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Dissertação especialmente elaborada para obtenção do grau de Mestre em Investigação Clínica

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“A impressão desta dissertação foi aprovada pelo Conselho Científico da Faculdade de Medicina de Lisboa em reunião de 18 de junho de 2024.”

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Abstract

Clinical research is the method upon which we assess, study, and develop new medicines to improve human health across all therapeutic areas. Oncology research has a particularly interesting evolution, as it has undergone a tremendous paradigm shift due to the advent of precision medicine. This brought Oncology researchers the challenging task of innovating clinical trial designs aiming to adapt to these new classes of drugs while improving research and healthcare quality.

In this dissertation, an extensive narrative review is conducted to follow Oncology's research journey, painting a picture beginning when radiotherapy and chemotherapy emerged, going through the most traditional methods, and ending with the dawn of targeted therapies and immunotherapies. Advancements in genetic sequencing have enabled the distinction of cancers by their genetic mutations, and to specifically target cancer cells based on these alterations rather than on their location. With this, several common aspects of trial designs shifted, and we are now presented with adaptive trials, seamless designs, master protocols, tissue-agnostic drugs, and biomarker-driven studies, that defy the traditional "one-size-fits-all" method and give room to adaptable and innovative clinical trials.

Overall, precision medicine changed forever oncology's landscape, and although it entails challenges such as protocol complexity and the need for solid statistical proficiency, this approach can be more efficient, reduce healthcare costs and attrition rates in research, and give patients a unique tailored approach, with fewer side effects, personalized for each patient-specific characteristics.

Keywords: Oncology, precision medicine, basket trials, seamless designs, master protocols

Resumo

A investigação clínica é o método através do qual se estuda e desenvolve novos medicamentos, com o objetivo de melhorar a saúde humana em todas as áreas terapêuticas, e procede-se normalmente através das seguintes fases distintas com diferentes focos, e aumento gradual de participantes:

- Fase I: centrada na avaliação da segurança e de parâmetros farmacocinéticos e farmacodinâmicos (*pharmacokynetic/ pharmacodynamic* - PK/PD) de resposta à terapêutica;
- Fase II: uma primeira avaliação de eficácia e dosagem;
- Fase III: avaliação decisiva de eficácia;
- Fase IV: estudos de vigilância pós-comercialização que pretendem obter informações adicionais sobre a segurança e a eficácia de um medicamento em situações reais (após aprovação para comercialização).

O desenvolvimento de medicamentos seguindo este processo é moroso, levando atualmente entre 7 a 10 anos, contempla uma grande sobrecarga burocrática a cada submissão de uma nova fase, e resulta em elevadas taxas de atrito – por exemplo, apenas cerca 5% dos medicamentos que foram submetidos a fase I conseguiram introdução no mercado entre 1991 e 2000. Em Oncologia, a Investigação Clínica teve uma evolução particularmente interessante ao ter passado por uma tremenda mudança de paradigma após o aparecimento da medicina de precisão, o que envolveu a alteração de pontos considerados essenciais na investigação clínica tradicional. Nesta dissertação, é realizada uma extensa revisão narrativa com o objetivo de acompanhar o percurso da investigação em Oncologia, desde o aparecimento dos ensaios aleatorizados, até aos dias de hoje.

Anteriormente, as terapias dependiam normalmente da classificação da patologia no órgão de origem, baseando-se muitas vezes numa abordagem citotóxica em que a divisão celular e a replicação do ADN são bloqueadas, originando efeitos secundários que se sentem de forma geral em todo o

organismo e não apenas nas células tumorais (baixos níveis de eritrócitos e leucócitos no sangue, queda de cabelo, etc.). O aparecimento da medicina de precisão possibilitou uma mudança de foco nos tratamentos em Oncologia, onde, com o avanço na sequenciação genética, se torna agora possível identificar mutações específicas de cada tumor, permitindo a investigação de novas terapêuticas independentemente da histologia e, possibilitando a criação de tratamentos mais individualizados, direcionados, com mais eficácia, e menos efeitos secundários.

Inicialmente, os primeiros tratamentos oncológicos recorriam à remoção de tumores com intervenções cirúrgicas. A descoberta dos raios-X no final do séc. XIX abriu caminho a novas possibilidades com tratamentos de radioterapia, e a própria investigação clínica misturou-se com a evolução das terapêuticas aquando da emergência dos ensaios clínicos randomizados e dos primeiros tratamentos com quimioterapia nos anos 40. Atualmente, métodos combinados destas três intervenções continuam a ser os mais utilizados na luta contra o cancro, mas a medicina de precisão tem crescido e conseguido o seu lugar como novo interveniente. A medicina de precisão baseia-se fundamentalmente em três pilares: terapias direcionadas (*targeted therapies*), imunoterapias, e biomarcadores.

As terapias direcionadas consistem em moléculas (muitas vezes anticorpos monoclonais) que atacam características específicas das células cancerígenas e bloqueiam atividades essenciais para o crescimento tumoral (por exemplo, estimulam apoptose ou bloqueiam a angiogénese), acabando por minimizar os danos nas células saudáveis.

A imunoterapia, por sua vez, destina-se a estimular a resposta imunitária do próprio organismo contra as células cancerígenas. Em situações normais, as células do sistema imunitário reconhecem e eliminam células anormais, mas as células cancerígenas possuem mecanismos que as tornam capazes de evadir o sistema imunitário. As classes mais estudadas desta terapêutica envolvem as células T, leucócitos cruciais na identificação e eliminação de células desconhecidas no organismo. As terapias com células CAR-T (*chimeric antigen*

receptor T cells) e com recetores de células T modificados, por exemplo, são terapias onde as próprias células T dos doentes são modificadas *ex vivo* para expressarem um recetor que reconhece um antigénio específico do tumor, tornando-se capazes de atacar a célula cancerígena. Os inibidores de *checkpoint* nas células T, por outro lado, atuam na interação entre as células T e as células cancerígenas, dando origem a uma resposta por parte do sistema imunitário, que antes não seria possível.

Os biomarcadores surgem como um grande apoio no diagnóstico, na deteção de respostas (adversas ou terapêuticas), na elaboração de critérios de elegibilidade em ensaios, na estratificação de participantes, e em prognósticos de segurança e eficácia.

Tudo isto significa também que o anterior desenho de ensaios clínicos, com um "tamanho único", já não se aplica tão bem à medicina de precisão. Afinal, não é possível analisar estas subpopulações genéticas através dos desenhos tradicionais, as toxicidades observadas nestas abordagens são mais tardias, e a as relações de dose-resposta que já são bem conhecidas para as terapêuticas tradicionais não se aplicam tão bem a estes novos agentes. Além disso, é um objetivo da medicina de precisão diminuir as taxas de atrito, bem como o tempo e os custos do desenvolvimento de novas terapêuticas. Assim sendo, foi instituída a desafiante tarefa de inovar o *design* dos ensaios fase precoce em Oncologia, de forma a que a investigação se consiga adaptar a estas novas classes de medicamentos.

Primeiro, houve uma alteração nos *endpoints* dos ensaios. A abordagem tradicional consistia em escalar doses dentro da janela da toxicidade limitadora de dose, atingindo eventualmente uma dose máxima tolerada (*maximum tolerated dose* - MTD) e uma dose recomendada para a fase II. No entanto, com os fármacos da medicina de precisão, uma maior dose não significa necessariamente uma maior eficácia. Em vez de (ou para além de) estabelecer uma MTD, o objetivo passa a ser encontrar a dose biológica ótima, e envolve incluir novos *endpoints* como por exemplo parâmetros de PK/PD (como área sob a curva), *endpoints* dependentes de biomarcadores, alterações no tecido

tumoral, entre outros, que possam melhor avaliar o mecanismo de ação e a resposta biológica destes novos agentes. Métodos como o *Pharmacological Audit Trail* (PhAT) foram desenvolvidos para acompanhar este desenvolvimento, avaliando quais os melhores *endpoints*, seguindo o comportamento farmacológico, e otimizando estratégias de dosagem. Os próprios métodos de escalada de dose sofreram alterações, sendo os tradicionais métodos baseados em regras pré-definidas (como o mais utilizado 3+3 e respetivas variações), substituídos por métodos baseados em modelos, fortemente sustentados por modelos farmacocinéticos e farmacodinâmicos, métodos estatísticos Bayesianos, e uma abordagem adaptativa para otimizar o equilíbrio entre eficácia e segurança. O Método de Reavaliação Contínua (*Continual Reassesment Method*) foi o pioneiro nesta nova abordagem, seguindo-se vários outros, e sendo neste momento o *Bayesian Optimal Interval* (BOIN) o mais utilizado.

Por último, os *master protocols* - ensaios *basket, umbrella* e plataforma - e os ensaios *seamless*, desenhos adaptativos, são os protagonistas da mudança de paradigma nos ensaios de fase precoce em Oncologia. Os ensaios *basket* consistem em desenhos em que uma mesma terapêutica é estudada em tumores de vários órgãos diferentes, que contém a mesma mutação. Os *umbrella,* contrariamente, centram-se num local/órgão e estudam diferentes intervenções que visam diferentes mutações nesse local. Os ensaios plataforma comparam os efeitos de várias intervenções com um grupo de controlo, com a principal caraterística das intervenções poderem ser adicionadas ou removidas durante o ensaio, e destes estudos poderem decorrer perpetuamente até ser desejado pelos investigadores. Os ensaios *seamless*, por sua vez, vieram preencher a lacuna do elevado tempo de desenvolvimento destes medicamentos, integrando várias fases dos ensaios (normalmente fase I e II) no mesmo protocolo de estudo, utilizando medidas adaptativas, e acelerando o desenvolvimento de medicamentos através do aumento da flexibilidade e de ajustes ao protocolo em tempo real.

Apesar de versáteis e eficientes, acabam por surgir alguns desafios com estes novos ensaios, uma vez que implicam desafios logísticos e regulamentares,

análises de dados em tempo real, monitorização contínua, constantes adendas ao protocolo, conhecimentos estatísticos sólidos, e treino muito específico de equipas de investigação. No entanto, é já possível ver frutos bem sucedidos deste trabalho, com tratamentos inovadores como o *pembrolizumab* e *laroctretinib* - inibidores das proteínas PD-1 (*programmed cell death protein 1*) e da TRK (*Tropomyosin receptor kinase*), respetivamente - que utilizaram novos designs, ultrapassaram desafios regulamentares, receberam designações de terapia órfão e revolucionária, e conseguiram aprovações aceleradas tanto pela *Food and Drug Admnistration* (FDA) como pela *European Medicines Agency* (EMA).

Portanto, apesar de exigente e em crescimento, a medicina de precisão mudou para sempre o panorama da oncologia e, embora acarrete desafios, esta abordagem consegue ser mais eficiente, reduzir custos dos cuidados de saúde e taxas de atrito na investigação clínica, e proporcionar aos doentes uma abordagem personalizada para as suas características específicas, com menos efeitos secundários, cumprindo o ideal a que a medicina de precisão se compromete de levar “o medicamento certo, ao doente certo, no tempo certo”.

Palavras-chave: Oncologia, medicina de precisão, ensaios *basket*, *seamless designs, master protocols*

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Abbreviations

ADME	Absorption, Distribution, Metabolism, and Excretion	**MHC**	Major Histocompatibility Complex
APC	Antigen-Presenting Cells	**MT-BOIN**	Multiple Toxicity BOIN
AUC	Area Under the Curve	**NCI**	National Cancer Institute
BiTEs	Bi-specific T-cell Engagers	**NGS**	Next-Generation Sequencing
BOIN	Bayesian Optimal Interval	**NME**	New Molecular Entity
BsAbs	Bispecific Antibodies	**MTD**	Maximum Tolerate Dose
CAR-T	Chimeric antigen receptor T-cells	**NSCLC**	Non-Small Cell Lung Cancer
CD3	Cluster of Differentiation 3	**NTRK**	Neurotrophic Receptor Tyrosine Kinase
CDK4/6	Cyclin-dependent kinase 4/6	**OBD**	Optimal Biological Dose
CEIC	Comissão de Ética para a Investigação Clínica	**PD**	Pharmacodynamics
CPI	Checkpoint Inhibitors	**PD-1**	Programmed Cell Death Protein 1
CRM	Continual Reassessment Method	**PD-L1/L2**	Programmed Cell Ligand 1/2
CTC	Circulating Tumor Cells	**PhAT**	Pharmacological Audit Trail
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4	**PK**	Pharmacokinetics
DLT	Dose Limiting Toxicity	**POC**	Proof Of Concept
dMMR	Deficient Mismatch Repair	**POM**	Proof Of Mechanism
DNA	Deoxyribonucleic Acid	**QoL**	Quality of Life
EMA	European Medicines Agency	**RCT**	Randomized Controlled Trial
ETV6	ETS Variant Transcription Factor 6	**RNA**	Ribonucleic Acid
EWOC	Escalation With Overdose Control	**RPTD**	Recommended Phase Two Dose
FDA	Food and Drug Administration	**TCR**	T-cell Receptor
GEP	Gene-Expression Profile	**TITE-BOIN**	Time-To-Event BOIN
HNSCC	Head and Neck Squamous Cell Carcinoma	**TRK**	Tropomyosin Receptor Kinase
IRB	Institutional Review Board	**USA**	United States of America
LD10	Lethal Dose 10%	**WHO**	World Health Organization
MAMS	Multi-Arm, Multi-Stage	**WW2**	World War 2
MSI-H	Microsatellite Instability-High		

I. Introduction

a. Overview of prevalence and incidence of cancer worldwide, economic burden, and quality of life

Cancer is a condition that encompasses more than 100 diseases, and it is defined by the uncontrolled growth and spread of abnormal cells. Cancer cells proliferate without signals and/or ignore signals for apoptosis.[1–3] Normal cells can become faulty due to several gene mutations and epigenetics modifications, either when active oncogenes are expressed, or when tumor suppressor genes are lost. Even more so, cancer cells can normally evade recognition by the immune system, making the repair of these default cells highly difficult. Thus, cancer cells multiply and take the place of the normal healthy cells, and, ultimately, are able to enter blood and lymph vessels and metastasize, multiplying outside the organ of origin and throughout the body. The traditional cancer treatments involve chemotherapy, radiation therapy, and surgery, individually or in combination, alongside biopsies of the tumor mass for morphological and histological analyses of the cancer, based on the location and cancer type.[4]

Cancer is a global leading cause of death and affects countries of all income levels.[5] Even though the more developed countries have implemented several strategies to lower the incidence and prevalence of cancer - such as improvements in early detection, diagnosis and treatment, encouragement of lifestyle modifications, and creation of preventative vaccines for certain cancers [6] - in 2020 there were around 19 new million cancer cases, and almost 10 million cancer deaths worldwide, according to the GLOBOCAN statistics. In fact, 22.8% of the total cancer cases, and 19.6% of the cancer deaths were registered in Europe, although it only represents around 9.7% of the global population.[7,8]

Lung (19.5%), colorectal (12.3%), breast (7.5%), and pancreatic cancers (7.4%) are the four most common cancer-related deaths [9], and according to the updated estimates for 2022 for European Union member states, before the age of 75, 31% of men and 25% of women are expected to receive a cancer diagnosis.[9] In low-income countries, as they go through economic transitions, the populations are starting to adopt lifestyles that increase the risk of cancers, such as high tobacco

use, physical inactivity, and excess body weight, as it already occurs in highly developed countries.[5] The number of cases and fatalities is then predicted to grow rapidly all around the world, with statistics indicating that there will be a 47% increase in the number of cases by 2040, as life expectancy has been increasing and populations growing, aging, and adopting lifestyle practices that enhance cancer risk.[7]

Besides concerns with mortality, it has become increasingly important to also attend to the quality of life (QoL) of cancer patients. There is no doubt that a patient's QoL is negatively impacted by cancer, and it varies with the nature of the disease, the type of treatment received, and the length of the illness. QoL is negatively impacted by the frequent hospital visits, treatment side effects, the negative emotions that follow the disease progression, and the various physical complaints.[10] Furthermore, the prevention and management of cancer places a significant financial burden on society. For instance, some of the patients are unable to continue working and rely on friends and family to support them during treatment, or in the last phases of the disease.[6] In 2018, the overall cost of cancer was €199 billion worldwide, and the disease also caused a €70 billion loss in productivity accounting for both early death and morbidity.[11]

b. Significance of clinical trial designs in advancing cancer treatment

i. Clinical trials as the tool for drug approval and new medicines

Every drug that is now prescribed to humans has undergone clinical trials before being authorized for marketing.[12] Preclinical studies are the basis for the discovery of novel medications, providing fundamental answers about the safety, and operating pathways of a drug, but clinical trials are the moment at which treatment is evaluated and approved (or not) in humans.[13]

Clinical trials provide a well-organized structure for thoroughly evaluating experimental treatments, offering an evidence-based perspective on their benefits and risks. They follow strict guidelines and procedures, guaranteeing

that the information gathered is reliable from a scientific perspective, representative of the larger patient population, and adhere to the ethical regulations of trials in humans.[14,15] This process is the principle followed in every therapeutic area, and Oncology is no exception. Clinical trials are essential not only to ensure optimized cancer care and management, but also to advance with new and groundbreaking treatments.[16] With a carefully planned clinical trial, researchers can evaluate the impact of experimental therapies on various aspects of cancer, such as tumor size, progression-free survival, overall survival, and many more.[17] Clinical trials are usually divided into phase I (first-in-human), phase II, phase III, and a possible phase IV study, increasing the number of participants as we progress through each phase. Each phase also involves a different objective: phase I is more focused on safety, pharmacokinetics/pharmacodynamics (PK/PD), and first-dosage assessments; phase II is an early look at efficacy and dose-ranging; and phase III is the ultimate test of efficacy.[18] In the field of Oncology, phase I clinical studies are especially significant. Usually, in other areas, first-in-human studies are conducted in healthy volunteers [18], but in Oncology, in order to define the optimal dose, the drug needs to be escalated until it reaches dose-limiting toxicity (DLT). The concept behind this, especially with chemotherapy, is that there is a linear relation between toxicity and efficacy, as the more toxic the treatment is, the more effective it is in treating cancer. Therefore, due to the toxicity that is commonly observed in preclinical studies, the first-in-human studies of new cancer therapies are already performed in patients with refractory cancers. From an ethical perspective, patients will potentially be able to get additional therapy and the results in these early patients will be the deciding point for the dosing of subsequent patients.[19,20]

Since these are studies conducted with humans, they are highly regulated by authorities such as the Food and Drug Administration (FDA) - in the United States of America (USA) - and the European Medicines Agency (EMA) - in Europe - not only to ensure a scientific assessment on the efficacy, safety, and data integrity along the trials but also for an ethical standpoint, ensuring the security and well-being of all participants.[21] In Portugal, all clinical trials are also subject to control

and revision by Autoridade Nacional do Medicamento e Produtos de Saúde - Infarmed and Comissão de Ética para a Investigação Clínica (CEIC).[22]

ii. Time and economic investment

Clinical trials are an expensive investment. According to previous research, in the USA, phase I clinical trials typically cost around US$3.4 million, phase II trials $8.6 million, and phase III trials $21.4 million (considering a timeline starting when the protocol is approved by the regulatory authorities and finishing when the final report is issued).[23] Moreover, the actual cost of a medicine reaching marketing authorization is believed to be between US$0.8 billion and US$1.0 billion (in the USA), if we account for all the research and development expenses of programs that failed.[24,25]

What is even more concerning is the rate of marketing approvals considering the investment being made. Worldwide, research and development spending of pharmaceutical and biotechnology companies has been escalating in the past decade and has increased from $137 to $244 billion from 2012 to 2022.[26] While one would expect this increase in expenditure would correlate to an increase in new molecular entity (NME) approvals, that is not exactly the case. In 2012, the number of NMEs approved per billion dollars spent was nearly cut in half every nine years since 1950.[27] 2022 was the year with the lowest number of approvals since 2016, with only 37 NME approvals by the FDA.[28,29] Simoens *et al* [30] has reported that clinical development is the most resource-consuming phase of pharmacological development, accounting for 50-58% of overall expenditures per NME, and Paul *et al* [31] reported that pharmacological development accounts for 63% of the development time, while drug discovery and preclinical development account for 33%, and submission to launch costs for 5%. Additionally, the clinical development time has increased in the last decade.[32,33] Previous studies show that between 2005 and 2009 the clinical development process took an average of 6.4 years [34], later 6.9 years between 2008 and 2013, and 7.5 years between 2014 and 2018.[32]

Attrition rates are also a very significant problem in Oncology, even more so than in other areas.[35–37] If we take into account every NME that enters phase I studies, up to 95% do not receive marketing authorization. This renders the process of developing new drugs exceedingly expensive and ineffective [24,38] and can be especially concerning to patients for several factors, one of them being toxicity and safety.[39]

In today's pharmaceutical landscape, the escalating costs and extended development timelines, coupled with a high rate of drug candidate failures, underline the vital need to enhance early-phase studies. These initial investigations are of paramount importance, serving as the gateway from laboratory experiments to clinical application. Not only do they mark the first introduction of a drug to human testing, but they also provide the crucial bridge between preclinical data on drug behavior and the beginning of human trials for innovative cancer drugs.

iii. Participant recruitment

Participant recruitment is one of the most crucial points in clinical development, and it undoubtedly impacts the course of the whole clinical trial.[40] The study's scientific validity depends on the patients' enrollment and continued participation, and it is decisive to enroll a large enough sample to obtain statistically meaningful results.[41,42] The whole process of recruitment involves various steps, such as identifying the eligible patients based on the study's eligibility criteria, obtaining their Informed consent, and conducting all the screening and enrollment procedures, which can be more or less demanding depending on the study protocol. However, participant recruitment is also a major barrier in clinical research, and it is widely recognized that research sites frequently fall short of the pre-established target of recruitment.[43] Chaudhari *et al* reported in 2020 that recruitment amounts to 30% of the development timeline of a clinical trial, but still, 11% of clinical research sites do not enroll a single participant and 37% of sites enroll fewer participants than anticipated.[42]

Even after surpassing recruitment challenges, we must also focus on participant retention. Participants have the right to withdraw from a study at any time and maintaining them can sometimes be difficult. Clinical trials can have a significant negative impact on a participant's life depending on how time-consuming they are, how the treatment is received by the participant, which arm the participant is participating in, and many other factors.[44] Clinical trials can be delayed for months due to setbacks in recruitment and participant losses.[43] The success of the recruitment and retention procedures depends on a well-planned recruitment approach, and the collaboration between the researchers, sponsors, medical professionals, and even the participants.

c. New classes of drugs and new strategies emerging that require new designs

The recent years have been transformative to Oncology, with the introduction of precision medicine.[45] These new classes of drugs such as targeted therapies or immunotherapies often work by interfering with specific molecules involved in cancer cell proliferation or by allowing the immune system to recognize and attack cancer cells, targeting specific molecular pathways involved in cancer growth and spread.[46] Precision medicine is also based on the use of biological biomarkers that can clarify various pathophysiological characteristics and reflect individual heterogeneity.[47] This allows for more tailored and effective therapies.

Diagnosis and therapeutics in traditional approaches (such as chemotherapy) are usually based on the pathological classification of the organ of origin of a tumor.[48] However, with the evolution of genomic tools and knowledge of genetic sequencing of tumors, it is now possible to differentiate cancers by identifying genetic mutations in them, which allows for precision therapies to target the tumors based on this sequencing, rather than on the organ or tissue of origin. This approach is called "histology-agnostic" or "tissue-agnostic" being that it removes the need for causality with the origin of the tumor.[49]

The advent of these new drug classes has enforced a rethinking of traditional cancer treatment approaches. The one-size-fits-all model ("phase I – phase II – phase III" trials) no longer "fits" with the precision medicine approach [50], since it

is not possible to analyze these genetic subpopulations through traditional clinical trial designs. Furthermore, in the quest to deliver more effective and economically viable pharmaceuticals to patients, it has also become crucial to expedite the drug development process, streamline research and development expenses, and make more informed decisions regarding drug progression, all while upholding rigorous standards.

By embracing these new trial designs, researchers can more efficiently identify the patient populations that benefit most from these novel treatments, accelerating the translation of scientific discoveries into meaningful clinical outcomes, and developing strategies that better apply to these new classes that have shown to be the future of Oncology care.

II. Objectives

One of the goals of this dissertation is to conduct a narrative/descriptive review of oncology clinical trial designs. We aim to paint a picture since the initial “phase I - phase II - phase III” designs in Oncology and explore all the shifts that occurred until the designs we see nowadays such as seamless designs and master protocols, with the introduction of targeted therapies, biomarkers, immunotherapy, and other new players.

It is also an objective to analyze the benefits and challenges that have arisen with these new early-phase clinical trial approaches. We will explore already existing cases of successful marketing authorizations with precision medicine and agnostic clinical trial designs, and gather information regarding development, regulatory approvals, difficulties in study protocol development, and other relevant factors in the scope of the paradigm shift of the clinical trials in the Oncology field.

III. Methodology

The information for this narrative literature review was acquired from major academic databases including PubMed, ScienceDirect, and Google Scholar, integrating keywords such as “oncology”, “oncology clinical trials”, “precision medicine”, “targeted therapies”, “immunotherapies”, “biomarkers”, “escalation methods”, “Bayesian design”, “master protocol”, “adaptive clinical trials”, “agnostic treatment”, “seamless designs”, and including articles published between 1989 and 2023, in English. The selection of articles was based on their abstracts and whether they provided insights into the development, execution, and assessment of the paradigm shift in early-phase clinical trials in Oncology or any of the included sections of this dissertation (i.e. introduction, discussion, etc.). Journal articles, systematic reviews, reports, and guidelines were among the several article types that were analyzed. Information was also gathered from textbooks and significant webpages such as the National Cancer Institute (NCI), European Medical Agency (EMA), Food and Drug Administration (FDA), World Health Organization (WHO), and ClinicalTrials.gov websites.

Following the selection of relevant studies, data were extracted to collect relevant information for this project, focusing on the traditional approaches to clinical research, the use of new designs and the rationale behind them, and the main outcomes, difficulties, and solutions arising from these new approaches. Traditional and modern approaches were compared, and several specific clinical trials were analyzed, emphasizing their designs and implications for oncology drug development. A critical review of general concerns regarding trial efficiency and regulatory issues of both approaches was conducted, leading to a final analysis and proposal of potential future directions for oncology research.

IV. Evolution of Early Phase Clinical Trials Designs

a. Early History/Traditional Trials design

i. Early attempts at cancer therapies and the emergence of Randomized Controlled Trials (RCTs)

Early in the history of cancer therapies, interventions were often based solely on removal surgeries, and usually grounded in theoretical ideas and empirical observations, as opposed to thorough scientific analysis.[51] In the first half of the 20th century, the field of cancer therapy witnessed an extraordinary turn with the dawn of radiotherapy and chemotherapy. The discovery of X-rays by Wilhelm Roentgen in 1895, and Marie Curie's further research on radioactivity paved the way for radiotherapy use in cancer patients.[52] Not much later, in the 1940s, chemotherapy emerged, following the accidental discovery of nitrogen mustard gas during World War 2 (WW2), and giving rise to a new major breakthrough for cancer therapeutics.[51] Trailing this event, the number of studies involving new drugs for cancer treatment with chemotherapeutic agents grew exponentially. In parallel, we witnessed the advent of Randomized Controlled Trials (RCTs), also around the 1940s, which incredibly coincided with these two hallmarks of cancer care. RCTs then transformed the way the studies of medical interventions were designed and evaluated by allowing researchers to upgrade the rigor of data about efficacy and safety, and to reduce the associated bias.[53,54] This methodology involved randomly assigning participants to different treatment and control groups, ensuring a more unbiased and reliable assessment of a therapy's efficacy.

Still, during the 1960s, surgery and radiotherapy were the basis for solid tumor treatment, which led to a stagnation of curability rates.[55] Around the 70s, chemotherapy drugs started being used in combination with the other therapies , creating the conventional cancer treatment we still see nowadays, and thus establishing the importance of early diagnosis and treatment, and the efficacy of

multimodal treatment.[54,56] Figure 1 summarizes the major milestones in cancer treatment history.

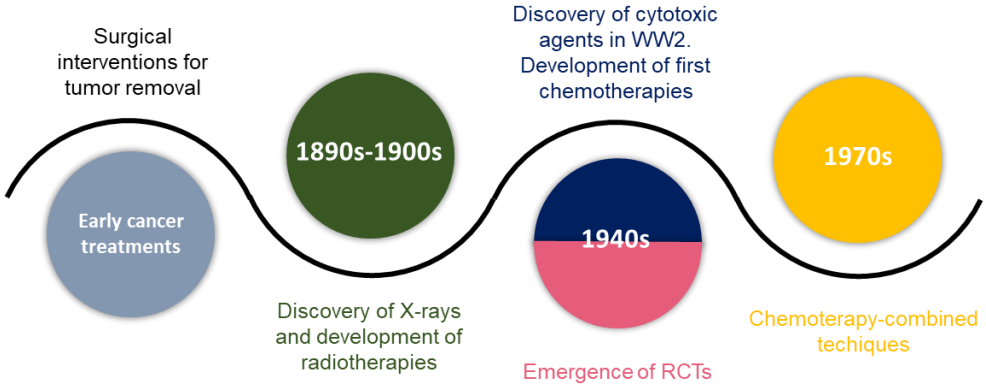

Figure 1: Timeline of the biggest milestones in cancer therapy. Adapted from Falzone et al.[51] WW2: World War 2.

The methodological accuracy introduced by RCTs has significantly improved the landscape of cancer care. These advancements have not only increased the efficacy of treatments but have also enhanced the understanding of cancer biology and the development of personalized treatment approaches.[51] The ongoing integration of novel technologies and therapeutics continues to build upon this historical evolution as we go through even one more breakthrough in cancer therapeutics with the arrival of precision medicine and tissue-agnostic therapies.

ii. Goal of the traditional first-in-human trials (phase I)

Phase I clinical trials are the interface between preclinical testing and human testing and aim prominently to evaluate the safety profile of the new therapeutic agent and define the dosage to be implemented in the phase II trials.[57] In these studies, preclinical data such as pharmacokinetics (PK), pharmacodynamics (PD), and toxicology are integrated into the study design and are fundamental components to achieving these study goals.[58]

The conventional drug development model in Oncology is typically designed to focus on defining the maximum tolerated dose (MTD)/recommended phase II dose (RPTD) and determining the schedules of administration.[59,60] MTDs and RPTDs are defined during trials by observing the dose-limiting toxicities (DLT) –

beyond the DLT, dose escalations are deemed unsafe for the participants.[61] Patients begin with a starting dose based on previous preclinical data, which is usually the dose at which 10% of the animals die (LD10), and the dosages are escalated, with toxicity used as the primary endpoint, until a certain number of adverse events prevents further dose escalation (depending also on their severity) and resulting on the DLT. MTD then represents the highest dose level at which the investigational product displays an acceptable level of toxicity.[62] This is the usual approach chosen with cytotoxic agents wherein higher doses represent greater therapeutic benefits, but also greater risk of severe toxic reactions. Chemotherapy agents have well-established PK/PD and dose–response relationships, and toxicity is a straightforward surrogate for activity (response is translated into a reduction in the size of tumors), making the MTD simple to determine.[58]

iii. Classic 3+3 design (advantages and disadvantages)

To determine the MTD/RPTD, phase I trials implement dosage increments, based on dose escalation methods. The traditional designs do so by following rule-based escalations, that administrate different dose levels to participants according to prespecified rules, based on observations of target events (usually DLT-related adverse events).[62] Among other less widely used, the most common method within the rule-based escalations is the 3+3 design.[63] This is a method built upon a 3-patient cohort, where the first cohort receives a starting dose based on preclinical data, and subsequent cohorts receive increasing dose levels. Usually, the dosage increment is set beforehand and based on the modified Fibonacci sequence.[64] In practice, this means that the first dose increase is 100% of the preceding dose, then 67%, 50%, 40%, and so forth of the prior doses, guaranteeing that dose increases are initially greater, but become smaller at higher dose levels.[65] The “rules” for the escalation of doses in 3+3 design are as follows (exemplified in Figure 2): 1) If none of the three first patients experience dose-limiting toxicity, the next higher dose will be administered; 2) If one of the first three patients experiences dose-limiting toxicity, the same dose will be administered again to three more patients; and 3) When two or more patients among the cohort experience dose-limiting toxicities the escalation will be

stopped. The RPTD is typically defined as the dose level immediately below the DLT.[62,65] These trials commonly report data for DLTs occurring only in a period of a few weeks, since most adverse events are acute and transpire during the first treatment cycles (usually each cycle consists of 4 weeks of treatment).[61]

The 3+3 strategy was pioneered in 1989 by Storer [66], and it is still widely used: in 2009, Le Tourneau [62] published a review in which 96.7% (n=171) of first-in-human trials in Oncology were developed following 3+3 escalation strategies. Later, more alternative rules based on this strategy emerged, for instance, the "2+4," "3+3+3," and "3+1+1" (also described as "best of five") rules. However, while this method is simple and safe to use, it still presents some drawbacks: first of all, it results in a wide range of patients being treated with low and probably subtherapeutic doses, implying that only a small number of patients actually receive doses there are close to the RPTD; second, although most DLTs are achieved during the first two weeks of treatment [67], it is possible that if a drug causes late or cumulative toxicity, a considerable number of participants has been treated at already toxic doses before any toxicity was observed. Furthermore, some statistical models have demonstrated that a trial implementing the 3 + 3 design only identifies the most accurate MTD/RPTD in 30% of trials.[60]

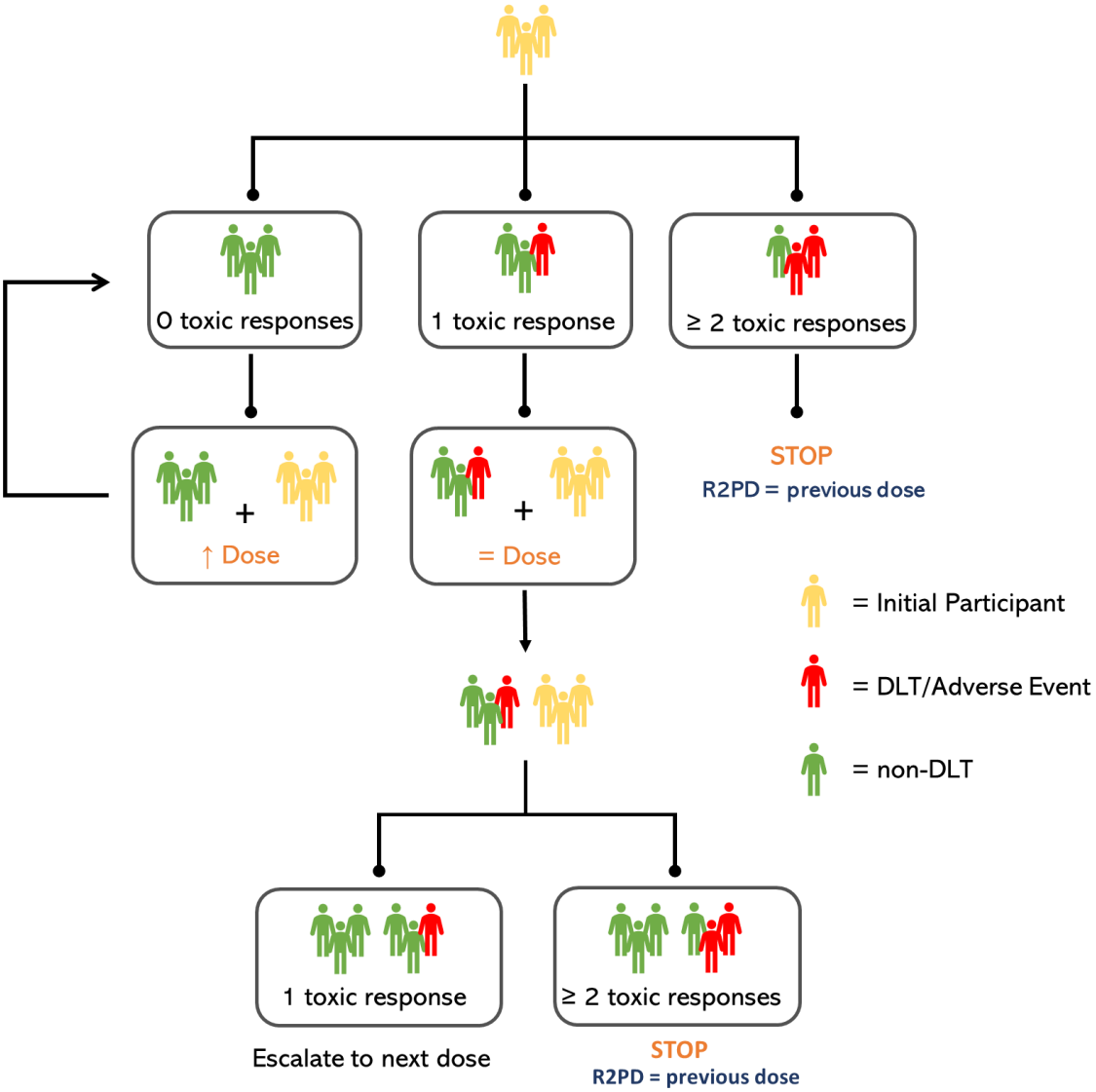

Figure 2: Illustration of the 3+3 escalation method. Adapted from Le Tourneau et al.[62]

iv. Limitations of the traditional designs

The traditional phase I – phase II – phase III designs, with early phases focused on obtaining RPTD and depending on DLT-related adverse events and rule-based escalation methods are considered the common paradigm in Oncology and are still widely applicable in new therapies, such as chemotherapy agents. The early-phase designs mentioned excel in offering a reliable safety evaluation, due to the conserving dose escalations and analysis of very small cohorts of patients.

However, this also results in insufficient patient representation in clinical trials, and ultimately, in uncertainty of what is the optimal treatment for the bigger number of patients possible.[68] There is also still a high rate of phase III failure [69], which indicates that early-phase trials have poor specificity for predicting benefits. This means that, ultimately, various patients are actually being submitted to sub-optimal therapies.[70] Traditional designs also often lack adaptability throughout the trial, and rigidly following pre-specified protocols hinders the incorporation of emerging data.[71]

b. Emergence and foundations of precision medicine

The FDA describes precision (or personalized) medicine as “an innovative approach to tailoring disease prevention and treatment that takes into account differences in people's genes, environments, and lifestyles”. Unlike the usual, transversally applied “one-site-size-fits-all” method, which accounts for the “average” patient, precision medicine aims to deliver “the right treatments to the right patients at the right time”.[72] Built upon scientific breakthroughs in the field of genetics and biology, and sustained by several approaches/intervenients (Figure 3), precision medicine is gaining the spotlight in many areas, especially in Oncology, where it aims to treat and prevent cancer with approaches based on new molecular biotechnology that considers tumor genomic variability, tumor environment, lifestyle, and morbidities of each patient to optimize patient care.[73]

i. Targeted Therapies

Targeted therapies are treatments that are directed at specific molecules involved in tumor growth and dissemination, and they are the cornerstone of precision medicine.[74] These drugs are substantially different from the cytotoxic methods we saw before with chemotherapy agents that block cell division and DNA replication. Targeted therapies consist of molecules that attack particular features of cancer cells while minimizing the harm to healthy cells.[75] This cutting-edge therapy is divided into two categories: small-molecule drugs, that act inside the cancer cells, and monoclonal antibodies, that act outside of cancer cells, attaching to specific receptors in the cell surface.[76] These therapies usually function through one of the following processes:

- Inactivation of tumor cell proliferation by interrupting growth signals.

- Regulation and stimulation of the immune system by marking cancer cells for easier identification and elimination by the immune system.

- Blocking angiogenesis around cancer cells, stopping the tumor blood supply, and incapacitating the tumor cells of growth.

- Triggering apoptosis by delivering toxic substances to cancer cells, for example, chemotherapy agents that will act directly, and only, on cancer cells.[74]

Regardless of what process is employed, targeted therapies are achieved with the expanding understanding of cancer biology and genomics, due to groundbreaking biotechnologies that have arisen. With the ability to develop drugs that selectively block or interfere with cancer cell's vital pathways, it has become possible to create individualized and effective treatments, which renders targeted therapies the central piece of precision medicine.[77]

ii. Immunotherapy

Another major intervenient emerges as a component of precision medicine. While the study of immunology dates back to the late 1800s [78], the identification of the acquired immune system and immune self-regulation (late 19th and mid-20th centuries, respectively [78,79]), along with the synthesis of the first monoclonal antibodies in 1975 [80], opened the door to a new age of immunotherapies.

Under normal physiological conditions the immune system recognizes and eliminates mutated cells, however, cancer cells have the ability to evade the immune system.[81,82] Immunotherapy works by modulating the immune system, making it more capable of recognizing and destroying these cancer cells. There are many approaches within immunotherapy, being TCR-engineered T-cells, CAR-T cells, bi-specific T-cell engagers (BiTEs), and checkpoint inhibitors, all involving the pathways of T-cells, the most innovative and studied therapies in

Oncology.[83,84] T-cells are leukocytes, crucial for the immune response, that identify and eliminate cells exhibiting irregular or unknown proteins. T-cells have receptors on their surface (TCRs) that bind to other cells [85], and whenever the interaction between the TCR and the ligand is unknown, the T-cell will trigger several processes to interfere and ultimately eliminate the cell. This is the process that is usually not accomplished with cancer cells.[83]

In TCR-engineered and CAR-T cell therapy, a patient's own T-cells are genetically modified *ex vivo* to express a receptor against a specific tumor antigen. With TCR therapy, T-cells express a synthetic TCR that recognizes membrane and intracellular proteins within the major histocompatibility complex (MHC).[86,87] In CAR-T cell therapy, T-cells are modified to express chimeric antigen receptors (CARs) that recognize and target specific antigens present on the surface of cancer cells.[88] Due to the difference in antigen recognition (intra/extracellular vs cell surface), TCR therapy is more directed to solid tumors [87], whereas CAR-T cells are mostly aimed at blood cancers, which more commonly express cell surface antigens.[89]

Bispecific T-cell engagers (BiTEs) are a specific class within bispecific antibodies (BsAbs), which are genetically modified antibodies that facilitate the binding of two molecules. In BiTEs' case, the antibodies bring tumor cells and T-cells to proximity by targeting CD3 (cluster of differentiation 3) in T-cells and tumor-specific antigens in cancer cells. By binding to CD3, BiTEs activate T-cells to target the tumor cells and ultimately eliminate them.[90]

In all the above approaches, the key aspect is to consider the genomic specificities of each tumor, so the modified cells can target the tumor cells with precision, stopping cancer proliferation and growth, and accomplishing an approach based on precise and personalized characteristics.

As for checkpoint inhibitors (CPIs), T-cells possess receptors on their surface that work as immune system checkpoints, such as the CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) and the programmed cell death protein 1 (PD-1) receptors. These checkpoints are "off" (inactive) whenever a healthy cell is

present and are “on” (active) when abnormal cells arise. [91] However, cancer cells can often exploit these mechanisms and escape T-cell activation. When CTLA-4 binds with the CD80 (or CD86) ligand in antigen-presenting cells (APCs) (such as macrophages, dendritic cells, and B cells) the interaction leads to an inhibitory signal that downregulates T-cell activation, stopping the immune response.[92] Similarly, when PD-1 binds to the ligands present in normal healthy cells (programmed cell ligand 1 (PD-L1) and programmed cell ligand 2 (PD-L2)) it sends signals to the T-cells, inhibiting their activity.[93] Cancer cells can express ligands that bind to these receptors and effectively break down the immune response, preventing T-cells from recognizing and attacking them. Checkpoint inhibitor drugs were then a huge breakthrough in cancer treatment, as they are able to prevent the interaction between the cancer cell ligands and these checkpoints, activating the T-cells that then target the cancer cells.[90] In 2018, James Allison and Tasuku Honjo jointly received the Nobel Prize in medicine for their work with immune checkpoints [94]: Allison for studying and first showing the CTLA-4 inhibition potential in treating cancer in the 1990s, and Honjo for discovering the PD-1 protein in 1992, and later in 2012 demonstrating the clear efficacy in the treatment of patients with different types of cancer.[95]

Although engineered T-cells, BiTEs, and CPIs are the most common approaches, there are other immunotherapy methods in place, such as synthetic interleukins and interferons, cancer vaccines, and immune system modulators.

Immunotherapy offers a chance of long-term control of cancer, as the immune system possesses a memory feature that allows the recognition and response to cancer cells if they reappear, potentially offering longer-term protection against cancer recurrence, unlike the traditional treatments that directly target cancer cells.[96] Nevertheless, due to the heterogeneity found in cancers and their microenvironment, patients respond differently to immunotherapy, and, although usually less severe than in chemotherapy, immunotherapy can cause immune-related complications such as tiredness, skin irritation, vomiting, diarrhea, and more serious side effects like immune-related organ impairment.[97,98] It is therefore essential to analyze the specific characteristics of every cancer to develop the most effective tailored treatment possible. Immunotherapy has also

shown improved results when paired with one or more of the traditional therapies (chemotherapy, surgery, and radiation therapy).[82]

It is notable to mention that immunotherapy differs from the targeted therapy approaches that involve monoclonal antibodies. Although they both do have immune-related functions, the antibodies in targeted therapy have as primarily focus the blocking of specific pathways or signaling involved in cancer growth, whereas immunotherapy aims to boost the body's own immune response against cancer cells by either activating immune cells or removing the restraints that prevent immune cells from attacking tumors.

iii. Biomarkers

Other grounds of precision medicine are biomarkers. A biomarker is defined as "a clear characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention." [99] This wide definition results from the various origins a biomarker can have, as they can derive from histologic, molecular, radiographic, or physiologic characteristics.[100] Biomarkers have distinct functions and can be grouped according to various subtypes:

Diagnostic Biomarkers

Diagnostic biomarkers identify or confirm the existence of a relevant condition/disease. Given that many diseases have genetic subgroups that differ significantly in their responses and prognoses, these biomarkers can help to confirm that a particular treatment is necessary. They can also serve as eligibility criteria for clinical trials that require a specific disease.[99,101] For example, the ETS Variant Transcription Factor 6 - neurotrophic tyrosine receptor kinase (ETV6-NTRK) translocation is a diagnostic biomarker in the context of infantile fibrosarcoma.[102] The ETV6-NTRK translocation refers to a genetic alteration where the ETV6 gene fuses with the NTRK gene and can result in an abnormal protein with oncogenic properties, contributing to the development of fibrosarcoma. This biomarker was also used as one of the eligibility criteria for

the NAVIGATE trial (a phase I-II study regarding the efficacy of larotrectinib in TRK fusion-positive cancers in adults, adolescents, and children - NCT02576431), and is used as criteria for treatment with larotrectinib (a highly selective TRK inhibitor for TRK fusion-positive cancers).[103,104]

Predictive Biomarker

When predictive biomarkers are present (or change) it indicates that a person is more likely to develop an effect from exposure to a medicinal or environmental agent. These biomarkers are also used as tools in clinical trial design to simplify the evaluation of the therapeutic effect of a specific drug. In these cases, the population is chosen based on the presence of a predictive biomarker on each participant, so that during the trial it is easier to analyze the effects of that therapeutic. These biomarkers are also used in the stratification of the participants based on the existence or not of a biomarker.[99,105]

Prognostic Biomarker

Prognostic biomarkers are analyzed in people who are already diagnosed with a certain condition in order to identify the probability of an event of interest, disease reoccurrence, or progression.[106] Prognostic biomarkers are used to identify higher-risk populations and are an integral part of anticipating the risk of an adverse event or undesirable outcomes in clinical trials. For example, BRAF gene mutations are prognostic biomarkers in the context of colorectal cancer.[107]

Pharmacodynamic/Response biomarker

Pharmacodynamic biomarkers are those in which levels change after contact with a medical or environmental agent.[99] The primary applications for this kind of biomarker are in early treatment development and clinical practice. This is the principle behind biomarkers that are surrogate endpoints: the biomarker is a substitute for an accurate measure of a patient's survival, quality of life, or symptoms. The surrogate endpoints based on pharmacological, physiological,

epidemiological, and other scientific evidence, forecast therapeutic benefit or harm.[108]

Safety Biomarker

A safety biomarker is measured before or after exposure to a medical or environmental agent to indicate the possibility, existence, and/or degree of toxicity as an adverse event.[109] For instance, hepatic aminotransferases are used as safety biomarkers when evaluating hepatotoxicity.[110]

Susceptibility/Risk Biomarker

Although the existence of this subtype is not uniformly accepted, the concept is that risk biomarkers indicate the possibility of developing a disease or medical condition in a person who does not currently have a clinically apparent disease, in contrast to prognostic biomarkers, which are investigated after a patient has already received a diagnosis. They are used broadly in epidemiological studies.[111]

Monitoring Biomarker

Monitoring biomarkers are assessed continuously allowing the assessment of disease progression (including new symptoms, effects, clinical worsening, etc.) and response to a condition or treatment. This subgroup ends up overlapping with some of the categories above such as safety or pharmacodynamic biomarkers, as “monitoring” is a very wide notion.[112]

Thus, biomarkers have proven to be extremely useful tools in clinical trial design. They support the development of eligibility criteria, the identification of therapeutic and harmful responses, and the stratification of patients, and are being more and more studied as new methodologies develop according to the changing paradigm of Oncology clinical research.

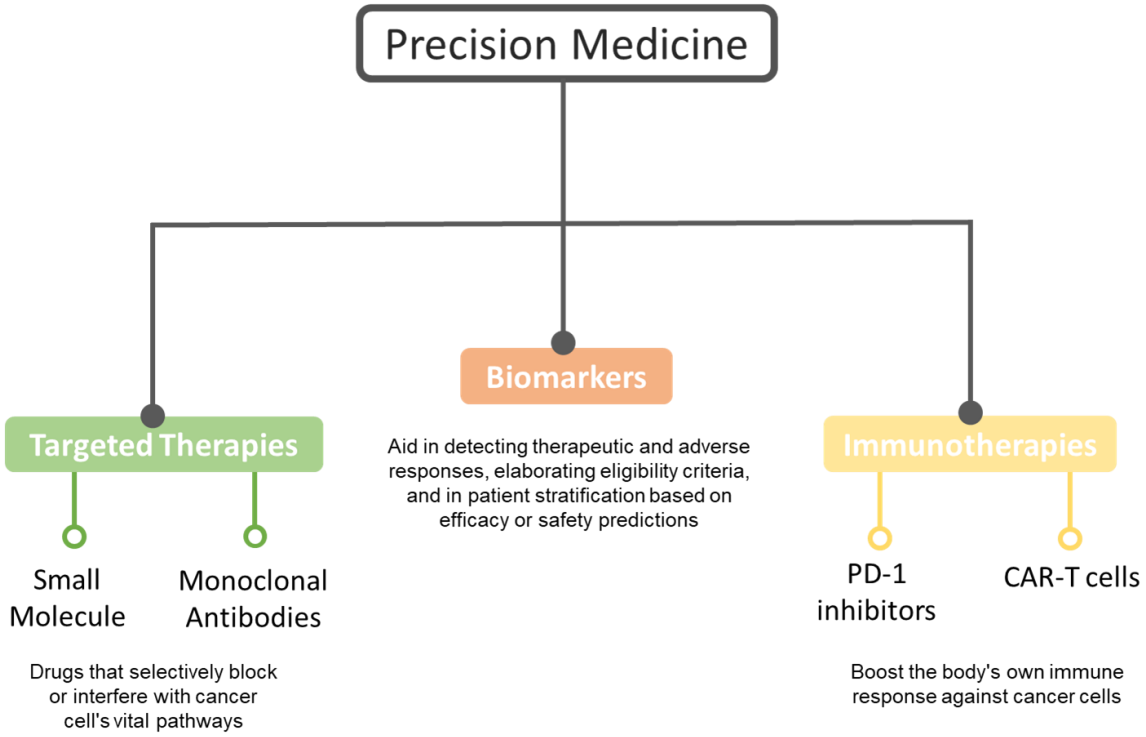

Figure 3: The three pillars of Precision Medicine.

V. Progress toward new designs

Plenty of landmarks led to this era of precision Oncology, starting back in the late 1980s, when the overexpression of the human epidermal growth factor-2 (HER2) was associated with breast cancer. This was followed by the first human genome sequencing in 2001, and, from this point, there have been increasing breakthroughs that allow the identification of intricate and distinct biological characteristics of carcinogenesis, such as the advent of next-generation sequencing (NGS) techniques that allow easier, more accessible, and massive DNA and RNA sequencing.[45] Another recent breakthrough is the emergence of liquid biopsies around the mid-2010s. With liquid biopsies, a sample of biological fluid (usually blood) is tested for various biomarkers, such as circulating tumor

DNA and circulating tumor cells (CTCs), providing a minimally invasive strategy for obtaining the tumor's genetic information.[113,114]

In conclusion, along the scientific process we have managed to understand the genomic heterogeneity of cancer, even within the same cancer type and same localization. This opened the door for precision cancer medicine to act, indicating that cancer therapies no longer need to be bound to tumor type, localization, or histology. Instead, histology-agnostic treatments, based on molecular alterations, biomarkers assessments, and immunology processes are the new steppingstones in cancer treatments, aiming to better the cancer care for each patient.[49]

But as these new treatments and intervenients arise, can the clinical trial design stay the same? These new drugs and new approaches, although bringing tremendous opportunities, also carry challenges. For example, it is not possible to analyze these genetic sub-populations by traditional clinical trial designs. Enrolling these highly particular populations is extremely challenging, and eventually, the Oncology first-in-human trials would face the same challenges as rare diseases, for example, where it is extremely difficult to identify a sufficient sample to ensure the validity of data regarding safety and efficacy. Furthermore, it has become of utmost importance to optimize the time spent on clinical trials and minimize the attrition rates between trial investments and the number of approvals, while still guaranteeing the safety and efficacy of the studied treatments, to fulfill the “right treatments to the right patients at the right time” ideal that arrives with precision medicine. All these factors culminate in the notion that the previous “one-size-fits-all” clinical trial designs, with a very rigid phase I – phase II – phase III strategy, is no longer the best possible option considering the paradigm change in early-phase clinical trials in Oncology.

a. Trial Endpoints Shift

The gold standard for early-phase clinical trial design and interpretation has therefore been based on the traditional cytotoxic agents, driven by predictable PDs and PKs, conventional toxicity and efficacy patterns, and based on dose-

escalating methods that ultimately achieve MTD and RPTD. However, precision medicine therapies have underlying differences related to dose-response pharmacodynamics and pharmacokinetics, and consequently require an adjustment of the conventional efficacy endpoints.[115]

Targeted therapies, for instance, have a more selective mechanism of action compared to chemotherapy agents, which leads to more prolonged and mild toxicity, often not observed within the typical DLT window.[58] Likewise, responses to immunotherapies follow nonlinear dose–response and dose–toxicity kinetics.[115] This means that, as responses may be delayed, and adverse events consequently also deferred, the typical dose-response relation is impaired. Can the drug not be effective despite showing toxicity? Is the drug actually toxic before reaching MTD, but we can't see it within the DLT window? [58,116] Additionally, in these cases, increasing doses does not follow the standard "higher dose – higher efficacy – higher toxicity" paradigm. Hence, traditional approaches lack the incorporation of pharmacodynamic and pharmacokinetic endpoints in early-stage clinical trials and may result in the non-acknowledgment of late toxicities. [117] The solution is to find alternative endpoints, such as biomarker-driven surrogate endpoints [115] and PK/PD measures such as area under the curve (AUC), level of target inhibition, or pathway alterations in tumor tissue, that can better capture the mechanism of action and biological response of these new agents. Rather than - or in addition to - establishing an MTD, the purpose is now to find the optimal biological dosage (OBD) by searching for PK/PD parameters that may work as surrogates for efficacy.[58]

However, as we already know, these drugs have found pitfalls, with high attrition rates and a vast majority of new therapies not reaching the market, despite the millions invested in the process.[118] Fortunately, new methods have arisen, namely the one envisioned by Paul Workman in the optimization of the clinical development process: the Pharmacological Audit Trail (PhAT) - a biomarker-driven strategy that documents with accuracy and reliability PK and PD aspects of drug development, in order to optimize the identification of the appropriate surrogate endpoints, and ultimately improve the quality of the research, offering

the investigators tools to draw informed decisions, and aid in early risk assessment and mitigation.[119]

PhAT enables more informative early-phase designs, encompassing steps from pre-clinical and clinical development. It consists of a set of questions (What is the status of the molecular target? Are sufficient drug concentrations achieved? Is activity achieved on the intended molecular target? How is the corresponding biochemical pathway modulated? Does this produce the desired biological effect? Does this correlate to a clinical response? [58,119]), organized into these key points (see also Figure 4):

1. *Defining target population*

A crucial initial step is to define patient subgroups that will likely respond positively to treatment. Predictive biomarkers and preclinical models such as patient-derived xenografts [120], among other strategies, are used to help define the population that will effectively respond to the proposed treatment.

2. *Describing pharmacokinetic (PK) characteristics*

With targeted therapies typically including oral ingestion, PK studies aim to understand how the body Absorbs, Distributes, Metabolizes, and Eliminates (ADME) drugs. The characterization of pharmacokinetics can aid in the clarification of toxicity profiles, the scheduling of oral intake agents, and the study of drug recommendations related to food consumption, concurrent medicine, and drug metabolism interactions.[118]

3. *Describing pharmacodynamic (PD) characteristics*

A vital aspect of the PhAT strategy is the use of PD biomarkers. Target engagement, inhibition of important pathways, and biological consequences are examples of frequently utilized PD indicators.[118] The focus at this point is to find proof of mechanism (POM) and proof of concept (POC) biomarkers. POM studies seek to demonstrate that a new drug reaches the targeted organ, interacts with the intended molecular target, and modifies the target cell's biology.[121] POC

studies provide the functional consequences, proving that the drug affects as expected the disease in question.[122]

4. *Identifying intermediate biomarkers of response*

Early detection of a patient's potential positive or negative effects from a treatment is crucial since it allows the treating physician to modify the course of care early on, improving patient outcomes, lowering avoidable toxicity, and increasing the effectiveness of healthcare. We can highlight, for example, the assessment of early response to treatment using CTC counts.

5. *Assess tumor response at resistance*

The likelihood of acquired resistance is becoming increasingly evident, so even in cases where there is a favorable initial response, it is crucial to evaluate how the treatment is progressing. Here, comprehending the mechanism of acquired resistance is the primary goal.

6. *Overcome Resistance*

Overcoming the resistance mechanisms is the final stage of a successful new drug, so it's critical to attempt to anticipate it as soon as feasible. One way to forecast resistance is through the analysis of certain mutations that can predict mechanisms of resistance. Combining other anticancer medications and looking into new targets or medications are some of the strategies usually used to overcome resistance.[118]

The PhAT's questions should be addressed as early in the clinical development process as possible and should not be viewed as isolated questions but rather as a component of a continuum.[58] PhAT principles allow the application of preclinical models that ensure a reduction in animal experimentation, and, ultimately, following PhAT's questions allows a reduction of drug attrition rates, speeding up and improving the quality of drug discovery and development.[120]

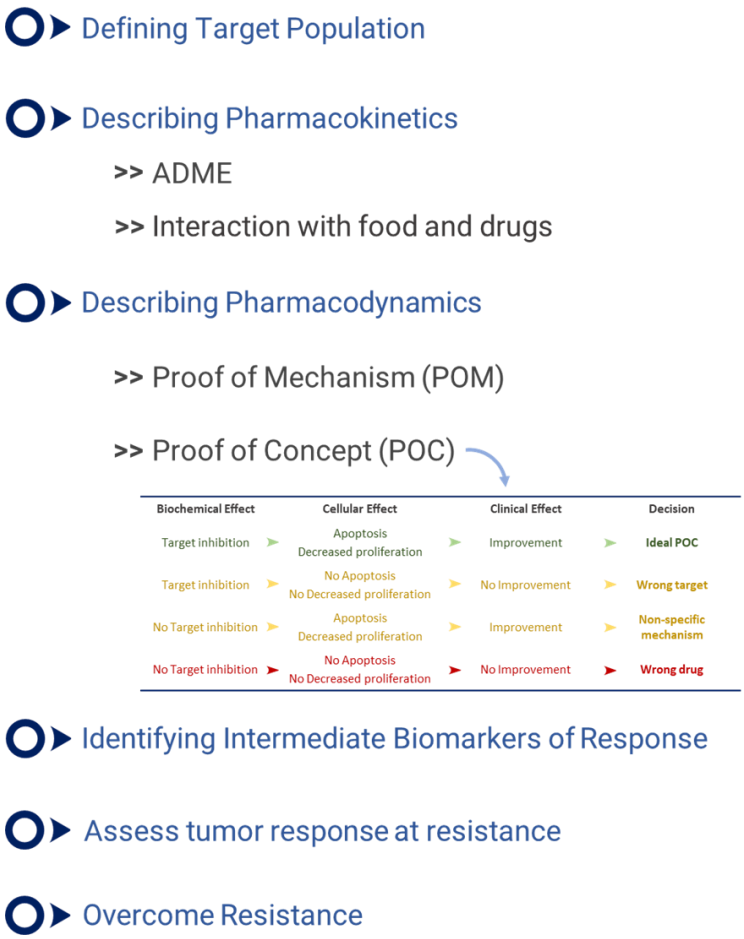

Figure 4: Key steps of the Pharmacological Audit Trail Strategy. Adapted from Mansinho et al [49] and Banerji et al.[118]

b. New methods of dose escalation

The arrival of precision medicine results in the reshaping of various components of clinical trials, and just like the trial endpoints have shifted, drug escalation methods have also started to transform.

The rule-based designs (such as the “3+3” design), although easy to follow and safe, are limited in their ability to find the MTD, and tend to treat a considerable number of participants with suboptimal doses. They follow this prefixed set of “rules” decided before the trial starts (usually called an escalation plan), and increase doses based on observed toxicities.[65] The most recent approaches, called model-based designs, have been constructed upon mathematical and statistical models that are adaptive, used to monitor the dose-escalation process based on estimations and probabilities that allow the dose-toxicity relationship to

be evaluated and updated as the trial progresses, and as more and more information accumulates.[65,123] Model-based designs integrate pharmacokinetic and pharmacodynamic models, Bayesian statistical methods, and adaptive dose adjustments to optimize the balance between efficacy and safety.[124] The key distinction between rule-based and model-based designs relies on the adaptability and flexibility of model-based designs, allowing real-time adjustments informed by accumulating data and prior knowledge. These latest strategies characterize the risk of toxicity as a function of the dose and one or more "parameters" (numbers or characteristics that affect the form of the dose-toxicity relationship).[125] Many of the models used in these trials are updated using Bayesian statistical methods and designs.

Bayesian designs are then based on the Bayes theorem, which establishes that we can obtain the probability of an event based on prior knowledge of conditions.[126] During the clinical trial, the information builds up with each new participant enrolled, and specific outcomes (DLT, PK/PKD endpoints, etc.) can be present or absent at a specific dose.[123] Thus, in Bayesian designs, each time a new participant is enrolled, the information from the previous participants is integrated, and a new estimation of probability distribution is accomplished with Bayesian inference, which can result in changes in the given dose.[65]

The first enacted model-based technique was the Continual Reassessment Method (CRM).[62] The CRM follows Bayesian methods, and usually employs the power or logistics regression model, with one or two parameters. In CRM, the study starts with a small cohort of patients that are treated at the dose considered the closest to the MTD, based on preclinical models or prior information. The statistical model is then updated with the observed toxicities every time a new participant enters the study, so the next dose is always determined based on the updated model. The trial stops when a prespecified condition is met, originally when six patients are assigned to the same dose.[62,65] Although this method allows for numerous dose escalations and de-escalations, proving to be an efficient method with undeniable adaptability, it was not well accepted due to safety concerns, since it can expose patients to concernedly high doses if the prespecified model is unfitting.[65]

Babb *et al*. [127] overcame this situation by presenting an alternative Bayesian approach called Escalation With Overdose Control (EWOC). The EWOC method is a modified CRM with extra steps regarding safety. In the EWOC, an upper boundary of toxicity is established beforehand, and the probability of administering a dose that exceeds this parameter is also assessed after each patient enrolls in the trial. If this probability ever exceeds the prespecified value, the dose escalation is stopped.[62,65] For example, in 2016, the ribociclib, an inhibitor of the cyclin-dependent kinase 4/6 (CDK4/6) was developed with an EWOC design, for patients with advanced solid tumors and lymphomas.[128]

An even more recent addition to the dose-escalation methods set is the Bayesian Optimal Interval (BOIN) design. The BOIN design actually falls within the scope of the model-assisted designs, a fairly new designation. These methods aim to conjugate characteristics both from rule-based and model-based designs, being easier and more transparent to apply in real-world trials (more similar to rule-based approaches) but using statistical, Bayesian, and adaptive methods as the source for the decision rules (as for model-based designs).[129]

BOIN designs provide great performance, equivalent to the more sophisticated model-based designs, but are similar to the 3+3 design in the sense that they are easy to perform.[130] The BOIN method works by comparing the observed DLT rate (hereinafter designated as $\hat{p}$, see equation below) in the current dose with previously set dose escalation and de-escalation boundaries. Usually, the upper and lower boundaries are designated λ_e and λ_d, respectively, and the design follows these steps:

1. The first cohort of patients is treated with the lowest dose.
2. The next dose is assigned according to (see also Figure 5):
    a. If the $\hat{p} \leq \lambda_e$, there is a dose escalation;
    b. If the $\hat{p} \geq \lambda_d$, there is a dose de-escalation;
    c. If the $\lambda_e < \hat{p} < \lambda_d$, dose is maintained;
3. The trial continues repeating these steps until the prespecified sample size is exhausted.

$$DLT_{rate} = \hat{p} = \frac{Total\ number\ of\ patients\ who\ experienced\ DLT\ at\ current\ dose}{Total\ number\ of\ participants\ at\ the\ current\ dose}$$

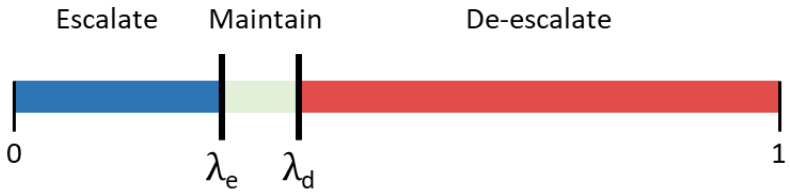

Figure 5.: Intervals of escalation and de-escalation according to the Bayesian Optimal Interval (BOIN) method. Adapted from Zhou et al.[132]

To determine the boundaries for dose escalation and de-escalation (λ_e and λ_d) it is necessary to first identify the highest DLT probability that is considered to be an under-dose ($\hat{p}_1$) and the lowest DLT probability that is considered to be an overdose ($\hat{p}_2$). According to Liu *et al*[131], the general guidance is that $\hat{p}_1$ and $\hat{p}_2$ should be: $\hat{p}_1$ = 0.6$\hat{p}$ and $\hat{p}_2$= 1.4$\hat{p}$ for general use. A table with the various λ_e and λ_d, depending on the DLT rate, is provided in several bibliography.[130,132]

Phase I trials using the BOIN design are carried out as a series of decision-making steps to determine the right dose for each recruited patient. The design reduces the likelihood of a mistaken decision regarding when to escalate or de-escalate doses. BOIN designs are recent - the statistical methodology of the BOIN was shared in 2015, by Liu and Yuan[131] – but have already started to be employed and are one of the most commonly used within Oncology designs with modern approaches. In 2021, the FDA considered the BOIN design a “fit-for-purpose” for dose determination, which has also helped in increasing the utilization and the importance of this approach.[133] Furthermore, the design has been classified as one of the top-performing designs for minimizing the risk of sub-therapeutic dosing.[134] Several extensions of the BOIN method have also already been explored, such as the multiple toxicity BOIN (MT-BOIN) and the time-to-event BOIN (TITE-BOIN).[129,133]

These model-based designs aim to reorganize the dose-finding process, reduce the number of patients exposed to suboptimal or toxic doses, and improve the

overall efficiency of early-phase Oncology trials. However, although simulation studies show that model-based methods achieve good estimations, reduce this exposure to suboptimal doses, and are efficient in finding an RPTD [62,124], these methods are not yet that broadly applied. For instance, in 2017, a review [135] of 1712 dose-finding clinical trials revealed that only 5.4% of these employed model-based strategies. This can be explained due to the model-based escalations implying some inherent challenges: they require careful planning, are time-consuming, involve a large workforce to assess real-time data during the trial, and impose biostatistical expert know-how and available software on the research site. Thus, operating with these designs is not as straightforward and easy as the classical rule-based methods. In addition, although showing better results, the model can still fail to reach the RPTD if the prior distributions for the parameters of the dose–toxicity curve are inadequate. This approach is only manageable by combining the knowledge of multidisciplinary teams, including statisticians, IT specialists, and clinicians.[62,123,136]

c. Adaptive Designs

The endpoints shift, the new dose-escalating methods, the new ways to pursue treatments - all ultimately culminate in a new way to create research protocols.

Recently, the idea of adaptive protocols has been surfacing all over clinical research, and certainly in Oncology research. Adaptive designs allow for the preplanned modifications of various aspects of the study protocol [137], such as enhancing the sample size throughout the study, facilitating dose escalation/de-escalation, stopping treatments or doses, changing the recruitment focus for a specific group of patients, modification of randomization, etc. The adjustments result from frequent interim analysis, with data being repeatedly examined to ensure the validity and integrity of the study (Figure 6).[138]

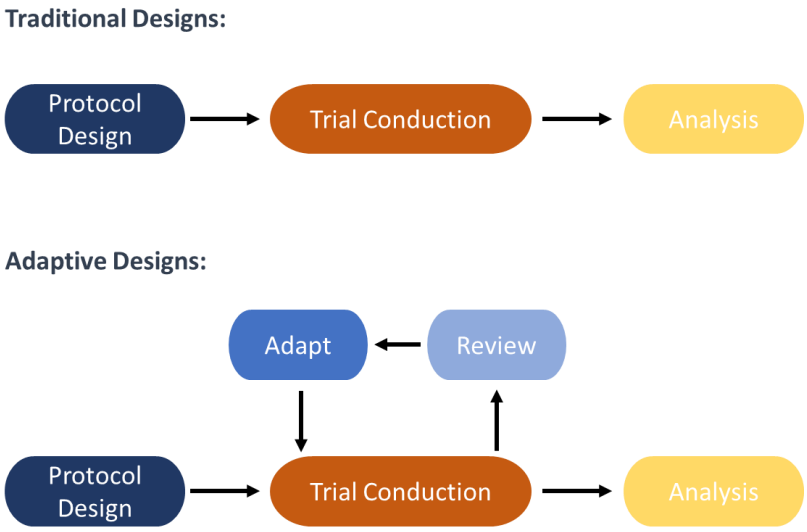

Figure 6: Differences in the clinical development process between the traditional designs and adaptive designs. Adapted from Pallmann et al.[137]

These new designs arise not only as a necessary adaptation following the dawn of precision medicine, but also to address the high attrition rates and slow bureaucratic processes that are involved in the clinical research progression, aiming to obtain accelerated approvals for new promising drugs, and improving the quality of the research itself. After all, although the Oncology research landscape is shifting, the final goal remains to improve the patient's health, care, access to treatment, and quality of life. Seamless designs and master protocols have been the key players in the Oncology research paradigm shift and are the last stop to understand what exactly is changing with the Oncology first-in-human trials.

i. Seamless Designs

Seamless designs are adaptive trial designs that work by integrating multiple phases of research, typically phases I and II, into a single, cohesive trial framework, allowing for flexibility of the protocol.[139] These seamless designs seek to establish safety and optimal dosages, while also learning about the potential activity of a novel agent, joining proof of concept with confirmation studies. The premise is that we can “seamlessly” transition between a dose-finding trial (typically phase I) and an efficacy assessment trial (typically phase II), all within the same protocol. This will therefore accelerate the evaluation of therapeutic

candidates, first by eliminating the gap (that can last up to several years) between phase I and phase II studies, but also by reducing the administrative burdens of a specific phase II submission.[36]

This idea, although appealing, raises new questions for all the clinical research stakeholders, as it affects the industry, regulatory authorities, institutional review boards (IRB), and investigators, regarding when to implement this type of trials, what additional safety monitoring is needed, and what type of statistical approach is required to ensure the validity of these data.[71]

For industries involved in drug development, seamless designs can reduce costs, minimize timelines, and improve efficiency in resource allocation. Regulatory authorities such as the FDA and the EMA have also been increasingly recognizing the value of seamless designs [72], with the FDA releasing in 2022 a Guidance for Industry first-in-human trials with dose-escalation goals that are followed by “three or more additional subject cohorts with cohort-specific objectives”. In this document, the FDA listed statistical and safety considerations for the Sponsor to “expedite the development of Oncology drugs and biologics” while respecting the safety and efficacy requirements of clinical research.[71,140]

There are, however, several challenges. The continuing evaluation and adaptability of the protocol implies more interim analyses, regular protocol amendments, and more frequent data reviews. While the goal is to lessen the administrative load, this enhances the complexity of the trial management, especially when multiple research sites are involved, as it also increases the training procedures for the study teams, the number of re-consents by the participants, and the dissemination of information among sites. Compared to conventional “sequential” trial designs, seamless designs call also for a more dynamic and adaptable approach in terms of monitoring activities. These types of studies usually require central and continuous monitoring, comprehensive risk evaluations, and adaptive decision-making based on emerging data and evolving patient status.[71,141] Although already important in traditional clinical research, it is in seamless designs of utmost importance to collaborate and communicate

closely with all the intervenients throughout the study to ensure compliance, safety, and data validity.

Based on the abstracts of the American Society of Clinical Oncology annual meetings from 2010 to 2017, during that time, 2.9% of the first-in-human trials were based on seamless designs.[71] These numbers are still low, as many sites lack the time, human resources, statistical expert knowledge, and funds to embark on the complex challenges of seamless designs. Despite that, this approach is indeed growing, and the scientific community has been working to overcome the difficulties posed by this new approach, in order to make the most of the benefits that come with this method. Overall, seamless designs are transforming the landscape of clinical trials, offering a more efficient, adaptive, cost-reducing, and accelerated approach to drug development.

ii. Master Protocols

Master protocols are another key player in this paradigm shift. Targeted therapies have brought a whole new set of tools to address cancers and tailor treatments according to the genetic characteristics of each tumor. Nevertheless, it is unfeasible to study these genetic subpopulations using conventional methods as these are highly unique patients with particular characteristics, leading to high heterogeneity and variability between them and their diseases. This leads to a scenario very similar to that of rare diseases. To target these concerns, clinical research evolved, and the master protocols emerged.

A master protocol is a single, flexible, comprehensive design developed to assess several hypotheses simultaneously. This infrastructure allows for the protocol to divide into several parallel sub-studies where characteristics such as patient selection, data collection and analysis, and study organization and management are shared between the different study arms.[142] Although some descriptions may vary, master protocols are commonly divided into basket, umbrella, and platform designs, based on characteristics of the population, studied therapies, tumor type, and design itself. [143]

Basket trials are designs where a targeted therapy is studied on multiple tumor sites (different organs, different diseases) that contain the same molecular alternations. This is, therefore, a histology-agnostic approach, where one single tumor mutation is targeted, regardless of tumor type and histology. (Figure 7. A) Umbrella trials, instead, typically focus on one tumor site/organ and study different interventions that target different mutations within that site. Usually, there are several cohorts of patients, according to the several different interventions analyzed.[144] (Figure 7. B) Both basket and umbrella designs employ a molecular screening process as the basis for patient recruitment. Platform trials, otherwise known as multi-arm, multi-stage (MAMS) trials, compare the effects of many interventions to a single control group. The main feature is that interventions can be added or removed during the trial, and that these studies can run perpetually until desired by the investigators.[142] (Figure 7. C)

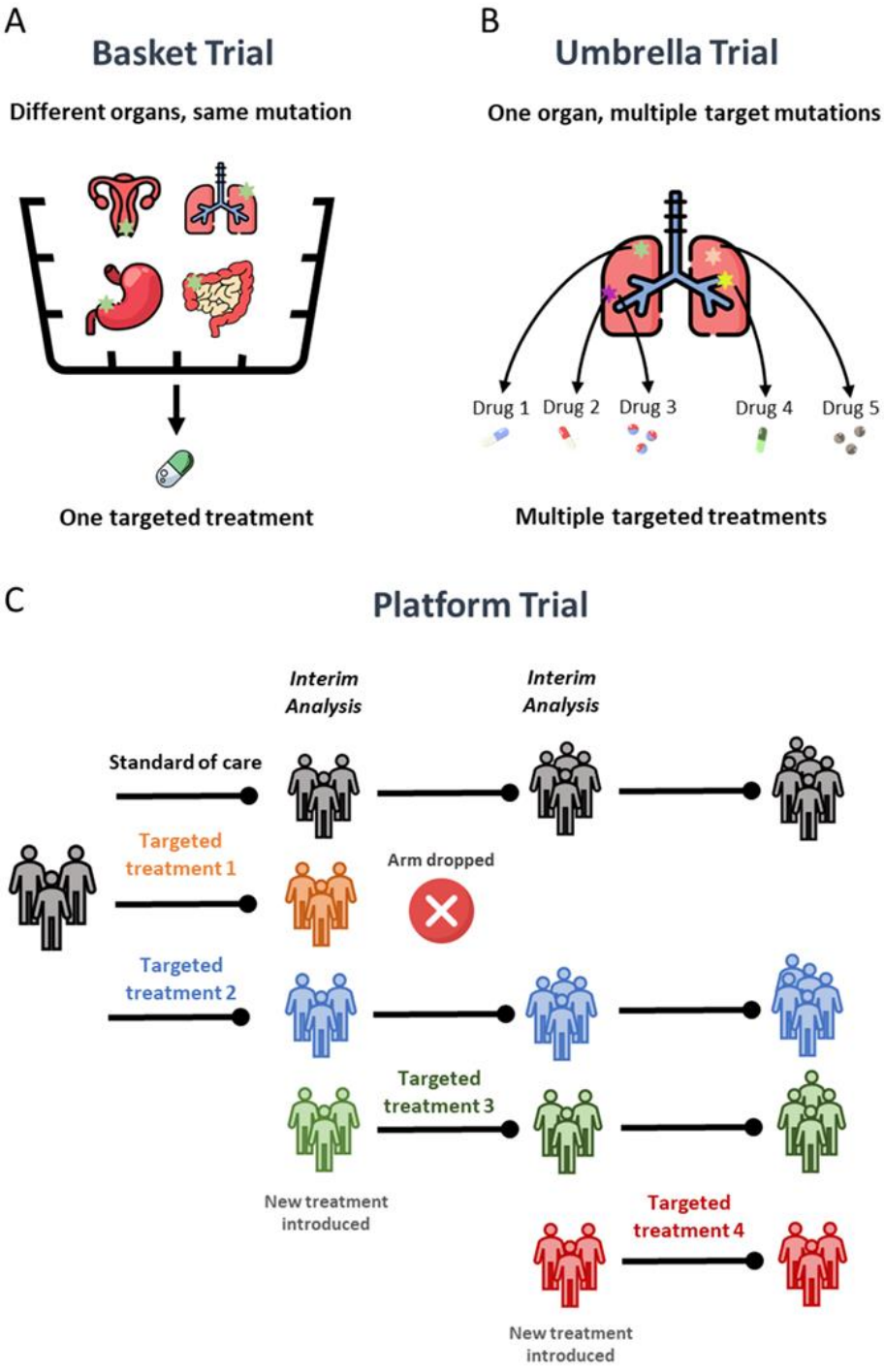

Figure 7: Overview of Master Protocol designs. A - Basket Trials. B – Umbrella Trials. C - Platform Trials. Adapted from Mansinho et al [49] and Park et al.[144] Image created using Flaticon resources.

As of today, there are several examples of trials that follow/followed a master protocol:

- ➔ The LIBRETTO-001 (NCT03157128) trial, for example, is an ongoing basket trial that started recruitment in 2017 and enrolls participants who have RET fusion-positive advanced solid tumors. This is an open-label study including multiple sites, that also encompasses two parts: a phase I study focused on dose escalation (already completed), and a phase II study on dose expansion (ongoing).[145,146]

- ➔ The BATTLE-2 Program (NCT01248247) was an umbrella study completed in 2020, designed to study targeted therapies on non-small cell lung cancers with *KRAS* mutations. The participants were separated according to *KRAS* mutation status and would enroll in one of four treatment arms (erlotinib, erlotinib + MK-2206, MK-2206 + AZD6244, or sorafenib). Prognostic and predictive biomarkers were evaluated using NGS for the tumor gene expression profiling.[147,148]

- ➔ Starting in 2010, the big I-SPY2 (NCT01042379) trial was one of the first platform trials in Oncology and is now the longest-running platform trial [149], still ongoing and having its estimated end in 2031. I-SPY 2 is a multicenter, open-label, adaptive, phase II trial with multiple experimental cohorts that evaluate new agents to treat large primary breast cancer. Over the last 14 years, 37 arms have already been studied, with more than 20 already closed.[150,151]

- ➔ Being these studies highly adaptable and flexible, there are even more complex designs that mix characteristics from the various master protocols subgroups, as is the example of the NCI-MATCH (NCT02465060) study. The NCI-MATCH trial started in 2015, aims to assess the efficacy of targeted therapies in patients with advanced refractory cancers, lymphomas, and multiple myeloma. The study encompasses umbrella and platform design characteristics, as there are several treatment arms and cohorts. Furthermore, the NCI-MATCH allowed for the removal or addition

of treatment arms along the study conduction, a typical characteristic of platform designs. With recruitment completed, NCI-MATCH counted with nearly 6000 patients submitted to screening and molecular testing, and over 1500 patients being assigned to one of 38 sub-studies, each with a therapy matched to a genomic alteration.[152,153]

Although sharing some of the challenges of seamless designs, master protocols have been rising, with a very significant increase between 2010 and 2019 (Figure 8).[142] One of the benefits of master protocols is that they avoid the enrollment of biomarker-negative patients, who would not benefit from the targeted treatment's exposure.[58] Master protocols can also result in less complexity for the sites due to the opportunity to originate designs that feature various cohorts that may include different patient populations, treatment modalities, or endpoints.[154] This adaptability enables researchers to tailor the trial design to specific research questions, clinical scenarios, and research site capabilities. Still, coordinating multiple treatment arms and patient cohorts within a single trial poses logistical challenges in terms of patient recruitment, enrollment, and data management, as well as there are still regulatory requirements concerning particularly these types of studies. Nevertheless, master protocols represent a promising approach to Oncology clinical trials, and the rapid increase in the publication of master protocols reflects their growing importance and impact in advancing cancer research.

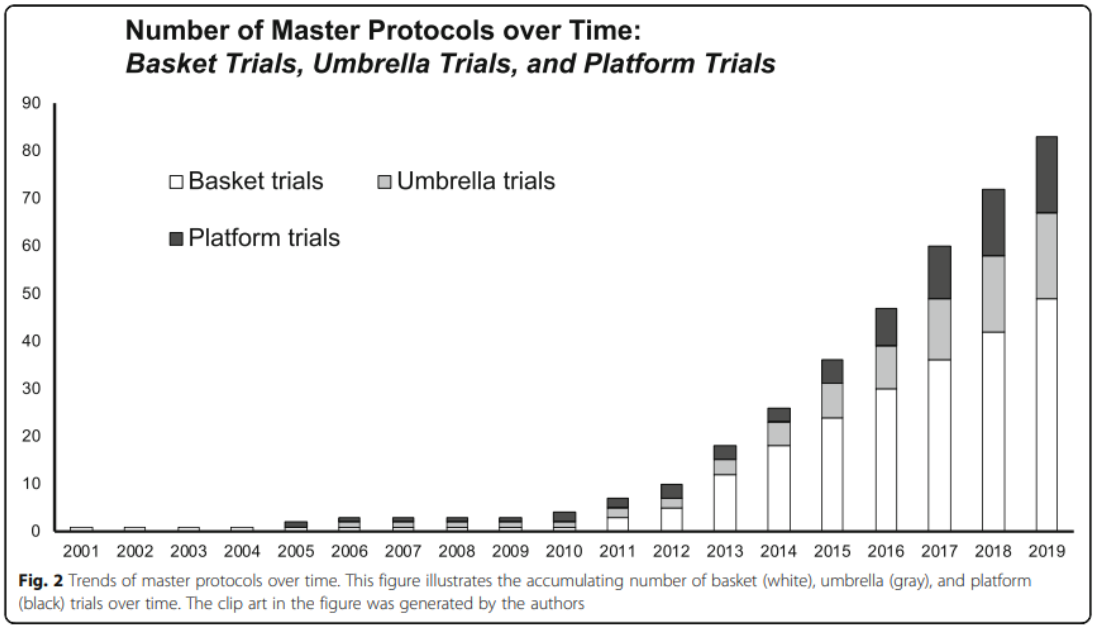

Figure 8: Evolution of Master Protocols over time (2001 – 2019). (Park et al, 2019 [142])

VI. Discussion

a. Exploring pembrolizumab and larotrectinib

In real-world practice, there are already several cases of targeted and immunotherapies that are successful, have received approval to be commercialized, and have forever changed the paradigm of Oncology clinical research. Two of these cases are pembrolizumab and larotrectinib – both exciting therapies, each with a different approach.

Pembrolizumab

Pembrolizumab (under the commercial name Keytruda) is a PD-1 inhibitor that was first approved in 2014 by the FDA, [155] and later in 2015 by EMA, for the treatment of advanced melanoma.[156] Afterwards, it was approved for other cancer indications including non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), urothelial carcinoma, and several more, being currently approved for more than 16 cancer types in the USA and 13 in Europe, and having two approvals for biomarker-defined tumors.[156,157]

Pembrolizumab was revolutionary in its course, representing the first tissue-agnostic drug approved in the USA [155,158] and Europe.[156] The regulatory journey of pembrolizumab also included an orphan drug designation, the first breakthrough therapy characterization by the FDA, and ultimately accelerated approval in the USA (see designations of regulatory terms in Annex 1).[159]

Hundreds of clinical trials have emerged of the studies with pembrolizumab across different types of tumors and stages of disease [157], each with its own design to address specific research questions and endpoints. The first of them was KEYNOTE-001 (NCT01295827), initially designed to be a dose-defining study, aiming to establish DLT and RPTD and describe PK characteristics. After the findings in the first cohort were that pembrolizumab was overall well received, with an acceptable side-effect profile and no DLTs reported [160], several expansion cohorts were initiated, adding also NSCLC indications to the study.[161] KEYNOTE-001 then resulted in a set of nested phase-2-like studies, contrary to the traditional phase I – phase II – phase III process [159], granting the study a unique evolution that led to the enrollment and treatment of 1260 patients.[162]

Besides groundbreaking, KEYNOTE-001 also served a purpose by being the pioneer in exposing what issues could arise from these types of designs: the inclusion of additional tumor sites, the rapid patient build-up in several different cohorts, and the testing of multiple hypotheses simultaneously resulted in various protocol amendments and high protocol complexity. The solid background achieved with strong preclinical studies, together with close and frequent interactions between sponsor and regulatory authorities were vital to solving the issues that arose. Building upon the success of KEYNOTE-001, the program expanded to include studies in other cancer types, including all lines of therapy, studying various stages of diseases, and later encompassing more innovative trial designs, focusing also on biomarker research.[159,163]

While KEYNOTE-001 was already innovative and portrayed characteristics that fall into the description of seamless trials, the subsequent trials further emphasized the paradigm shift in Oncology trial designs. KEYNOTE-010 (NCT01905657) and KEYNOTE-024 (NCT02142738), for instance, are examples

of biomarker-driven trials, where patient selection was based on PD-L1 expression to identify those most likely to benefit from treatment.[164,165] KEYNOTE-028 (NCT02054806) was a phase Ib open-label, multicohort basket trial [166] evaluating pembrolizumab in patients with PD-L1 positive advanced solid tumors, regardless of the tumor site, in 20 cohorts of patients. It was an exploratory study in which endpoints included the association between pembrolizumab efficacy and two inflammatory biomarkers, the T-cell–inflamed gene-expression profile (GEP) and PD-L1 expression.[167–169] The later, still recruiting, KEYNOTE-158 (NCT02628067), started in 2015 and is also a basket trial [166] that enrolls patients with various solid tumors harboring microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR), regardless of the tumor's origin, to evaluate pembrolizumab.[170,171]

Currently, 181 results can be found after searching for “KEYNOTE”, “Pembrolizumab” and “Industry” on ClinicalTrials.gov [172] proving the massive platform that was ensembled with the KEYNOTE trials, and the pioneering discovery that pembrolizumab was. Pembrolizumab is currently the most used oncologic medication for solid tumors and was considered the second best-selling drug globally after the estimated sales of the third quarter of 2021 amounted to $4.534 billion.[157]

Larotrectinib

Larotrectinib (under the commercial name Vitrakvi) is a small highly selective TRK inhibitor, used to treat solid tumors that have a fusion of the neurotrophic receptor tyrosine kinase (NTRK) gene.[173,174] Previous research had shown that the NTRK genes, which encode for TRK proteins, result in a chimeric TRK fusion protein when abnormally fused to other genes. This protein can lead to uncontrolled cellular growth and progression of tumor cells, and although considered rare, NTRK fusions occur in cancers in numerous different tissues. Larotrectinib works by blocking the action of these chimeric proteins, thus slowing down cancer proliferation.[175,176]

The regulatory journey of larotrectinib is also a remarkable accomplishment. It began with designations of orphan drug both in the USA and Europe, in 2016, and later breakthrough therapy by the FDA. In 2018, larotrectinib received accelerated approval from the FDA, and in 2019 it was granted a conditional marketing authorization and additional monitoring status by EMA, for both adults and pediatric populations (see designations of regulatory terms in Annex 1).[177]

The approvals for larotrectinib have their foundation in three open-label, single-arm, multicentric trials that followed each other, aiming to prove the safety and efficacy of the treatment: the phase I LOXO-TRK-14001, the phase II NAVIGATE trial, and the phase I/II SCOUT trials.[177,178] LOXO-TRK-14001(NCT02122913) began recruitment in 2014 and involved an initial dose escalation phase followed by an expansion phase for efficacy, in adults with solid tumors having a NTRK fusion. [179] NAVIGATE (NCT02576431) was a basket phase II study that followed LOXO-TRK-14002 and enrolled adults with locally advanced or metastatic solid tumors with confirmed NTRK gene fusion, regardless of tumor type, with the primary objective of studying larotrectinib efficacy.[180] The SCOUT trials were performed on the pediatric population and were phase I/II studies. Phase I of the study aimed to establish the safe dose of larotrectinib for children, drug absorption and metabolism, and evaluate on an initial basis the cancer response. Phase II aimed to seek out how effectively and how long various cancers reacted to treatment.[181]

It was the second time a cancer treatment based on a biomarker across different types of tumors was approved both in the USA and in Europe. The trials that led to laroctrecitnib's approvals involved groundbreaking aspects that paved the way for the new trial design in Oncology:

- The tissue-agnostic, biomarker-driven approach, that allowed for a basket design in which the participants were enrolled based on a specific mutation, regardless of tumor site and histology;

- Seamless designs in which phase I and II studies were performed sequentially within the same protocol;

- Open-label designs where both researchers and patients were aware of the treatment being received, allowing for real-time monitoring of safety and efficacy, and availability of data for interim analyses;

- The primary endpoints included not only MTD and RPTDPTD, but also overall response rate, duration of response, progression-free survival, and pharmacokinetic parameters.

Overall, these aspects enabled a thorough evaluation of the safety and efficacy of larotrectinib. The basket trial allowed for a variety of tumor types to be studied underneath a single protocol, as well as the enrolment of only patients who could benefit from the treatment, leading to a more personalized approach.

b. Challenges of the modern era of precision Oncology, and possible solutions

With the door opened by pembrolizumab and larotrectinib, several precision medicine drugs followed, each with different approaches and designs, encompassing more or less of the innovative aspects of the new clinical trial designs. The landscape of Oncology clinical research has forever changed and experienced a tremendous evolution, and the new approaches have come to solve several challenges that have arisen with precision medicine, such as patient heterogeneity, the need for accelerated marketing authorizations, and the vast economic burden of cancer care. It is noticeable by comparing the cases of the development of pembrolizumab and larotrectinib with the usual 7-10 years for approval of a new drug, that modern approaches had a faster development, quickly started being studied in other indications and populations, and therefore had smaller attrition rates comparing to the traditional methods. However, that does not mean that the modern methods did not bring some challenges with them.

Adaptive trials are versatile and efficient, yet they carry logistical and regulatory challenges and require thorough planning and solid statistical expertise. They are

time-consuming, requiring real-time analysis, constant data reviews, and ongoing monitoring.[71] Plus, they demand a significant workload from the sites and research teams, being time and training usually a pitfall at research sites. This is also evident in participant recruitment and retention: the more complex a protocol is, the more difficult it is to gather the necessary participants and ensure they remain on the trial. Since biomarkers are difficult to identify and validate, biomarker-driven research also adds obstacles with sample size, sensitivity, reproducibility, and regulatory compliance.[182]

This in no way suggests that we should abandon modern approaches to Oncology trials. Precision medicine has proven time and time again its efficacy and necessity, allowing patients worldwide to receive better care, minimize side effects, reduce healthcare costs for both patients and the healthcare systems, and overall improve quality of life. In fact, it is now a goal of the Oncology research community to overcome these inherent challenges of research advancement and develop new strategies to enhance quality and performance. Overcoming these obstacles is only possible with the cooperation of researchers, physicians, regulatory entities, and industry partners to collectively create standardized measures, support and equip study research teams, and encourage transparency and communication among all stakeholders involved. In order to improve, we must leverage the potential that cutting-edge technology like artificial intelligence, liquid biopsies, and next-generation sequencing presents us daily. Other interesting approaches include decentralized clinical trials and collaborations with patient associations, to try to lessen burden on the participants and enhance patient engagement.

In overview, the challenges encountered with novel approaches to Oncology clinical trial designs are multifaceted and require collaborative efforts, creative problem-solving, and capable teams to overcome them. By embracing personalized medicine, adaptive trial designs, and innovative endpoints, it is possible to steer around the complexities of modern Oncology clinical research.

VII. Conclusions

Clinical research itself has come a long way since RCTs were implemented. Nowadays, around the world, various governments and scientific communities have agreed on guidelines for ethical, safe, and quality clinical research, that can improve people's health and well-being in all imaginable conditions and diseases.

Oncology research, however, has a particularly interesting evolution, and the last years have seen several groundbreaking accomplishments. Precision medicine forever changed Oncology's landscape, with a clear goal to enable patients everywhere to have tailored, efficient, and rapidly accessible treatments. This brought Oncology researchers the challenging task of innovating clinical trial designs, endpoints, eligibility criteria, escalation methods and even modifying the regulatory views on these medicines, especially when it came to early-phase stages of drug development. Seamless designs that deconstruct the "one-size-fits-all" phase I – phase II – phase III clinical trials, adaptive trials that allow flexibility and real-time analysis of safety and efficacy data, master protocols that enable histology-agnostic designs, and biomarker-driven studies that allow for the enrollment of patients that will most likely benefit from a specific treatment are now part of the day-to-day of Oncology research. Although traditional approaches like chemotherapy and surgery still cover most of the cancer treatments applied today, we have seen the growth of many exciting new drugs, either in monotherapy or combined with traditional approaches, that have already revolutionized how we treat cancer. Some challenges do arise given the complexity and the different layers of these new approaches, but the collaboration of multidisciplinary teams, the oversight of regulatory entities, a strong scientific background, and the constant evolution of cutting-edge technologies can make room for this area to grow and enable the full potential of precision Oncology toward improved, personalized treatments, hopefully lessening the burden and improving the quality of life of cancer patients worldwide.

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Annex I

Table 1: Summary of designations given by the regulatory entities FDA and EMA in the context of drug approvals. Content of designations was gathered from the FDA and EMA official websites (https://www.fda.gov/ and https://www.ema.europa.eu/en/homepage, respectively)

	Designation	Regulatory Entity
Orphan Drug	Drugs intended for the treatment, diagnosis, or prevention of rare diseases or conditions, defined as those affecting fewer than 200,000 people in the United States or 5 in 10,000 people in the European Union. Drugs with orphan drug designation are eligible for incentives such as protocol assistance, access to centralized marketing authorization, fee reductions, and market exclusivity.	FDA and EMA
Breakthrough Therapy	Granted to expedite the development and review of drugs that show substantial improvement over existing therapies for serious or life-threatening conditions. Drugs with breakthrough therapy designation receive intensive guidance from the FDA and may benefit from additional interactions and support throughout the development process. This designation is based on preliminary clinical evidence demonstrating improvement in efficacy or safety compared to existing therapies.	FDA
Accelerated Approval	Expedited approval of drugs that address unmet medical needs for serious or life-threatening diseases. This pathway is designed to provide earlier access to promising therapies based on surrogate endpoints, such as laboratory measurements or other clinical markers, that are likely to predict clinical benefit. Accelerated approval comprises post-marketing requirements, including the completion of post-marketing confirmatory trials and the submission of periodic safety reports.	FDA
Conditional Marketing Authorization (CMA)	Expedited approval of medicines that address unmet medical needs for serious or life-threatening diseases. It allows for the approval of a medicine based on less comprehensive data than is normally required, provided that the benefits of immediate availability outweigh the risks of less complete data.	EMA
Additional Monitoring	Additional monitoring is granted to medicines that already have marketing approval, many times a CMA. Drugs under additional monitoring are identified by a black inverted triangle symbol alongside their names in the product information, on their packaging, and on EMA's website. The purpose is to continuously monitor the safety of these medicines by collecting and analyzing information about their safety profile.	EMA