growth, causing the cancer progression. The innate immune response can limit cancer progression but can also be pro-tumorigenic. Tumor-associated macrophages can cause chronic inflammation in the tumor microenvironment and promote tumor growth because of the release of growth factors and cytokines (these can also be pro or anti-tumorigenic depending on the type that is produced). In many cancers it is found an inflammatory response like in a chronic inflammation, which results in lots of innate immune cells, like tumor-associated macrophages promoting angiogenesis, cell proliferation (just like in a wound healing process) and even metastasis [20,21].

4. Advanced metastatic melanoma

4.1. Pathophysiology

During the cutaneous defense mechanisms, melanocytes (melanin-producing cells) are a very important cell type, located in the basal layer of the epidermis. The exposure to UVR causes keratinocytes to produce the melanocyte stimulating hormone (MSH) that binds the melanocortin receptor 1 (MC1R) on the melanocytes, which are responsible for the production and release of melanin. Melanocytes synthesize melanin in melanosomes transported into keratinocytes, conferring protection from UVR. This protection comes from the ability of melanin to block a wide spectrum of UVR, operating as a shield for these radiations, preventing more DNA alterations [22]. Skin cancers have complex mechanisms involving multiple pathways and the immune system. The steps can be briefly divided into:

(1) initiation, an irreversible step of DNA mutations due to genotoxic effects in normal cells;

(2) promotion, a reversible stage of clonal expansion;

(3) progression, transformation of the initial cells into carcinoma malignant cells [5].

4.2. Melanomagenesis

Melanoma results of a process called melanomagenesis or melanoma tumorigenesis, an abnormal proliferation of atypical melanocytes in the epidermis, that can spread to the dermis and subcutaneous cellular tissue, capable of eluding the immune system, invading underlying tissues and metastasize to other organs by lymphatic pathway or blood [23].

Although the exact sequence of events leading to melanomagenesis is not well known, it involves a multistep process of genetic mutations that interfere with cell proliferation, differentiation, and death [5–7].

There are five main steps during melanomagenesis, easily distinguished because the melanocytes produce melanin that makes their proliferation perceptible and the lesions formed observable:

(1) melanocytic nevi with melanocytic hyperplasia, aberrant differentiation;

(2) melanocytic nevi with aberrant differentiation and melanocytic nuclear atypia, melanocytic dysplasia;

(3) radial growth phase of primary melanoma (RGP);

(4) vertical growth phase of primary melanoma (VGP);

(5) metastatic melanoma.

Melanocytic dysplastic nevi (MDN) are normally biologically stable, but some have histological atypia and can progress to melanoma; they are believed to have pre-malignant potential, being potential precursors and risk factors for melanoma, although without metastatic capacity. Classification of the radial (nontumorigenic) or vertical (tumorigenic) growth phases is an attempt to distinguish metastatic melanomas from less severe forms, revealing the tumor progression. The first step to the melanocytic proliferation is represented by the hyperplasia of melanocytic nevus in the dermoepidermal junction. A group of cells remain stable and the other group continues to grow and can follow two pathways – either differentiation or aberrant differentiation leading to melanocytic dysplasia. It may be considered that the first and early stage of melanoma happens only in the third phase – radial growth phase, where a thin intraepidermal tumor is formed. RGP of primary melanoma cells progression is sequential and focal and they grow indefinitely and independently, with partial growth autonomy. This capacity is crucial for the tumor to grow, since in the preceding steps the lesion growth was limited and self-controlled. Metastatic melanoma starts to develop when the cells acquire autonomy during the transition to vertical growth phase, the most crucial step in melanomagenesis. The different cell types and distribution in the two last phases allow a subclassification of melanoma based on the histology.

The primary metastatic sites for melanoma are the lymph nodes and the secondary are commonly skin, subcutaneous soft tissue, lung and brain. The thicker the primary tumor, the greater the risk of metastases. Melanoma metastases in the skin are normally represented by dermal nodules of different sizes without epidermal connection and lymphocytic response [24–26].

4.3. Mutations in malignant melanoma

Melanoma is one of the types of cancer most associated with genetic mutations. Mutations in MM were fundamentally reported in two oncogenes: *BRAF* and *NRAS*. Mutations in *BRAF* were found in approximately 60% of melanomas and mutations in *NRAS* were found in 15–30% of melanomas [16]. These genomic alterations and mutations in genes that activate some pathways in melanoma, can lead to the aberrant activation and dysregulation of two of these main pathways: Mitogen Activated Protein Kinase (MAPK) signalling pathway, also known as RAS/RAF/MEK/ERK and Phosphoinositol-3-Kinase (PI3K) pathway.

The MAPK pathway is involved in the transduction of extracellular signals to the nucleus, leading to the expression of key genes in cell proliferation, differentiation and survival. It represents the most commonly aberrant activated pathway in MM, about 90% of the cases [22]. BRAF, mutated in more than half of MM cases, is a signalling serine/threonine protein kinase at the top of the MAPK pathway, linking extracellular signals to the interior of the cell, controlling cellular growth, proliferation, differentiation, migration, and apoptosis. Mutations in the *BRAF* gene can lead to the expression of mutant BRAF protein that is always active and no longer requires extracellular signals [27]. This leads to the constitutive activation and increase of some MAPK signalling processes, such as uncontrolled cell proliferation, invasion, metastasis, survival, angiogenesis and apoptosis inhibition, which are steps involved in melanoma development [28]. *RAC1*, *PPP6C* and *STK19* are also on the list of mutated oncogenes in MM [5].

The PI3K pathway is involved in cellular homeostasis, representing the second most frequently activated pathway in MM [22]. Figure 2 describes the stages of melanomagenesis and the mutations occurred during the formation of this tumor [6].

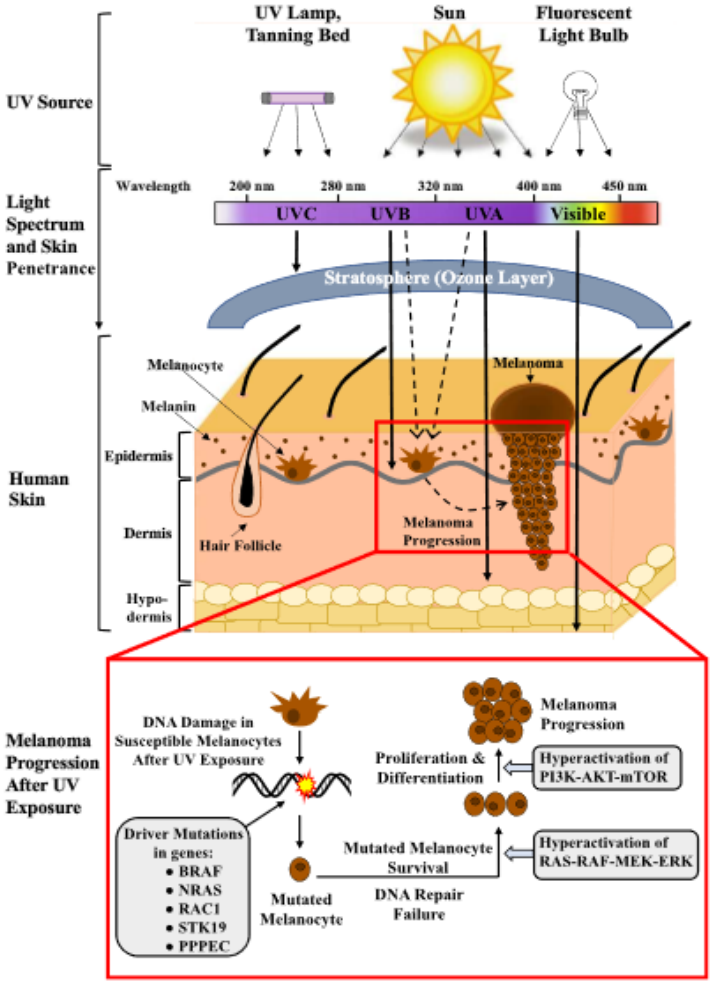

Figure 2 – Stages of melanomagenesis and mutations in melanoma [6]

4.4. Prognostic factors

The histopathologic factors that can be considered predictive for melanoma include linear depth, histologic level, mitotic rate, inflammatory (lymphocytic) response, and ulceration. The clinical characteristics with prognostic significance include tumor site, sex and age.

Primary tumour lesions located in contiguous areas (BANS) – upper back, posterior arm, posterior neck, and posterior scalp are more likely to metastasize than those ones located in extremities. Otherwise they metastasize more, BANS lesions tend to be ignored longer than non-BANS because such lesions normally don´t get thicker, in contrast to the non-BANS lesions. This could happen because this subset of BANS lesions can metastasize early or the events leading to that metastization are different from the most part of other melanoma subsets [29].

Gender is also a factor to be considered, since it constitutes an important prognostic factor and independent predictor of survival in patients with MM. It is known that male patients

have a worse outcome when compared to females, having a worse disease-free survival (DFS), distant disease-free survival (DDFS), and overall survival (OS). However male melanoma patients have worse primary tumor characteristics than females, the reasons for this gender difference are not well clarified yet [30]. According to other studies, the different prevalence in both sexes must be analysed in relation with age, since the incidence rate of melanoma depends on the age – until 40 years old, the incidence is higher in women than in men but by 75 years of age, the incidence is almost 3 times higher in men comparing to women [4].

Primary melanomas become thicker, with higher mitotic rates and are more likely to be ulcerated with the increased of age. The first reason why patient age has not been incorporated into staging systems yet is because there is not a well-defined limit of patient age that signals a worse prognosis. Then, it is not known if old people are disadvantaged due to factors related to age – declining immune competence and other comorbidities or factors associated to the disease itself – or due to differences in the biological behaviour of melanoma. Plus, since melanoma among teenagers and children is much less common, it is not possible to do a valid comparison between this group and the older population group, which means that patient age might not be a truly independent prognostic factor [31].

Thickness is the most important predictor of metastatic risk in the primary MM, followed by histologic ulceration (Tumor [T] and Nodal [N] classifications) and mitotic rate. Melanoma ulceration is the absence of an intact epidermis covering an important portion of the primary melanoma based on microscopic examination of the histologic sections. It is important to define the stage of the MM because it improves the clinician-patient and clinician-clinician communication, the clinical decision-making and the prognostic of the disease. The staging is needed for clinical trials, eligibility, stratification, analysis and report the data.

4.5. Possible classifications of metastatic melanoma

According to the *American Joint Committee on Cancer* (AJCC), these are the possible classifications for MM, performed after the biopsy of the primary melanoma and the evaluation of the lymph nodes:

4.5.1. T-Category (Tumor Classification)

Criteria including tumor thickness (measured to the nearest 0.01 mm) and presence or absence of ulceration in all subcategories. In the stage T1, mitoses (<1 VS ≥1 mitosis/mm²)

are also considered to measure the tumor mitotic rate. In the Table 1, the AJCC T-Classification is represented [32].

Table 1 – AJCC T-classification

STAGE	BRESLOW THICKNESS (mm)	DEFINITION
Tis[1]	Not applicable	Not applicable
TX[2]	Not applicable	Not applicable
T0[3]	Not applicable	Not applicable
T1	≤1.00	a: No ulceration and <1 mitosis/ mm² b: Ulceration or ≥1 mitosis/mm²
T2	1.01-2.00	a: No ulceration b: Ulceration
T3	2.01-4.00	a: No ulceration b: Ulceration
T4	>4.00	a: No ulceration b: Ulceration

[1]Melanoma *in situ*

[2]Primary tumor thickness cannot be assessed (eg: diagnosis by curettage)

[3]No evidence of primary tumor (eg: unknown primary or completely regressed melanoma)

4.5.2. N-Category (Nodal Classification)

Criteria including regional lymph node disease as well as non-nodal regional disease (satellites, microsatellites and in-transit metastasis (ITM)).

A non-nodal regional disease occurs when there is a regional spread of the tumor via lymphatic vessels in the dermis or subcutaneous tissue but outside of nodal basins.

In the AJCC staging system, satellite lesions and in-transit metastases are in the same group, considered a manifestation of intralymphatic disease. For this reason, it is not necessary to distinguish these two lesions from a clinical point of view, since the tumor biology treatment and prognosis are similar. The difference is that satellite lesions are skin or subcutaneous lesions within 2 cm of the primary tumor, considered intralymphatic extensions of the primary mass; in transit metastases are any skin or subcutaneous metastases that are more than 2 cm from the primary lesion but are not beyond the regional nodal basin, they are located prior to reaching the regional lymph nodes. Lesions occurring within 2 cm of the primary tumor are classified as satellite metastases. A microsatellite is a microscopic cutaneous and/or subcutaneous metastasis adjacent or deep to, but discontinuous from a primary melanoma.

In N-classification is considered also the number of tumor-involved regional lymph nodes (#LNs) (1 versus 2/3 versus ≥4) and tumor burden – clinically occult (detected by sentinel lymph node biopsy) versus clinically evident (detected by clinical examination or radiographic imaging) nodal metastases. The N-category designation is "a" if the tumor is

clinically occult and "b" if the tumor is clinically evident. In the presence of microsatellites, satellites, or in-transit metastases, the N-category designation is "c". The presence or absence of satellites or in transit metastases is considered in an independent way of the number of lesions [33–35].

After categorizing the tumor in the different categories, we can define it in a pathological stage group – stages 0 to IV. Stage 0 is *in situ* (intraepithelial) melanoma – proliferation of malignant melanocytes that are restricted to the epidermis. Stages I and II are localized melanomas without lymph node involvement, stage III is for nodal metastases and stage IV for distant metastases. In the Table 2 and in the Figure 3 are represented the different stages of the disease [36].

Table 2 – AJCC stage 0 to II subgroups

T	N	M	PATHOLOGICAL STAGE GROUP
T0	N0	M0	**0**
T1a	N0	M0	**IA**
T1b	N0	M0	**IA**
T2a	N0	M0	**IB**
T2b	N0	M0	**IIA**
T3a	N0	M0	**IIA**
T3b	N0	M0	**IIB**
T4a	N0	M0	**IIB**
T4b	N0	M0	**IIC**

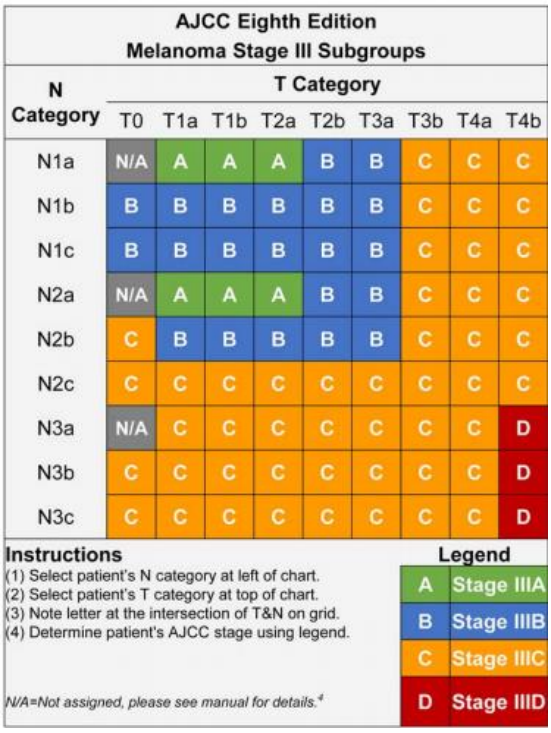

Figure 3 – AJCC stage III subgroups based on T and N categories *[36]*

4.5.3. M-Category (Distant Metastases Classification)

M1 is defined by anatomic site of distant metastases and serum lactate dehydrogenase (LDH) level, for patients with distant metastases.

Patients are divided into 4 M subcategories according to the anatomical site(s) of metastasis: M1a, M1b, M1c, M1d. Category M1a includes patients with distant metastasis to skin, subcutaneous tissue, muscle, or distant lymph nodes, regardless of serum LDH level. Category M1b includes patients with metastasis to lung (with or without concurrent metastasis to skin, subcutaneous tissue, muscle, or distant lymph nodes and regardless of serum LDH level). Patients with metastases to any other visceral site(s) (exclusive of the central nervous system (CNS)) are designated as M1c. Patients with metastases to the CNS (involving the brain, spinal cord, leptomeninges, or other components of the CNS) are designated as M1d (irrespective of the presence of metastatic disease at other sites).

LDH constitutes a clinically significant factor associated with response, – elevated is an adverse prognostic factor – progression-free survival and overall survival in immunotherapy treatment [37]. Baseline serum LDH is an established and independent prognostic factor for survival [38].

5. Treatment of the advanced metastatic melanoma

5.1. Evolution in the treatment

In the last eight years, treatment for advanced melanoma has progressed, with some new agents approved. All the agents are shown to significantly prolong the survival times of patients with advanced metastatic melanoma [39]. Generally, these agents can be divided into two groups:

(1) targeted therapies – selective inhibitors of the MAPK that inhibit the oncogene *BRAF* and MAPK pathway directly, orally administered;

(2) immunotherapies – immune checkpoint inhibitors targeting cytotoxic T lymphocyte associated antigen-4 (CTLA-4) and **programmed cell death protein-1 (PD-1)** administered via IV to indirectly activate the immune system, like nivolumab and pembrolizumab [27].

The development of immunology and immunotherapy has changed the way to look at and to treat metastatic melanoma [40]. Melanoma is a type of cancer that has always led immunotherapy research because the most part of the times chemotherapy – which was a standard therapy before 2011 [41], was not effective and due to the fact that immune system

plays an important role in regulating this cancer [42]. Patients with advanced metastatic melanoma present tumor immune tolerance and a tumor microenvironment that is propitious to the progression of the disease because their immune response to the tumor is supressed. Immunotherapy helps to overcome this immune suppression [40].

Before these two types of treatment agents described above, patients with inoperable metastatic disease had a median survival time between 8 to 12 months. Today, because of the use of one or both of these therapies, survival time is at least 24 months [39]. Approximately 42-47% of patients with *BRAF* mutations treated with targeted therapy have more 3 years survival and 46-53% of patients treated with immune checkpoint inhibitors have more 4 years survival [41].

There are other therapies that can be used to help with the treatment, although they are not mainstay therapies like systemic therapy. These include surgery, radiation therapy and chemotherapy, which is well tolerated but with low response rates [43].

The first immunotherapeutic approaches were cytokine therapy, like interferon alpha (IFN-α) and interleukin-2 (IL-2). IFN-α2 was the first immunotherapy approved by US Food and Drug Administration (FDA) for the adjuvant treatment of melanoma of stage III patients, in 1995. Three years later, IL-2 was approved [44]. Although, these agents were not associated with durable responses or enough improvements in survival for the most part of the patients. These therapies were also often associated with severe side effects and toxicity [42,43].

5.2. Mutation status of tumor

Since approximately 40-50% of patients with advanced metastatic melanoma have a *BRAF* mutation, the presence or absence of this mutation should be one of the first parameters analysed in these patients. In over 90% of the cases, this activating mutation consists in the substitution of valine to glutamic acid at amino acid 600 (*BRAF* V600 mutation). For patients with *BRAF* mutations, *BRAF* inhibitors are an important achievement in the treatment [41,45].

6. Drugs used for the treatment of advanced metastatic melanoma

6.1. Immune checkpoint inhibitors

During acute infections or vaccinations, naive T-cells are activated and differentiate into effector T-cells in 1 or 2 weeks. When the inflammation is resolved, the most part of the activated T-cells die, but some subsist and turn into memory T-cells, that downregulate the

activation programme of effector T-cells. These memory T-cells also have a self-renewal capacity that is antigen-independent, which is a type of stem cell-like. The development of persisting memory T-cells occurs, after the effector phase, in the absence of antigen stimulation or inflammation. This aspect is different in chronic infections and cancer, because in these pathologies, T-cells are persistently exposed to antigen and/or inflammatory signals. In these situations, memory T-cell differentiation is altered – this altered differentiation state is called T-cell exhaustion. T-cell exhaustion is characterized by T-cell dysfunction, causing loss of the effector functions, upregulation and co-expression of inhibitory receptors, altered expression of transcription factors and antigen-independent memory T-cell responsiveness. T-cell exhaustion avoids control of infections and tumours, but this dysfunction can be reversed by targeting CTLA-4 and PD-1.

Inhibition of immune checkpoints seems to be the key in melanoma treatment, resulting in increased activation of the immune system. Immune checkpoints are negative regulators of T-cell activation, and in combination with co-stimulatory pathways have an important role in modulating T-cell activity and in the maintenance of self-tolerance.

Immune system under physiological conditions modulates the duration and amplitude of its response, protecting the organism from collateral tissues damage and maintaining self-tolerance (prevention of autoimmunity). This modulation is mediated by "stop signals" called immune checkpoints that are turned on when specific receptors on T-cells like CTLA-4 or PD-1 interact with their ligands. This interaction inhibits the activity of T-cells and blocks the immune response. Tumours escape from the control of the immune system and immune checkpoints can be hyperactivated in many cases, as an immune resistance mechanism. Most cancer cells express the ligands for PD-1, meaning that the interaction between these ligands and their receptors blocks the activity of T-cells and reduces immunosurveillance.

Immune checkpoint inhibitors are monoclonal antibodies able to inhibit the "stop signals", restoring the anti-tumor activity of the immune system. These drugs block CTLA-4 or PD-1 receptors on T-cells by blocking the binding site for their ligands. Immunosurveillance is restored and cancer cells are recognised and killed by the immune system [42,46,47].

6.1.1. Cytotoxic T lymphocyte-associated antigen 4

The cells of the innate immune system – dendritic cells, macrophages, neutrophils and natural killer (NK) can differentiate a tumoral cell from a healthy cell and start the elimination of the first ones by themselves or call for the intervention and activation of the adaptive immune system cells – T and B-cells. Antigen presenting cells (APC) – dendritic cells,

macrophages and B-lymphocytes make the communication between both systems. When APC identify a foreign molecular pattern like the ones presented in tumoral cells, they activate T-lymphocytes helper (TH or TCD4+ lymphocytes) in the initiation phase. APC and class II major histocompatibility complex (MHC II) molecules present the foreign antigen to the T-cell receptor (TCR). This T-cell recognize peptide fragments of intracellular proteins expressed on the surface of the APC, bound to MHC molecules.

Immune checkpoint pathways regulate activation of T-cells during an immune response in a process called peripheral tolerance, to prevent autoimmunity that occurs when these mechanisms fail [48]. However, the activation of the initiation phase of the cellular immunity doesn´t occur only due to the connection antigen-MHC II molecules with TCR, but also it needs co-stimulatory signals, requiring the presence of a co-stimulatory molecule (B7). This activation will upregulate cytotoxic T-lymphocyte antigen 4 (CTLA-4). The CTLA-4 receptor on TH lymphocyte is a negative regulator of T-cell activation that competes with a cluster of differentiation (CD28) receptor for their shared ligand, B7 (known also as CD80). CTLA-4 has stronger affinity for B7 than CD28, which means that in the absence of the complex CD28:B7, there is a downregulation of T-cell activity. CD28, on the other hand, is an enhancer for T-cell activation, which means that levels of CD28:B7 complex leads to the proliferation of T-cells, increased T-cell survival and differentiation through the production of growth cytokines like interleukin-2 (IL-2). CTLA-4 and CD28 are transmembrane proteins that regulate the T-cell inactivation/activation process, respectively. CD28 is a critical molecule in the T-cell activation and its inhibition can decrease or stop it, while CTLA-4 functions like a "brake" on the activated immune system [20,49]. The relative amount of CD28:B7 binding versus CTLA-4:B7 binding determines whether a T-cell is activated or not [50].

CTLA-4 is an important negative regulator of T-cell activity and it was the first targeted immune checkpoint inhibitor used in the clinic. Anti-CTLA-4 antibodies block CTLA-4 signalling, prolonging T-cell activation and T-cell proliferation, increasing anti-tumoral immunity and regression in pre-established melanomas [51].

For these reasons, there were developed two humanized anti-CTLA-4 monoclonal antibodies – Ipilimumab and Tremelimumab. Data suggests that anti-CTLA-4 therapy induces durable anti-tumor immunity, even after the discontinuation of the treatment [42].

6.1.2. Programmed cell-death 1

After the success of the first treatment with immune checkpoint inhibitors through the inhibition of CTLA-4, there have been further developments in immunotherapy targeting

immune checkpoints. Both CTLA-4 and PD-1 are negative regulators of T-cell immune function.

A second co-inhibitory pathway uses the programmed cell death receptor 1 (PD-1), which is an immune-inhibitory receptor present on activated T cells that belongs to the CD28/CTLA-4 receptor family.

It regulates T-cell activation through binding to its ligands, programmed death ligand 1 (PD-L1 or B7-H1) and programmed death ligand 2 (PD-L2 or B7-DC) [52]. These ligands are expressed according to the tumor microenvironment, suggesting that anti-PD-1 therapy is more specific to the tumor, having a better response with less adverse effects comparing to anti-CTLA-4 [42]. When PD-1 binds to its ligands, which are often present on activated tumor cells, the ability of the T-cell to produce an effective immune response is downmodulated.

Similar to CTLA-4, PD-1 binding inhibits T-cell proliferation, interferon-γ (IFN-γ), tumor necrosis factor-α and IL-2 production, reducing T-cell survival [50]. These are called immune checkpoints, and they have the power to optimize or inhibit the activation of the initiation phase. Like CTLA-4, PD-1 is also a negative regulator of T-cell activation and serves as a brake, which can prevent T-cells from killing cancer cells [44,49,53].

Melanoma is one type of cancer in which the progress made with anti-PD-1 monoclonal antibodies – Nivolumab and Pembrolizumab – is well noticed, being approved for advanced metastatic melanoma treatment. These antibodies direct against PD-1 to restore or increase the antitumor immune response and to regress the tumor [42,46].

In 2015, after an assessment made by the European Medicines Agency's Committee for Medicinal Products for Human Use, Nivolumab (Opdivo®) was the first PD-1 immune checkpoint inhibitor approved by the European Commission for the treatment of advanced metastatic melanoma in adults [54].

6.1.3. Differences between CTL-4 and PD-1

Although they have the same negative regulation function of T-cell activation, CTLA-4 and PD-1 have different timing of downregulation, signalling mechanisms and anatomic locations of immune inhibition.

CTLA-4 is considered the "leader" of the immune checkpoints, regulating T-cell proliferation firstly in lymph nodes, early in the immune response, while PD-1 regulates T-cell proliferation firstly in peripheral tissues, later in the immune response. CTLA-4 blockade affects the immune priming phase by supporting the activation and proliferation of effector T-cells, regardless of TCR specificity. On the other hand, PD-1 blockade acts during the effector

phase to restore the immune function of T-cells in the periphery that have been turned off following extended or high levels of antigen exposure. PD-1 expression is a mark of "exhausted" T-cells that have experienced high levels of stimulation or reduced CD4+ T-cell help.

Another difference is based on the anatomic locations of the inhibition, since CTLA-4 is limited to T-cells while PD-1 is expressed on activated T-cells, B-cells, and myeloid cells. The distribution of PD-1 ligands is also different from those for CTLA-4. Also, the B7 ligands for CTLA-4 are expressed by professional APCs, which typically reside in lymph nodes or spleen.

PD-1 ligands (PD-L1 and PD-L2) are expressed by antigen-presenting cells and other immune cells, and can also be expressed on nonimmune cells, including tumor cells. PD-L1 and PD-L2 are more widely expressed – PD-LI is expressed on leukocytes, on nonhematopoietic cells, and in nonlymphoid tissues, and can be induced on parenchymal cells by inflammatory cytokines (IFN-γ) or tumorigenic signalling pathways; it is found on many different tumor types, associated with an increased number of tumor-infiltrating lymphocytes and poorer prognosis. PD-L2 is primarily expressed on dendritic cells and monocytes but can be induced on a wide variety of other immune cells and nonimmune cells, depending on the local microenvironment. PD-1 has a higher binding affinity for PD-L2 than for PD-L1, and this difference may be responsible for differential contributions of these ligands to immune responses [46,50,55].

Both PD-L1 and PD-L2, but PD-L1 especially, are overexpressed in tumor cells, while PD-1 is highly expressed on T-cells in patient tumours. PD-L1 tumor expression and PD-1 T-cell expression are related with tumor aggressiveness and poor clinical outcome. The frequent overexpression of PD-1/PD-L1 in tumours and the fact that it is correlated with tumor aggressiveness and poor prognosis, identify this as a candidate target for monoclonal antibody therapy [56]. In the Figure 4 are represented both CTLA-4 and PD-1 immune checkpoints regulating different components during the immune response [47].

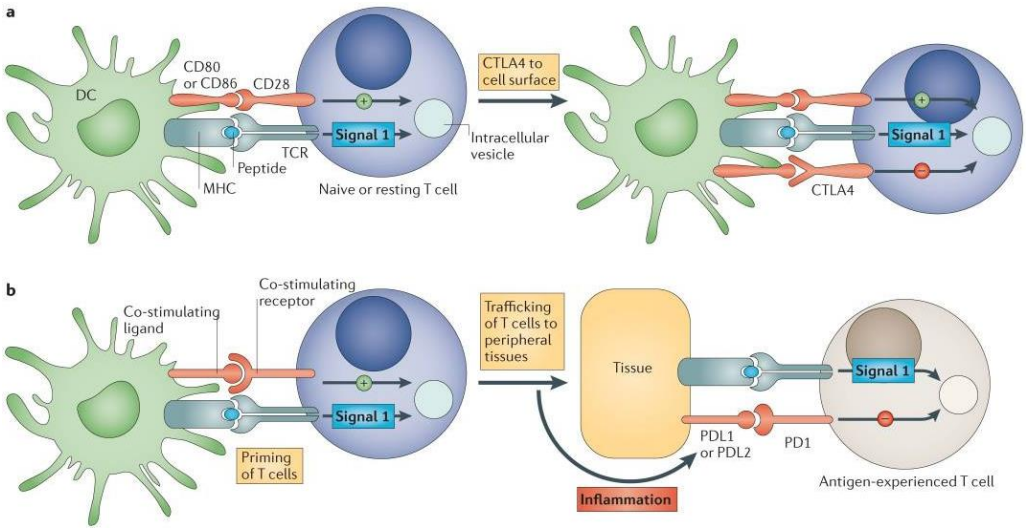

Figure 4 – CTLA-4 and PD-1 immune checkpoints regulate different components during the immune response

6.1.4. Mechanism of action of Anti-PD-1 immunotherapy

First line therapies for advanced metastatic melanoma include immunotherapy with anti-PD-1 antibodies (alone or in combination with anti-CTLA-4 agents) or targeted therapy with BRAF inhibitors in cancers with BRAF V600 mutation [42].

Nivolumab and Pembrolizumab are anti-PD-1 monoclonal antibodies and immune checkpoint inhibitors. They are immunoglobulins G4 (IgG4) monoclonal antibodies, that inhibit selectively programmed cell death-1 (PD-1) activity by binding to the PD-1 receptor, blocking the bind of the ligands PD-L1 and PD-L2. This way, the negative T-cell activation and PD-1 pathway-mediated inhibition of the immune response are stopped, allowing the antitumor immune response. Cancer cells can produce proteins (PD-L1 and PD-L2) that attach to PD-1 receptor, blocking T-cell activity and don´t allow them to attack the cancer. As these drugs attach the receptor instead of the other proteins, the ability of the immune system to kill cancer cells is increased. In Table 3 is established the comparison between the two Anti-PD-1 antibodies used in the clinic – Nivolumab and Pembrolizumab [57–60].

Table 3 – Comparison between Nivolumab and Pembrolizumab

	Nivolumab	**Pembrolizumab**
Comercial name	Opdivo®	Keytruda®
Pharmacotherapeutic group	Antineoplastic agents, monoclonal antibodies	Antineoplastic agents, monoclonal antibodies
Mechanism of action	Humanised monoclonal antibody, IgG4 with a variable region against PD-1. It binds to PD-1 receptor and blocks its interaction with PD-L1 and PD-L2	Humanised monoclonal antibody, IgG4 with a variable region against PD-1. It binds to PD-1 receptor and blocks its interaction with PD-L1 and PD-L2
Antibody structure	γ1 heavy chain and κ light chain	γ4 heavy chain and κ light chain
Manufacturing	Produced in hamster ovary cells by recombinant DNA tecnology	Produced in hamster ovary cells by recombinant DNA tecnology
Therapeutic indications	**1) Monotherapy** **i.** advanced unresectable or metastatic melanoma (BRAF V600 wild-type or BRAF V600 mutation-positive) **ii.** adjuvant treatment of adults with melanoma with involvement of lymph nodes or metastatic disease with complete resection **2) Combinated with ipilimumab** **i.** the combination of nivolumab with ipilimumab is established only in patients with low tumour PD-L1 expression, increasing progression-free survival (PFS) and overall survival (OS) in these cases	**Monotherapy** **i.** advanced unresectable or metastatic melanoma in adults **ii.** adjuvant treatment of adults with stage III melanoma and lymph node involvement with complete resection
Pharmaceutical form	Concentrate for solution for intravenous (IV) infusion	Concentrate for solution for IV infusion
Recommended dose and infusion time	**1. Monotherapy** 240 mg every 2 weeks over 30 minutes or 480 mg every 4 weeks over 60 minutes **1.1. Monotherapy in adjuvant treatment** 3 mg/kg administered intravenously over 60 minutes every 2 weeks **2. In combination with ipilimumab** a) 1 mg/kg nivolumab + 3 mg/kg ipilimumab every 3 weeks for the first 4 doses b) then in monotherapy 240 mg every 2 weeks or at 480 mg every 4 weeks	**Monotherapy** 200 mg every 3 weeks or 400 mg every 6 weeks over 30 minutes.
Treatment duration	**1. Monotherapy or adjuvant tretament** Until disease progression or unacceptable toxicity **2. In combination with ipilimumab** Maximum 12 months	**Monotherapy** Until disease progression or unacceptable toxicity.
Clearance	≈ 0,2 L/day	≈ 0,2 L/day
Terminal half life	26,7 days	26 days
Time to steady state	12 weeks when administered at 3mg/kg every 2 weeks	18 weeks when administered at 2mg/kg every 3 weeks

Note: Models of dose/exposure relationships for efficacy and safety show that there are no clinically significant differences in efficacy and safety between a Nivolumab dose of 240 mg every 2 weeks, 3 mg/kg every 2 weeks, and 480 mg every 4 weeks. The same is applied to Pembrolizumab where there are no significant differences between a dose of 200 mg every 3 weeks, 2 mg/kg every 3 weeks and 400 mg every 6 weeks as monotherapy [57,58].

6.2. Predictive biomarkers of the response to checkpoint inhibitors

There are patients that can have more benefits than others using therapy with checkpoint inhibitors. Considering this fact and the inherent toxicity of these treatments in some patients, it is useful to identify some biomarkers that translate the potential benefit to a specific patient [42].

In the treatment with Ipilimumab, the most used biomarker is the elevated LDH level, a worst outcome in patients treated with this molecule. The high levels of LDH are the result of high disease load and cell turnover, since tumours that grow fast are normally less vascularized, meaning that the cells have more difficulty in using oxygen, opting for anaerobic glycolysis as an alternative way to obtain energy. For this process, LDH is needed to convert pyruvate into lactate, so its level is increased. Also occurs the accumulation of lactate and a decrease in pH, which negatively affects the normal function of the lymphocytes [42,61].

More importantly, when it comes to PD-1 inhibitors, the expression of PD-L1 in the tumor microenvironment is an objective biomarker for them use and response. However, a patient with a negative PD-L1 tumor should not be excluded right away for the use of anti-PD-1 therapy, because there is a percentage of these negative patients that still obtain some benefit with this therapy. PD-L1 expression alone is not enough to exclude patients for the therapy but is useful to choose monotherapy or combination therapy. Patients with positive tumours for PD-L1 have the same benefit either with monotherapy with Nivolumab or combination therapy with Nivolumab and Ipilimumab. On the other hand, patients with negative tumours for PD-L1 have more benefit with the combination therapy, meaning that the toxicity and the treatment-related adverse events of the combination therapy cannot be avoided in this group, unlike what happens in patients with PD-L1 positive tumours [42,62].

7. Clinical trials

7.1. Established end-points

Some clinical trials have been done to evaluate a few parameters about the therapies with Nivolumab and Pembrolizumab. There are some special precautions to consider when doing clinical trials with anti-PD-1, since in these cases it is hard to know the perfect end-point and to evaluate the tumor response. Normally, in other clinical trials, the end-point is based on the parameter overall survival (OS), because it is simple, easy and practice to evaluate and interpret clinically. OS is defined as the time from randomization to death from any cause. The

main differences between other clinical trials comparing to anti-PD-1 trials, that make harder to consider OS the end-point, are that this parameter requires a large sample and big follow-up duration. Patients can die from any non-cancer cause and they can do other therapies after progression, which make those parameters not reliable [63,64]. So, to evaluate possible alternatives to OS as an end-point, a meta-analysis has been performed to analyse if overall response rate (ORR) and progression-free survival (PFS) could be considered end-points [65]. ORR was defined as the proportion of patients who had a complete response (CR) or partial response (PR) at the best overall response. PFS is defined as the time from randomisation to the first event (progressive disease or death from any cause) and ORR is defined as the proportion of confirmed CR or PR at the best response. All these definitions were based on the conventional Response Evaluation Criteria in Solid Tumours (RECIST) criteria, which uses the OS as end-point [63]. Strong associations between PFS/ORR and OS were not observed, but these criteria are useful in randomised studies [56].

7.1.1. Randomised phase III study: Nivolumab VS chemotherapy

The safety and efficacy of Nivolumab (Opdivo®) were evaluated in a phase III randomised study. A group of patients was treated with Nivolumab administered intravenously over 60 minutes at 3 mg/kg every 2 weeks and the other group was treated with chemotherapeutic agents, such as dacarbazine, carboplatin and paclitaxel (Table 4) [57].

Table 4 – Randomised study: Nivolumab VS chemotherapy

	Nivolumab	**Chemotherapy**
ORR	27.2%	9.8%
Median durations of response	31.9 months	12.8 months
PFS HR	1.03	

The PFS hazard ratio (HR) is frequently used in randomised clinical trials in oncology to evaluate the treatment effect based on time-to-event. The HR is an estimate of the ratio of the hazard rates between the experimental group (Nivolumab) and a control group (chemotherapy) during all the study.

If the HR is 1, it means that the efficacy of the experimental treatment is the same as the efficacy of the control treatment. If the experimental treatment is better than the control treatment, HR<1. If the experimental treatment is worse than the control treatment, HR>1 [66]. In this study, PFS HR is approximately 1, so PFS here doesn´t give an idea of the benefit of Nivolumab compared to chemotherapy.

The OS obtained for both groups did not represent a statistically significant difference [57]. On the other hand, the differences between ORR and median durations of response in the two groups demonstrate the better efficacy of Nivolumab compared to chemotherapy.

7.1.2. Randomised phase III study: Nivolumab in combination with Ipilimumab or Nivolumab as monotherapy VS Ipilimumab as monotherapy

The safety and efficacy of Nivolumab 1 mg/kg in combination with Ipilimumab 3 mg/kg or Nivolumab 3 mg/kg VS Ipilimumab 3 mg/kg monotherapy were evaluated in a phase III randomised study. Patients were randomised to receive Nivolumab in combination with Ipilimumab, Nivolumab in monotherapy or Ipilimumab in monotherapy [57].

The results presented in the Table 5 were based in patients with a PD-L1 expression ≥5%.

Table 5 – Randomised study: Nivolumab + Ipilimumab VS Nivolumab VS Ipilimumab

	Nivolumab + Ipilimumab	Nivolumab	Ipilimumab
Objective response	59%	45%	19%
ORR	74%	59%	21%
Median durations of response	NR	31.1 months	18.2 months

NR=not reached

Table 6 contains the results of OS HR and PFS HR for Nivolumab + Ipilimumab VS Nivolumab; Nivolumab + Ipilimumab VS Ipilimumab and Nivolumab VS Ipilimumab in monotherapy [57].

Table 6 – Randomised study: Comparison between OS HR and PFS HR in Nivolumab in combination with Ipilimumab and in monotherapy

	OS HR	PFS HR
Nivolumab + Ipilimumab VS Nivolumab	0.99	0.87
Nivolumab + Ipilimumab VS Ipilimumab	0.59	0.35
Nivolumab VS Ipilimumab	0.60	0.41

The results of the OS HR demonstrate that the combination Nivolumab + Ipilimumab is related to a lower risk of death compared to the treatment in monotherapy.

Talking about the combination therapy arms compared to the monotherapy arms, since OS HR <1 in both, it means that the treatment in combination provides a lower risk of death, especially compared to Ipilimumab in monotherapy. In this case, OS HR=0.59, meaning approximately a 41% lower risk of death on the combination treatment compared to

Ipilimumab in monotherapy. Combination therapy compared to Nivolumab in monotherapy is the arm that contains less significative differences in terms of overall survival/ risk of death.

Comparing the treatments in monotherapy, OS HR=0.60, meaning approximately a 40% lower risk of death in the treatment with Nivolumab comparing to Ipilimumab.

About the PFS HR, the main difference is related to the combination therapy arm and Ipilimumab. PFS HR=0.35 means that the treatment with Nivolumab and Ipilimumab provides approximately a 65% lower risk of an event such as progressive disease or death from any cause comparing to Ipilimumab in monotherapy. Nivolumab provides a 59% lower risk of an event compared to Ipilimumab in the monotherapy treatments.

In conclusion, both arms containing Nivolumab demonstrated a significant PFS and OS benefit and greater ORR compared with Ipilimumab alone. Combined Nivolumab (anti-PD-1) and Ipilimumab (anti-CTLA-4) show improved anti-tumour responses in advanced metastatic melanoma because there is a dual blockade of PD-1 and CTLA-4 that results in synergistic anti-tumour activity [57].

7.1.3. Randomised phase III study: Pembrolizumab VS chemotherapy

The safety and efficacy of Pembrolizumab (Keytruda®) were evaluated in a phase III randomised study. A group of patients was treated with Pembrolizumab administered intravenously at a dose of 2 mg/kg every 3 weeks and the other group was treated with chemotherapeutic agents, such as dacarbazine, carboplatin or paclitaxel (Table 7) [58].

Table 7 – Randomised study: Pembrolizumab VS chemotherapy

	Pembrolizumab	**Chemotherapy**
ORR	22%	5%
Median durations of response	22.8 months	6.8 months
PFS HR	0.58	

Comparing the results obtained with Pembrolizumab VS chemotherapy and Nivolumab VS chemotherapy obtained above, they corroborate the fact that both anti-PD-1 drugs are more effective than chemotherapy.

7.1.4. Randomised phase III study: Pembrolizumab VS Ipilimumab

Unlike what happens with Nivolumab, Pembrolizumab has no studies done in combination with Ipilimumab. With Pembrolizumab, the studies just compare the safety and efficacy of this drug and Ipilimumab in monotherapy, not in combination [67].

The results presented in Table 8 were based in patients with a PD-L1 positive expression [58].

Table 8 – Randomised study: Pembrolizumab VS Ipilimumab

	Pembrolizumab	**Ipilimumab**
ORR	40%	14%
OS HR	0.63	
PFS HR	0.53	

The results obtained are like the ones obtained with Nivolumab, showing that Pembrolizumab is also associated with a lower risk of disease progression and risk of death. The conclusion of these studies is that anti-PD-1 therapy is more effective than therapy with anti-CTLA-4 agents.

8. Safety and tolerability

Immune checkpoint inhibitors block the PD-L1 pathway, increasing the action of the immune system, recovering the immune deficiency induced by the tumor microenvironment. This change may generate some adverse effects in several organs. Nevertheless, the toxicity pattern resulting from immunotherapy is different from that one resulting of the classical treatments like chemotherapy agents or targeted therapy. Plus, since immunotherapy is still a "novel therapy", there is little experience in recognize and manage the resulting toxicity, meaning that these toxic effects are still a challenge for clinicians and a barrier to develop new effective combinations. Generally, the toxicity associated with this therapy can be controlled with corticosteroids or immune modulators, but sometimes it can lead to interruption of the treatment or fulminant and fatal events [68,69].

The concepts of safety and tolerability are intrinsic to each drug and are important to mention when the subject is toxicity and adverse effects of a drug. According to European Medicines Agency, safety is associated to the risk of a drug for the subject, evaluated by laboratory tests, vital signs, clinical adverse events (diseases, signs and symptoms) and other special safety tests (eg: electrocardiograms). Tolerability is defined as the degree to which adverse effects can be tolerated by the patient. In real-world studies, to obtain the degree of confidence in the safety and tolerability of a certain drug, it is necessary to carry out an exposure in a patient-number-dependent way [70,71].

8.1. Adverse events and toxicity grades

The National Cancer Institute (NCI) created the NCI Common Terminology Criteria for Adverse Events, a descriptive terminology which can be used to report an Adverse Event (AE).

An AE is defined as "any unfavourable and unexpected sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure".

There is a grading (severity) scale provided for each AE. The term grade is referent to the severity of the AE, disposed from grade 1 through 5. The clinical descriptions of severity for each AE are represented on the Table 9 [72].

Table 9 – Classification of AE by grade according to NCI

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL*
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**
Grade 4	Life-threatening consequences; urgent intervention indicated
Grade 5	Death related to AE

Activities of Daily Living (ADL)

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

In patients treated with anti PD-1, approximately 2 in 3 had at least one adverse event and 1 in 7 patients had one grade 3 adverse event [73]. The most frequent adverse reaction is fatigue (16-24%) that constitutes the most common grade 3 adverse event [42]. Other frequent adverse reactions are rash (17%), pruritus (13%), diarrhoea (13%) and nausea (12%). The most part of the adverse reactions were mild to moderate (Grade 1 or 2) [57].

It is recommended the interruption of the treatment with PD-1 blocking agents if the adverse events are grade 2 or higher. The risk-benefit relation should be considered and for that it is important to have a multidisciplinary team composed of oncologists, internists, clinical immunologists and other specialists.

In severe cases, immunotherapy must be permanently discontinued, while, in other cases it can be continued once the immune-related adverse event is resolved [68].

In Table 10 are presented some of the adverse reactions in patients treated with Nivolumab or Pembrolizumab in monotherapy or in combination, divided by system organ

class and by frequency. For the frequency, the three classes are very common (≥1/10); common (≥1/100 to <1/10) or uncommon (≥1/1000 to <1/100) [57,58].

Table 10 – Adverse reactions in patients treated with anti PD-L1 immunotherapy divided by system organ class and frequency

		Nivolumab monotherapy	Pembrolizumab monotherapy	Nivolumab + Ipilimumab	Pembrolizumab + Chemotherapy
Infections and infestations	**Common**	Upper respiratory tract infection	Pneumonia	Pneumonia, upper respiratory tract infection	Pneumonia
	Uncommon	Pneumonia, bronchitis	----------	Bronchitis	--------
Blood and lymphatic system disorders	**Very Common**	Neutropaenia	Anaemia	------	Neutropaenia Anaemia Thrombocyto-paenia
	Common	---------	Thrombocytopae-nia Lymphopaenia	Eosinophilia	Lymphopaenia Neutropaenia Leukopaenia
	Uncommon	Eosinophilia	Eosinophilia Neutropaenia Leukopaenia	-------	Eosinophilia
Immune system disorders	**Common**	Infusion related reaction Hypersensitivity	Infusion related reaction	Infusion related reaction	Infusion related reaction
	Uncommon	-------	Sarcoidosis	Sarcoidosis	--------
Endocrine disorders	**Very Common**	---------	Hypothyroidism	Hypothyroidism	--------
	Common	Hypothyroidism Hyperthyroidism	Hyperthyroidism	Adrenal insufficiency Hyperthyroidism Thyroiditis	Hypothyroidism Hyperthyroidism
	Uncommon	Adrenal insufficiency Hypophysitis Thyroiditis Diabetes Mellitus	Hypophysitis Thyroiditis Adrenal insufficiency	Diabetic ketoacidosis Diabetes mellitus	Hypophysitis Thyroiditis Adrenal insufficiency
Metabolism and nutrition disorders	**Very common**	------	Decreased appetite	Decreased appetite	Decreased appetite
	Common	Decreased appetite	Hyponatraemia Hypokalaemia Hypocalcaemia	Dehydration	Hyponatraemia Hypokalaemia Hypocalcaemia
	Uncommon	Dehydration Metabolic acidosis	Type I diabetes mellitus	------	Type I diabetes mellitus
Nervous system disorders	**Very common**	------	Headache	Headache	Dizziness Neuropathy peripheral Dysgeusia Headache
	Common	Peripheral neuropathy Headache Dizziness	Dizziness Neuropathy peripheral Lethargy Dysgeusia	Peripheral neuropathy Dizziness	Lethargy
	Uncommon	Polyneuropathy Autoimmune neuropathy	Epilepsy	Polyneuropathy Autoimmune neuropathy	Epilepsy
Vascular disorders	**Common**	Hypertension	Hypertension	Hypertension	Hypertension
	Uncommon	Vasculitis	------	------	------
Respiratory, thoracic and	**Very common**	------	Dyspnoea Cough	Dyspnoea	Dyspnoea cough
	Common	Pneumonitis Dyspnoea	Pneumonitis	Pneumonitis	Pneumonitis

mediastinal disorders		Cough		Pulmonary embolism Cough	
	Uncommon	Pleural effusion	------	Pleural effusion	------
Gastrointestinal disorders	**Very common**	Diarrhoea Nausea	Diarrhoea, Abdominal pain Nausea Vomiting Constipation	Colitis Diarrhoea Vomiting Nausea, Abdominal pain	Diarrhoea Nausea Vomiting Constipation Abdominal pain
	Common	Colitis Stomatitis Vomiting Abdominal pain Constipation Dry mouth	Colitis Dry mouth	Stomatitis Pancreatitis Constipation Dry mouth	Colitis Dry mouth
	Uncommon	Pancreatitis Gastritis	Pancreatitis	Intestinal perforation Gastritis Duodenitis	Pancreatitis
Skin and subcutaneous tissue disorders	**Very common**	Rash Pruritus	Rash Pruritus	Rash pruritus	Rash Alopecia Pruritus
	Common	Vitiligo Dry skin Erythema Alopecia	Severe skin reactions Erythema Vitiligo Dry skin Alopecia Eczema Dermatitis acneiform	Vitiligo Dry skin Erythema Alopecia Urticaria	Severe skin reactions Erythema Dermatitis acneiform Dry skin
	Uncommon	Erythema Psoriasis Rosacea Urticaria	Lichenoid keratosis Psoriasis Dermatitis Papule Hair colour changes	Psoriasis	Psoriasis Dermatitis Eczema Hair colour changes Lichenoid keratosis Papule Vitiligo
Musculoskeletal and connective tissue disorders	**Very common**	--------	Musculoskeletal pain Arthralgia	Arthralgia	Musculoskeletal pain Arthralgia
	Common	Musculoskeletal pain Arthralgia	Pain in extremity Myositis Arthritis	Musculoskeletal pain	Pain in extremity Myositis Arthritis
	Uncommon	Polymyalgia rheumatica Arthritis	Tenosynovitis	Arthritis Myopathy, Myositis Rhabdomyolysis	Tenosynovitis
Hepatobiliary disorders	**Common**	------	------	Hepatitis	Hepatitis
	Uncommon	Hepatitis	Hepatitis	------	------
Renal and urinary disorders	**Common**	-------	------	Renal failure (including acute kidney injury)	Nephritis Acute kidney injury
	Uncommon	Nephritis Renal failure (including acute kidney injury)	Nephritis	Nephritis	-------
General disorders and administration site conditions	**Very common**	Fatigue	Fatigue Asthenia Oedema Pyrexia	Fatigue Pyrexia	Fatigue Asthenia Oedema Pyrexia
	Common	Pyrexia Oedema	Influenza-like illness Chills	Oedema Pain	Influenza-like illness Chills
	Uncommon	Pain Chest pain	-------	Chest pain	-------

8.2. Autoimmune-mediated adverse events

The type and severity of side effects in a patient receiving immunotherapy is dependent of the treatment type, the dose, the cancer stage and the health of the person before the treatment – in patients with previous autoimmune disorders, anti-PD-1 therapy can exacerbate their condition [42,74].

Immune checkpoints have an important role in the maintenance of tolerance to self-antigens. Treatment with inhibitors of immune checkpoints block the immune checkpoint pathway and active the cellular immunity, causing autoimmune-mediated adverse events [42,73].

However, the autoimmune toxicity caused by anti-PD-1 therapy is less severe and less frequent comparing to the toxicity caused by cytokine therapy like Ipilimumab. In more than half of the patients treated with cytokines, approximately 70% will have some immune-related adverse event (irAE), being a quarter of these cases severe. The most frequent events are related to the skin (44%) like rash, pruritus and vitiligo, flowed by events related to gastrointestinal tract (35%) such as diarrhoea; then the endocrine system (thyroid disorders such as hypothyroidism, hyperthyroidism, hyperglycemia, thyroiditis…) and hepatic system (hepatitis). PD-1 inhibitors are better tolerated with less autoimmune toxicity, although in some cases irAE´s are present [42,75].

About 24% of pneumonitis cases were grade 3 or higher adverse event in severity, being the most common cause of treatment-related death in patients treated with PD-1 inhibitors.

Nivolumab demonstrated to be related with higher incidence of adverse events (moderated or severe) compared with Pembrolizumab, but the mechanism remains unclear [73]. However, toxicity increases when the treatment is done in combination with Ipilimumab. In these cases, almost all the patients had an adverse event, more than a half of these being grade 3 or higher [42].

It is important to instruct the patient before the beginning of the treatment with PD-1 inhibitors, giving information about the most common adverse effects and how can they recognize them. The clinician should monitor closely possible signs and symptoms because the autoimmune-mediated adverse reactions could be fatal if not detected early [73].

8.2.1. Immune-related skin adverse reactions

Severe rash has been observed with combination therapies and, less commonly, with monotherapies. Therapies in monotherapy or combination should be discontinued until the adverse reaction recover to grade 0-1 for grade 3 rash or be permanently discontinued for grade 4 rash. To treat the rash, it should be used a high-dose corticosteroid (1 to 2 mg/kg/day methylprednisolone equivalents) [57,58].

8.2.2. Immune-related colitis

Severe diarrhoea or colitis has been observed in monotherapy and combination therapies. Patients should be monitored for diarrhoea and additional symptoms of colitis, such as abdominal pain, mucus or blood in stool. The discontinuation of the treatment is done in the same way as the previous in 8.2.1 [57,58].

8.2.3. Immune-related endocrinopathies

Severe endocrinopathies, including hypothyroidism, hyperthyroidism, adrenal insufficiency, hypophysitis, diabetes mellitus, and diabetic ketoacidosis have been observed in all the therapies. Patients should be monitored for clinical signs and symptoms of endocrinopathies and for hyperglycaemia and changes in thyroid function – at the start of treatment and during treatment. Patients may complain of fatigue, headache, mental status changes, abdominal pain, unusual bowel habits and hypotension or nonspecific symptoms.

For hypothyroidism, therapy should be discontinued, and thyroid hormone replacement should be initiated. For hyperthyroidism, after the discontinuation of the treatment should be initiated antithyroid medication. If there is an acute inflammation of the thyroid, corticosteroids at the same dose as in 8.2.1. should also be considered. For diabetes, insulin replacement should be initiated, and it should be monitored the level of blood sugar [57,58].

8.2.4. Immune-related hepatitis

Severe hepatitis has been also observed. Patients should be monitored for signs and symptoms of hepatitis such as transaminase and total bilirubin elevations. For grade 2 transaminase or total bilirubin elevations it can be done a non-permanent discontinuation, initiating corticosteroids at a dose of 0.5 to 1 mg/kg/day methylprednisolone equivalents until 2 mg/kg/day if needed. For Grade 3 or 4 transaminase or total bilirubin elevation, Nivolumab

or Nivolumab in combination with Ipilimumab must be permanently discontinued, and corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day methylprednisolone equivalents [57,58].

8.2.5. Immune-related pneumonitis

Severe pneumonitis is the most common cause of treatment-related death in patients treated with these kinds of drugs. Patients should be monitored for signs and symptoms of pneumonitis such as radiographic changes, dyspnoea, and hypoxia. For grade 2 pneumonitis, corticosteroids should be initiated at a 1 mg/kg/day dose until 2 to 4 mg/kg/day. For grade 3 or 4, the treatment should be permanently discontinued and the initial dose of corticosteroids used is from 2 to 4 mg/kg/day [57,58].

8.2.6. Immune-related nephritis and renal dysfunction

Severe nephritis and renal dysfunction have been observed with monotherapy treatment and in combination. Patients should be monitored for signs and symptoms of nephritis or renal dysfunction. Most patients have an asymptomatic increase in serum creatinine. For Grade 2 or 3 serum creatinine elevation, after the cessation of the therapy in corticosteroids should be initiated at a dose of 0.5 to 1 mg/kg/day and increased until 1 to 2 mg/kg/day if necessary. For Grade 4 serum creatinine elevation, the treatment must be permanently discontinued, and corticosteroids should be initiated at a dose of 1 to 2 mg/kg/day [57,58].

9. Concluding Remarks

Advanced metastatic melanoma is a type of cancer whose incidence has grown in the last years, currently being considered a global health threat. Due to this fact, in the last eight years there has been a breakthrough in melanoma therapy, with more studies and trials to be carried out and consequently, increasing the number of new agents approved for the therapy of this type of cancer.

The development of immunotherapy and immunology was crucial when it comes to melanoma treatment, since the most part of the times this type of tumor does not respond well to the conventional chemotherapy. Plus, in patients with advanced metastatic melanoma, the immune response to fight the tumor is supressed, being immunotherapy essential to overcome this suppression.

Immune checkpoint inhibitors like anti-PD-1 agents – Nivolumab (Opdivo®) and Pembrolizumab (Keytruda®) – have been shown to be the most effective therapeutic agents for melanoma treatment, increasing the overall survival, the progression free survival and the duration of response.

Nivolumab and Pembrolizumab are two similar drugs, belonging to the same pharmacotherapeutic group and having the same mechanism of action, pharmaceutical form and therapeutic indications. These two drugs differ only slightly in the heavy chain of the antibody structure, – they both have a κ light chain but Nivolumab has a γ1 heavy chain while in Pembrolizumab there is a γ4 heavy chain – and in the posology. In randomised clinical trials, excellent results were obtained regarding the effectiveness of both drugs, but it is still unknown which of the drugs is more effective and which one could be more advantageous considering the individual conditions and comorbidities of the patient. There are no real-world studies comparing the two drugs.

In terms of adverse events and toxicity, despite of all the autoimmune-mediated adverse events related and described, these still constitute a challenge to clinicians and patients and a barrier to the development of effective combinations. Further toxicity studies are also required. In the future, real world studies should be done comparing both drugs in terms of efficacy, effectiveness, safety, tolerability and toxicity.

Overall, immunotherapy with anti-PD-1 agents represent a viable and still promising therapeutic for the treatment of advanced metastatic melanoma, with more combinations and forms of treatment to be discovered.

10. Future Perspectives

Despite all the advances in MM therapy in the past years, almost 50% of patients still do not get the expected results with those therapies. Data on which would be the most appropriate therapy for all melanoma patients are still insufficient. Currently there is ongoing research on various therapeutic strategies for new immunomodulatory agents or using the treatment options already available to test different combinations, improving tolerability and maintaining the efficacy of those agents. Some immunomodulatory receptors are being studied because they exist not only on the lymphocyte surface but also in the tumour's microenvironment, suggesting they can be useful targets and play an important role in increasing the antineoplastic function of the immune system [42,76,77].

A new clinical approach is currently being studied in clinical trials, consisting of the use of checkpoint inhibitors together with metabolic modulation of the tumor microenvironment, since the tumor cells use metabolic pathways as a strategy to create an immune-suppressive microenvironment and protect themselves. This has been tested by targeting an enzyme called indoleamine 2,3-dioxygenase (IDO), which is used in the catabolism of tryptophan to be transformed into kynurenin, inhibiting T-cell proliferation. Epacadostat, the first selective oral IDO inhibitor is under investigation in clinical trials. A phase III study involving combination treatment with Pembrolizumab and Epacadostat suggests clinical benefit and an acceptable grade of toxicity. This study was done because IDO is overexpressed in tumours that express PD-L1, suggesting that this may be a potentially interesting combination. However, the synergistic effect of this combination needs to be confirmed with more clinical trials.

Another clinical approach being tested is the combination treatment with multiple immune checkpoint inhibitors. The success of this therapy stems from the fact that T-cells co-expressing more than one type of co-inhibitory molecule are less susceptible to respond comparing to the T-cells expressing PD-1 alone. Studies involving combinations of anti-PD-1 agents with other checkpoint inhibitors (anti-lymphocyte activation gene-3 (LAG-3) and anti-T-cell immunoglobulin and mucin domain-3 (TIM-3)) or with co-stimulatory antibodies of the tumor necrosis factor receptor (TNFR) family (anti-TNFR-4 (OX-40) and anti-glucocorticoid-induced TNFR-related protein (GITR)) are in progress [76,77].

Oncolytic virotherapy in combination with anti-PD-1 is another therapy in investigation. The concept of "viral oncolysis" means destruction of a tumor cell following by viral infection. Oncolytic virotherapy includes oncolytic viruses that infect tumor cells causing their lysis and inducing immunogenic death, increasing host immunity and antitumor immune responses. Immunogenic cell death may activate both innate and adaptive immune system,

increasing the antitumor efficacy. These viruses can also self-propagate and spread to nearby tumor cells, inducing multiple antitumor mechanisms. Oncolytic herpes simplex viruses are DNA viruses that exhibit tropism for some cancer cell types. These viruses are interesting because they are easy to manipulate genetically, being promising alternatives for melanoma treatment. The recently approved Talimogene Laherparepvec (T-VEC) is being studied in clinical trials in combination with anti-PD-1 with great results [76,78].

The upcoming years will be promising in terms of the emergence of new therapies and in the improvement of the current agents for advanced metastatic melanoma [79].

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