UNIVERSIDADE D
COIMBRA

Rodrigo Marques das Dores

Relatórios de Estágio e Monografia intitulada "mRNA-based Cancer Vaccines and Delivery Strategies" referentes à Unidade Curricular "Estágio", sob a orientação do Doutor Luís Maria Marques dos Santos Bimbo, da Doutora Ana Catarina Pinto e da Doutora Claúdia Cristina Silvestre 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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Setembro de 2022

Declaração

Eu, **Rodrigo Marques das Dores**, estudante do Mestrado Integrado em Ciências Farmacêuticas, com o nº **2017257053**, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatório de Estágio e Monografia intitulada "**mRNA-based Cancer Vaccines and Delivery Strategies**" apresentado à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade curricular de Estágio Curricular.

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Coimbra, 14 de agosto de 2022.

Rodrigo Marques das Dores

(Rodrigo Marques das Dores)

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Parte I

Relatório de Estágio Curricular em Indústria Farmacêutica

Bluepharma – Indústria Farmacêutica, S.A.

Research & Innovation

Sob orientação da Doutora Ana Catarina Pinto

e supervisão do Doutor António Lucas Nunes

10 de janeiro a **31 de março de 2022**

Resumo

O curso e a área das Ciências Farmacêuticas, pela sua abrangência, multidisciplinaridade e exercício profissional, constituem, indiscutivelmente, uma área científica de enorme relevo no âmbito das Ciências da Saúde atuais, nomeadamente na Indústria. Através da Unidade Curricular "Estágio Curricular", é dada a oportunidade aos alunos de MICF de adquirirem conhecimentos e aptidões fulcrais para o exercício farmacêutico em Indústria Farmacêutica, preparando-os para uma possível entrada no mercado de trabalho na referida área. Estando na base de desenvolvimento de medicamentos a serem usados pela população, a Indústria Farmacêutica é um dos pilares dos cuidados de saúde. Este estágio alimentou e solidificou conhecimentos prévios relativos ao dito setor (provenientes de outras unidades curriculares), permitindo ainda a introdução de novos conceitos através de uma dinâmica letiva inteiramente prática.

Palavras-chave: Indústria Farmacêutica; Estágio Curricular; Instrução prática

Abstract

Pharmaceutical Sciences' course and overall area, through its diversity, multidisciplinarity and professional applications, constitutes (undoubtably) one of the most relevant scientific areas in the current Health Sciences field, namely in Pharmaceutical Industry. The "Curricular Internship" Unit allows for the acquisition of crucial knowledge and skills for pharmaceutical activity, preparing the student for the pharmacist's labour market. Pharmaceutical Industry stands as the medicine development basis and, therefore, represents one of the Healthcare pillars nowadays. This internship leveraged and solidified pre-existing knowledge related to the area (from other curricular units) while allowing for the introduction of new concepts based on an entirely practical approach.

Key-words: Pharmaceutical Industry; Curricular Internship; Practical Education

Abreviaturas

BLPH – Bluepharma

COVID-19 – *Coronavirus-related Disease* 2019

IF – Indústria Farmacêutica

MICF – Mestrado Integrado em Ciências Farmacêuticas

S.A. – Sociedade Anónima

UC – Universidade de Coimbra

1. Introdução ao Estágio em Indústria Farmacêutica

Tendo iniciado o curso de Ciências Farmacêuticas em Mestrado Integrado em 11 de setembro de 2017, as disciplinas que me foram lecionadas e os conhecimentos científicos que adquiri de forma multidisciplinar e sob as mais diversas perspetivas da atividade farmacêutica, são, de certa forma, postas à prova (ainda que igualmente sobre um modelo de aprendizagem) na derradeira Unidade Curricular de "Estágio Curricular". Esta unidade destaca-se assim por permitir idealmente reunir conhecimentos prévios de outras restantes numa vertente genuinamente prática.

Após ter tido a oportunidade de efetuar um estágio em Farmácia Hospitalar no verão de 2021, considerei ser também ideal um estágio em Indústria Farmacêutica, completando assim os *Big 3* da atividade farmacêutica. Essa chance de uma aprendizagem mais completa foi me atribuída através da **Bluepharma** – Indústria Farmacêutica, S.A. que, dada a forte ligação desta com a Faculdade de Farmácia da Universidade de Coimbra, continuamente permite a estudantes de MICF alimentar o seu ego farmacêutico enquanto fornece força jovem para uma Indústria que requer frequentemente mudanças para persistir. O referido estágio destaca-se principalmente pela minha obstinação pessoal em enveredar por este setor farmacêutico que já persiste previamente à iniciação do próprio curso de MICF.

Este contacto com a IF iniciou-se primeiramente com a entrevista de estágio em novembro de 2021, tendo absorvendo desde cedo alguma da dinâmica deste setor. O estágio propriamente dito começou no dia 10 de janeiro de 2022, tendo sido dado como concluído no dia 31 de março do mesmo ano e foi efetuado inteiramente no Edifício-sede da BLPH em São Martinho do Bispo (Coimbra), no Departamento de Investigação e Inovação sob a tutela e supervisão da Doutora Ana Catarina Pinto e Doutor António Lucas Nunes.

De forma a relatar o estágio, uma **análise SWOT** (*Strenghts*, *Weaknesses*, *Opportunities & Threats*) é o modelo adequado e escolhido, permitindo uma análise sob diferentes perspetivas (Pontos Fortes e Fraquezas relativas ao estágio efetuado + Oportunidades e Ameaças externas ao estágio) da atividade farmacêutica em contexto de Indústria.

2. Bluepharma – Indústria Farmacêutica, S.A.

Sendo uma empresa privada genuinamente portuguesa, a Bluepharma encontra-se sediada em São Martinho do Bispo (Coimbra) e nasceu em 2001, herdando a infraestrutura da antiga BAYER. Atualmente com o lema "*With eyes set on the Future*", a Bluepharma está presente em mais de 140 territórios, com mais de 230 clientes globalmente, com elevada proporção de exportação (88%). [1] De facto, em 21 anos, a empresa evoluiu desde um "simples" edifício de produção de medicamentos um grupo farmacêutico integrando 20 empresas, que cobrem a grande maioria das fases da cadeira de valor do medicamento [1]. Estando intimamente ligada com a Cidade dos Estudantes e com a própria Universidade de Coimbra, a Bluepharma mostra-se como um espelho deste meio de excelência na Ciência. De facto, direcionado a sua atividade segundo 5 pilares: Investimento, Inovação, Internacionalização, Parcerias e Qualidade [1], a Bluepharma já atingiu uma reputação internacional notável. A BPLH oferece atualmente ao mundo desde inovação tecnológica e desenvolvimento de produto até produção e comercialização de medicamentos genéricos, enquanto estimula a superação dos colaboradores segundo elevados padrões de qualidade e melhoria contínua e reforça a coesão de uma empresa "de pessoas para pessoas". [2]

Divida por diversos departamentos e setores, a Bluepharma tem como força de ataque à inovação o Departamento de Investigação e Inovação (*Research & Innovation*) liderado pelo Doutor António Lucas Nunes. Este departamento, trabalha intimamente com outros departamentos/setores, mas com a mais alta autonomia separando-se a sua atividade em 2 unidades – Inovação Tecnológica (*Technological Innovation*) e Apoio Científico (*Scientific Affairs*). O meu estágio foi inserido na unidade de Apoio Científico, sob a orientação da Doutora Ana Catarina Pinto, mas com versatilidade de acompanhamento e aprendizagem das atividades referentes também à unidade de Inovação Tecnológica. A unidade que me acolheu destaca-se por ter a missão de dar o suporte científico, não só internamente ao departamento, mas potencialmente a todos os departamentos da empresa permitindo orientar o desenvolvimento destes, com base em literatura científica e regulamentar. A dinâmica deste dito departamento passa muito pela multidisciplinaridade de trabalho, com interligação entre apoio científico, analistas e formuladores.

3. Análise SWOT

A. Pontos fortes (*Strenghts*)

i) Integração e Equipa Técnica

Algo presente já desde o dia de apresentação de estágio foi, de facto, o amigável ambiente que permite ao estagiário sair da sua zona de conforto e integrar-se na equipa como mais um membro do departamento. Os esforços e as ações dos outros colegas de departamento foram constantes de forma a permitir a minha integração nos processos e dinâmica do grupo, o que permitiu criar um ambiente favorável à minha aprendizagem e trabalho. Nunca obstante da autonomia possibilitada na realização das tarefas diárias, a disponibilidade quase permanente de contacto com os tutores e supervisores de estágio possibilitou uma almofada de apoio que permitia a resolução de dúvidas, existindo 2 reuniões semanais de acompanhamento que permitiam uma otimização constante do meu projeto decorrente.

ii) Melhora Contínua (Formações)

A Bluepharma possui um Sistema de Gestão e Garantia de Qualidade bem definido e altamente regido pelo conceito de Melhora Contínua. Uma das estratégias relativa a este conceito de qualidade são as formações, tanto internas como externas. As formações internas em que participei iniciaram-se logo após a sessão de boas-vindas e estenderam até às últimas semanas de estágio. Estas permitiram a aquisição dos mais diversos conceitos da atividade farmacêutica em Indústria e o mais correto trabalho a desenvolver na empresa. As formações externas foram desenvolvidas maioritariamente através do evento dinamizador *Journal Club* com palestrantes convidados, apresentando temas recentes e relevantes para a IF e representando uma fonte de conhecimento adicional.

iii) Tema de Projeto Desenvolvido

O objetivo fulcral do meu estágio na BLPH foi o levantamento de informação sobre um tema em saúde muito relevante e recente - *"Overview of cancer immunotherapy strategies*" – com os objetivos iniciais de levantamento do estado de arte do tema através de pesquisa bibliográfica, mapeamento das estratégias e identificação dos *key players* na dita área, compilação da informação para consulta futura e visando criar um trabalho sobre o dito tema na vanguarda dos avanços científicos sob diferentes perspetivas (farmacêutica, tecnológica, mercado, etc.). Assim, esse trabalho que se alinhou com os interesses da empresa em inovação, encheu-se da maior relevância possível uma vez que, para além de possuir valor

acrescido no patamar dos Cuidados de Saúde atuais e futuros na área oncológica, ajustava-se ao meu tema de monografia que estaria concomitantemente a desenvolver. Contrariamente a temas bem estabelecidos, mas pouco atuais, este projeto permitiu, pela sua contemporaneidade e possível implicação em projetos futuros, outro nível de motivação e eficiência de trabalho.

iv) Reuniões Kaizen Diárias e de Planeamento Semanal

Também como ferramenta de Melhoria Contínua, existem diariamente reuniões *Kaizen* com o objetivo de planeamento e discussão relevante de temas internos ao departamento. Nestas reuniões de 15 minutos aproximadamente, decorrentes em todos os departamentos/setores da empresa, discute-se agenda e planos de trabalho, indicadores de atividade, ações de melhoria, problemas e lições aprendidas e comunicações gerais relevantes, proporcionando um tempo de avaliação do trabalho do departamento visando a melhoria contínua individual e coletiva. Semanalmente e em dia definido, o *Kaizen* diário dá lugar a uma reunião de planeamento com o objetivo de exposição do status de trabalho e de projetos decorrentes (com discussão de *outcomes* semanais) e ainda o planeamento semanal até à reunião seguinte. Estas reuniões diárias e semanais permitiam a inclusão e participação de outros colegas nos trabalhos desenvolvidos por essa pessoa e ainda a possibilidade de discussão e *brainstorming*, algo muito útil considerando a elevada quantidade de projetos desenvolvidos simultaneamente no departamento. Estas reuniões ofereceram-me a possibilidade de desenvolver os meus conhecimentos em diversas áreas (tecnologia, estatística, engenharia, química, etc.).

B. Fraquezas

i) Fraca rotatividade de tarefas

Onde claramente a dinâmica de estágio pecou mais foi na falta de rotatividade de tarefas que senti. Tendo-me sido proposto um estágio com um objetivo bem delineado, o meu trabalho focou-se muito em trabalho de pesquisa por computador e discussão/ desenvolvimento da informação recolhida, pelo que foi exigido que passasse a maioria do tempo em ambiente de escritório. Uma alternativa a este regime de trabalho que sugeriria seria a rotatividade com trabalho em ambiente laboratorial com um horário definido (quando possível).

ii) Duração do estágio

Com um tempo de estágio inferior a 3 meses, a realização deste e as aprendizagens permitidas foram limitadas por este prazo um algo breve. Tendo consistido o meu estágio num trabalho de vertente de pesquisa e elaboração de um projeto na área de assuntos científicos, o ponto 1) destas Fraquezas foi também incitado por este aspeto, uma vez que a prolongação da duração de estágio para além dos 3 meses poderia permitir distribuir mais rotativamente tarefas de diferentes vertentes da IF, não tendo eu tido possibilidade de contactar com outras demais. Adicionalmente, defendo que, tendo todas as atividades do exercício farmacêutico teoricamente o mesmo valor, as horas de estágio requeridas para os setores de Farmácia Comunitária, Indústria Farmacêutica e Farmácia Hospitalar deveriam ser equivalentes, não se verificando domínio de Farmácia Comunitária sobre os outros setores (que retira conteúdo de aprendizagem a estes últimos).

C. Oportunidades

i) Apoio ao Desenvolvimento de Medicamentos

O projeto desenvolvido poderá servir de suporte científico para o desenvolvimento de medicamentos por parte da Bluepharma num futuro não muito distante, permitindo a comercialização de mais um medicamento desenvolvido internamente e especificamente numa área tão relevante e rentável como o tema do projeto de estágio. De facto, através da pesquisa sistemática do estado da arte e da *pipeline* em desenvolvimento para imunoterapia oncológica, a Bluepharma poderá identificar oportunidades de desenvolvimento de medicamentos que auxiliem no crescimento da empresa no mercado deste tipo de medicamentos.

ii) Ciência em 1ª mão

Tendo sido o alvo do meu trabalho de pesquisa um tema científico muito atual permitiu que tivesse escrito e estudado a ciência mais contemporânea possível. De facto, a metodologia de recolha de informação não consistiu somente em artigos já bem estabelecidos (com alguns anos), mas em informação extremamente recente com maior valor (meses/semanas ou até mesmo dias após a sua publicação). Assim, através de vertente de elaboração de um trabalho de revisão/discussão deste tema foi-me concedida a oportunidade de rever e escrever material científico em primeira mão, seguindo a linha da frente de ciência a ser criada "diariamente".

D. Ameaças

i) Incerteza associada à pandemia COVID-19

O estágio na BLPH decorreu durante os primeiros meses de 2022, quando a pandemia COVID-19 ainda se fazia sentir consideravelmente. Assim, a incerteza associada ao rumo futuro desta pandemia e às possíveis mudanças de regime de trabalho, possivelmente causadas por esta, foram uma constante ameaça durante o decorrer do estágio (principalmente durante o 1° mês). Proveniente de decisões anteriores ao meu início de estágio, o regime misto de teletrabalho/trabalho presencial (através de 2 turnos) foi exercido pelos meus colegas de trabalho, à exceção do meu horário fixo de 8 horas presenciais. Devido a casos positivos no departamento e a este regime por turnos, o meu próprio trabalho ficou ligeiramente condicionado, tendo sido mais notório na impossibilidade de acompanhamento de trabalho laboratorial. De forma geral e sucinta, a incerteza relativa ao futuro próximo intimamente relacionado com o rumo/estado pandémico impactou a capacidade de planeamento e organização do meu estágio.

ii) Elaboração concomitante da monografia de estágio

Sendo também um fator externo ao estágio de IF propriamente dito (embora ambos inseridos no plano de Estágio Curricular), a produção da Monografia de Estágio foi uma ameaça à elaboração ideal do trabalho de estágio. O fundamento desta ameaça prende-se com o facto de o tema de ambos os trabalhos ser semelhante pelo que, por vezes, o objetivo da pesquisa de estágio a ser efetuada era contaminada pela monografia a ser desenvolvida fora do estágio. Também sendo ambos trabalhos de pesquisa e elaboração, criava-se frequentemente uma sobrecarga uma vez que após as 8 horas de estágio diárias, seguiam-se mais horas de elaboração da monografia, tendo isto muitas vezes impactado níveis de cansaço e aptidão ideal de pesquisa consequentemente.

4. Conclusão/Considerações Finais

Seguindo o curso de Ciências Farmacêuticas (na Universidade de Coimbra) um modelo de Mestrado Integrado, os 5 anos permitem uma vastidão de conhecimentos em diversas áreas do exercício farmacêutico e outras, e ainda oportunidades de aprendizagem prática através de estágios curriculares como o de Indústria Farmacêutica. Através da forte ligação da Bluepharma com a UC, foi-me atribuído o privilégio de poder estagiar durante 3 meses nesta empresa farmacêutica e numa área tão valorosa como a de Investigação e Inovação Farmacêutica.

Deste estágio e do projeto desenvolvido durante este, retiro considerações extremamente positivas. De facto, estes 3 meses promoveram de forma exponencial o meu crescimento pessoal pelo contacto direto com mercado de trabalho, tornando-me num futuro profissional de saúde mais maturo e experiente. O contacto com diversas áreas da IF permitiu abrir o leque da multidisciplinaridade de trabalho enquanto aprimorava o meu método de trabalho (previamente muito embrionário). Este projeto de pesquisa e síntese, tendo sido de facto o cerne do estágio, permitiu o enriquecimento dos meus conhecimentos teóricos, o desenvolvimento da capacidade de pesquisa que, após a conclusão do estágio, tornou-se clara a sua elevação a um patamar muito mais profissional devido ao projeto desenvolvido, e ainda a fomentação de autonomia, fundamental para o sucesso no mercado de trabalho. Estando inserido num departamento multifacetado, o trabalho em equipa foi também ponto de evolução pela inserção num coletivo profissional. Esta introdução aos processos de Indústria, altamente marcada por uma dinâmica exigente regida pela Qualidade, promoveu a adaptação de conhecimentos prévios relativos a este setor uma vez que mesmo processos práticos, claramente lecionados noutras unidades curriculares, eram realizados de forma diferente (mais rigorosa) e exigiram uma reformulação de conceitos. Todos estes aspetos serão certamente úteis para o meu futuro no mercado de trabalho principalmente relativos a metodologia de trabalho, excelência e rigor, empenho, versatilidade e espontaneidade.

O enunciado anteriormente aparece em concordância com o objetivo geral dos Estágios Curriculares e, relativamente ao meu estágio em Indústria Farmacêutica, é seguro afirmar que os objetivos foram de facto cumpridos.

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Parte II

Relatório de Estágio Curricular em Farmácia Comunitária

Farmácia de Celas

farmáciadecelas

4 de abril a **29 de julho de 2022**

Estágio sob orientação da Drª Claúdia Silvestre

Resumo

O curso de Ciências Farmacêuticas, atualmente sob formato de Mestrado Integrado, refina todas as unidades curriculares na derradeira Unidade "Estágio Curricular", onde o estágio em Farmácia Comunitária se apresenta como o mais proeminente na capacitação de futuros farmacêuticos. Em território português, o exercício farmacêutico profissional é marcado por uma constante evolução de métodos de trabalho, visando primeiramente a (melhor) satisfação das necessidades dos utentes. Atualmente e em diversas situações, a Farmácia Comunitária representa o único porto de abrigo para cuidados de saúde acessível, sublinhando o papel crucial que o farmacêutico tem como o profissional de saúde mais próximo da população, e tendo sido este fator ainda mais evidenciado pela pandemia COVID-19. O papel do farmacêutico comunitário destaca-se pela elevada qualificação deste para um aconselhamento diferenciador e irrepreensível sob os mais diversos contextos em saúde. Assim, um Estágio em Farmácia Comunitária acarreta-se de elevada importância no aprofundamento, expansão e consolidação de conhecimentos, através de um regime de aplicabilidade destes de forma prática, capacitando o aluno como profissional de saúde ideal.

Palavras-chave: Farmacêutico Comunitário; Estágio Curricular; Formação Prática.

Abstract

The Pharmaceutical Sciences' course, currently under an Integrated Masters format, refines all other curricular units into the ultimate unit: *"Curricular Internship*", on which Community Pharmacy's internship stands as the most preeminent in future pharmacists' qualification. In Portugal, the pharmaceutical professional activity is characterized by constant evolution in work method, aiming the (most adequate) satisfaction of patients' needs. Nowadays, Community Pharmacy represents the only safehouse for healthcare services on several situations, highlighting the crucial role pharmacists have as the community's most convenient and close healthcare professionals, even more noticeable during COVID-19 pandemic. The pharmacists' importance is based on his differentiating and impeccable counseling, under the most diverse healthcare contexts. Concluding, a Community Pharmacy's internship elevates itself to a higher ground supported by its knowledge's deepening/expansion/reinforcement regimen, through practical applicability, readying the student as the future's ideal healthcare professional.

Keywords: Community Pharmacist; Curricular Internship; Practical Training.

Abreviaturas

API – Active Pharmaceutical Ingredient

CE – Contraceção de Emergência

CHUC – Centro Hospitalar e Universitário de Coimbra

COVID-19 – Coronavirus Disease 2019

DCI – Denominação Comum Internacional

DGS – Direção Geral de Saúde

id – in die

MedDRA - Medical Dictionary for Regulatory Activities

mg - miligramas

MICF – Mestrado Integrado em Ciências Farmacêuticas

mmHg – milímetros de mercúrio

MNSRM – Medicamento Não Sujeito a Receita Médica

MSRM – Medicamento Sujeito a Receita Médica

PIM – Preparação Individualizada da Medicação

PV – Prazo de Validade

PVF – Preço de Venda à Farmácia

PVP – Preço de Venda ao Público

SARS-CoV-2 – Severe Acute Respiratory Syndrome Coronavirus 2

SNS – Serviço Nacional de Saúde

SWOT – *Strenghts, Weaknesses, Oppoortunities & Threats*

TA – Tensão Arterial

1. Introdução

Atualmente o papel do farmacêutico, em Portugal, prende-se fulcralmente por um serviço de apoio à população e, tendo as Farmácias de Oficina representado frequentemente as únicas instituições de saúde de proximidade e a primeira linha em termos de oferta de cuidados de saúde, justificou-se a posterior alteração de designação para Farmácia Comunitária. O exercício farmacêutico português estabeleceu-se em 1449, quando os farmacêuticos eram nominados de "boticários", com uma atividade então muito focada na preparação oficinal de medicamentos/substâncias medicamentosas. A evolução e metamorfose farmacêutica ao longo das épocas mostrou-se singular e fascinante, culminando no presente e largo espetro de atividades, tendo na Farmácia Comunitária o papel mais determinante como pilar do Serviço Nacional de Saúde (SNS), especialmente notável nas últimas três décadas. Assim, o farmacêutico comunitário contemporâneo, como especialista máximo do medicamento, tem contribuição privilegiada (próxima da população) em áreas como a gestão e aconselhamento terapêuticos, administração de medicamentos, determinação de parâmetros, deteção precoce de doenças e promoção de estilos de vida saudáveis. [1]

O plano curricular do presente curso de Mestrado Integrado em Ciências Farmacêuticas (MICF), realizado na Faculdade de Farmácia da Universidade de Coimbra ao longo de 5 anos letivos, permite aos alunos desenvolver competências tanto teóricas como práticas relativas a diversas áreas, sendo este curso fortemente marcado pela sua multidisciplinaridade, exigência/rigor, aplicabilidade e abrangência. O "Estágio Curricular", a derradeira disciplina do curso, preenche-se de importância uma vez que culmina idealmente todas as disciplinas previamente lecionadas e permite a aplicação de conhecimentos de tais disciplinas segundo uma perspetiva prática em ambiente profissional. Assim, foi-me dada a oportunidade de efetuar um estágio curricular em Farmácia Comunitária, tendo recaído sob a Farmácia de Celas a escolha como instituição acolhedora. [2] O meu estágio em vertente de farmácia comunitária decorrido na "Farmácia de Celas", sob a orientação da Doutora Claúdia Silvestre, iniciou-se no dia 4 de abril de 2022 e teve em 29 de julho de 2022 o seu dia de término.

Com o objetivo de avaliar e relatar o dito estágio, uma análise **SWOT** é o método escolhido para o efeito. Este tipo de análise permite uma avaliação global do estágio efetuado e, por conseguinte, dos conhecimentos idealmente adquiridos ao longo de 10 semestres de curso e a sua aplicabilidade em contexto profissional. Esta análise SWOT é feita com base em 4 vertentes: Pontos Fortes (***S**trenghts*), Pontos Fracos (***W**eaknesses*), Oportunidades (***O**pportunities*) e Ameaças (***T**hreats*), conforme pormenorizado em secção subsequente.

2. Farmácia de Celas

Embora o nome possa originar equívocos, este está intimamente ligado à história da instituição. Agora estabelecida na Estrada de Coselhas (Coimbra), esta farmácia esteve primordialmente localizada na Rua Doutor António José de Almeida, em Celas, desde a sua fundação em 1957 sob o nome de "Farmácia Montes Claros". Em 2002 iniciou-se um processo de metamorfose: a transferência de localização primeiramente para a Alameda Armando Gonçalves (Celas) e, posteriormente em 2012, para a atual localização em Coselhas, perpetuando e homenageando a localização antecessora através da atual denominação – Farmácia de Celas. [3] Aliada à proximidade fulcral às Circulares de Coimbra (vias de acesso ao CHUC, Hospital Pediátrico, CUF e Hospital da Luz), esta farmácia oferece à população de Coimbra e visitante dos hospitais os mais diversos serviços farmacêuticos sob um compromisso tenaz de dedicação e profissionalismo, visando um estado ótimo de Saúde para todos os seus utentes concordante com o seu próprio lema de "*Cuide de si. Aqui*".

Sob a atual Direção Técnica da Doutora Cláudia Silvestre, reúne-se uma equipa que consegue conciliar experiência farmacêutica com eficiência/simpatia de atendimento e procura da vanguarda de conhecimento. Com admiráveis condições de funcionamento, a equipa opera, sob uma metodologia de melhoria contínua "*Kaizen*" [4], em diversos espaços como gabinetes de atendimento, *backoffice*, Laboratório de Manipulação, com os balcões/área de atendimento funcionando como a cara da farmácia *per se*. Estando atualmente aberta à população com o horário de 8h30-20h00 (2ª a 6ª feira) e 9h-13-30 (sábados), são diversos os serviços disponibilizados para além do atendimento/aconselhamento farmacêutico entre os quais: Preparação Individualizada de Medicação (PIM), Administração de Injetáveis, Medicamentos Manipulados, Entrega ao domicílio, Medicação de Parâmetros Bioquímicos, Consultas de Nutrição, Podologia, Espaço Veterinário, Testes Rápidos COVID-19 e Farma Express. [3]

Relativamente à minha experiência como estagiário, adquiri desde cedo conceitos teóricos, tendo constantemente oportunidade de os aplicar de forma prática e sob um regime de aprendizagem contínua. O meu raio de aprendizagem consistiu principalmente em trabalho de *backoffice*, atendimento em balcão, marketing, e organização e gestão farmacêutica. Devido ao contexto pandémico, o processamento/comunicação de testes imunocromatográficos de deteção de SARS-CoV-2 foi outra área explorada, permitindo a aquisição de experiência adicional relativamente a estágios curriculares de anos anteriores a 2020. O plano de estágio foi previamente delineado pela orientadora Drª Claúdia Silvestre e contou com o auxílio de todos os colaboradores para a mais proveitosa realização deste.

3. **Análise SWOT** (*Strenghts, Weaknesses, Opportunities & Threats*)

A. Pontos Fortes (***Strengths***)

i) Integração e Equipa Técnica

Desde o primeiro dia de estágio, o apoio constante disponibilizado por toda a equipa técnica da Farmácia de Celas (diretora técnica, farmacêuticas, técnicas auxiliares e estagiárias) foi admirável. Este suporte estabelecido desde cedo, permitiu-me o esclarecimento de todas as dúvidas e a elucidação dos processos laborais internos e do setor, resultando numa ótima introdução ao mundo farmacêutico profissional e numa instauração de conhecimentos e práticas fulcrais para o futuro. Desde a receção de encomendas, processamento de testes de deteção de SARS-CoV-2, trabalho de *backoffice* geral, manipulação de medicamentos, até aconselhamento a utentes, visando uniformizar o trabalho exercido (segundo os postulados do Manual de Gestão de Qualidade interno), foram algumas das áreas mais trabalhadas. O ambiente de entreajuda, intercomunicação e camaradagem tornou-se assim uma base para o êxito do estágio e para desenvolvimento de carácter pessoal/profissional.

ii) Multidisciplinaridade de atividades/projetos

A atividade na farmácia comunitária demonstrou ter uma enorme multidisciplinariedade, tanto no balcão de atendimento como no *backoffice*, tendo tido a oportunidade de contactar de forma prática com diversas áreas como Marketing, Parâmetros Bioquímicos, (testes de glicémia, triglicéridos, tensão arterial etc.), administração de injetáveis/vacinas não incluídas no Plano Nacional de Vacinação Contabilidade, Gestão Farmacêutica e Nutrição.

Relativamente às diversas funções que desempenhei sob supervisão, sublinho com distinção o setor do ***backoffice***. Embora seja altamente subvalorizado, aprendi que este representa o principal pilar de uma farmácia comunitária organizada, permitindo um atendimento mais eficiente. A receção de encomendas incorpora a conferência da mesma, entrada e contabilização dos produtos com recurso ao software SIFARMA (com verificação/correção de prazos de validade), averiguação de preços de custo (líquido), Preços de Venda às Farmácias (PVF) e PVPs (quando aplicável e segundo margens de lucro tabeladas internamente), e ainda a triagem de produtos reservados (faturados ou não faturados). Após este processo, prossegue-se o armazenamento de produtos, exceto daqueles a serem devolvidos devido a validades inadequadas *versus* a sua rotação na farmácia (normalmente com PV < 1 ano). A arrumação é feita por divisões definidas segundo o tipo de produto (MSRM, MNSRM, dispositivo médico, produto alimentar, produto veterinário, etc.), segundo a fórmula

farmacêutica ou forma de administração (injetáveis, retais, pós, soluções orais, ex.), segundo condições de armazenamento (frigorifico para medicamentos entre 2-8ºC), segundo indicações terapêuticas (lineares de aconselhamento rotulados, ex.), e ainda segundo a rotação de produtos (gavetas especializadas para medicamentos com maior rotação, denominadas *cockpit*, permitiam o fácil acesso a estes e a diminuição dos tempos de atendimento).

O **atendimento ao balcão** consolidou os meus conhecimentos de aconselhamento farmacêutico através de aprendizagem observacional e prática, e análise dos procedimentos do Manual de Gestão de Qualidade. Aqui foi visível a minha evolução em termos de autonomia, espírito critico, profissionalismo e desenvolvimento de método de trabalho, solidificando o meu papel como futuro farmacêutico. Aqui destaco 2 vertentes do atendimento que fomentei com auxílio à experiência adquirida em atendimento e aos conceitos transmitidos por colegas: trabalho em SIFARMA e aconselhamento farmacêutico (farmacológico e não farmacológico), exemplificado com Casos Clínicos que apresento anexados (ponto 4.)

Assim, os conhecimentos adquiridos permitem preparar o estagiário para a vida profissional em diversos setores farmacêuticos.

iii) Comunicação em lingua inglesa

A proximidade aos grandes centros hospitalares de Coimbra, sendo alguns destes dos mais reputados do país, permite um elevando fluxo demográfico à Farmácia, sendo uma considerável percentagem de utentes, estrangeiros. Face às barreiras linguísticas, a língua inglesa foi sempre a usada para aconselhamentos nestas situações. A necessidade de comunicar em inglês permitiu-me aplicar e ainda afinar o meu discurso em língua inglesa, com especial atenção para termos médicos e a sua concordância com a uniformização MedDRA.

iv) Diversidade no tipo de utentes

A proximidade com grandes centros hospitalares de Coimbra e com as Circulares Internas e Externas da cidade possibilita o direcionamento de um considerável fluxo de pessoas para a farmácia, procurando obter a medicação após consulta médica nos referidos centros. Assim, este fluxo de utentes de diversos locais do país (não somente utentes locais) possibilitou-me uma vasta diversidade de atendimentos consoante as mais diversas necessidades clínicas que me foram apresentadas. Este fator evitou (felizmente, na minha opinião pessoal) monotonia de atendimentos. Exemplificando este fator profícuo, destaco o caso de medicamentos para tratamento da infertilidade, cuja procura destes na farmácia era considerável devido à proximidade a consultas de Medicina da Reprodução.

B. Pontos Fracos (*Weaknesses*)

i) Conhecimento limitado

O fator que sinto que mais impactou negativamente o meu estágio foram, de facto, as lacunas de conhecimentos que foram evidenciadas principalmente aquando da realização de atendimentos ao utente, como na efetivação de planos de complementaridade, mas também em atividades de *backoffice* como processos de faturação. A áreas científica onde verifiquei maior desconhecimento foi Cosmética e Dermofarmácia, agravado por ser uma das áreas mais requeridas em termos de aconselhamento. Essa lacuna de fundamentação teórico-prática criou assim repetitivamente a necessidade de auxílio no aconselhamento por parte de outras colegas, pondo em causa a "autonomia" referida na secção anterior. Relativamente às causas das tais lacunas, algumas serão devidas deficiente interiorização de matéria teórica lecionada em unidades curriculares, porém outras, como as atividades de faturação (contabilidade) pecam por não constar no plano curricular de MICF.

ii) Formações externas

Devido ao elevado fluxo de utentes, contexto pandémico e à dinâmica da farmácia, não existiu grande possibilidade de formações externas, especificamente as formações temáticas ("Cuidados da Pele na Gravidez", etc.). Estas ditas formações permitiriam o aprofundamento e/ou consolidação de conhecimentos relativos a farmacoterapia, farmácia clínica, aconselhamento diferenciado, dispositivos médicos, gestão farmacêutica e (substancialmente) dermocosméticam. Considero que a realização de (mais) formações externas temáticas permitiria a divulgação de conhecimento novo/atualizado, sendo este potencialmente útil no atendimento ao utente, podendo ter representando um trunfo no estágio.

C. Oportunidades (*Opportunities*)

i) Formação mais completa com o SIFARMA

Considerando fatores com potencial favorável de melhoria, destaco a oportunidade de um maior aprofundamento dos conceitos de funcionamento do sistema informático SIFARMA (versão 2000 e/ou nova) da Glintt®, ferramenta de gestão e atendimento de 90% Farmácias Comunitárias em Portugal, a ser ministrado em unidades curriculares uma vez que considero que o plano curricular somente se foca em algumas funcionalidades do referido sistema. A possibilidade de um curso intensivo sobre os novos módulos de atendimento e outras funcionalidades do SIFARMA seria uma valiosa oportunidade de melhoria, facilitando o cumprimento dos objetivos ideais de estágio.

ii) Protocolos de formações externas alinhados com a universidade

Com o objetivo de igualar oportunidades de aprendizagem externa, sugeria a elaboração de protocolos universais de formações obrigatórias durante o estágio curricular, independentes da farmácia de estágio e por parte da Faculdade de Farmácia, funcionando como palestras de professores convidados (como se verifica em diversas outras unidades curriculares do curso). Estas formações tornariam assim o estágio mais dinâmico e completo.

D. Ameaças (*Threats*)

i) Pandemia COVID-19

Tendo o estágio decorrido ainda com normas vigentes de segurança e profilaxia (DGS) no âmbito da COVID-19, a utilização de equipamento de proteção individual e cuidados profiláticos foram fatores constantes durante o estágio. Defendo que esta situação se tornou uma ameaça na medida em que pôde ter prejudicado especialmente atendimentos a utentes em balcão, uma vez que frequentemente se verificaram dificuldades de comunicação entre o utente e o farmacêutico devido ao uso de máscaras e barreiras físicas de proteção. Assim, existia o risco de compreensão errónea da solicitação de cuidados por parte do utente e de dificuldades na transmissão do aconselhamento por parte do farmacêutico, especialmente em atendimentos a população geriátrica. De forma a colmatar este problema, um discurso mais pausado e cuidado permitia um atendimento adequado às necessidades do utente e com menor probabilidade de erros.

ii) Medicamentos Esgotados

Sendo a farmácia comunitária o local para levantamento de medicação, cuja toma de uma grande desta se mostra como imprescindível para a manutenção diária de um estado de saúde do utente, medicamentos sem disponibilidade no mercado (esgotados) impossibilitaram frequentemente um atendimento proveitoso, causando a procura de outras farmácias e a privação da oportunidade de aprendizagem inerente a cada atendimento. Assim, a reduzida ou até nula disponibilidade de produtos verificou-se uma distinta ameaça para a farmácia, pondo em causa o ideal apoio à população e potenciais vendas, e ainda para um entrave para o estágio.

iii) Prescrição por receita manual

No decurso do estágio, aprendi que uma das tarefas mais comuns (e fulcrais) se prendia com aviamento de receitas/prescrições médicas. Existindo atualmente ainda vários tipos de receituários, contactei com prescrição eletrónica desmaterializada ou *"sem papel"* (a mais

habitual), prescrição eletrónica materializada e ainda prescrição manual provenientes de diversos centros de cuidados de saúde, como o CHUC, Hospital da Luz, mas também clínicas dentárias, veterinárias e privadas. A receita manual por vezes apresentada na farmácia correspondia a forma menos comum de receituário, uma vez que somente é opção de prescrição segundo justificações bem estabelecidas e específicas. Uma das justificativas do racional por de trás da transição para receitas eletrónicas foi-me apresentado claramente durante o percurso do estágio – **fraca legibilidade** da prescrição. Frequentemente, face a caligrafia confusa e "apressada", atendimentos morosos tinham lugar uma vez que uma "descodificação" do conteúdo da receita (tanto letra como algarismos numéricos) era necessária mesmo com auxílio de outras colegas mais experientes. Assim, este tipo de receituário criava um sentimento de "insegurança" no dito atendimento, evidenciando o perigo de erros de medicação e ameaçando o mais correto funcionamento do estágio e da farmácia. Assim, defendo e incentivo a extinção de receitas manuais (e a transição para receitas eletrónicas), permitindo a perpetuação de legibilidade ideal em prescrições e a minimização de erros consequente. Face à possibilidade de falhas informáticas, existiriam sistemas eletrónicos e/ou manuais não-manuscritos de *backup*.

4. Casos Clínicos Práticos

Foram diversas as situações práticas em farmácia em que foram requeridos atendimentos mais atentivos/personalizados, e aconselhamentos farmacológicos e não-farmacológicas em resposta a uma necessidade do utente. Destaco aqui cinco situações exemplares onde apliquei conhecimentos que adquiri em estágio e, previamente, em faculdade (sob supervisão), presentes em **Anexo**.

5. Considerações Finais

Sumarizando 4 meses de estágio em farmácia comunitária, confesso que senti um aprofundamento admirável de bases previamente interiorizadas ao longo dos 5 anos de curso académico, enriquecendo o meu potencial como futuro profissional de saúde. O balanço claramente positivo do estágio foi resultado de uma mistura de fatores tanto relativos a mim, à Farmácia de Celas e à Faculdade de Farmácia da Universidade de Coimbra. Destaco então o papel fulcral de toda a equipa da Farmácia de Celas e, principalmente, à direção técnica, uma vez que se comprometeram com dedicação a me proporcionarem um ambiente de aprendizagem exemplar. De facto, a entreajuda constantemente disponibilizada permitiram um meio de trabalho de excelência, e o profissionalismo e personalização de atendimento foram contagiantes no desenvolvimento pessoal de metodologias de trabalho digno de um farmacêutico, organização, comunicação interpessoal, autonomia de atuação e, particularmente, a expansão de horizontes.

O estágio curricular permitiu-me então obter conceitos do exercício farmacêutico em mundo real, expandindo os conceitos em faculdade, e concluir sobre o papel importantíssimo do farmacêutico comunitário atual e a necessidade da procura constante da vanguarda (melhoria contínua) tanto de conhecimento como de ação.

Assim, concluo que os objetivos delineados de estágio foram certamente cumpridos, uma vez que, através da aquisição de alicerces práticos, cresci a nível profissional e fomentei o meu potencial como farmacêutico, enquanto desenvolvi aptidões pessoais, como paciência, compreensão, frieza, compaixão, entre outras, e expus falhas a serem trabalhadas futuramente com empenho contínuo.

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7. Anexos

- **CASO I**

Um homem (adulto) dirige-se ao balcão de atendimento da farmácia pedindo aconselhamento relativo a "dor de garganta" ligeira a moderada que relata ter desde os 2 dias anteriores. Refere que procurava umas pastilhas para a dita dor. Tendo isto ocorrido numa época crescente em termos de casos positivos de infeção por SARS-CoV-2, foi informado ao utente que poderia ser sintoma de COVID-19 e questionado se tinha tido algum contacto de risco e/ou feito auto-testagem para despiste da dita doença pandémica. [5] O utente confessou que esteve com um contacto positivo 5 dias antes, mas justificando que tinha apresentado um autoteste negativo no dia seguinte ao contacto e que não seria COVID-19 devido a essa razão. Foi-lhe explicado que através do período de incubação e perfil de desenvolvimento carga viral da variante dominante do vírus SARS-CoV-2, após somente 1 dia do contacto de risco, a carga viral seria ainda potencialmente insuficiente para deteção em testes rápidos de antigénio, pelo que após 2 a 5 dias já poderia testar positivo. [6] Assim, foi-lhe aconselhada a toma de pastilhas anti-inflamatórias para dor de garganta (Strepfen® Mel e Limão) para tomar de 4 em 4 horas [7] (sem comer nada durante aproximadamente 30 minutos idealmente, evitando a "lavagem" do API da zona de atuação e a perda de eficácia), e ainda um Teste Rápido de Antigénio por imunocromatografia a ser efetuado na farmácia. O utente testou positivo à presença de SARS-CoV-2, sendo aconselhado iniciar o isolamento de 7 dias (contando desde o início dos sintomas) e, em caso de febre/dores corporais, a toma de paracetamol 500 mg, de acordo com as normas vigentes da DGS para tratamento sintomático de COVID-19.

- **CASO II**

Uma mulher jovem desloca-se à farmácia, questionando sobre o risco de engravidar devido a uma relação sexual não protegida. Foi questionada sobre quando decorreu o ato sexual de risco, pelo que confessou ter sido **3 dias** antes, e ainda sobre o uso de algum contracetivo hormonal, tendo respondido que usava uma pílula combinada, porém, devido às festividades da Queima das Fitas, confessou se ter esquecido de tomar em 2 dias e ainda ter vomitado uma vez alguns minutos após ter tomado a medicação (nos últimos 7 dias). Foi ainda questionada a fase do ciclo menstrual em que se encontrava, tendo confessado que teve a hemorragia de privação há cerca de 2 semanas, inserindo-se num potencial período fértil. Assim, concluiu-se e foi explicado detalhadamente à utente que, de facto, existia o risco de gravidez. Ela solicitou então contraceção de emergência. Visto que a relação de risco ocorreu cerca de 72 horas antes, levonorgestrel 1,5mg podia não ser eficaz, sendo então ideal a toma de ellaOne®

(acetato de ulipristral 30mg em comprimido revestido), uma vez que tem indicação aprovada até 120 horas após o contacto. Após a exclusão de contraindicação por hipersensibilidade ao fármaco ou asma não controlada, foi selecionada definitivamente a CE. Posteriormente foi aconselhada então a toma mais breve possível da CE, o retomar imediato do método de contraceção hormonal habitual e ainda proteção adicional com preservativo durante 14 dias após o uso do acetato de ulipristal (na eventualidade de outras relações sexuais durante este período). [8]; [9]

- **CASO III**

Uma mãe e o filho chegaram à farmácia procurando levantar a medicação prescrita em receita. Na referida receita (prescrita para o filho de 15 anos) constatava somente um medicamento, prescrito por DCI. Após trazer o medicamento em questão – Avamys® – para o balcão, a mãe reconhece a caixa e apresenta-se descontente uma vez que no ano anterior foi prescrito o mesmo medicamento para a rinite alérgica do filho e confessou não ter dado resultado. Foi questionado ao utente se tinha seguido a posologia que o médico tinha recomendado (2 pulverizações em cada narina/dia), tendo confessado que achava que pensava ser 2 pulverizações totais (1 por narina) e não 2 por narina, estando aqui presente uma potencial causa da falha terapêutica. Sendo um medicamento administrado com recurso a um dispositivo de pulverização nasal, questionei se lhe tinha anteriormente sido explicado o "exercício respiratório" que deveria efetuar ao administrar o medicamento, tendo também confessado que somente pulverizava o spray nasal sem outros cuidados. Expliquei que antes de administrar o spray propriamente dito na narina, deverá ser feita uma inspiração nasal (para presença ideal do princípio ativo no local de ação devido) seguida do "puf" ainda durante a dita inspiração contínua. Após isto, sublinhei que era importante seguir-se uma expiração somente pela boca, evitando que o API volte a sair para o exterior (algo que o utente confirmou desconhecer). [10] Tendo a mãe agradecido pelo aconselhamento relativo à técnica de administração, queixou-se depois de que, sempre que vai de férias, o filho se costuma coçar bastante, sendo indicativo de pele atópica (sofrendo de rinite alérgica, a atopia é provável também a nível da pele) e pediu algo para aliviar - aconselhei Rovinex® (levocetirizina) a tomar *id* durante o período das férias, podendo tomar a qualquer hora do dia (2ª geração de anti-histamínicos H1 apresentam uma probabilidade de causar sonolência muito menor do que os de 1ª geração) [11], e cuidados hidratantes para pele atópica (gel lavante e creme pós-banho).

- **CASO IV**

Uma senhora idosa polimedicada entra na farmácia e pede para lhe ser medida a tensão arterial, para comparar com o seu tensiómetro pessoal. São medidos os valores de tensão arterial, e encontram-se dentro dos valores recomendados para o perfil de utente em questão.[12] Confessou também que tinha vindo a sofrer algumas tonturas ao levantar-se, podendo indicar sintomas de hipotensão. Temperaturas elevadas nessas semanas podem explicar, pelo menos parcialmente, estes eventos. Foi questionada sobre medicação e outros produtos que tomava. Aqui, a utente referiu que iniciou na quinzena anterior uma nova medicação anti-hipertensora: "4 comprimidos por dia". Após consulta do histórico medicamentoso, foi possível concluir que a posologia prescrita do dito medicamento era, no entanto, "2 id (2 comps)", tendo sido interpretado erradamente como "2 comprimidos 2x/dia" e não como "1 comprimido 2x/dia". Assim, o doente estava a tomar o dobro da dose diária prevista, podendo explicar os aparentes sintomas de hipotensão (juntamente com as temperaturas elevadas referidas anteriormente). Após contactado o médico, foi clarificada a verdadeira posologia prescrita para com o utente e aconselhada a ingestão diária adequada de água e um controlo atento dos valores de TA após a retificação da posologia.

- **CASO V**

Uma mulher dirige-se ao balcão da farmácia queixando-se de "irritação da garganta" e alguma "dor em engolir alimentos" que já lhe durava desde o dia anterior. Foi discutida a possibilidade de corresponder a sintoma de COVID-19, porém foi feito teste rápido no dia em questão e no anterior (ambos negativos). A utente pediu pastilhas de Strepfen®, uma vez que já tinha tido sucesso terapêutico em situações semelhantes anteriores. Ao trazer o medicamento solicitado para o balcão, questionou-se a utente sobre se estava grávida ou amamentar, pelo que ela respondeu que de facto estava grávida de 5 meses. Através dessa questão, uma contraindicação contra o Strepfen® solicitado foi apresentada: "Não aconselhado o uso durante a gravidez". [7] Assim, sabendo deste facto, o produto aconselhado foi então 1 pastilha de Thymotabs® 4x/dia (suplemento alimentar sob a forma de pastilhas com sabor a laranja), uma vez que este tem uso aprovado na gravidez e amamentação no auxílio e suavização da inflamação. [13]

Parte III

Monografia

"mRNA-based Cancer Vaccines and Delivery Strategies"

Sob a Orientação do Professor Doutor Luís Maria Marques dos Santos Bimbo

List of Abbreviations

APC – Antigen Presenting Cells

AuNP – Gold Nanoparticle

CAR – Chimeric Antigen Receptor

CEA - Carcinoembryonic antigen

CMV - Cytomegalovirus

CNE – Cationic Nano-Emulsion

COVID-19 – Corona Vírus-related Disease 2019

CQA – Critical Quality Attribute

CTL – Cytotoxic T Lymphocytes

DC – Dendritic Cell

DMPE - 1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine

DNA – Deoxyribonucleic acid

DODAP – 1,2-dioleoyl-3-dimethylammonium-propane

DODMA - 1,2-dioleyloxy-3-dimethylaminopropane

DOPE - Dioleoylphosphatidylethanolamine

DOTAP - 1,2-Dioleoyl-3-trimethylammonium propane

DOTMA - N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride

DSPC - Distearoylphosphatidylcholine

EC – European Comission

ECIS - European Cancer Information System

EGFR - Epidermal Growth Factor Receptor

eIF4E - Eukaryotic translation initiation factor 4E

EU – European Union

Fas-L – Fas Ligand

FDA – Food and Drug Administration

GMP – Good Manufacturing Practice

HER - Human Epidermal Growth Factor Receptor

HIC - Hydrophobic Interaction Chromatography

HPLC – High Performance Liquid Chromatography

HPV – Human Papillomavirus

i.d. - intradermal

i.m. - intramuscular

ICI – Immune Checkpoint Inhibitor

IEC - Ion Exchange Chromatography

IFN - Interferon

IL – Interleukin/Ionizable Lipid

IO – Immuno-Oncology

IPC - Ion Pair Chromatography

IVT – *In vitro* Transcribed/Transcription

LDH - Layered Double Hydroxide Nanoparticle

LNP – Lipid Nanoparticle

MA – Market Authorization

mAb – Monoclonal Antibody

MAGE - Melanoma Antigen Gene

MD - *Medicinae Doctor*

MHC – Major Histocompatibility Complex

mRNA – messenger Ribonucleic Acid

MSNP - Mesoporous Silica Nanoparticle

MUC-1 - Mucin 1

MW – Molecular Weight

NK – Natural Killer

nr – non-replicating

nsP – Non-structural Protein

NTP - Nucleoside Triphosphate

NY-ESO- 1 - New York Esophageal Squamous Cell Carcinoma 1

OAS - Oligoadenylate Synthase

ORF – Open Reading Frame

OSR – Overall Survival Rate

PABP - Poly(A)-Binding protein

PAMAM - Poly(amidoamine)

PAMP - Pathogen-Associated Molecular Pattern

PAP/PSA - Prostatic Acid Phosphatase/Prostatic Specific Antigen

PCV – Personalized Cancer Vaccine

PEG - Polyethylene Glycol

PEI - Polyethylenimine

PKR - Protein Kinase R

PLGA - Poly(lactic-co-glycolic acid)

PLL – Poly(α-l-lysine)

PO – Precision Oncology

PRR – Pattern Recognition Receptor

PSD – Particle Size Distribution

Ras - Rat Sarcoma vírus protein

RIG-I - Retinoic acid-inducible gene I

s.c. - subcutaneous

saRNA – Self-amplifying RNA

SEC - Size-exclusion Chromatography

siRNA – Small-interfering RNA

SPIO - Superparamagnetic Iron Oxide

ss – Single Stranded

TAA – Tumor Associated Antigen

taRNA – trans-amplifying RNA

TCR – T Cell Receptor

TFF – Tangential Flow Filtration

TLR – Tool-Like Receptor

TME – Tumor Microenvironment

TNS - 2-p-toluidinylnaphthylene-6-sulfonate

TSA – Tumor Specific Antigen

USA – United States of America

UTR – Untranslated Region

VEGF - Vascular Endothelial Growth Factor

WHO – World Health Organization

WT1 - Wilms tumor 1 protein

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Abstract

mRNA-based technology applied to vaccines' platform, contradicting large public assumption, isn´t quite truly a novel technology. COVID-19 pandemic created an unmet need where these vaccines could finally mark their stand, and they did. Now taking the scientific community's spotlights, one main question emerged: "Can mRNA vaccines be proven effective in other therapeutic fields?". The continuous demand for (more) effective and safe oncology drugs, strongly encourages the employment of mRNA technology in cancer vaccines, reenforcing the current immunotherapy's arsenal. Vaccines containing mRNA excel mainly in safety, versatility and feasibility, while also still facing some challenges – *in vivo* efficacy. The development and enhancement of delivery strategies and systems aims to overcome mRNA's intrinsic *in vivo* instability, one of the main sources of effectiveness issues, and represents a crucial optimization point in cancer vaccines' development. Currently, the most promising delivery systems are dendritic cells' transfection (highly employed in clinical trials) and formulated mRNA, on which LNPs stand as the most auspicious and versatile systems due to flourishing nanotechnology advances. Lamentably, an extensive delivery optimization seems to be mandatory in order to achieve effectiveness, swerving from an "*one size fits all*"-type system. Nevertheless, cancer vaccines with optimized delivery represent a highly encouraging option, mainly as adjuvant therapy, working in combination regimens with increasingly more favorable outcomes but not representing the stand-alone "cure" *per se*.

Keywords: mRNA, cancer vaccines, immune-oncology, delivery systems

1. Introduction

The COVID-19 outbreak, which stunned the planet, brought not only unprecedented panic and global health risks, but also (on a much brighter side) the (re)-dawn of mRNA technology as one of the most promising healthcare tools in recent decades. It was mostly thanks to COVID-19 vaccines containing mRNA encapsulated within lipidic structures, such as Pfizer-BioNTech vaccine (Comirnaty®) [1], that was undoubtedly shown, with real-word evidence, the promising value of mRNA as a therapeutic agent and further supported the concept of its use in oncology. After these recent breakthroughs, one major question emerged and remains as the most crucial in this setting: "Can mRNA-based vaccines do for cancer what they did for COVID-19?".[2]

1.1. Cancer's Background, Hallmarks & Burden

The concept of "cancer", "tumors" and "malignancies" have haunted humanity for centuries due to its unpredictability and mortality rates. Cancer isn´t a disease in *stricto sensu*, but an umbrella term for life-threatening diseases that are characterized by an uncontrolled growth of transformed malignant cells which, when left untreated, can disseminate throughout the body and severely compromise it.[3]; [4] Aiming a more complete disease understanding, researchers created **hallmarks** to distil cancer's complex pathology mechanisms. These cancer hallmarks grew from six originally proposed in the turn of the century: apoptosis evasion, self-sustaining proliferative signalling, growth suppression evasion, angiogenesis triggering, inexhaustible replication potential, and tissue's invasion and metastasis.[5]; [6] Naturally, through the expansion of cancer mechanisms' understanding, other hallmarks emerged throughout the years increasingly refining the oncology field, such as the currently-validated capabilities - cellular metabolism deregulation and **immune response evasion** [6]; [7] (evidencing immunotherapy as promising therapeutic strategy), and the proposed emerging capabilities - phenotypic plasticity and senescent cells.[7] Treating cancer remains the most challenging healthcare area since conventional therapies, such as surgery, radiotherapy and chemotherapy, stemmed considerable outcome improvements but still deficit on disease recurrence prevention in a significant number of patients. This is highly due to the aberrant cells immunosuppressive milieu and its diverse portfolio of evasion-mechanisms resulting in reduced tumor-targeted immune responses[8] and the consequential perpetuation of a substantial gap in cancer care and burden.

The **burden** sustained by oncologic problems begets the need for a solution already overdue for generations. Oncology advances have patched holes but never truly found a global therapeutic answer: the "cure" *per se*. In fact, according to recent statistics, **1,918,030** new cancer cases and **609,360** cancer-associated deaths are estimated for **2022**, in USA only [9]; of which deaths, 42% are considered related to preventable causes, and therefore representing a direct improvement opportunity.[10] Such considerably high impact on mortality/morbidity is greatly due to elevated mutation rates and metastasis phenomenon of aberrant cells which result in frequent therapeutic failure with current conventional therapies such as chemotherapy, surgery, radiotherapy, and targeted therapy. The **challenges** remain on removing completely the tumor, avoiding severe side effects by off-target drug toxicity and undesirable immune responses, and reducing the likelihood for infection events (due to debilitated immune system).

Focusing on the numbers on which cancer burden is reflected (by global regions), in terms of cost and mortality/morbidity, and specifically by analyzing historical incidence and mortality statistics in **Europe** (2004-2012) it is clear the existence of an age-related cut-off (50/60 years of age), specific cancer-types prevalence, and an overall male-sex prevalence, in terms of both incidence and mortality, according to ECIS database. In the latest update from ECIS in 2020, the EU-27's average on incidence and mortality was 568,9 and 263,5 (per 100.000), respectively (age-standardized rate - Both sexes, all sites but non-melanoma skin, all ages) with some countries having a considerable relative change (+26% incidence – Ireland; +34% mortality - Slovakia, e.g.). Also, in both incidence and mortality, the European male gender continues to present a higher count (**Fig.1** and **2**). When looking at Survival Estimates, the European Average stands at 54,15% (Relative Survival - Both sexes, all Sites, 15+ years, 2000-2007) and decreases as the age at diagnostic and follow-up interval increases.

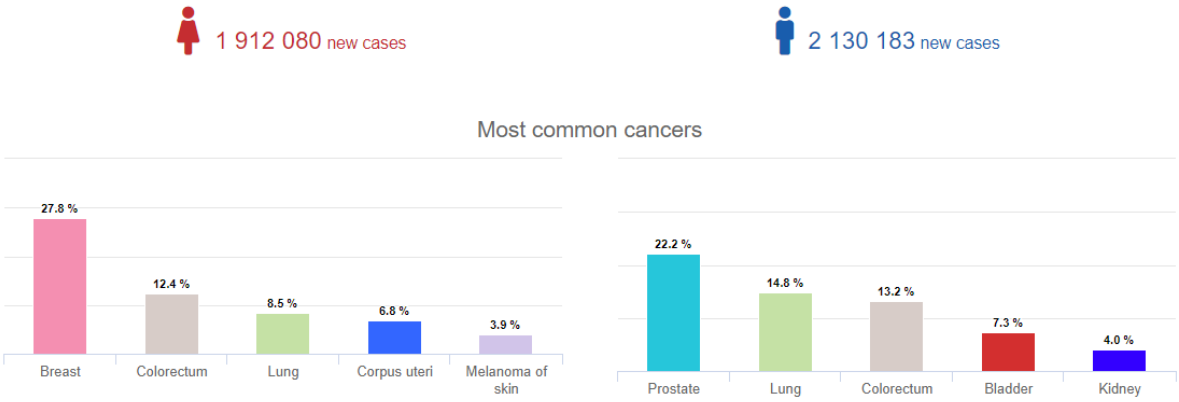

Fig.1 - Overall **Incidence** Top 5 Cancer types (Europe, Both sexes, All ages, 2020). Infographic's source: ECIS - European Cancer Information System

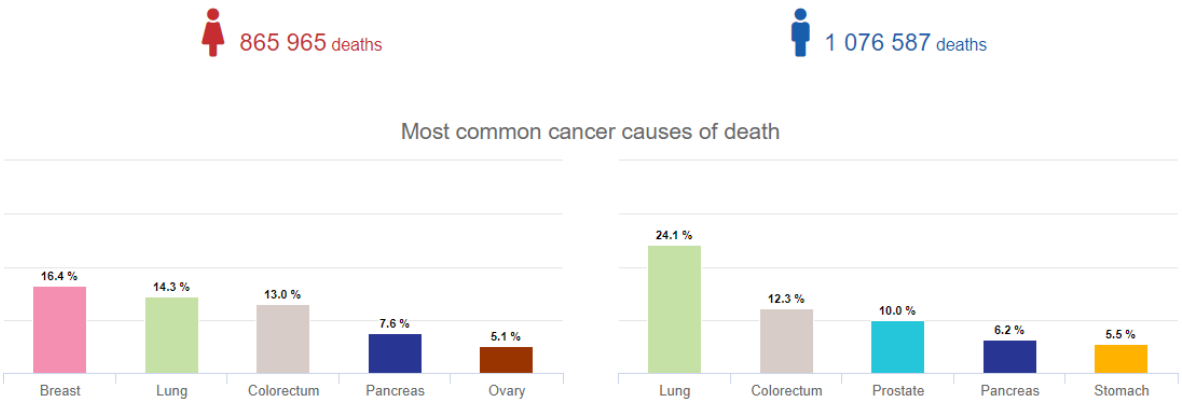

Fig.2 - Overall **Mortality** Top 5 Cancer types (Europe, Both sexes, All ages, 2020). Infographic's source: ECIS - European Cancer Information System

On the other side of the Atlantic, the burden of cancer in the **United States** seems to be comparable to Europe's. According to National Cancer Institute, an estimated 1,806,590 new cases of cancer were diagnosed, and 606,520 individuals would perish from the disease, in USA in 2020, although these numbers were projected prior to COVID-19 pandemic (cancer treatments/screenings were vastly postponed) so the burden in 2020 was probably greater. Similar to Europe, the most frequent cancer types were breast, lung, prostate, colorectal and skin melanoma, and the male gender reported higher mortality rate (2020's data). Also, when differentiating by ethnicity/race, african-american men report the most cancer-related deaths and Asian/Pacific Islander women, the least. The most incident and lethal cancer types in USA are arranged in **Fig.3** and **Fig.4**, respectively (2022's projections).

Thus, **worldwide** cancer burden is reflected in the **19,292,789** new cancer cases and **9,958,133** cancer-related deaths, in 2020, globally, according to International Agency for Research on Cancer 2020 December report (WHO). According to Our World Data, cancer occupies the 2nd place in worldwide leading causes of death (behind cardiovascular diseases) [11] with cancer incidence rates normally appearing to be higher in developed/high-income countries (according to National Cancer Institute), as this could be the result of higher average life expectancy and healthcare diagnostic capability.. Oncology continuous progress have reduced this burden over the years; however, annual new cases are expected to reach over 28.0 million and deaths over 16 million worldwide, in 2040, indicating the need for oncology improvements as soon as possible.[12]; [13]

In terms of **COST** to society, cancer also takes a punch. In 2020, the estimations of direct annual medical costs in oncology care were **$80.2 billion** in the USA (hospitals, clinics, ambulatory, etc.).[10] As the cancer "disease" represents a highly-debilitating condition, the

(indirect) costs caused by loss of productivity appear as a $94.4 billion lost, due to cancer-related deaths in individuals aged 16 to 84 (**-$190 000 per cancer death**, approximately).[14] Altogether, around **175 billion** dollars are "wasted" every year due to cancer's impact.

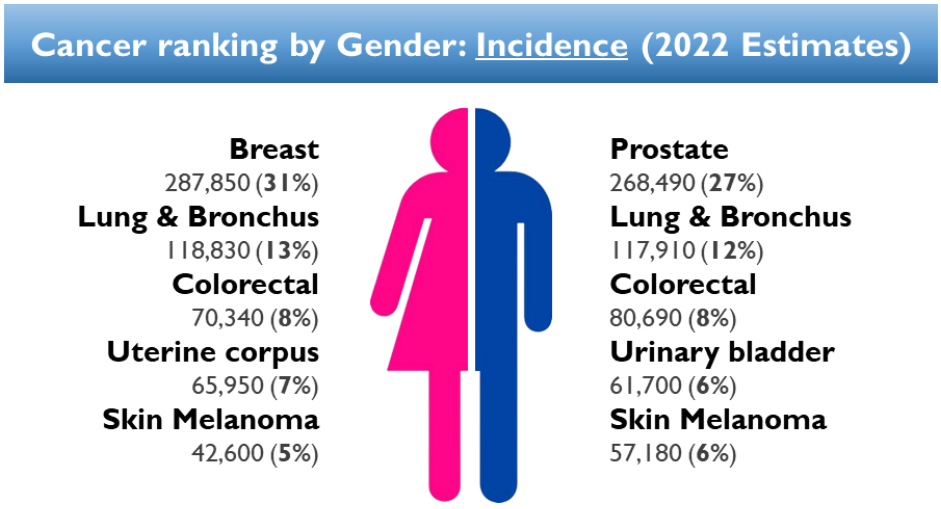

Fig.3 – Top 5 most incident cancer types by gender, according to 2022 estimated numbers for USA (cancer.org). Breast, Prostate, Lung & Bronchus and Colorectal cancers, and Skin melanoma occupy the top spots in overall expected incidence rates. Adapted from ref. [9]

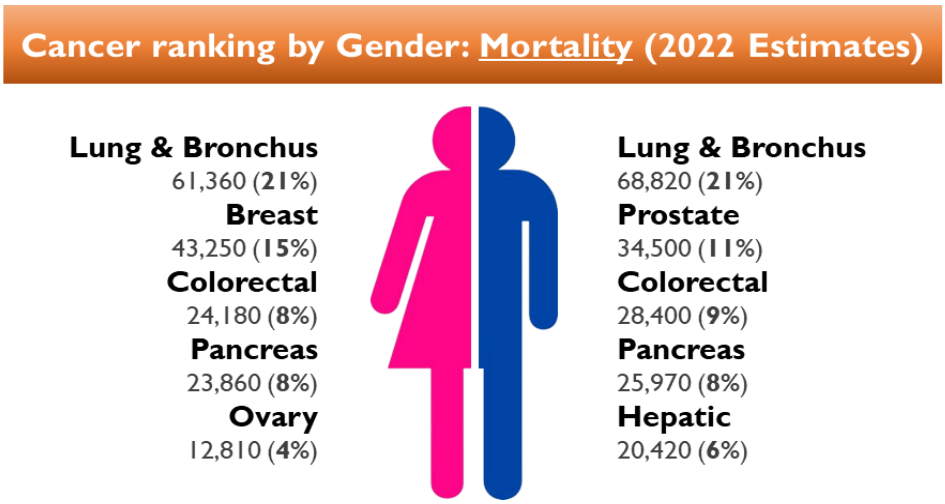

Fig.4 – Top 5 most lethal cancer types by gender, according to 2022 estimated numbers in USA (cancer.org). Lung & Bronchus, Colorectal, Pancreas, Breast and Prostate cancers occupy the top spots in overall expected mortality rates. Adapted from ref. [9]

Moreover, with greater diagnostic capability and continuous demographic growth, cancer incidence is expected to increase so burden-related data is actually underestimated.[10] This clearly shouts the need for new (effective) treatments to address unmet therapeutic gaps and reduce the depicted burden. Immunotherapy definitively possesses the potential to fill such gaps, justifying its current status and emphasizing its (expected) standard-of-care role in oncology [15], where mRNA may be an decisive tool.

1.2. mRNA Technology in Immuno-Oncology

Although oncology is currently the most research health area (and will continue to be for the foreseeable future)[16]; [17], cancer care until approximately a few decades was majorly supported by surgery (1st line, when applicable) and systemic treatments such as chemotherapy (game-changer, but the side effects are considerable), radiotherapy (mostly used in combination with other therapies) and targeted therapy (most recently).[18] This way, it could be said that oncology used to have 4 pillars, until a 5th therapy – **Immunotherapy** – emerged and (re-)revolutionized cancer care. This 5th artillery introduction is greatly due to the development and introduction of Precision Oncology's concept, on which targeted therapy is also based on.

With the due clinical basis, Precision-Oncology (PO) is considered a landmark in Oncology as it relies on medicines which act on very specific targets (biochemical pathways and/or mutations, or targets enhance the patient's immune system precise and specifically).

PO can be categorized in: [19]

- Targeted therapy - blocking the growth and spread of cancer by interfering with specific molecules involved in the growth, progression and spread of cancer;
- **Immuno-oncology/Cancer Immunotherapy** – enhancing/guiding the individual's own immune system to target cancer cells;
- Other therapies associated with predictive biomarkers - predicting tumor response to a certain therapy by informing on which molecular alterations cancer growth is dependent on. Predictive biomarkers can be used to select which targeted therapy is likely to have an effect before even starting the treatment.

Note: Immunotherapy can also be targeted, but targeted therapies work directly on the tumor while immunotherapies work to boost the immune system.

Cancer Immunotherapy or **Immuno-Oncology** (IO) is indeed a relatively recent and exciting oncology subfield, especially when looking at its research pipeline and potential. This therapy changed and continues to change our perspective on cancer treatment. The fundamental therapeutic principle is: rather than targeting tumor cells directly (like in radiotherapy or chemotherapy), **immuno-oncology** aims at **harnessing the patient's own immune system power and stimulating it in order to trigger anti-tumor immune responses and consequently eradicate tumors or at least slow their**

progress [20] with potential to remain effective for long duration beyond on treatment's term, seeking this way a memory-like ability. So, it can be said immunotherapy doesn´t treat cancer *per se*, as the individual's immune system does "all the work", so immune-deficient patients must be sadly excluded from these treatments, representing a therapeutic limitation.

> "*Cancer immunotherapy: we are not directly treating the cancer at all. Actually, our efforts are to enable and empower the immune system to do the killing*."
>
> - Eric Walk, MD, College of American Pathologists

Compared to the "conventional" therapies, immunotherapy has the upper hand due its targeting enhancement, side effects reduction (in some therapies) and ultimately "unique patterns of clinical benefit".[21] Research and clinical evidence show immunotherapy is improving outcomes and survival rates for some patients, including kidney, lung cancer and metastatic melanoma patients (some of the most cancers of the highest incidence rates) thanks to these new treatment options which already have reached, in some cases, the 1st line treatment status.

The continuous and accumulating evidence supporting the immune system's role to treat cancer is contraposed with tumor's portfolio of different strategies to escape immune surveillance such as secretion of different cytokines (IL-10, IL-4, TGF-β, IL-35, etc.) [22] and VEGF, expression of Fas-L (undesirable pro-apoptotic action on activated T cells), MHC downregulation (crucial protein in antigen presentation), secretion of myeloid-suppressor and regulatory cells, representing the most critical hurdle to immunotherapy effectiveness.[8]

In 2020, over 4 700 immuno-oncology products were reported to be in development [23], divided in the most relevant and/or promising IO strategies: Immune Checkpoint Inhibitors, CAR-T Cell Therapy, Monoclonal antibodies (mAbs), Cancer vaccines (where mRNA-based vaccines hold the highest promising value), Oncolytic viruses, Cytokines (mainly as adjuvants/combination) and Autologous NK Cells.

1.2.1. Cancer Vaccines

With vaccine usefulness more than proven with infectious diseases, the hypothesis of using this technology to treat cancer represents a promising oncology therapy. Following immunotherapy's principles, these vaccines aim at instructing the immune system on the scouting, recognition and elimination of aberrant cells based on the administration of the most

adequate immunostimulant component - **antigen** (with optimized formulation). This is a much more difficult task (comparing to bacteria and virus-caused diseases) due to the cancer cells' resemblance to "healthy" non-aberrant cells, and also due to the individuality of tumor antigens which appeals for a personalized approach, not the main "one size fits all" pharmaceutical development approach. As a result, more sophisticated approaches are required in cancer vaccines' effective development. Cancer vaccines mechanism of action is illustrated in **Fig.5** and is based on the delivery of Tumor-Specific Antigens (**TSA**s) or Tumor-Associated Antigens (**TAA**s) (the 2 categories of "shared antigens") [24] to APCs which, after antigen processing and presentation by MHC-I/II molecules, triggers an anti-tumor specific immune response resulting in antibody-mediated (humoral response) and T-lymphocyte-mediated action, with CD8+ T cell-mediated cytotoxic activity as the common and final step in aberrant cell elimination. Ideally, only cancer cells are targeted and with long duration of effect.[25] Vaccine-based immune-oncology besides triggering the general immune system specifically, is characterized by the ability to turn the so called "cold" tumors (resistant to therapy) into "hot" tumors, increasing their susceptibility to other therapies (synergy effect).[24]

Cancer vaccines can be divided in two types: preventive/prophylactic and therapeutic.[26] The preventive vaccines focus only on viral infection-caused cancers and are commonly used in vaccination plans (prophylaxis). On the other side, **therapeutic** cancer vaccines aim at treating tumors after diagnosis through the activation of specific CD8+ CTLs. This strategy is based on the interaction of MHC-I epitopes with tumor antigens, which optimally elicits both time-persisting innate and adaptive immune responses.[25] Sadly, only one therapeutic cancer vaccine is currently FDA-approved: Provenge® (sipuleucel-T) for prostate cancer (withdrawn from the EC-regulated market on MA holder decision).[27] The extremely low quantity of approvals of therapeutic cancer vaccines is disappointing since they are the most relevant vaccine type to battle cancer. Nevertheless, to date there are several therapeutic vaccine strategies in development for immuno-oncology. The highlighted ones are: Peptide-based vaccines; Tumor Cells-based vaccines; Dendritic Cells-based vaccines (Provenge® is DC-based); Microbial-based vaccines; Exosome-based vaccines; and Nucleic acid vaccines (DNA/**mRNA**).[15] All these have their advantages and challenges in terms of effectiveness, safety, manufacturing, storage, tunability, etc. but **mRNA-based cancer vaccines** should here be highlighted and the basis behind such statement will be addressed in the following sections (**2.** and **3.**).

- Neoantigen Personalized Vaccines

The development of a cancer vaccine must overcome additional challenges compared to infectious disease-directed vaccines, greatly based on aberrant cells and "normal" cells similarity. Although it represents the most employed antigen type, non-specific "shared antigens" (TAAs) application presents several challenges, such as the incomplete identification of TAAs in certain tumors (limited therapeutic indications), the patient-associated intrinsic variability in antigen expression (resistance), and the presence of some TAAs in "normal/healthy" cells. Therefore, the employment of these antigens might result in poor immune response and overall low vaccine effectiveness.[28] In carcinogenic events, the formed tumor cells retain most of the original and endogenous proteins and these are tolerated by the immune system and some of these undergo mutational events, resulting in new and highly-unique molecular epitopes.[29] The discovery of these exclusively tumor cell-expressed antigens, **neoantigens**, originated the concept of precision cancer vaccines targeting patients' tumor cells while sparing healthy cells from immune attack (reducing side effects probability).

Neoantigens can be divided in **TSA**s (Tumor-Specific Antigens) or **personalized neoantigens**.[30]; [31] This latter type allows for a patient-centered specificity (the highest theoretically) but requires tumor biopsy to identify and characterize the most adequate neoantigen, and then test it for immunogenicity profile in order to achieve optimized specific anti-tumor response. SAHIN et al.[31] observed specific T Cell responses with reduction of progression rates in a RNA-based poly-neoantigen suspension vaccine's clinical trial. Nowadays, the main challenge remains at optimizing the complex and burdensome manufacturing pipeline for these vaccines. Neo-epitope prediction and identification are achieved using next-generation sequencing data (bioinformatic tools) and, all together, the production under GMP takes several months and is costly.[29] The neoantigen approach is also limited by the existence of low mutational burden tumors, which hinder neo-epitopes identification. Overall, although currently lacking feasibility (highly-dependent on tumor biopsy and whole-exome sequencing,[28] the exquisite specificity inherent to this approach offers a level of targeting which is still out of reach of other therapies and, so, the focus on optimization strategies will enable the clinical use of neoantigens.

As the overall vaccine field (including mRNA vaccine platform) is highly driven by target (antigen) identification and therapeutic evaluation, some targets under study in clinical trials are here highlighted in view of the fact that some of these can support forthcoming available cancer vaccines:[26]

Table I - Most studied antigens in cancer vaccine research and development

Most relevant vaccine targets under evaluation in clinical trials					
5T4	Folate-related proteins	EGFR	Mesothelin	PAP/PSA	Telomerase
CEA	MAGE antigens	HER2	MUC-1	Neoantigens (personalized approach)	WT1
CMV-related antigens	P53	HPV-related antigens	NY-ESO-1	Ras	Survivin

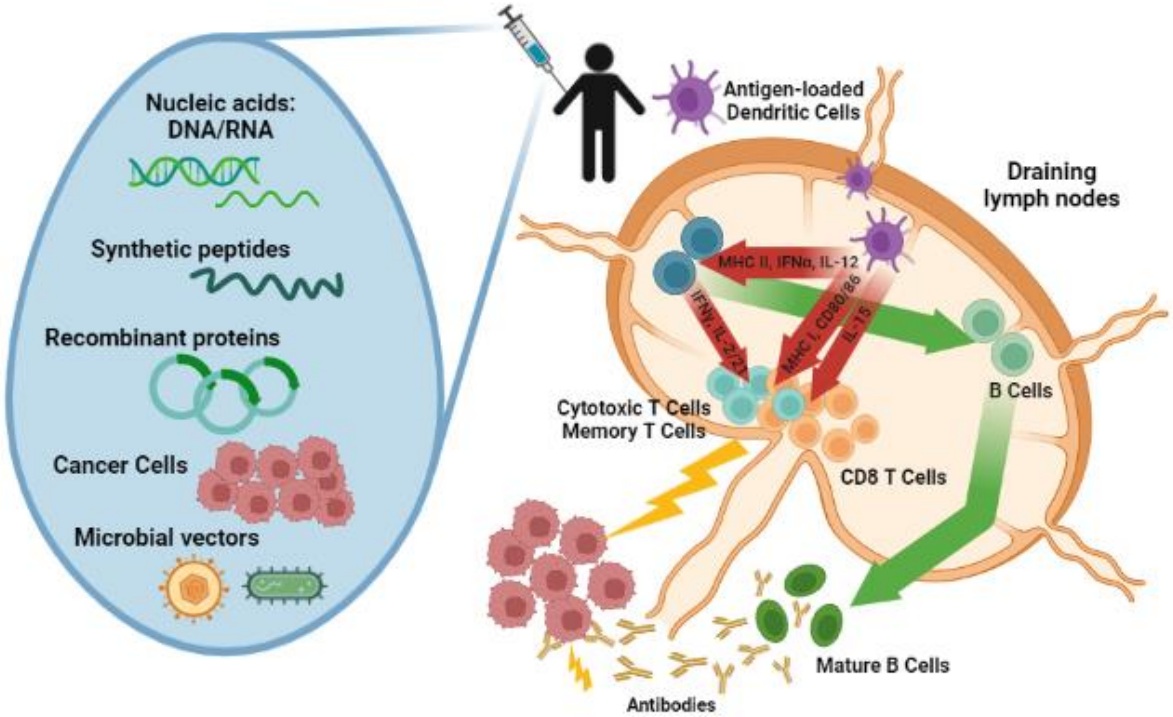

Fig.5 - **Cancer vaccine platforms and mechanism of action**. With continuous development and elucidation of tumor-related immune pathways, more optimized vaccine designs will emerge in immune-oncology monotherapy or in combination regimens, as IO stands now as the 4th pillar in [32] oncology (alongside with surgery, chemotherapy and radiotherapy). IFN - Interferon; IL - Interleukin; MHC - Major Histocompatibility Complex; TCR - T-cell Receptor. (Figure constructed using BioRender® software). Adapted from ref. [24]

2. mRNA-based Cancer Vaccines

Although people recognize mRNA therapeutics as a novel therapy due to its involvement in COVID-19 vaccines as active compound, this nucleic acid biotechnology dates back, in fact, to 1990.[32] Messenger RNA is a crucial intermediate on protein synthesis which is transcribed from a DNA sequence and then translated to correspondent amino acids sequences, by cellular enzymatic machinery, forming chain-specific proteins. The potential immunogenic profile of the targeted proteins (antigens) sustains synthetic mRNA's employment in (cancer) vaccine's field. **IVT** or **synthetic mRNA** structural elements are in fact similar to naturally transcribed eukaryotic ss-mRNA, as illustrated in **Fig. 6**, consisting of (5' to 3')[33]; [34]:

- **5' cap** – This cap serves as a translation initiator, critical for *in vivo* cytosol protein expression. This component accomplishes this by binding to the cytosolic Eukaryotic Translation Initiation Factor 4E (eIF4E).[35]
- **5' Untranslated Region** (UTR)
- **Coding Region/Open Reading Frame** (ORF) (including sequence of interest)
- **3' UTR**
- **3' Poly-adenosine tail** – The poly(A) tail consists on a multiple adenosine monophosphate chain (100 - 250 residues long) and protects the nucleic acid from nuclease action through poly-(A)-binding protein (PABP), improving the molecule's stability and efficacy.[36]

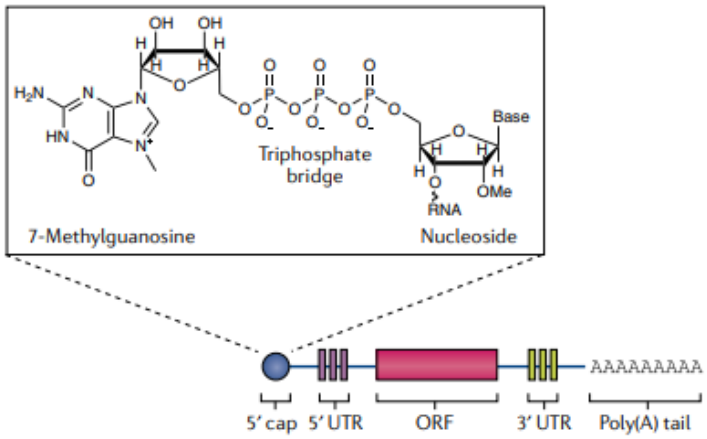

Fig.6 – IVT mRNA structural components. 5' cap is characterized by a guanosine methylation modification, as illustrated (5' to 3´ → left to right). Adapted from ref. [37]

Both flanking UTRs have an active part on translation process efficiency based on UTRs' sequences involvement in ribosome recognition, recruitment, and mRNA trafficking. Beside this, they are also responsible for regulating transcription and mRNA stability. [28]

Currently, mRNA can be divided in 2 main types: **non-replicating/conventional mRNA** (analogous to naturally occurring mRNA) with unmodified/modified nucleotide chain, and **self-amplifying/replicon RNA (saRNA)**; and has 3 major healthcare applications: prophylactic vaccines, therapeutic vaccines and therapeutic drugs, of which therapeutic vaccines are the most interesting in oncology (notably, Personalized Cancer Vaccines [PCV] which employ neoantigens).[28]; [38] Although the current marketed application of mRNA in oncology still stands only with prophylaxis, the therapeutic value holds the greatest promise. The most relevant therapeutic applications (currently) could be summarized in vaccination against infectious diseases, cancer immunotherapy, protein replacement, gene editing and regenerative medicine/cell engineering, however, as frequently observed in Pharma sector, the success in translating the clinical potential into market approvals is the most onerous challenge, requiring extensive optimization in mRNA-based products development.[39]

Although the conventional mRNA is a much simpler and straight-forward molecule, it requires higher doses than self-amplifying RNA (64-fold lower dose reported in a study)[40] and also reports a shorter half-life *in vivo*.[41] In fact, saRNA in this aspect has an edge over mRNA, due to one apparently small difference: the addition of viral replicase genes (nsP1,2,3,4) - viral replication machinery - to the ORF sequence enabling intracellular RNA amplification and abundant protein expression without viral-related safety concerns. This functional gene is usually derived from ss-RNA viruses, being alphaviruses the most common sources, such as Sindbis and Semliki-Forest viruses [28] (others: flaviviruses and picornaviruses).[42]

Nevertheless, a novel mRNA type recently appeared – a novel bipartite vector system – **trans-amplifying RNA (taRNA**), divided in an antigen-of-interest template and another consisting on the replicase system.[43] The template containing the vaccine antigen is derived from an alphaviral saRNA, but with the replicase deleted in order to form a transreplicon structure. The replicase-like system is controlled by another molecule - standard saRNA or an optimized non-replicating mRNA in a trans setting. This allows for an upgrade on the saRNA platform (safer and more cost-effective) although this technology still stands on its beginnings. [43]; [44]

The idea of *in-vitro* constructed/transcribed RNA had the premise of being able to **originate proteins (in-vivo) after administration**, following the same molecular mechanism, and even at an early-stage was confirmed protein production in mice.[32] Sadly, these results, although incredibly promising, weren´t followed by meaningful investment due to lack of *in vivo* efficacy, even after nucleotide chain optimization (modified mRNA).

"Pure" mRNA is, in fact, considered a double-edged sword as discerned in this section, characterized firstly by diverse clinical and pharmaceutical **benefits**: [45]; [46]

- **SAFETY** – No risk of infection or mutagenesis – opposing to virus-based vaccines' potential for viral infection and plasmid vector's risk of chromosome insertion. mRNA can also be regulated in order to control *in vivo* half-life and intrinsic immunogenicity (molecule's safety profile allows therefore for multiple administrations);
- **FLEXIBILITY** – as long as the targeted antigen's structure is known, it's theoretically possible to encode any protein and take up arms against an almost-infinite quota of diseases by way of immunotherapy (infectious diseases or **cancer**, e.g.) or protein-substitution therapy (Diabetes, e.g.). Moreover, by purposely altering a small portion of the encoded antigen, mRNA backbone characteristics aren´t necessarily affected, as reported;[47]
- **EFFICACY** – Easily-achievable stability and translation efficacy – through various molecular and formulation modifications, exploiting the use of adequate functional groups and carriers for its appropriate uptake and in-cell translation;
- **PRODUCTION** – mRNA-based vaccines have the most convenient potential for production requirements due to IVT high yields, permitting a fast, low-cost and a efficiently scalable manufacturing (theoretically). This enables even a low-resource company to manufacture elevated amounts of product in a profitable manner, which is desirable in cancer setting (large mRNA quantities required for cancer care).

These pillars were particularly vital for mRNA platform assignment during COVID-19 vaccines' development, leveraging the global distribution of safe, effective and much-needed vaccines in record time. However, this biotechnology platform doesn´t exist without its **challenges**, the other side of the mentioned "sword":

- Intrinsic molecular *in vivo* **instability** – In normal physiological conditions, mRNA is highly susceptible to degradation from extracellular RNases in blood and tissues (enzymatic hydrolysis) and undergoes extensive hepato-renal clearance, being normally excreted via kidneys only minutes after being administered. This reduces its half-life *in vivo* down to a few minutes and up to an hour [48]; [49] compromising an ideal half-life time (not permitting the molecule to reach the cellular target) and consequential efficacy, and therefore requires protection from such ubiquitous degradation

mechanisms. However, this can also be viewed as a benefit which allows increased safety due to low time of exposure, as referred previously.[50]

- Inherent difficulty in cellular uptake – Cellular uptake consists on different and simultaneous uptake mechanisms. Besides undergoing degradation easily, is also considerably difficult to mRNA molecules to diffuse trough the lipid bilayer cell membrane since naked RNA possess a considerable weight, large size and (dense) negative charge, making this process thermodynamically unfavorable. Particles smaller or equal to 200 nm are internalized via clathrin-mediated endocytosis (in approximately 30 minutes). In contrast, particles bigger than 200 nm but smaller than 1000 nm can take several hours to entry.[48]
- Adverse immunogenicity/toxicity – even though, as mentioned, mRNA has low potential for highly adverse immunogenic events, as an external compound exogenous mRNA can nevertheless produce detectable immune responses through the activation membrane-bound Tool-Like Receptors (TLRs)-dependent and -independent pathways. The most commonly triggered receptors are TLR7 and TLR8 located in intracellular vesicles, but also cytosolic Protein Kinase R (PKR) and Retinoic acid-inducible gene I (RIG-1) (which can be favorable or not, depending on idealized effect). [28]; [48]

Such challenges have met, in **delivery systems**, a solution. In fact, by improving delivery, mRNA could be protected from degradation proteins and cellular permeation problems would be solved. This premise highlights the development/optimization of appropriate delivery systems as one of the main focus of mRNA-based vaccines maturing, as both physical delivery methods and carrier systems have been and still are being developed.

Being both genetic-based vaccines and nucleic acids, mRNA and DNA have various common benefits/challenges, but RNA differentiates itself on cellular site of action with only having to cross the cellular membrane in order to originate protein (on the other side, DNA needs to get to the nucleus, requiring crossing another barrier – nuclear membrane), making vaccine delivery easier in this aspect. RNA is also much safer relatively to genome integration mutagenesis (no oncogenicity potential) and due to mRNA-encoded transitory protein expression (small $t_{1/2}$ - 2/3 days).[50] Also related to safety, since manufacturing occurs in an *in-vitro* cell-free environment, cell/viral impurities are avoided.[41]

- mRNA-based formulations' STORAGE and STABILITY **challenges**

In addition to the mentioned mRNA molecule intrinsic handicaps, mRNA vaccines formulation, as wonderful as they appear to be, they do have one major flaw based on the molecule's natural instability – the need for cold-chain storage. This requirement was also reported in COVID-19 mRNA vaccines (initially required storage conditions of -80 °C, then 4 °C for only one month).[51] In developing countries, this challenge is exacerbated since ultracold freezers' availability is low. So the challenge here would be to improve stability in order to reduce the need for such rigorous storage and distribution conditions.[52]

Moreover, although *in-vitro* assays showed continuously favorable results, the translation to *in-vivo* efficacy remains the **biggest challenge** to mRNA therapeutic use which is rooted on RNA intrinsic challenges (stability, delivery, etc.).[45]

2.1. Vaccine Feasibility & Real-World Usefulness

The laboratorial process of obtaining synthetic mRNA is **IVT**, using a linear template-DNA, which includes the protein of interest sequence, and a T7, T3 or Sp6 phage RNA-polymerase in optimal conditions. Obviously, behind the selection of the DNA template there is solid immunopeptidomics/proteomics research, [53] optimally resulting in a RNA sequence with ORF of interest, 5'- and 3´-flanking UTRs, 5'-cap and 3'-poly-adenosine tail as its structural constituents, imitating naturally transcribed eukaryotic mRNA molecule (fully processed).[45] The simplicity and low equipment burden stand out as the major contributing factors for the feasible mass production of mRNA vaccines, edging over conventional vaccines' production.

As a novel technology, it still lacks a well-established manufacturing method allowing for a variety of steps' combinations even more differentiated with scale-up procedures. Nonetheless, these vaccines production starts with mRNA manufacturing, which is divided in **Upstream** processing and **Downstream** processing. Upstream consists of obtaining the proper mRNA molecule through enzymatic reaction. Downstream, on the other hand, comprises mRNA purification procedures. After purified mRNA is obtained, formulation is the next production step, closing with fill-to-finish steps.[54] A crucial aspect never to forget consists on scale-dependent procedures. In **lab scale**, upstream processing is normally a one-step reaction (then digestion by nucleases and precipitation – purification) and with **large scale** a two-step reaction is usually required.

2.1.1. **mRNA Manufacturing**: From Upstream to Downstream

- Upstream mRNA Production[45]

mRNA production (similar to both nrRNA and saRNA) process is through *in vitro* transcription (IVT) which utilizes as starting materials: linear DNA template (previously synthesized); (T7, SP6 or T3) RNA-dependent RNA polymerase; nucleotide triphosphates substrates; polymerase cofactor $MgCl_2$; pH buffer (with polyamine); and antioxidants.[55]

This process is so relatively simple that it can be standardized for any antigen of interest. It only takes a few hours to complete (whereas for conventional vaccines it is rather time-consuming) and the process' yield is considerably high (mg of RNA per mL of reaction medium).[56] The mRNA capping process can call for another reaction step. If an cap analogue is used for capping, this can still be established in one step (lab-scale). On the other hand, if an alternative capping method using vaccinia capping enzyme and a methyl donor (substrate) is employed, a secondary enzymatic reaction is done (large scale).

The transposition of IVT to large industrial scale remains as the dominant challenge as the fulfilment of Good Manufacturing Practices in this area is significantly difficult due to scarce availability of large amounts of certified and appropriate materials (mostly biologics).[45]

- Downstream processing[45]

The next manufacturing step is downstream processing. The reaction mixture is filled with diverse impurities, such as NTPs, enzymes, aberrant mRNAs and others, so in this step the produced mRNA is isolated and then purified. The lab scale methods consist in digestion by DNAses before LiCl precipitation. Purification is crucial since reportedly allowed a 10 000-fold increase in protein product after a reverse phase HPLC purification.[57] In fact, chromatography is the most relevant purification method in pharmaceutical industry setting due to its favorable versatility, sample selectivity, scale-up ability and cost-effectiveness. The most commonly used types of chromatography for this purpose are Size Exclusion Chromatography (SEC), Ion Pair reverse-phase Chromatography (IPC), Ion Exchange Chromatography (IEC), Affinity Chromatography; and for removal of small size impurities: Tangential Flow Filtration (TFF) and Core bead chromatography (previous removal of DNA by DNase digestion/denaturing agents). In order to enhance even more the purification yield, hydrophobic interaction chromatography (HIC) can be putted into use.[41]

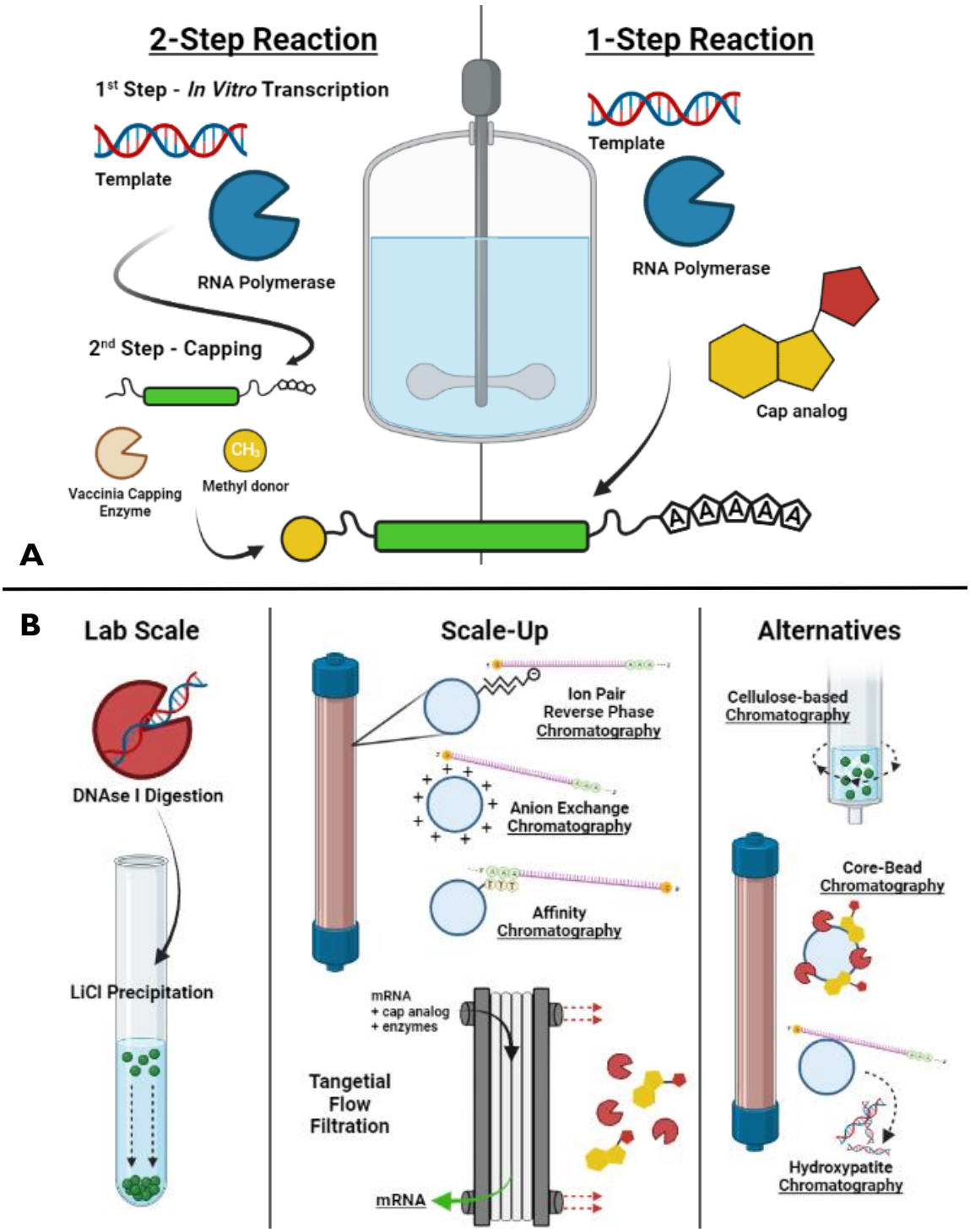

Fig. 7 (A + B) - Schematic representation of the production and purification steps of mRNA vaccine manufacturing process. mRNA production (**A**) can be performed in a one-step enzymatic reaction, where a capping analogue is used, or in a two-step reaction, where the capping is performed using vaccinia capping enzyme. mRNA purification process at lab scale consists of DNAse I digestion followed by LiCl precipitation. Purification (**B**) at a larger scale is obtained using well-established chromatographic strategies coupled with tangential flow filtration. Alternatively, new types of chromatography can be used to complement the standard purification. (Figure constructed using BioRender® software). Adapted from ref. [41]

2.2. mRNA's immunogenicity in Cancer: desirable or not?

Since cancer vaccines' primer objective is triggering an immune response, mRNA's inherent immunogenicity doesn´t seem like a limitation but an advantage, theoretically increasing the anti-tumoral immune response. This effect *in vivo* is shown to not be so linear, displaying both beneficial or detrimental results.[28] The innate immune system detects exogenous PAMPs (such as IVT mRNA) via PRRs, which are widely expressed in APCs (the target cell for vaccination). IVT mRNA (single- or double-stranded) can be recognized by both membrane, endosomal and cytosolic PRRs, being the most relevant TLR-7/8, RIG-I, OAS and PKR, resulting in type I interferon pathway-related immune response. [28]; [49]; [58] This production of type I INF can enhance beneficially APC activation/maturation, antigen presentation and overall robust immune response. This increase can however become excessive and cause immune-related adverse events. On the other hand, the mentioned intracellular pathways can also inhibit antigen translation and expression, resulting in weaker response. Although these paradoxical effects of type I INF signaling are still in debate nowadays, it is clear that mRNA-based vaccine purity, sequence optimization (all regions), administration route and delivery system have to undergo tuning in order to tilt this "Janus" effect to the beneficial side and also improve translation efficiency, which can only be truly confirmed in clinical trials.[28]

3. *"Shaking off the dust on mRNA vaccines"* – Importance of Systematic Research

As referred previously, mRNA, for the surprise of many, isn´t actually a novel technologic platform. 1990 was the year where the hypothesis of using this nucleic acid molecule in vaccines emerged and, given the protein expression in mice skeletal muscles witnessed in this study, this mRNA employment was considered plausible.[32] But, ironically, from a chaotic pandemic environment, mRNA rose from oblivion as a miracle backed by its benefits which overstep those of conventional or DNA vaccines. mRNA stood out by its antigen production specificity, precise immune response (humoral, cellular, and innate) and ease of production. [59]

The present research (and analysis) paper can be useful in the context of this emerging technology by pinpointing mRNA-base cancer vaccines' relevant aspects (both positive and negative) as a promising tool for cancer care. This work can also support a vaster spectrum of delivery system since LNPs took the spotlight, considerably undervaluing many other systems with potential to leverage equal or even better clinical outcomes.

3.1. Methodology

Supporting the construction of the present research and critical analysis work, several papers and clinical trials were consulted and analyzed. The referred literature research took place between the dates of 20th December 2021 and 12th June 2022 with the objective of collecting information on mRNA technology associated with cancer vaccines and strategies/systems to delivery such mRNA *in vivo*. Open-access journals were the preferred databases (Frontiers, PubMed, Elvesier, etc.) although the accession of some paper was only enabled via institutional access. The research was firstly focused on a few non-specific articles in order to attain an adequate level of knowledge to interpret future research in the best way possible. The literature topics' information was gathered in the following order: cancer pathology, current burden and unmet therapeutic needs, immuno-oncology concepts, mRNA technology (principles of action, advantages and disadvantages), mRNA in therapeutic cancer vaccines, delivery systems employed or potentially employed in cancer vaccines. Fortunately, the literature retrieval was facilitated due to the increasingly broader access to papers enabled by COVID-19-related rise on information demand (especially concerning mRNA vaccine technology).

4. Delivery Strategies for mRNA-based cancer vaccines

As expounded in the "challenges" section, in order to allow adequate mRNA cellular uptake several delivery barriers must be crossed trough different strategies. Indeed, adequate delivery of mRNA is nowadays one of the most challenging steps in mRNA vaccine development. These delivery strategies can be interpreted as a truck (carrier) which transports raw materials (mRNA) to a factory (cell) conducive to the product manufacturing (immunogenic protein). Although some possess more usefulness and clinical feasibility than others (see **Appendix I**), the current delivery systems include mainly: Lipid-based Delivery; Polymer-based Delivery; Peptide-based Delivery; Virus-Like Replicon Particle; Cationic Nanoemulsion; Dendritic Cell incorporation [60], which can be grouped in 3 main strategies: a) **Naked mRNA direct injection**, b) **Dendritic cells transfection**, c) **Formulated mRNA**; all with their inherent advantages and limitations illustrating the need for delivery optimization to enhance delivery efficiency, cell targeting and pharmacotechnical vaccines aspects (such as administration route). Although apparently with inferior relevance, delivery strategies also extend to administration approaches, of which the highlighted are physical delivery strategies such as electroporation and gene gun. [41]

4.1. Naked mRNA

This administration strategy was in fact the basis of the proof-of-concept trial in 1990 by Wolff and colleagues.[32] By injecting it directly (without any carrier) into the subject, target-site immune response are commonly observed. Physical delivery methods can thus be employed, such as electroporation (which consists of applying electric pulses locally on a cell membrane resulting in pore formation), gene gun (using a pneumatic pump into the targeted cell) and microinjection (via microneedles).[61] Some advantages are inherent to this approach such as preparation feasibility, relatively simple formulation process, favorable storage and cost-effectiveness ratio. These "pure" mRNA formulations, through lyophilization, are reportedly stable for 10 months on 4°C conditions (in adequate buffer – trehalose 10%) and only require solvent reconstitution and buffer dilution, such as the well-known Ringers Solution.[60]; [62] However, the unprotected molecule won´t allow for a consistent response due to target tissue-associated extracellular exonucleases (RNAses), resulting in unpredictable pharmacodynamic effects and irregular pharmacokinetic profile. In fact, after naked mRNA intravenous administration the reported half-life for non-formulated mRNA is estimated to be less than 5 minutes.[61] Also, relating to mRNA intrinsic inability to cross the lipid bilayer, its cellular uptake should be impracticable, but it does trigger some immune response resulting in the "How?" question. The theorized mechanism-related hypothesis consists of macrophage and dendritic cells-reported macropinocytosis, justifying the poor but detectable pharmacodynamic profile.[63] This hypothesis supports the efficacy observed after naked-mRNA subcutaneous, intramuscular and intranodal vaccinations, due to APCs availability in locus and low mRNA amount needed.[61]

4.2. *Ex Vivo* Loading of Dendritic Cells and Transfection

Dendritic cells, the most efficient professional Antigen-Presenting Cells (APCs), have demonstrated their therapeutic potential for cancer immunotherapy for some years now through its unmatched immune response stimulation capacity and ubiquity. Dendritic cells have the highly effective capacity to process and present antigens (formed after mRNA translation) to CD8+/CD4+ T-cells using MHC I/II molecules, triggering an antigen-specific response. In this immune-oncology approach, DCs are extracted from the individual and, in a laboratorial setting, naked mRNA is loaded into those cells (*ex vivo* method). This transfection process is predominantly accomplished based on mild electroporation method, but also using lipidic carriers can be employed. After this, DCs undergo phenotype characterization (although cumbersome), followed by the re-administration of these now transfected/activated APCs into

the autologous organism, resulting in cell-mediated I antigen-specific anti-tumoral response.[63] In **Appendix I**, a table of current clinical trials using mRNA-based cancer vaccines was constructed. By analyzing the formulation types of the tabled vaccines, a trend is clear: **DC-based vaccine** is the most widely employed mRNA vaccines type in oncology trials. This clearly states the major contribution these formulations can have in the future.

4.3. Formulated mRNA

Through the formulation of mRNA with a delivery system/carrier, the objective consists of ribonucleic acid molecule's protection, favorable intracellular transport, specific cell targeting and the prevention of early and unwanted mRNA immunogenic effects.[61]

4.3.1. Viral-based Systems

The earliest employed nucleic acid's delivery systems were viral-based, using recombinant viruses (retrovirus, lentivirus, adenovirus, etc.). Although they allow for a good transfection ability, they are limited in terms of favorable safety profile (much based on its inherent immunogenicity) despite being genetically modified to nullify undesirable replication.[61] These vectors have a major role in DNA delivery, but in mRNA its usefulness is considered insignificant. In fact, when focusing on mRNA molecules delivery strategies, non-viral systems have the spotlight all to themselves, supported by a distinguishably favorable safety profile, simple, cost-effective and highly reproductible manufacture processes (opposing to the intrinsic variability in viral-based products).

4.3.2. Chemical-based Systems

Currently, these systems possess the most promising value and represent the vanguard of mRNA delivery research. Such systems overcome some of the viral-based limitations and are composed of synthetic, semi-synthetic or natural substances which stand out based on its biocompatibility and tunability characteristics. The evolution and continuous development of this delivery platform, as the result of advances of multiple areas like nanotechnology and nucleic acid chemistry, allowed for the establishment of several established methods. The most relevant nowadays are:

A) Lipid-based systems

Characterized by increased RNA stability, greatest biocompatibility profile and biodegradability, the lipid-based systems can be divided in Liposomes, Lipoplexes, Lipopoliplexes and, the currently most studied delivery system, Lipid Nanoparticles.[49]

Liposomes are composed of phospholipids grouped in a spherical structure which can be either unilamellar or multilamellar. These phospholipids possess a polar hydrophilic head and a non-polar hydrophobic tail, resulting in the formation of a thermodynamically stable vesicle with an aqueous core. As the (cationic) lipids are amphiphilic, they have the ability to electrostatically encapsulate the negatively-charged mRNA with its positively-charged amine group head during the vesicle assembly. Some of the most widely used lipids are DOTMA, DOTAP, DOPE, etc. and their function as carrier-forming lipids is supported by their biodegradability, encapsulation efficiency, safe profile, easy formulation and overall efficacy (mRNA delivery efficiency + adjuvant-like activity).[49]; [64] Liposome-based formulations in cancer immunotherapy are indeed very promising as a lot of trials reported favorable clinical benefits, tumor regression and survival rates after mRNA vaccines leveraging this delivery technology, even as early as 1999 with ZHOU et al. preclinical trial with mRNA-codified melanoma antigen glycoprotein 100.[65]

Lipoplexes, which nomenclature originated from "lipid and nucleic acid complexes", are formed after cationic liposomes interact electrostatically with mRNA, resulting in liposome's structural rearrangements. The overall structure changes into a more compact liposome.[49]; [64]

Lipid Nanoparticles (LNPs), as a delivery strategy, are even now the most promising tool for mRNA therapy optimization, also constitutes all of the (currently approved) COVID-19 mRNA vaccines formulation.[41] Due to their potential for reducing unfavorable immunogenicity/toxicity, LNPs constitute the most clinically advanced delivery systems for nucleic acids owing to several unique benefits, such as: formulation ease, biocompatibility, sizeable payload capacity, modulatory components and decades of extensive research background. As lipid-based particles, LNPs are usually divided in **4 constituting lipids**, as followingly disclosed.[28]; [37]; [49]; [61]; [66]

i. <u>Ionizable lipids (ILs)</u>

Initially, Cationic Lipids like DOTMA/DOTAP or Lipofectamine were used due to its positive charge which facilitated mRNA (negatively charged) encapsulation, via electrostatic interaction. But, faced with cationic lipid-associated toxic responses, the development of Ionizable Lipids overcame these safety limitations and buried cationic lipids as the main mRNA delivery and LNPs most important components. The major advantage in ionizable lipid utilization rests on its pKa-dependent activity and interaction with physiological environment pH levels. ILs in acidic conditions (pH lower than pKa) possess positive charge so, in

formulation procedure in acidic conditions, interacts with the negatively-charged mRNA capturing it. After administration, systemically ILs have a neutral charge (low interaction potential), although after suffering cellular uptake, they become in the endosomes (acidic environment) promoting the lipid fusion with the endosomal membrane, therefore allowing for the mRNA release in cytosol. This pH-dependent mechanism stands as majorly favorable delivery feature, but due to the rational lipid design approach's slow pace it was only after optimization of the first ionizable lipids (DODAP and especially DODMA) which eventually resulted in DLin-MC3-DMA creation and its application on the first approved LNP-containing formulation: ONPATTRO® (siRNA) that ILs finally made their stand. Nowadays, in order to optimize this lipid design rationale, academia and industry are increasingly using Combinatorial Reaction strategies, aiming at new entities discovery, lipid libraries and structure–activity relationship datasets construction: [37]

- C12- 200
- $503O_{13}$
- $306O_{i10}$
- OF-02
- TT3
- 5A2-SC8
- SM-102 (used in the COVID-19 Moderna's vaccine)
- ALC-0315 (used in the COVID-19 Pfizer/BioNTech's vaccine)

Note: Although the commercially approved are the lipids in LNPs (COVID-19) vaccines, lipids used in mRNA vaccines for oncological purposes would be those with targeting characteristics not so much the referred non-targeted "systemic" ones.

Combinatorial Reaction strategy also allowed for the definition of IL's Critical Quality Attributes (**CQAs)**: pKa, surface charge (at pH = 5) and hemolytic activity (at pH = 5,5).[37]

In Immuno-oncology/vaccine field, LNP development should be primarily focused on improving delivery specificity to appropriate target immune cells. There are some functional group-modified lipids which can target T cells *in vivo* and stimuli-responsive formulations which have the auspiciousness for anti-tumoral immune response effective trigger, some of which I highlight here: 11-A-M; 93-O17S; A18-Iso5-2DC18.[37] Nevertheless, clinical studies are required to confirm these claims since many other effective *in vitro*/*in vivo* therapies fail in human setting.[37]

ii. Cholesterol

Being a versatile molecule, cholesterol aids membrane stabilization of LNPs (filling gaps) and reportedly helps in their fusion with the endosomal membrane after cellular uptake.[37]

iii. Helper phospholipids

As the name suggests, these lipids have a major auxiliary role in the formation of the nanoparticle structure. Optimally, they modulate the nanostructure's membrane fluidity, promote transitions in lipids that facilitate endosome membrane fusion and can modulate specificity of target organ delivery. Several factors must be considered on helper lipid optimal choice, such as cargo RNA size and selected ionizable lipid; Examples: DSPC (used in referred COVID-19 mRNA vaccines [both] and ONPATTRO®); unsaturated lipids - A6 and 4A3-Ci; zwitterionic lipids - 9A1P9 (enhanced endosomal escape).[37]

iv. PEGylated lipid

This lipid is composed by two chemical entities: anchoring lipid (DMPE, DMG, e.g.) + polyethylene glycol (PEG); This compounded lipid function rests on PEG hydrophilic profile and allows for: LNP stabilization (like cholesterol), particle size regulation and increase of LNP half-life. This component is also highly flexible since it permits to regulate efficacy, systemic circulation time and cellular uptake through PEG chosen molecular weight - 350 to 3,000 Da and anchoring lipid tail length - 10 to 18 carbons (usually there is direct proportion between MW/length and circulation times; and the inverse for cellular uptake).[37]

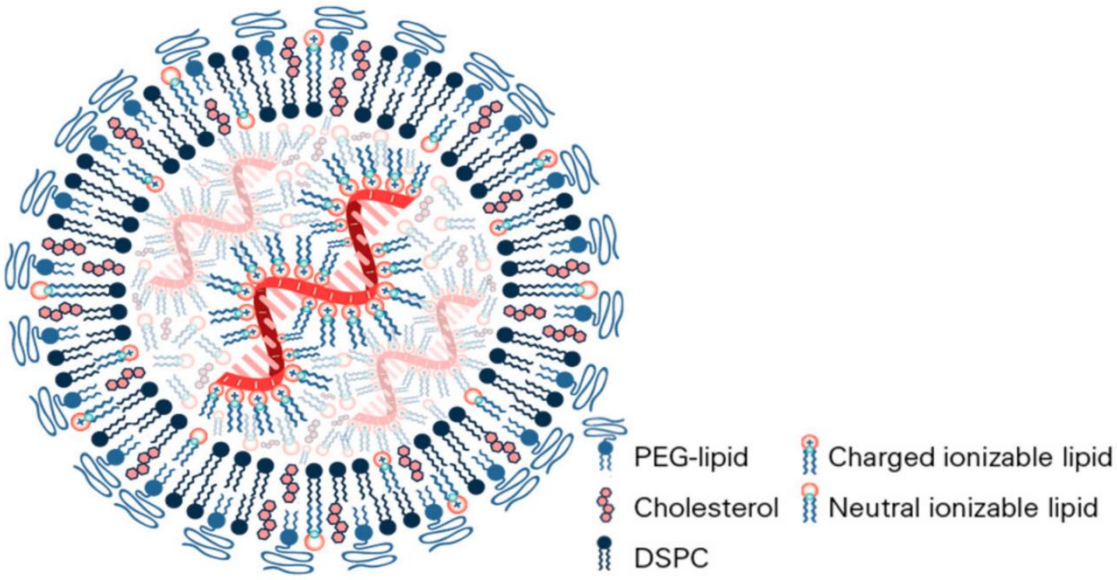

Fig.8 – mRNA-loaded lipid nanoparticle structural components. Normally, the numbers of mRNA molecules complexed by ionizable lipids vary from 1 to 10, occupying the particle's core. Adapted from ref. [67]

➢ **LNPs Formation Methods** [41]; [67]

The early/conventional LNP manufacturing methods consisted in Thin Film Hydration Method (+ Extrusion) and Ethanol-injection Method. These methods were limited in terms of scalability, particle size heterogenicity and poor encapsulation efficiency. Aiming to overcome these challenges, Rapid Mixing-based methods were introduced and are currently the methods used in GMP-compliant LNP production (also utilized in COVID-19 mRNA vaccines production). These novel methods are **Microfluidic Mixing** and **T-Junction Mixing** methods and both consist of mixing an (hydrophobic) lipid-containing ethanol phase with an mRNA-containing aqueous phase (containing a pH4 buffer), supported by hydrophobic and electrostatic interactions. Although T-mixing/T-junction mixing is the generally chosen method for industrial large-scale batches, both these processes allow for particles with similar nanosize (<100 nm, as intended) and morphologies. The interplay between environmental pH and lipid pKa is the major LNP's assembly driver, although the 3:1 water/ethanol ratio in the mixed solution, essential for ionizable lipid protonation (triggering mRNA complexation), and PEG hydrophilic effect (defining the final thermodynamic stability) are also crucial for the particle assembly. The assembly interactions and stepwise reaction scheme are represented in **Fig.9**:

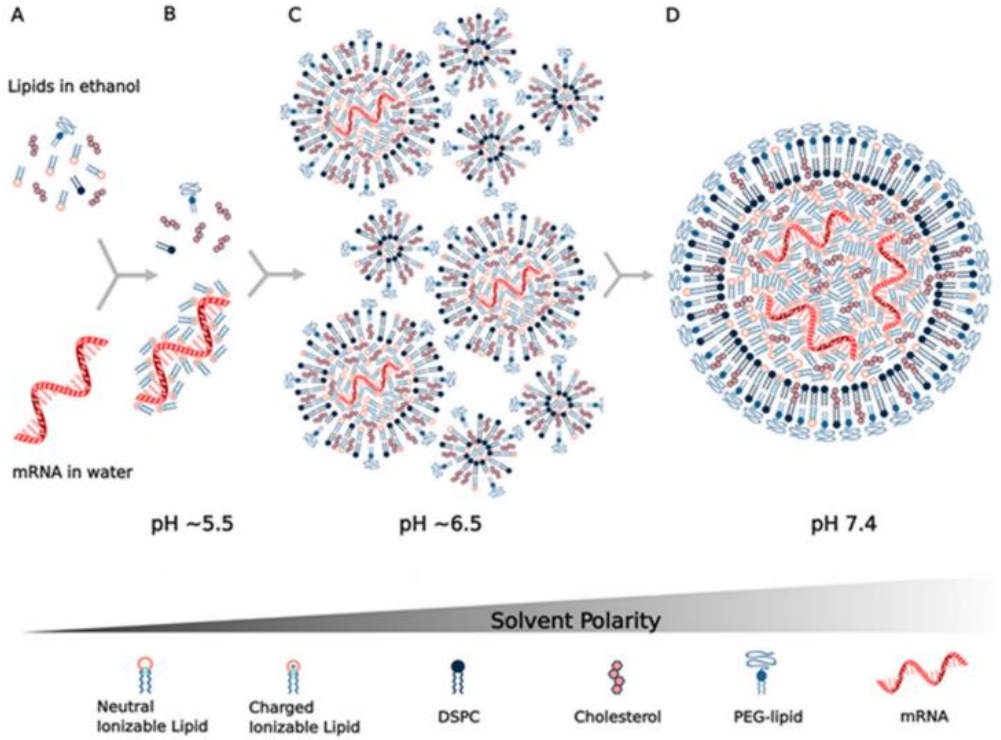

Fig.9 – **LNP-based mRNA formulation production steps**. Currently, the lipid nanoparticle construct loading mRNA is based on 2 GMP-complying methods: microfluidic and T-junction rapid mixing. **A)** As raw materials, 4 component lipids (ionizable, helper, PEGylated and cholesterol) in ethanol mix and mRNA in aqueous buffer solution (pH≈4) are used and mixed. **B)** After mixing, at pH≈5,5, the ionizable lipid becomes protonated (positive-charged) based on its pKa. **C)** Subsequently,

the ionizable lipid binds to mRNA (negative charge due to the anionic phosphate groups in nucleic acids' backbone) via electrostatic interaction and then encapsulates the therapeutic molecule via hydrophobicity phenomenon. **D)** After this, pH is gradually increased (using dialysis, dilution or filtration) resulting in ionizable lipid neutralization and its hydrophobicity increase, ensuing a nano-sized thermodynamically stable and bilayered particle. Other lipids (helper and cholesterol) are incorporated during the process due their also hydrophobic profile at adequate pH level, while PEG-lipid-associated hydrophilicity allows it to incorporate itself externally on the particle. Adapted from ref. [67]

Moreover, by adopting a Quality by Design approach, the manufacturing of LNP-mRNA formulations must stand accordingly to the following **CQAs**: Particle size distribution (PSD) and polydispersity index of LNPs; Encapsulation efficiency; Potency; Structure; Zeta-potential; pH; Osmolality; Lipid content; LNP-mRNA transfection. [67]

B) Polymer-based delivery systems: Polyplexes and polymeric nanoparticles

Other widely used delivery structures are polymer-based structures, especially leveraged on its favorable biocompatibility and biodegradability profile.[49] The polymers employed for this setting are usually cationic, even though neutral/anionic polymers are also being studied as delivery systems. Cationic polymers can complex nucleic acids via electrostatic interactions (similar to the previously referred ionizable lipids) and "encapsulate" them, forming polyplexes or polymeric nanoparticles, and freeing these molecules through the theoretical mechanism "Proton Sponge Hypothesis" where in which the polymer (buffering ability) causes increase in osmotic pressure and causes endosome escape. The employed monomers are divided in natural polymers (chitosan, hyaluronic acid, e.g.) and synthetic polymers (PEI, PLGA, PLL, e.g.).

Natural polymers such as chitosan and hyaluronic acid leverage their activity mainly on their low immunogenicity and biocompatibility. **Chitosan**, a cationic and linear polysaccharide copolymer (D-glucosamine + N-acetyl-D-glucosamine), complexes mRNA with high affinity via electrostatic interaction, furthermore the efficiency of this process can be modulated by chitosan's molecular weight selection and deacetylation and amine/phosphate ratios.[49] **Hyaluronic acid**, on the other hand, interacts with RNA molecules via non-covalent bonds (hydrogen bonds and Van der Walls forces) since it is an anionic polymer. This polysaccharide's utilization is emphasized in cancer vaccines due to its favorable affinity for CD44 receptors (overexpressed in tumor cells, in some cancers).[49]

The polymer-associated charge density can be a double-sided sword due to favorable mRNA complexation capacity and potentially unfavorable toxicity and systemic aggregation, just like observed in polyethylenimine (**PEI**). PEI polymer, although the most studied cationic polymer, fails to fulfil its expectations due to the mentioned toxicity limitations. As PEI-based nanoparticles' activity is highly dependent on molecular weight (the highest the weight, the highest the transfection efficiency, and the highest the toxicity events), as well on amine/phosphate ratio [49], some optimizing solutions would be: use of the low molecular weight form, PEGylation, cyclodextrin conjugate, disulfide bonds, addition of other polymers and core-shell formation with liposomes.

PLGA, poly(lactic-co-glycolic acid) copolymer, and **PLL**, poly-L-lysine, are other polymers studied in mRNA delivery, but haven´t presented promising results so far.[49]

This technology can also make use of functional polymers such as pH-responsive (**PR** - similar mechanism to ionizable lipids in LNPs), pH-responsive charge-altering releasable transporters, differing from the first on its mRNA release mechanism – safe self-degradation at cytosolic pH, and biodegradable polymers (**BD** - less toxic): [37]; [41]

- Poly(aspartamide)s [PR] (with ionizable aminoethylene side chain)
- Poly(β-amino ester)s [BD]
- Poly(amidoamine)s [BD] → they form hyperbranched spherical dendrimers which allow for an effective mRNA complexation (due to high peripheric amine density)

These polymers have the advantage of being highly versatile and modulable through side chains/length modifications and ratio changes, and easily synthesized by Michael Reaction, resulting in optimal mRNA encapsulation, stability, biocompatibility and organ-selective delivery (if desirable).

Dendrimers, as polymeric molecules, consist of a polymer-based core with a large presence of branching units and terminal groups. [61] These macromolecules which possess desirable hydrophilic and biocompatibility/safety profiles have shown their promising value as mRNA delivery systems. In fact, the tunability associated with these molecules, resulted in modified dendrimers with homogenous particle-size distribution of which, polyamidoamine (PAMAM)-derived dendrimers are highlighted due to their favorable results in pre-clinical studies using dendrimer-based nanoparticles for vaccination purposes.[68]

C) Peptide-based delivery systems [37]; [63]

Although less relevant, these systems delivery ability is mostly supported by peptide cationic or amphipathic amine groups. These functional amine groups (positive charge) bind to mRNA electrostatically and able allow for its nano-complexation. Arginine-rich **protamine** peptides are the most relevant tools in this setting, but the attention given to RALA structural peptide motifs, which are rich in arginine, alanine and leucine, is also well-justified. This polypeptide component allows DCs targeted delivery (very favorable in vaccination) for conformation modification according to different pH levels (such as in endosomes) and consequentially mRNA breaking free off the endosome.

Nevertheless, protamine-mRNA easily achievable complexes (positively charged at neutral pH) also demonstrated a promising adjuvant-like action: TLR7/8 activation. This capacity is the base of RNActive® vaccination platform. Focusing on novel cell-penetrating peptides, here I also highlight PepFect14 due to its promising ovarian cancer cells' targeting activity.[69]

D) Cationic Nano-emulsion (CNE)

Belonging also to first mRNA delivery systems, are systems based on Oil/Water (O/W) emulsion at nanoscale. Similarly to previously mentioned compounds, cationic profile is key in mRNA encapsulation and delivery (electrostatic interaction). That said,1,2-dioleoyl-3-trimethylammonium propane (DOTAP) (cationic lipid) is here used as component in the emulsion's oil phase to complex the negatively-charged mRNA, allowing for the genesis of CNEs (90-130nm Ø). Currently, there are some promising CNE-based formulations, such as LION®, a self-amplifying RNA formulation constituted by an inorganic-based DOTAP/Squalene emulsion (favorable stability). Also, as a functional CNE component, Superparamagnetic Iron Oxide nanoparticles (SPIO – 15 nm), located into the hydrophobic phase, offer adjuvant-like activity. Where this formulation really stands out is relatively to its stability profile, especially compared to the approved mRNA vaccines. It has been shown favorable colloidal stability for (at least) 3 months in 4° - 25 °C temperature conditions. This attribute holds a major edge over the approved vaccines which require cold-chain storage and distribution (particularly in low-income countries where these requirements are practically unfeasible), but this enhanced stability role still needs to undergo further elucidation.[63]

Squalene-based nano-emulsions are here highlighted: these, yet again, cationic formulations have a distinct manner of mRNA capturing – surface adsorption. Composed by a squalene core and a stabilizing lipid carapace which adsorbs the mRNA but the endosomal release

mechanism remains uncertain. Also, similar to RNActive® platform, some squalene-based formulations have an adjuvant-like ability, causing cytokines secretion at injection site and increasing antigen uptake by mobilized APCs.[37]

E) Inorganic Nanoparticles

Mainly as novel alternative delivery systems, inorganic-based systems present favorable stability, tunability and biocompatibility supporting its promising value as mRNA nanocarriers, overcoming some challenges in cationic polymers' use such as poor transfection efficiency, adverse events, and systemic inactivation. The currently most relevant are gold, mesoporous silica, calcium phosphate and layered double hydroxide nanoparticles.[49]

- Gold Nanoparticles (AuNPs)

Gold-based nanoparticles already exist in the pharmaceutical market for some time mainly in Lateral Flow Immunoassays diagnostic devices (such as in SARS-CoV-2 antigen detection kit tests). Its therapeutic use is here described as a vaccination platform for nucleic acid delivery, leveraged on its favorable tunability/controllability relatively to nanoparticle's surface and size. As the surface of these particles is highly-optimizable, it allows for the addition of functional and targeting groups/moieties modulating the particle's toxicity, pharmacokinetics, efficacy and overall stability. As an example of these modifications and its impact, the nanoparticles' coating with polymers such as PEI, PLL and amine-alkyl thiols has shown to increase transfection efficiency and overall vaccine efficacy.[49]; [61] In terms of manufacturing, this delivery platform has a favorable scalability with low heterogeneity profile. These attributes have set up AuNPs' use in pre-clinical trials for cancer vaccination.

- Mesoporous Silica Nanoparticles (MSNPs)

MSNPs consist on nanoscale silica particles which possess a honeycomb-like structure with hollow channels, useful in mRNA loading and controlled release, which became desirable (inorganic) delivery systems promoted by silica's intrinsic (chemical and thermal) stability, extended drug-encapsulation/loading ability and favorable modularity/tunability.[70] The optimization processes permitted by surface functionalization can be both of non-covalent and covalent nature [70], such as the polymeric coating with PEI and/or PAMAM which increases mRNA's binding affinity.[49]

- Calcium Phosphate (CaP) Nanoparticles

Calcium phosphate - $Ca_3(PO_4)^2$ - is an endogenous compound found in some animal's teeth and bones, so its biocompatibility and safety are naturally favorable. Electrostatic interaction between nano-CaP particles and (negatively charged) mRNA results in the nucleic acid's complexation with high encapsulation efficiency.[49] Once again, surface functionalization enhances nanoparticles' stability and delivery efficiency, especially with cationic polymers' addition (PEI, e.g.).

- Layered Double Hydroxide Nanoparticles (LDHs)

Consisting of a sandwich-like structure, LDHs are two-dimensional nanomaterials composed of intercalating positively charged layers and anionic interlayers, offering great biocompatibility profile, feasibility and degradability in endosomes' acidic conditions (potential for favorable endosomal escape). mRNA's loading capacity is based on the anion exchange between interlayers and mRNA, backing the recent claims of LDHs as potential RNA delivery carriers.[49]

Note: The **saRNA** formulations require a special attention relatively to the employed delivery strategies. Comparable with nr-mRNA, nonviral systems such as cationic lipid-based, polymer-based systems, as well as electroporation, may be utilized in saRNA vaccines but due to the addition of nsP1-4 replicase gene sequence these nucleic acids are much longer than the corresponding "conventional" mRNA. This can negatively impact the delivery system's effectiveness and therefore must be optimized.[43]

4.4. Routes of mRNA vaccine administration

When discussing vaccine delivery and although carrier methods possess the spotlight since pharmaceutical technology enhancements are majorly related to them, how the vaccine is administered has a marked influence on the vaccine's efficacy, safety and even in the choice of the most adequate delivery strategy. With current knowledge and clinical trials information, the most prominent routes of injection for cancer vaccines are still the local ones – **intra-muscular** (i.m.), **subcutaneous** (s.c.) and **intradermal** (i.d.). Just like with the mRNA-based COVID-19 vaccines (approved at 27/02/2022), the **intramuscular** route is the most frequently chosen in clinical trials. This choice is based on technological aspects such as flexible injection volume, easy dosing and a (more or less) favorable safety profile [71], and not so much

on efficacy profile. Recent studies have hinted for the vaccination route impact on antigen presentation/trafficking and overall innate and adaptive immune responses. It is evidenced that the immunogenicity varies significatively due to variation in magnitude and duration of the mRNA-derived protein expression. Nevertheless, the overall immune response seems to be comparable between i.m and s.c. routes. In cancer vaccines, as the wanted outcome is the elimination of cancer cells and CD8+ cell activation, systemic injection (intravenous) is reported to have a more desirable impact on T Cell activation levels. All together i.m. and s.c. are still the most relevant routes of mRNA cancer vaccine administration, as the systemic route is more susceptible to adverse events (delivery methods have the potential to avoid this by optimizing the targeting aspect), and other potentially appealing routes such as intranasal and intranodal (high APC titre *in locus*) are also being investigated.[28]

5. "How to select the optimal delivery strategy?" – The Key question

In the previous section, several mRNA delivery strategies were showcased and all of them had indeed the potential for nucleic acid delivery (at least *in vitro*), remaining the most crucial question: "Which one do we choose?". The most adequate choice doesn´t follow exactly a linear approach since the most adequate *in vitro* could not correspond to the most adequate *in vivo* (real word setting). In order to select a specific delivery system/component, it's the reported performance which ultimately has the biggest impact on the selected system and can only be assessed through well-designed pre-clinical and clinical studies.[67] In order to study performance, three main multifactorial and interrelated determinants should be assessed: 1) **potency** (efficiency of appropriate delivery and intracellular release); 2) **adjuvant-like ability** (supporting and boosting the immune response); and **3) safety** (absence or reduction of adverse events caused by exaggerated efficacy and/or off-target action). And within the referred main determinants, several performance-indicating factors should be individually examined (the numbers indicate the according main determinant[s] involved): [67]

- Dose - **1**,**2**,**3**

Following the pharmacodynamics/pharmacokinetics basic principles, it's clear the relationship between effective and toxic systemic minimal concentrations which create the concept of therapeutic window, guiding the therapeutic dose range. Aiming at the determination of the required dose, 2 indicators are employed – neutralizing antibodies titres/T cell responses (compared to convalescent plasma) and magnitude/frequency of adverse events. Delivery systems' potency have here a major influence on determining

adequate mRNA doses. Without delivery system, doses vary from 1 to 100 μg [67] (saRNA < unmodified RNA < modified RNA) but, using LNP, a 0,1μg saRNA dose was possible.[72] Truly, the dawn of mRNA-loaded LNPs allowed for a 10-fold decrease (approximately) in dose compared to earlier delivery systems. The dose reduction could allow for potency maintenance while reducing adverse events, lowering the raw material's quantity required for manufacturing and ultimately lowering the overall vaccine unitary cost.

- Potency and Delivery Efficiency -**I**

Focusing on LNPs, the most frequently reported features which determine the delivery system's potency/efficiency is **pKa** (most relevant), **IL morphology** and **PEGylated lipid concentration/size**. pKa, measured using TNS dye-binding assay, is crucial since its relationship with pH determines the lipid charge and allows the bilayered structure disruption and cytosolic mRNA release, as previously mentioned. The optimum range for pKa must be elucidated according to wanted delivery mechanism. With Onpattro®, for example, was determined an optimal pKa of 6,4 for MC3-based LNP allowing an efficient hepatic delivery. A coned shape derived from branching of ILs is presumed to cause greater endosome disruption/release, and therefore greater delivery efficiency (molecular shape hypothesis). PEG lipid, by influencing LNP's size and structure, is also a variable in the nanoparticle potency: a higher PEG lipid concentration corresponds to smaller LNPS, leading to faster PEG shedding and facilitated endosomal release. This hints for the need to find an equilibrium in this shedding rate (formulating PEG concentrations/alkyl tail size).

- Endosomal Release -**I**

Although endosomal release has been already addressed in the previous aspect, after the endosome is formed several pathways can take place. The desirable one consist of endosome release and mRNA ribosomal translation, but nevertheless the majority of mRNA still undergoes lysosomal degradation, exosome incorporation, and even exocytosis (in equal extent to cytosolic release) so only a small percentage of mRNA follows the therapeutic path. In order to optimize the favorable endosomolytic mechanism (and increasing delivery potency), the critical variables consist, once again, on **pKa** and **ionizable lipid** (cone-shaped) **morphology**.

- Charge- & Ligand-mediated Targeting – **1**,**3**

The targeting aspect on the formulation pharmacokinetics corresponds to a major variable in overall vaccine performance since the ideal in cancer vaccination would be, usually, APCs (DCs mainly) delivery and not a specific organ (as in the case of cytotoxic drugs). It is reported that targeting can be modulated by particle charge and targeting-ligands. A positively-charged LNP, for example, tends to target the lungs while a negatively-charged one tends to target the spleen (which can be favorable in vaccination). Additionally, neutral-charged systems majorly target the hepatocytes (via Apo-E), which can be a trouble if a rapid metabolization takes place, reducing the therapeutic effect of the vaccine. Even if such vaccine is administered via intramuscular, systemic distribution and expression is still considerable so targeting aspects remain crucial in efficacy and safety terms.

- Adjuvanticity of the Delivery System – **2**

As a foreign body, the delivery system itself has the potential to exhibit an adjuvant-like activity which would enhance the core vaccine efficacy even higher. In several preclinical trials, LNPs seem to amplify T- and B-cell immune responses after formulated mRNA injection.[73]; [74] Thus, this characteristic should also be an optimization determinant.

- Injection Site Reactions, Safety, Tolerability & Reactogenicity – **3**

As mentioned earlier, the immunogenicity associated of vaccines can, in some cases, become exaggerated and cause (severe) adverse events. With LNPs a similar situation can be observed. By reducing the required dose adverse events would also decrease, so this aspect should be optimized in formulation selection although there is a scarcity of clinical studies in order to correlate properly with animal studies.

6. Critical Analysis

➔ "How can we fit mRNA vaccines in current immuno-oncology?"

Naturally when aiming to introduce a novel therapeutic strategy, based on a benefit/risk comparative analysis, mRNA cancer vaccines must be disclosed on a face-off analysis against the current leaders in cancer immunotherapy: Immune Checkpoint Inhibitors.[75] ICIs have reached the "elite" status in cancer immunotherapy mostly due to their unprecedented clinical outcomes and OSRs, which allowed them to lead the pack in terms of global product approvals and development pipeline.

I've highlighted several aspects to help guide mRNA cancer vaccines draft in the current clinical setting: mRNA cancer vaccines (and overall therapeutic cancer vaccines) don´t seem to significatively approach the effectiveness level of ICIs; mRNA vaccines show much more favorable safety profile compared to ICIs' (immune-related adverse events considerable risk); the mRNA platform is more versatile in terms of molecular targets; manufacturing process is less burdensome and more cost-effective comparing to ICIs' (monoclonal antibodies-related onerous production), as IVT mRNA vaccines' ease of production stands as its one of greatest advantages. The mRNA technology already has its safety proven, usually only with cases of injection site reactions being reported. [28] The adequate designing and employment of a delivery system/strategy in mRNA vaccines could further increase the technology's safety (and efficacy). This additional safety profile enhancement over ICI's encourages even more these vaccines utilization.

Considering this and recalling the "*Can mRNA-based vaccines do the same for cancer as they did for COVID-19?*" puzzle, mRNA vaccines don´t seem to represent the idealized cancer "cure" *per se* and would only occupy the 2nd line treatment arsenal in oncology. Nonetheless, cancer immunotherapy's clinical effectiveness doesn´t come from the use of individual therapy but from combination regimens, which employ several synergistic mechanism of action, allowing to overcome TME's intrinsic resistance strategies.[76] So, mRNA place might be as an potent and effective combination constituent beside the current stars – ICIs – as well as cytokines, monoclonal antibodies, and even oncolytic viruses. This latter consideration also appears to be aligned with the current clinical research panorama, since a great portion of clinical trials employing mRNA cancer vaccines probes its synergetic use with other drug products (see Appendix I) and prospects the importance of combination therapy in oncology furthermore. Personalized cancer vaccines encoding neoantigens have, amongst all types of therapeutic vaccines, the most promising value due to its highly-specific individualized action (which ICIs lack), and therefore would have tremendous impact along a non-specific therapy.

Whilst my "research + analysis" endeavor was being carried out, the designing of combinatory regimens protruded as the most crucial strategy employing mRNA cancer vaccines. Nevertheless, I deemed one other aspect as key: intervention timing. These vaccines can elevate their potential when employed just in the right time, especially following surgery which represents a common 1st line treatment [77]; [78] (where cancer cells are removed to minimum quantities). A temporally well-designed combination regimen clearly states the difference between therapeutic success and failure.

7. Conclusions and Final Remarks

With the currently available oncology arsenal, the eradication of cancer shifts even more from dream to reality mainly owing to immunotherapy's recent establishment. However, immuno-oncology still doesn´t quite fulfil all its promise (with response rates lower than expected). The development of cancer vaccines might be the key to unlock higher response and eradication rates, turning resistant ("cold") tumors into responding ("hot") tumors, essentially in combination regimens with conventional or other immunotherapies.[24] The mRNA vaccine platform, equipped its advantageous versatility, feasibility and safety profile, stands as one of the most promising strategies for vaccine cancer care. mRNA's inherent fragility underpins the current optimization and development focus – delivery strategies. Numerous studies have employed diverse delivery systems/strategies, leveraging nanotechnology to delivery immunotherapeutic mRNA represents nowadays the most valuable approach, especially with the use of LNPs which already have their effectiveness proven in the Real World, although the most clinically employed delivery system seem to be transfected dendritic cells. Nonetheless, there is still much work to be done with nanotechnology (LNPs mainly), optimizing their complexation and endosomal release abilities, efficacy and safety profiles and even storage requirements.[63] mRNA-based vaccination in current oncology holds great promise not as a stand-alone "cure", but as an effective adjuvant therapy which can definitively reenforce immunotherapy's combat power (especially due to its antigen-encoding flexibility). A well-designed development and research approach concerning safety and effectiveness (where delivery strategies are crucial) are key to obtain the most of this technologic platform and revolutionize, once again, cancer therapy's paradigm.

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Appendix 1– Recruiting, Active, Completed and Terminated Clinical Trials employing mRNA-based cancer vaccines. ClinicalTrials.gov and PubMed® were the chosen databases, counting a total of **37** clinical trials combined (09/04/2022). In both research platforms the "advanced search" terms employed were: "cancer", "mRNA" and "vaccine". By analysing the research's results, a tendency is noticed – Transfected Dendritic Cells as a delivery system is the most employed strategy

Identification	Status	Phase	Indication	Formulation Type	Combination	Opened	Sponsor	Study Results
NCT03897881 (KEYNOTE-942)	Active, not recruiting	II	High-Risk Melanoma	**Lipid**-formulated mRNA Personalized vaccine (i.m.)	Pembrolizumab	2019	ModernaTX, Inc.	No results available to date
NCT03313778 (KEYNOTE-603)	Recruiting	I	Solid Tumors	**Lipid**-formulated mRNA Personalized vaccine (i.m.)	Pembrolizumab	2017	ModernaTX, Inc.	No results available to date
NCT03480152	Terminated (2019) -slow accrual	I/II	Melanoma; Colon Cancer; Gastrointestinal Cancer; Genitourinary Cancer; Hepatocellular Cancer	**LNP**-formulated mRNA Personalized vaccine (i.m.)	/	2018	National Cancer Institute (NCI)	Good safety profile and favourable immune response
NCT03164772	Completed (2021)	I/II	Metastatic NSCLC	**Protamine**-formulated mRNA (**RNActive®**) encoding NY-ESO-1, MAGE-C1, MAGE-C2, TPBG, survivin, MUC1 (i.d.)	Durvalumab; Tremelimumab	2017	Ludwig Institute for Cancer Research	No results available to date
NCT03083054	Active, not recruiting	I/II	Myelodysplastic Syndromes; Acute Myeloid Leukemia	Autologous **Dendritic Cells** electroporated with WT1 mRNA	/	2017	University of Campinas, Brazil	No results available to date
NCT02366728	Completed	II	Glioblastoma; Astrocytoma, Grade IV; Giant Cell Glioblastoma; Glioblastoma Multiforme	Human CMV pp65-LAMP mRNA-pulsed autologous **DC**s	/	2015	Gary Archer Ph.D., Duke University	Increased migration of the DC vaccine to draining lymph nodes observed
NCT02285413	Completed (2015)	II	Melanoma	Tyrosinase, gp100 mRNA-loaded **DC**s (i.d. and i.v.)	Cisplatin	2011	Radboud University Medical Center	No results available to date

Appendix I – Recruiting, Active, Completed and Terminated Clinical Trials employing mRNA-based cancer vaccines. (Continuation)

NCT02140138	Terminated (2016)	II	Prostate Carcinoma	**Protamine**-formulated mRNA (**RNActive®**) PSA, PSMA, PSCA, STEAP1, PAP and MUC1 (i.d.)	Radical prostatectomy	2014	CureVac AG	No results available to date
NCT01995708	Active, not recruiting	I	Multiple Myeloma	CT7, MAGE-A3, and WT1 mRNA-electroporated Langerhans-type **Dendritic Cells** (s.c.)	Standard-of-care	2014	Memorial Sloan Kettering Cancer Center	No results available to date
NCT01734304	Completed (2018)	I/II	Acute Myeloid Leukemia	WT1, PRAME, and CMVpp65 mRNA-electroporated **DC**s (i.d.)	/	2013	Ludwig-Maximilians - University of Munich	No results available to date
NCT01530698	Completed (2014)	I/II	Melanoma	gp100/tyrosinase mRNA-electroporated **DC**s (single-step treatment) (i.n.)	Two-step DC treatment	2010	Radboud University Medical Center	No results available to date
NCT01456104	Active, not recruiting	I	Melanoma	Langerhans-type **DC**s with Trp2 mRNA	/	2011	Memorial Sloan Kettering Cancer Center	No results available to date
NCT01446731	Completed (2015)	II	Prostatic Neoplasms	PSA, PAP, survivin, hTERT mRNA-loaded **DC**s (i.d.)	Docetaxel	2011	Inge Marie Svane	No results available to date
NCT01334047	Terminated	I/II	Recurrent Epithelial Ovarian Cancer	Autologous **Dendritic Cells** loaded with amplified cancer stem cell mRNA + hTERT and Survivin mRNA	/	2011	Steinar Aamdal	No results available to date
NCT01278940	Completed (2006)	I/II	Malignant Melanoma	Whole tumor mRNA-transfected **DC** vaccine (i.d. or i.n.)	IL-2	2002	Oslo University Hospital	No results available to date
NCT01278914	Completed	I/II	Prostate Cancer	mRNA transfected **DC**s (route: ?)	/	2011	Oslo University Hospital	No results available

Appendix I – Recruiting, Active, Completed and Terminated Clinical Trials employing mRNA-based cancer vaccines. (Continuation)

								to date
NCT01197625	Active, not recruiting	I/II	Prostate Cancer	Tumor mRNA + hTERT, survivin TAA mRNA loaded in **DC**s (i.d.)	/	2010	Oslo University Hospital	No results available to date
NCT01066390	Completed (2013)	I	Melanoma	TriMix + TAAs mRNA- transfected **DC** vaccine (i.v. and i.d.)	/	2010	Bart Neyns	No results available to date
NCT00978913	Completed (2013)	I	Breast Cancer, Malignant Melanoma	hTERT, survivin and p53-enconding mRNA **DC** vaccine (i.d.)	/	2009	Inge Marie Svane	No results available to date
NCT00961844	Terminated (2012)	I/II	Metastatic Malignant Melanoma	**DC**s transfected with hTERT-, survivin- and tumor cell derived mRNA (i.v.)	Temozolomide	2009	Steinar Aamdal	No results available to date
NCT00929019	Terminated (2016)	I/II	Uveal Melanoma	Autologous **DC**s electroporated with gp100 and tyrosinase-encoding mRNA (i.v. and i.d.)	/	2009	Radboud University Medical Center	No results available to date
NCT00923312	Completed (2012)	I/II	Non-Small Cell Lung Cancer	**Protamine**-formulated mRNA (**RNActive**®) PSA, PSMA, PSCA, STEAP1, PAP and MUC1 (i.d.)	/	2009	CureVac AG	No results available to date
NCT00890032	Completed (2014)	I	Recurrent Central Nervous System Neoplasm	Brain Tumor Stem Cell derived mRNA-loaded **DC**s (i.d.)	/	2009	John Sampson	No results available to date
NCT00846456	Completed (2013)	I/II	Brain Tumor; Glioblastoma	Tumor Stem Cell derived mRNA-transfected **DC**s (i.d.)	/	2009	Oslo University Hospital	No results available to date

Appendix I – Recruiting, Active, Completed and Terminated Clinical Trials employing mRNA-based cancer vaccines. (Continuation)

NCT00834002	Completed (2007)	I	Acute Myeloid Leukemia (AML)	WT1 mRNA-electroporated autologous **DC**s (i.d.)	/	2005	University Hospital, Antwerp	No results available to date
NCT00639639	Active, not recruiting	I	Malignant Brain Neoplasms	Cytomegalovirus pp65-LAMP mRNA-loaded **DC**s (i.d.)	Tetanus toxoid; Therapeutic autologous lymphocytes	2006	Gary Archer Ph.D.	No results available to date
NCT00626483	Completed (2016)	I	Malignant Brain Neoplasms	Cytomegalovirus pp65-LAMP mRNA-loaded **DC**s (i.d.)	Basiliximab; GM-CSF	2007	Gary Archer Ph.D.	No results available to date
NCT00514189	Terminated (2009)	I	Leukemia	Autologous **DC**s loaded with AML lysate + mRNA (i.d.)	/	2007	M.D. Anderson Cancer Center	No results available to date
NCT00510133	Completed (2011)	II	Acute Myelogenous Leukemia	hTERT mRNA with LAMP-1 targeting sequence (i.d.) **DC**s	/	2007	Asterias Biotherapeutics, Inc.	No results available to date
NCT00243529	Completed (2009)	I/II	Melanoma Stage III/IV	Autologous **DC** mRNA-loaded vaccine	/	2004	Radboud University Medical Center	No results available to date
NCT00228189	Completed (2010)	I/II	Colorectal Cancer; Liver Metastases	CEA mRNA-transfected **DC**s (i.d. and i.v.)	/	2003	Radboud University Medical Center	No results available to date
NCT00204607	Completed (2007)	I/II	Malignant Melanoma	**Protamine**-formulated mRNA vaccine encoding MART1, tyrosinase, gp100, MAGEA1, MAGE-A3 and surviving (i.d.)	GM-CSF (s.c.)	2004	University Hospital Tuebingen	No results available to date

Appendix I – Recruiting, Active, Completed and Terminated Clinical Trials employing mRNA-based cancer vaccines. (Continuation)

NCT00204516	Completed (2012)	I/II	Malignant Melanoma	**Protamine**-formulated mRNA vaccine encoding MART1, tyrosinase, gp100, MAGEA1, MAGE-A3 and surviving (i.d.)	GM-CSF	2007	University Hospital Tuebingen	No results available to date
NCT01302496	Completed (2013)	II	Malignant Melanoma Stage III and IV	MAGE-A3, MAGE-C2, tyrosinase and gp100 mRNA- loaded **DC**s (with TriMix) (i.v./i.d.)	Ipilimumab	2011	Bart Neyns Universitair Ziekenhuis Brussel	Meaningful response in stage III/IV melanoma patients
NCT01915524	Terminated (2016)	I	Non-Small Cell Lung Carcinoma	**Protamine**-formulated mRNA (**RNActive**®) PSA, PSMA, PSCA, STEAP1, PAP and MUC1 (i.d.)	Radiotherapy	2013	CureVac AG	Potential observed for wide immune response trigerring
NCT01676779	Completed (2016)	II	Malignant Melanoma Stage III and IV	MAGE-A3, MAGE-C2, tyrosinase and gp100 mRNA- loaded **DC**s (with TriMix) (i.v./i.d.)	/	2012	Universitair Ziekenhuis Brussel	Well-tolerated adjuvant activity for melanoma patients
NCT04335890	Active, not recruiting	I	Uveal Metastatic Melanoma	gp100, tyrosinase, PRAME, MAGE-A3 and IDO mRNA-loaded IKKb-matured **DC**s (i.v.)	/	2020	Hasumi International Research Foundation	No results available to date