INSTITUTO POLITÉCNICO DE LISBOA

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA

Update on mTOR signalling inhibitors – A systematic review

Beatriz Ribeiro Santos de Matos João

Advisers:

Ana Marques Ramos, PhD – Escola Superior de Tecnologia da Saúde de Lisboa

Priscila Gomes Mendes, PhD – Escola Superior de Tecnologia da Saúde de Lisboa

Master in Clinical-Laboratory Technologies

Lisbon, July 2024

INSTITUTO POLITÉCNICO DE LISBOA

ESCOLA SUPERIOR DE TECNOLOGIA DA SAÚDE DE LISBOA

Update on mTOR signalling inhibitors – A systematic review

Beatriz Ribeiro Santos de Matos João

Advisers:

Ana Marques Ramos, PhD – Escola Superior de Tecnologia da Saúde de Lisboa

Priscila Gomes Mendes, PhD – Escola Superior de Tecnologia da Saúde de Lisboa

Jury:

President: Edna Soraia Ribeiro, PhD – Escola Superior de Tecnologia da Saúde de Lisboa – ESTeSL-IPL;

Arguer: Vânia Marina Gonçalves, PhD – Instituto Nacional de Saúde Doutor Ricardo Jorge – INSA;

Master in Clinical-Laboratory Technologies

Lisbon, July 2024

Dedicated to Grandmother Teresa, Uncle Zé and Grandfather Diamantino

Acknowledgments

I would like to thank Professor Doctor Ana Marques Ramos as well as Professor Doctor Priscila Mendes, for all the guidance, availability and support during the development of this dissertation.

To my family, especially my parents and my sister, who helped me overcome the difficulties and made the process happier and lighter. Thank you for the support and unconditional love, not only during this phase of my life, but during these challenging 23 years. Without you nothing would be possible, thank you very much.

General Index

Index of Tables

Index of Figures

Abstract

Background: Currently, therapies used to treat cancer have not demonstrated sufficient effectiveness, resulting in an increase in the incidence rate as well as mortality, making it necessary to develop new approaches [1] [2] [3]. Alterations in the mTOR signalling pathway have already been identified in several types of cancer, allowing proliferation and survival of cancer cells as well as drug resistance, being the mTOR inhibitors one of the possibles answers for the treatment of malignant tumors [4] [5] [6].

Aims: In this systematic review, the objective was to clarify whether mTOR inhibitors are more effective and safer than placebo or the respectively standard therapy for the treatment of cancer in randomized clinical trials (RCT).

Methods: Articles were collected from three databases, PubMed, SCOPUS and Web of Science, those being randomized controlled trials between January 2019 and December 2023, and the results were collected, analysed and discussed evaluating the efficacy and safety of mTOR inhibitors as a treatment in cancer patients, when compared with placebo or standard therapy. This entire process was carried out through the Rayyan systematic review's web application.

Results: Ten articles were analysed, where it was possible to observe that Everolimus combined with standard therapy had the best results in terms of efficacy, and Samotolisib demonstrated to be the inhibitor with less effect in the treatment of cancer. mTORi showed fewer positive results in safety compared to standard therapy.

Conclusions: mTORi present good results in terms of efficacy, but safety is their biggest limitation. As a future perspective, studies comparing the mTORi efficacy and safety in RCT with the real-world practice results of effectiveness are necessary. Future studies should aim to apply a meta-analysis of the results, with homogenous and standardized data in terms of the disease, mTORi treatment-related parameters, and assessed outcomes. Moreover, a cost analysis of mTORi could contribute to highlight the most efficient solutions to real-world clinical practice.

Keywords

mTOR inhibitor, cancer, treatment, efficacy, safety

Resumo

Contexto: Atualmente, as terapias utilizadas para tratar o cancro não têm demonstrado eficácia suficiente, resultando num aumento da taxa de incidência e mortalidade, tornando necessário o desenvolvimento de novas abordagens [1] [2] [3]. Alterações na via de sinalização do mTOR já foram identificadas em diversos tipos de cancro, permitindo a proliferação e sobrevivência das células cancerígenas, bem como a resistência aos medicamentos, sendo os inibidores de mTOR uma das possíveis respostas para o tratamento de tumores malignos [4] [5] [6].

Objetivos: Nesta revisão sistemática, o objetivo foi clarificar se os inibidores de mTOR são mais eficazes e seguros do que o placebo ou a respetiva terapia *standard* para o tratamento do cancro em ensaios clínicos randomizados (RCT).

Métodos: Os artigos foram retirados de três bases de dados, PubMed, SCOPUS e Web of Science, sendo ensaios clínicos randomizados entre janeiro de 2019 e dezembro de 2023, e os resultados foram retirados, analisados e discutidos avaliando a eficácia e segurança dos inibidores de mTOR como tratamento em pacientes com cancro, quando comparados com placebo ou com terapia *standard*. Todo este processo foi realizado através da aplicação destinada a revisões sistemáticas, Rayyan.

Resultados: Foram analisados dez artigos, onde foi possível observar que o Everolimus combinado com a terapia *standard* apresentou os melhores resultados em termos de eficácia, e o Samotolisib demonstrou ser o inibidor com menos efeito no tratamento do cancro. Os mTORi apresentaram resultados menos positivos na segurança em comparação com a terapia *standard*.

Conclusões: Os mTORi apresentam bons resultados em termos de eficácia, mas a segurança é a sua maior limitação. Como perspetiva futura, são necessários estudos comparando a eficácia e segurança dos mTORi em ensaios clínicos randomizados com os resultados da prática real da efetividade. Estudos futuros devem ter como objetivo aplicar uma meta-análise dos resultados, com dados homogéneos e padronizados em termos da doença, parâmetros relacionados com o tratamento com mTORi e *outcomes* avaliados. Além disso, uma análise de custos dos mTORi poderia contribuir para destacar as soluções mais eficientes para a prática clínica do mundo real.

Palavras-chave

Inibidor mTOR, cancro, tratamento, eficácia, segurança

List of Acronyms and Abbreviations

4EBP1 – Eukaryotic translation initiation factor 4E-binding protein 1

AE – Adverse Events

AMPK - AMP-activated protein kinase

AR-v7 – Androgen Receptor Splice Variant

ATP – Adenosine triphosphate

AKT – protein kinase B

DNA – deoxyribonucleic acid

eIF4E – Eukaryotic translation initiation factor 4E

ER – Estrogen receptor

FKBP12 – 12-kDa FK506-binding protein

GTPase – Guanosine triphosphate-binding proteins

HER2 – Human epidermal growth factor receptor 2

HR – Hormone receptor

mLST8/Gβ1 – Target of rapamycin complex subunit LST8/ G protein beta subunit-like

mRNA – messenger Ribonucleic Acid

mSIN1 – mammalian stress-activated protein kinase (SAPK)-interacting protein 1

mTORi – mTOR inhibitors

PI3K – phosphatidylinositol 3-kinase

PSA – Prostate-specific antigen

PTEN – phosphatase and tensin homolog

RCT – Randomized clinical trials

S6K1 – ribosomal protein S6 kinase

TAK-117 – Selective PI3Kα isoform inhibitor

TSC1 – Tuberous sclerosis 1

TSC2 – Tuberous sclerosis 2

1. Framework

This dissertation is included in the master's program in Clinical-Laboratory Technologies, at the Escola Superior de Tecnologia da Saúde de Lisboa of the Instituto Politécnico de Lisboa. This is a systematic review that studies the efficacy and safety of mTOR inhibitors in the treatment of cancer patients, through randomized controlled trials. Current standard therapies, such as chemotherapy, have not demonstrated the desired results, mainly due to drug resistance [1] [2]. mTOR inhibitors can be an asset in the treatment of cancer, as they have properties favorable to the decrease of the activity of the mTOR signaling pathway, normally hyperactivated in several types of cancer, inhibiting the growth, proliferation and survival of cancer cells [3] [4]. The dissertation consists of 8 chapters: **background**, a theoretical introduction was divided in topics such as cancer, the mTOR signaling pathway, mTOR inhibitors, chemotherapy, randomized controlled trials, and the importance of new pharmacological therapies; **relevance**; **aims**, addressing the research question and research aims; **methods**, referring eligibility criteria, information sources, search strategy, selection process, data collection process, data items, risk of bias assessment, registration and reporting; **results and discussion**, where both the study selection and its results are presented, and discussion of them, regarding the study characteristics, treatment-related parameters, efficacy and safety outcomes, the results of the risk of bias are also presented and discussed; **conclusions**; **limitations and future perspectives; references**; and **appendices**.

2. Backgorund

2.1 Cancer

2.1.1 Pathogenesis

Currently, cancer is one of the diseases that continues to affect a large part of the population in all age groups, and in most cases, due to inefficiency and/or lack of treatments, increasing the probability of disease progression and consequently the death of the patient [1] [5] [6] [7] [8].

According to the World Health Organization, cancer is defined as *"... the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs...",* these abnormal cells are called cancer cells and develop from normal cells [9]. Changes in these cells are

generally derived from mutations occurring in proto-oncogenes, enabling the growth and survival of cells, becoming oncogenes, in tumor suppressor genes, allowing uncontrolled proliferation of these cells, and in DNA repair genes, allowing duplications and deletions in chromosomes [10]. This process of transformation of normal cells into cancerous ones is called carcinogenesis, and is divided into 4 phases, initiation where mutations in genes occur, promotion wherein proliferation leads to cell growth, progression where the last changes occur, both at a genetic and phenotypic level, and finally metastasis where cells spread from the initial location to other parts of the body [10] [11] (Figure 1).

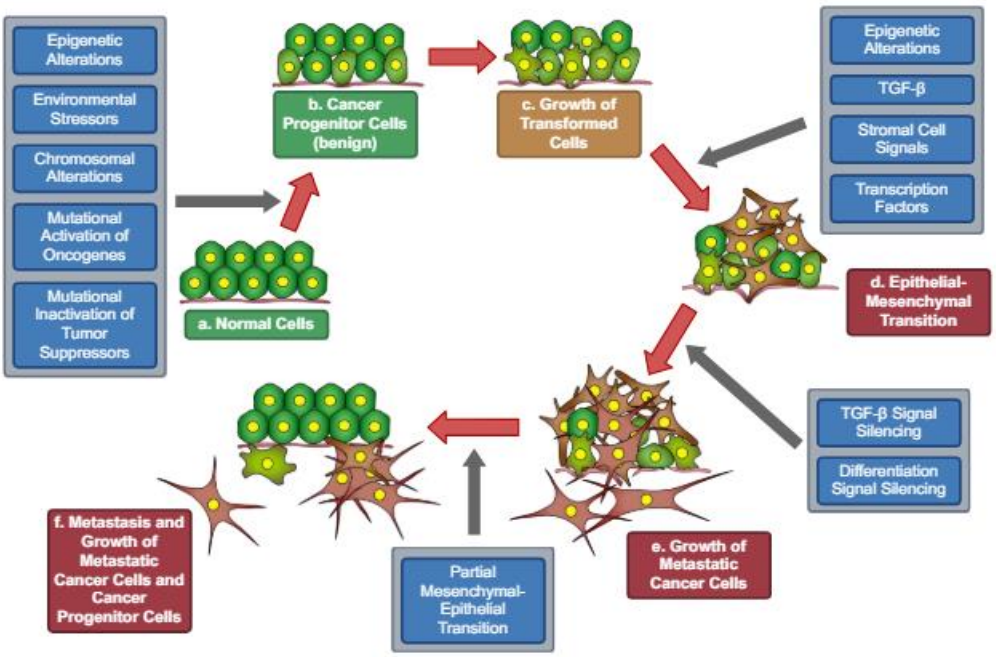

Figure 1 - *Cancer Development [10].*

Cancer cells have very different characteristics when compared to normal cells, these are defined by their ability to ignore the process of apoptosis, to multiply uncontrollably without the need for signals, to spread to any other part of the body, to ignore and deceive the immune system and also to adapt to different conditions, this being one of the most important and worrying capabilities, as its unpredictability makes treatment more difficult as a result [12]. The mechanisms that cancer cells acquire when developing a neoplasm are called Hallmarks and consist of 8 biological capabilities. **Sustaining proliferative signalling** in which cells can ignore signals, through the production of their own growth factors or by stimulating normal cells that correspond with growth factors. **Evading Growth Suppressors**, in which cells can overcome processes designed to reduce cell growth and proliferation. This happens through the inhibition of contact mechanisms, which allow the suppression of cell proliferation by cell-to-cell contact, by eliminating the tumor suppressor Merlin involved in cell adhesion, or on the

other hand by suppressing the activity of the liver kinase 1 (LKB1), making epithelial-mesenchymal transition more conducive due to the disorganization of the epithelial structure caused. This ability may also occur through the suppression of the transforming growth factor-beta (TGF-β) pathway, inhibiting its antiproliferative function. **Resisting cell death** through the loss of the tumor suppressor TP53, whose function is to lead to apoptosis in cells with irreparable DNA damage. **Enabling Replicative Immortality** occurs due to the presence of telomerase activity, normally absent in normal cells, preventing the senescence process. **Inducing Angiogenesis** in which the formation of blood vessels occurs, allowing the transport of substances essential to the survival of cells and thus their proliferation. **Activating Invasion and Metastasis** occurs thanks, for example, to the suppression of the E-cadherin protein, encoded by the cadherin.1 (CDH1) tumor suppressor gene, unbalancing cell-to-cell adhesion allowing invasion and metastasis. **Deregulating cellular energetics** where cells can alter their metabolism, and finally **Avoiding immune destruction** in which cells use proteins on their surface to prevent their destruction by the immune system [13] [14] (Figure 2).

Along with the Hallmarks characteristic of cancer, 2 additional abilities acquired as a result. **Genome Instability and Mutation** that arises given the ability of cells to carry out genetic mutations to promote tumor progression. And **Tumor-promoting inflammation** in which cells take advantage of the inflammation mechanisms arising from infections, promoting their proliferation and resistance to cell death [13] [14] (Figure 2).

One of the mechanisms through which these Hallmarks are possible is through mTOR.

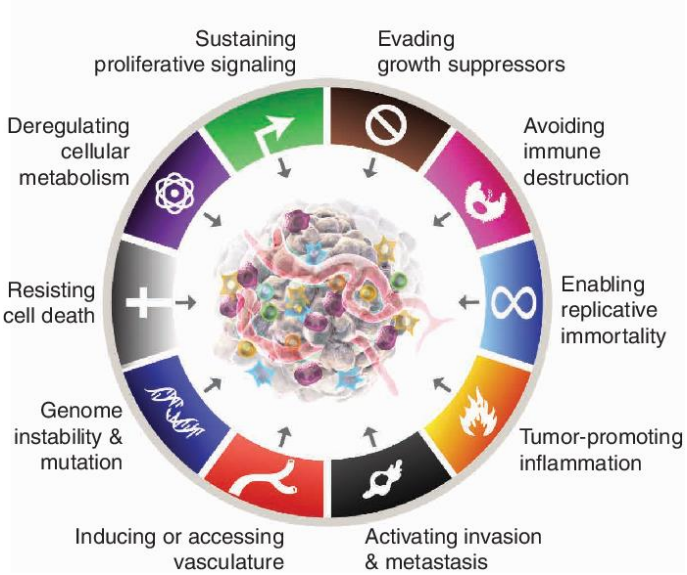

Figure 2 - *Hallmarks of Cancer (8 capabilities and 2 additional abilities). Adapted from [15].*

2.1.2 Categories of cancer

Cancer is a disease that can originate in different types of cells and is therefore classified into categories. Carcinoma, where the disease begins in epithelial tissue, such as skin cancers and adenocarcinomas [16]. Sarcoma is when the cancer starts in bone, cartilage or muscle, for example osteosarcoma and soft tissue sarcoma. Leukaemia originates from white blood cells, leukocytes, produced in the bone marrow. Lymphoma, derive from specific white blood cells, called lymphocytes, causing alterations in the lymphatic nodes, as Hodgkin and non-Hodgkin lymphomas. And Myeloma, arise from a type of immune cell, plasmacyte, causing for example IgG myeloma [16].

2.1.3 Diagnosis

There are several techniques aimed at diagnosing cancer, depending on the specific markers for each neoplasm. One of the least invasive is liquid biopsy, where blood collection allows the detection of cancer cells as well as the verification of the number of their constituents, and circulating-tumor DNA (ctDNA) analysis, which verifies the presence of mutations, epigenetic alterations, amplifications, deletions, translocations and gene fusion, through real-time PCR, digital PCR, BEAMing and NGS. Imaging tests are also a technique that helps in the diagnosis as well as in the definition of the stage of the disease (TNM) by assessing the extent of the primary tumor (T), whether the lymph nodes are affected (N), and finally whether there is evidence of metastasis (M), the most used being magnetic resonance imaging (MRI), computed tomography (CT) scan, positron emission tomography (PET), ultrasound, and X-ray. Tissue analysis obtained by biopsy is an invasive technique that allows the immunohistochemistry method to be performed, capable of detecting specific diagnostic biomarkers through the antibody-antigen binding, such as prostate-specific antigen (PSA) for prostate cancer, carcinoembryonic antigen (CEA) for colorectal cancer and breast cancer gene 1 (BRCA1) for breast cancer, as well as histology that allows the identification of the type as well as the degree of differentiation of cells [17] (Figure 3).

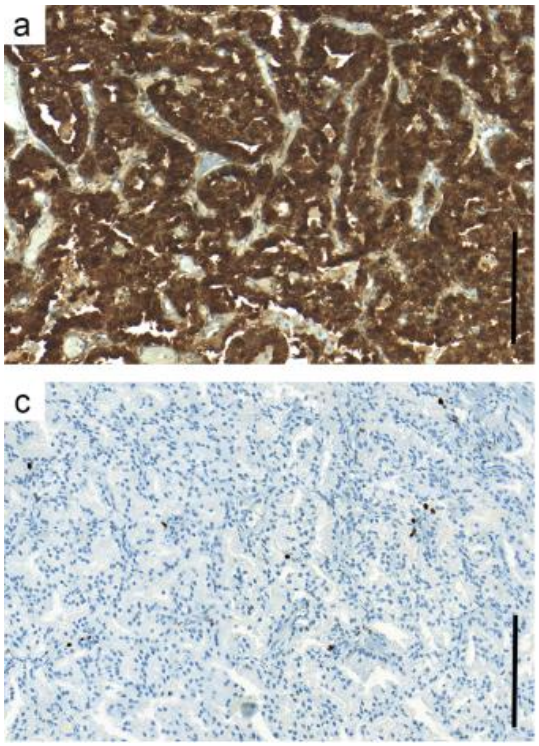

Figure 3 - *Tissues sections from two patients showing immunostaining for PSA. High PSA immunoreactivity (a) and low PSA immunoreactivity (c). Adapted from [18].*

2.1.4 Risk Factors

Cancer can be triggered by genetic characteristics, such as mutations in the TP53 gene [19], occupational, such as exposure to asbestos [20], and environmental, such as excessive UV radiation [21], as well as by lifestyles, such as tobacco use, obesity, lack of physical activity, sedentary lifestyle, poor diet and alcohol as well as infections caused by viruses, including Epstein-Barr virus, hepatitis B virus (HBV) and human papilloma virus (HPV) [22].

2.1.5 Epidemiology

Global data from 2022 indicate that more than 19 million patients were diagnosed with some type of neoplasia, with more than 9.7 million cases resulting in death, and with a prevalence (1 year) of 14.1 million of the cases [23]. Europe has an incidence of more than 4 million new cases, with a mortality percentage of 43%, and with a prevalence (1 year) of 3.5 million of the cases [24]. In Portugal, the incidence is around of 69 000 new cases, 47% of which result in death, with a prevalence (1 year) of 52 506 of the cases

[25]. Currently, the types of cancer with the highest incidence worldwide are breast, lung, colorectal, cervix uteri and thyroid cancer in females, and lung, prostate, colorectal, stomach and liver cancer in males, with the highest mortality rates are breast, lung and colorectum cancer [23] [26].

2.2 mTOR signalling pathway

The mammalian target of rapamycin, better known as mTOR is a protein discovered during a study of resistance to the immunosuppressive drug rapamycin [4] [27].

The mTOR signalling pathway has a very important role as regulates cell survival, growth and proliferation, acting on processes such as transcription, translation, ribosomal biogenesis, autophagy and actin organization [4] [28] [29] [30].

This pathway is made by two complexes, mTORC1 formed by mTOR, mTOR regulatory protein (RAPTOR), the Target of rapamycin complex subunit LST8/ G protein beta subunit-like (mLST8/GβL) and DEP domain-containing mTOR-interacting protein (DEPTOR) and the 40kDa substrate protein kinase B, AKT (PRAS40), and mTORC2 formed by mTOR, by rapamycin-insensitive comparison of mTOR (RIPTOR), mLST8/GβL, DEPTOR, PROCTOR and by mammalian stress-activated protein kinase (SAPK)-interacting protein 1 (mSIN1), a protein essential for the integrity of mTORC2 [30-35] (Figure 4).

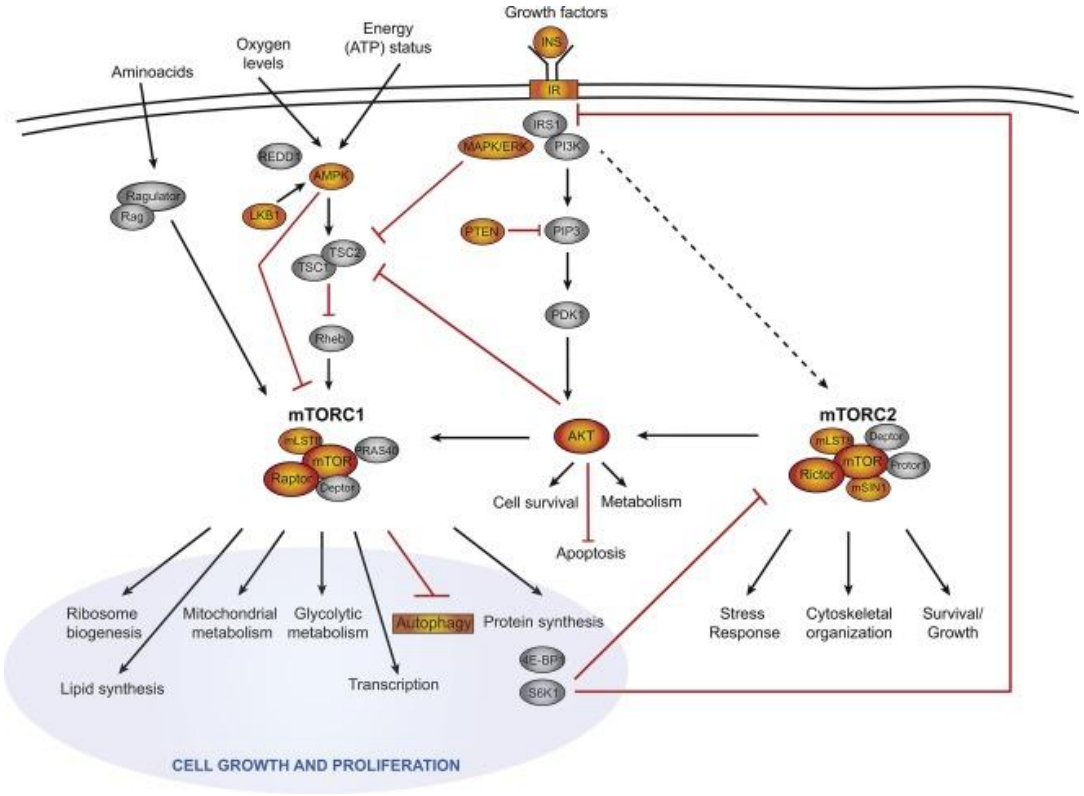

Figure 4 - *PI3K/AKT/mTOR signalling pathway [36].*

Both complexes, mTORC1 and mTORC2, regulate cell growth according to the nutrients and growth factors present, and cell survival and proliferation [32].

This signalling pathway works through signals, also called upstream regulators, that establish a connection with receptors present on the cell membrane, generally Insulin-like growth factors (IGFs), and are then mediated through the PI3K/AKT signalling pathway [31] [32] [37].

Activation of the mTORC1 complex occurs when growth factors, through phosphatidylinositol 3-kinase enzymes, cause the activation of the AKT substrate. This in turn will inactivate and phosphorylate the tuberin protein, encoded by the TSC2 gene, which allows the union of mTOR with the PI3K/AKT pathway together with TSC1, stimulating the Guanosine triphosphate-binding protein (GTPase) activity of the Ras homologue-enriched in brain protein (Rheb), establishing a direct connection with mTOR. mTOR phosphorylates eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), allowing the initiation factor, eIF4E, to trigger translation. This also requires the phosphorylation of ribosomal protein S6 kinase (S6K1), as it promotes the translation of mRNA [28] [30] [33]. Amino acids, who are sensored by Rag GTPases, have an important role in the regulation and activation of the mTOR complex 1. Stress is another upstream regulator, but in this case, it acts in the inhibition of mTORC1. Low levels of oxygen (hypoxia) and energy increase the expression of the regulated in development and DNA damage-response 1 protein (REDD1), activating AMPK through the LKB1-AMPK complex, enhancing GTPase activity of the Rheb protein resulting in the inhibition of the mTORC1. DNA damage inhibits the expression of this complex through the signalling of the AMPK-TSC2, caused by activation of p53 [37]. Inflammation is a positive regulator of mTORC1, through the connection between the tumor necrosis factor (TNF) and TSC1-TSC2 proteins [33] [35] [38].

mTORC2 is, in turn, the complex with the least knowledge about its functioning. The growth factors also regulate this complex, being activated when the expression of mSIN1, which has inhibitory characteristics when insulin is not present, is decreased through the connection of the insulin receptor substrate 1 and the PI3K. The GTPases Ras can also increase the expression of this complex. It is also believed that the activation can occur due to the phosphorylation of the mTOR and Rictor caused by AMPK, when there are low energy levels [28] [32] [33] [35] [37] [38]. Until now, the response to

stress signals, such as hypoxia and DNA damage, has only been verified in the mTORC1 complex, and further studies are needed to confirm the possible similarity in the functioning of the two complexes [37].

On the other hand, the two complexes can influence each other's expression. mTORC1 can inhibit the mTORC2 by blocking the insulin receptor substrate 1, letting mSIN1 decrease the expression of the complex, as well as mTORC2 can activate the mTOR complex 1 through the phosphorylation of the AKT [33] [35].

When deregulation of the mTOR signalling pathway occurs, it is related to mutations in proto-oncogenes or the inhibition of tumor suppressor genes present in this pathway, hence its presence in most of the cancer hallmarks [4]. Alteration of the mTOR signal can occur through the overexpression of signals such as growth factors, which amplify the activity of the PI3K/AKT signalling pathway, hyperactivation of the proto-oncogenes S6K1, 4EBP1 and Eukaryotic translation initiation factor 4E (eIF4e), which contributes to cellular transformation and alters translation, mutation of the AKT proto-oncogene, leading to aberrant expression of mTORC1, thus altering processes such as apoptosis and loss of the phosphatase and tensin homolog (PTEN) tumor suppressor gene, promoting tumor progression [4] [28] .

All these factors reveal that the mTOR signalling pathway is related to the survival and proliferation of cancer cells, promoting the disease. Mechanisms for inhibiting this pathway in patients with abnormal expressions of this protein prove to be an added value in the treatment of the disease, isolated or in combination with standard treatment [31] [37].

2.3 mTOR inhibitors

The mTOR inhibitors can block the mTOR by binding to important constituents in the signaling pathway activity. There are three types of mTOR inhibitors, 1st, 2nd and 3rd generation, which act in different ways [39] (Figure 5).

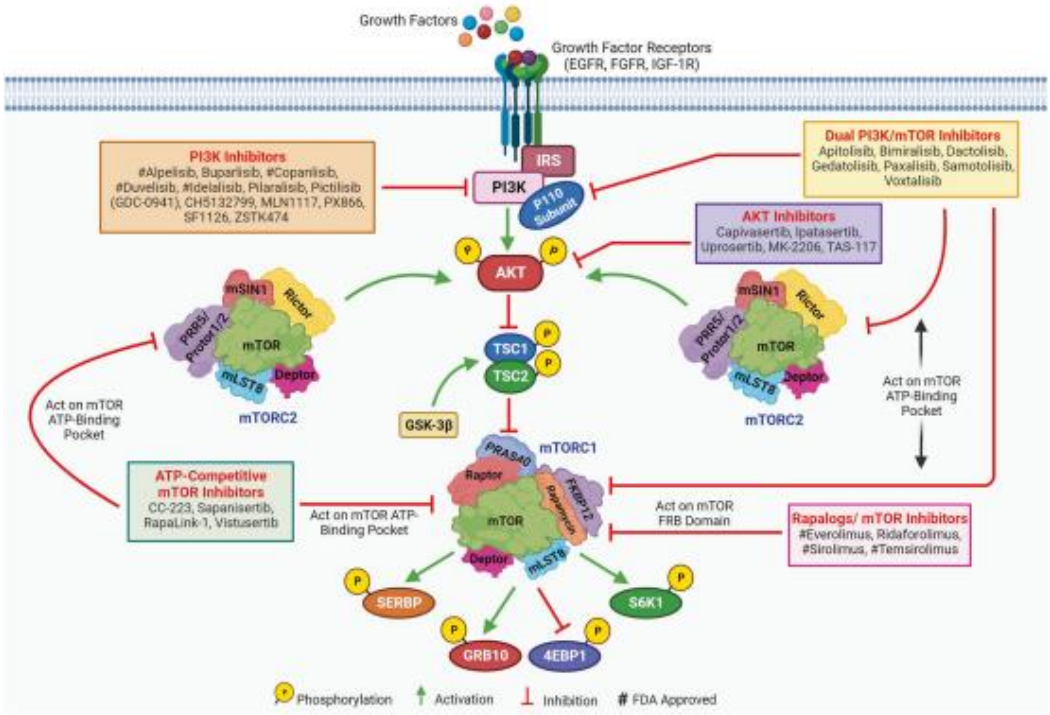

Figure 5 - *Pharmacological targeting of the mTOR signalling cascade in human malignancies [5].*

2.3.1 1st Generation

Rapamycin is a 1st generation mTOR inhibitor, also known as rapamune or sirolimus, it is a drug initially known only for its immunosuppressive properties, acting to inhibit B and T lymphocytes, but over time it was possible to realize its usefulness in inhibition of the mTOR signalling pathway [28] [40]. It acts mainly on one of the mTOR complexes, mTORC1, inhibiting some functions of this complex, making the proliferation of cancer cells impossible and causing their apoptosis. It is believed that long-term exposure to this drug may also inhibit the second complex. Due to problems in solubility and pharmacokinetic properties, other inhibitors called rapalogs were developed from rapamycin, with more advantageous characteristics and the ability to act directly on the activated mTOR kinase [31] [40] [41] [42]. These inhibitors attach to FKBP12, an intracellular binding protein, to bind to mTOR and initiate its inhibition through decreasing the phosphorylation of 4EBP1 and S6K1, inhibiting the PI3K/AKT signalling pathway. These mechanisms affect translation, and as consequence the cell cycle, proliferation, survival and angiogenesis [28] [31] [40] [41]. Rapalogs directly affect mTORC1 but indirectly mTORC2, since the binding of these inhibitors with FKBP12 can retain metabolic substances necessary for this complex [28] [40].

The best-known rapalogs are Temsirolimus, Everolimus, Ridaforolimus, Umirolimus and Zotarolimus [42].

Although these inhibitors present many advantages, there are always some associated limitations. Due to the unpredictability of cancer cells, adaptation through other signalling pathways is something to consider. Since rapalogs directly inhibit only one of the complexes, cells can therefore resort to mTORC2. This also happens, interestingly, as the total loss of mTORC1 activity causes an increase in signalling of the PI3K/AKT pathway, acting to promote the tumor [5] [31] [41].

2.3.2 2nd Generation

There are also 2nd generation inhibitors, divided into two types.

ATP-competitive mTOR kinase inhibitors (TKI's) bind directly to the ATP-binding site, managing to completely inhibit both complexes. They present better efficacy when compared with rapalogs in cell proliferation and survival, since total inhibition prevents excessive PI3K signalling, and in turn reduces the probability of a feedback loop [32] [40] [42].

This excessive inhibition may represent a limitation, as the loss of B and T lymphocytes can promote tumor development, as well as affect normal cells. The toxicity associated with these inhibitors raises doubts about their use as a treatment [41].

One of the causes of alterations in the mTOR signalling pathway is the overexpression of PI3K, following which mTOR/PI3K dual inhibitors (TPdIs) emerge. These 2nd generation inhibitors in addition to inhibiting both mTOR complexes, they also completely block the PI3K pathway [31] [41] [42].

The total blockade of mTOR plus the inhibition of PI3K signalling, can lead to an increase in adverse events, which may involve glucose metabolism, ultimately damaging other systems unrelated to the disease [31] [41].

2.3.3 3rd Generation

3rd generation inhibitors are still a little unknown, but they have been demonstrating that they could be the future. The best known are called RapaLinks, these consist of the combination of two types of inhibitors, rapamycin and ATP-competitive

mTOR inhibitors, linked by carbon chains, the “linker” [40] [42]. This connection allows a single inhibitor to present the advantages of its two constituents, being able to attach to FKBP12 and bind to mTOR, while binding to the ATP-binding site of mTOR, completely inhibiting the two complexes [40] [42] (Figure 6).

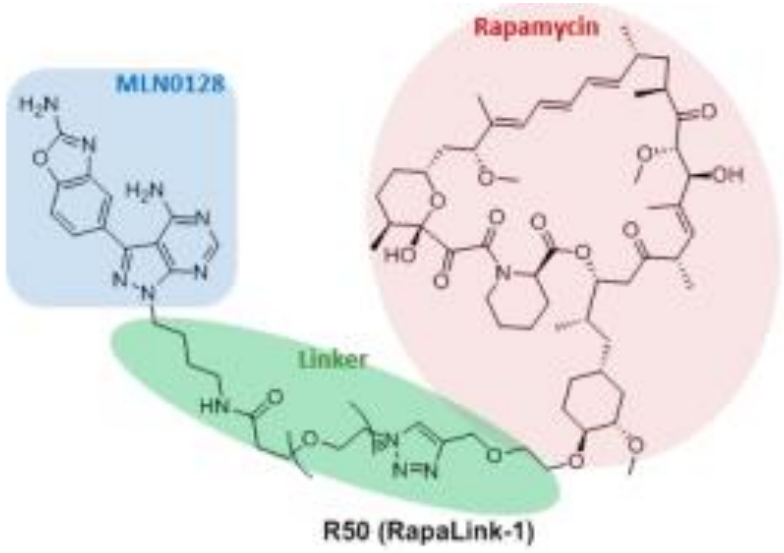

Figure 6 - *Structures of dual binding site inhibitor RapaLink-1 [39].*

2.3.4 Limitations of mTORi

These inhibitors, despite having several advantages, are also associated with some limitations. Cancer resistance to these agents can occur by activation of the pathway through mTORC2, mutations in the FRB domain, mutations in the mTOR kinase domain, changes in the eIF4E/4E-BP ratio, alteration in cell metabolism as well as negative suppression of the feedback loop through other signaling pathways [43].

Adverse events of various types of severity and not present in chemotherapy, such as metabolic toxicities, hyperglycemia, hyperlipidemia, and hypophosphatemia, are also associated with the use of mTOR inhibitors [44] [45].

2.3.5 mTOR inhibitors in cancer treatment

There are already some mTOR inhibitors approved as cancer treatments. In the United States of America, Sirolimus was the first inhibitor approved for organ transplants in 1999 [5] [40]. Everolimus (1st generation) is approved in renal cell carcinomas (2009), breast carcinomas (2012) and in neuroendocrine tumors (2016) [5] [40]. This inhibitor is

being tested alone in phase I trials to prostate cancer and combined with standard therapies in phase II trials to endometrial and kidney cancer [5] [40]. In Portugal, Everolimus is used in the treatment of renal cell carcinomas, neuroendocrine tumors, pancreatic neuroendocrine tumors and positive human epidermal growth factor receptor 2 (HER2+) breast cancer [46]. Temsirolimus (1st generation) use in renal cell carcinomas (USA and Portugal) and in mantle cells lymphoma (Portugal) is approved since 2007 [40] [47], and is being tested for relapsed acute lymphocytic leukemia (phase I trial), recurrent ovarian and endometrial cancer (phase I trial) and for mestastatic castration-resistant prostate cancer [5] [40] . Ridaforolimus (1st generation) is approved for sarcoma since 2011 (USA) and is being tested for hematologic malignancies (phase II), endometrial and breast cancer (phase II trials) [5] [40]. Vistusertib (2nd generation- mTORC1/mTORC2 inhibitor) is in clinical trials for estrogen receptor (ER) positive breast cancer, as well as ovarian cancer and lymphoma [5] [40]. Sapanisertib (2nd generation-mTORC1/mTORC2 inhibitor) is also still being tested for thyroid and endometrial cancer, renal cell carcinoma, bladder cancer and sarcoma [5] [40]. Samotolisib (2nd generation dual PI3K/mTOR inhibitor) is being tested for mestastatic castration-resistant prostate cancer (phase II trial) [5] [40]. Alpelisib, a PI3K inhibitor, was approved in 2020 for positive hormone receptor (HR+) breast cancer (Portugal) [48] and was also approved in 2022 for the treatment of breast cancer (USA) [49].

2.4 Chemotherapy

Currently, chemotherapy is one of the most widely used therapies in the treatment of cancer. Its objective is to eliminate cancer cells as well as to block cell proliferation, through different mechanisms dependent on the type of drug administered. They can act in the breakdown of DNA, through its destabilization, in the inhibition of DNA and RNA as well as their enzymes, or in the blocking of cell division. They can be classified as alkylating agents, antimetabolites, antimicrotubular agents and antibiotics [50].

Resistance to this therapy can happen due to the heterogeneity associated with these cells or through the production of *p*-glycoprotein, which ends up eliminating the treatment administered. Adverse events are also present, most of which are nausea, vomiting, mucositis, fatigue, diarrhea, constipation and neurotoxicity, although there are medications to control them *a posteriori* [51].

2.5 Randomized Controlled Trials

Randomized controlled trials (RCT) are prospective longitudinal studies, where participants are randomized into two groups, the intervention where they receive the new treatment, and the control where they receive the standard treatment of the pathology under study or placebo [52] (Figure 7).

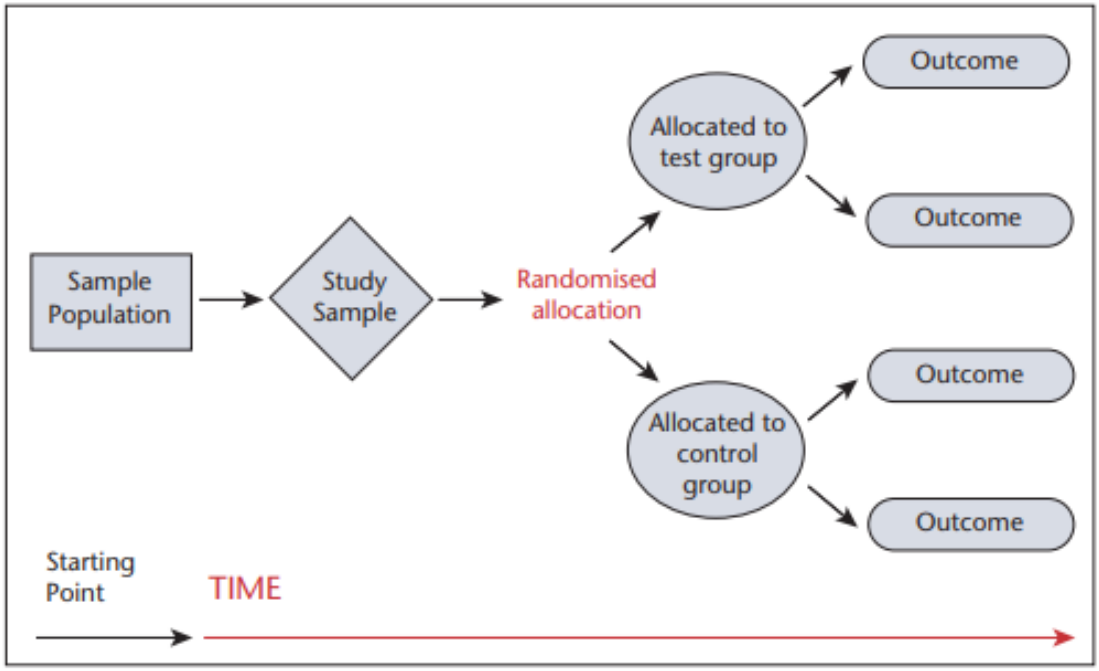

Figure 7 - *Diagrammatic representation of a Randomized controlled trial [52].*

This type of study allows comparing the outcomes and thus evaluating the effectiveness of the new intervention by answering a specific research question [52]. Withdrawal of participants, loss to follow-up, costs, and ethical questions such as research-practice distinction, consent, disclosure, and oversight may be some limitations of RCTs [52] [53].

2.6 Importance of new pharmacological therapies

These innovations are extremely important as the standard therapies currently used, mostly chemotherapy and radiotherapy, do not provide the desired results. In fact, a study predicts that within 50 years (2020-2070), if new therapies with a higher percentage of efficacy than the current ones are not developed, the increase in premature death will be mainly due to cancer [54]. Estimates predict increases between 53% and 400% in the number of new cases, in countries with very high and low human

development indices (HDI), respectively, reaching a global incidence of around 34 million in 2070 [54] (Figure 8).

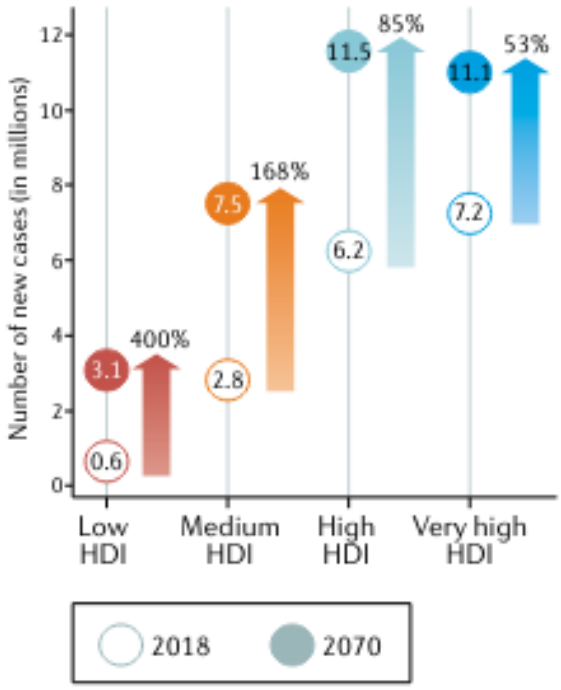

Figure 8 - *Changes in new cancer cases between 2018 and 2070 [54].*

In addition to efficacy, there is another factor associated with current therapies that needs to be overcome: costs. The economic burden associated with this disease is a topic that is not very valued, but it worsens the experience of the disease [55] [56]. A study revealed that in 2018 Europe will have spent around €199 billion on cancer care, with €70 million productivity loss, caused by mortality and morbidity [57].

The lack of desired response to standard therapies for the treatment of cancer, together with the presence of adverse events, thus reflect low effectiveness as well as low safety profiles of current pharmacological treatments [1] [6] [8].

All these factors together make the development of new therapies urgent and fundamental for the progress of cancer treatment. Given that mTOR has critical roles in tumor progression, as previously mentioned, mTOR inhibitors are promising in cancer therapy. Several mTOR inhibitors have been approved to treat human cancer and many more are being evaluated in preclinical and clinical studies.

3. Relevance

Currently, the standard therapies used to treat cancer have not demonstrated sufficient effectiveness, as literature indicates that chemotherapy has a response rate of 11.9%, estimating that between 2022 and 2045 the number of new cases as well as the number of deaths, will rise from 20 million to 32.6 million and from 9.74 million to 16.9 million [1] [6]. 90% of chemotherapy failures is due to drug resistance, by mechanisms such as genetic alterations, epigenetic modifications, altered drug metabolism and activation of survival pathways, making it necessary to develop new approaches to overcome this problem [7] [8].

Alterations in the mTOR signalling pathway have already been identified in several types of cancer, allowing proliferation and survival of cancer cells as well as drug resistance, and specific targeted therapies for these mutations are essential [1] [4] [6]. mTOR inhibitors (mTORi) inhibit cell proliferation and induce apoptosis, with the aim of blocking the signalling pathway [4] [5] [58]. The action of these drugs has immense potential, both in immunosuppression and in the treatment of malignant tumors [4] [5] [58]. According to the literature, the properties of mTORi identified in preclinical studies have led to the development of clinical trials for different types of cancer, to verify the efficacy and effectiveness of the inhibitors as a treatment [4] [5].

This systematic review will allow to synthesize the most recent mTORi considered in randomized clinical trials, using updated information to evaluate the efficacy and safety of these drugs in cancer treatment, thus contributing to a better understanding of new potential approaches.

4. Aims

4.1 Research Question

To define a structed research question, the acronym PICOS was used (**Table 1**).

Table 1 – *PICO(S) of the research question:*

Population	Cancer patients
Intervention	Administration of mTORi, isolated or in combination
Comparator	Placebo or standard therapy
Outcomes	Efficacy and safety of mTORi in the treatment of cancer
Study Design	Randomized clinical trials

Therefore, this study proposes to answer the following research question: “Are mTOR inhibitors effective and safe in cancer patients in randomized controlled trials for the treatment of cancer, when compared to placebo or standard therapy?”.

4.2 Research Aims

The present project aims to clarify whether mTOR inhibitors are more effective and safer than placebo or the respectively standard therapy for the treatment of cancer in randomized clinical trials.

To attain the major goal, there are specific aims to be taken in account, such as: collect, analyse and discuss the results from randomized controlled trials that include the inhibition of mTOR in cancer treatment, evaluating 1) efficacy, including overall survival, overall response rate, partial response, stable disease, progression-free survival and time to progression, and 2) safety, including adverse events.

5. Methods

The present systematic review was developed considering the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA 2020) guidelines [59].

5.1 Eligibility criteria

The studies were selected according to the following **inclusion criteria**: original articles, studies reporting efficacy and safety outcomes of mTOR inhibitors (mTORi) in cancer treatment patients; studies comparing mTORi with standard treatment or placebo; and only randomized clinical trials will be included. Regarding the **exclusion criteria**, the following were considered: studies including other comorbilities; studies that did not refer mTOR inhibitors as cancer treatment; in vitro and in vivo preclinical studies; review articles, expert opinions, book chapters; and studies prior to 2019.

5.2 Information sources and Search strategy

This review used the biomedical electronic databases MEDLINE, via the PubMed platform, SCOPUS, and Web of Science to develop a highly sensitive search strategy to identify and select eligible studies. The search was only limited by the publication date, namely between January 2019 to December 2023, since there were already some systematic reviews related to mTOR inhibitors prior to 2019. A comprehensive research expression was developed using descriptors related to three terms (Cancer, mTOR inhibitors, and clinical trials) and their synonyms combined with Boolean operators “AND” and “OR” to identify and select the eligible studies. In addition, the keywords were inserted in the MeSH Database to find the MeSH term. **Appendix A** presents the search strategy developed for each database.

5.3 Selection Process

The retrieved articles were exported to a Systematic Reviews Web Application (Rayyan QCRI, Cambridge, MA, USA). After the exclusion of duplicates, both titles and abstracts of retrieved studies were analysed to select potentially eligible articles. Full text of potentially eligible articles was assessed, and eligibility criteria were applied

throughout this process to decide whether a study is included or excluded. The selection process, including the reasons for excluding studies, was presented as a PRISMA flow diagram

5.4 Data Collection Process

Data was extracted from each included studies, which considered article identification (first author's name and year of publication); population, disease and treatment-related parameters; and efficacy and safety outcomes. In each included study, information was collected from the text, tables, and/or graphs and inserted in a customized data extraction document Excel software (Microsoft, Redmond, WA, USA) by the same reviewer. The included studies were descriptively analysed and presented in a tabular format.

5.5 Data Items

During the process of data collection, several parameters were considered, which were extracted and organized: Population, data of interest to be extracted were related to country, sample size, gender, age, and cancer type were considered; intervention, isolated mTORi administration or in combination with standard therapy, including dose and frequency and duration of the treatment; comparator, placebo or standard therapy; outcomes of efficacy, such as overall survival, overall response rate, partial response, stable disease, progression-free survival and time to progression; Outcomes of safety, such as adverse drug reactions; and study Design: randomized clinical trial, including phase and experimental groups.

5.6 Risk of Bias Assessment

All the selected studies were assessed to evaluate their methodological quality and potential for risk of bias. To evaluate the quality and risk of bias in randomized controlled trials, the Risk of Bias 2 (RoB 2) checklist was used [60]. We derived domain-specific and overall quality grading for each study as follows: low risk (green), some concerns (yellow), and high risk of bias (red) using ROBVIS tool [61].

5.7 Registration and Reporting

The protocol for this systematic review was submitted and registered in the International Prospective Register of Systematic Reviews - PROSPERO.

6. Results and Discussion

6.1 Study Selection

Through the established search expression, 4886 articles were obtained from the three platforms, MEDLINE (via PubMed), Scopus and Web of Science. After removing duplicates, the eligibility criteria were applied, as previously described, resulting in 10 final articles. This entire process as well as the reasons for exclusion are described and presented in **Figure 9**.

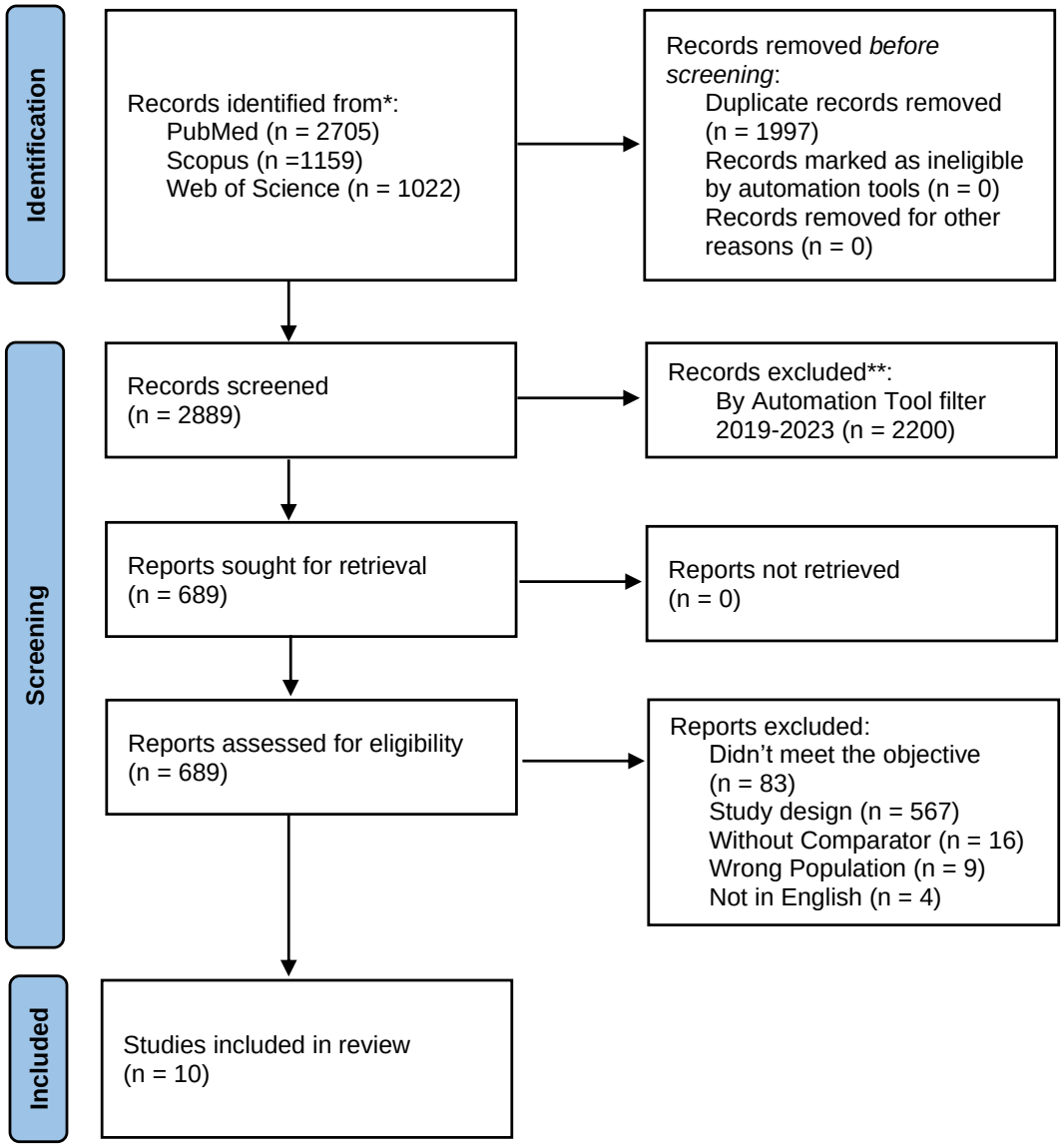

Figure 9 - *PRISMA 2020 flow diagram representing the selection process [59]*

6.2 Study Characteristics

The characteristics of each article included this systematic review, are presented in **Table 2**.

It is possible to verify that seven (70%) are phase II clinical trials, two (20%) carry out phase I and II studies, and one (10%) is a phase III study. All trials have a relevant number of participants, with article [71] being the one with the largest number (1499 participants), thus making its results more robust and reliable. Three articles present large differences in the distribution of genders, male and female, a characteristic that can alter the interpretation of the results [63] [70] [71]. Only two articles include underage participants [62] [64]. The types of cancer included are rhabdomyosarcoma [62], head and neck squamous cell carcinoma [63], fibrolamellar carcinoma [64], breast cancer [65], endometrial carcinoma [66] [69], pancreatic neuroendocrine tumors [67], prostate cancer [68] and renal cell carcinoma [70] [71], all in advanced, metastatic or recurrent stages, except one article which does not mention the stage of the disease in which the participants are [62].

Table 2 –*General characteristics of each randomized clinical trial.*

Authors, year [Reference]	RCT-related parameters			Participants-related Parameters			
	Country	**Phase**	**Sample size (N)**	**Gender** Male (%) Female (%)	**Age**	**Cancer Type**	**Stage**
Mascarenhas L, et al. 2019 [62]	USA	II	86	M (52,3) F (47,7)	<30 years	Rhabdomyosarcoma	-
Seiwert TY et al., 2020 [63]	USA	II	80	M (85) F (15)	36-83 years	Head and neck squamous cell carcinoma	Recurrent or metastatic
El Dika I et al., 2020 [64]	England	II	28	M (50) F (50)	14-41 years	Fibrolamellar carcinoma	Metastatic/ advanced
Fan Y et al., 2021 [65]	USA	II	199	F (100)	44 years (mean)	Breast cancer	Advanced
Slomovitz BM et al., 2022 [66]	USA	II	74	F (100)	30-89 years	Endometrial cancer	Stage III or IV
Kulke MH et al., 2022 [67]	England	II	150	M (56) F (44)	21-86 years	Pancreatic neuroendocrine tumors	Advanced
Sweeney CJ et al., 2022 [68]	USA	Ib/II	129	M (100)	45-91 years	Prostate cancer	Metastatic Castration-Resistant
Heudel P et al., 2022 [69]	USA	I/II	73	F (100)	37-88 years	Endometrial cancer	Metastatic or recurrent
Choueiri TK et al., 2022 [70]	England	II	95	M (76) F (24)	35-81 years	Renal cell carcinoma	Advanced (II, III, IV)
Ryan CW et al., 2023 [71]	England	III	1499	M (69.5) F (30.5)	50-66 years	Renal cell carcinoma	Intermediate-high and very high risk of recurrence

6.3 Treatment-related parameters

The **Table 3** describes the treatment and its characteristics for each article, with information regarding the mTOR inhibitor used, as well as whether it was combined with another therapy or isolated, as well as the comparator, doses, frequency and duration.

The articles in this case present some differences, as expected. Around seven articles (70%) studied 1st generation mTOR inhibitors, the rapalogs Temsirolimus and Everolimus, two (20%) studied ATP-competitive mTOR inhibitors (TKIs), Samotolisib and Vistusertib and finally one (10%) studied two mTOR inhibitors, Everolimus and Sapanisertib, rapalog and TKI respectively. The inhibitors present differences in their mechanisms of action and their inhibition targets, therefore, data will be aggregated to elucidate their efficacy and safety.

Most articles present two treatment regimes, apart from two articles that present three [64] [70]. Three articles have as intervention mTORi combined with standard therapies and control with the same standard therapies [62] [65] [66] [69], while two articles have as intervention mTORi isolated and as control the same mTORi combined with standard therapies [63] [67]. One article ends up providing a more complete analysis comparing the mTOR inhibitor isolated with its combination and with standard therapy alone [64]. One article compares two types of mTOR inhibitors individually, Everolimus and Sapanisertib, also comparing with Sapanisertib plus TAK-117, an inhibitor of the PI3K signalling pathway [70]. Finally, two articles use placebo as a control, in one the comparison is between mTORi and placebo, but both combined with standard therapy [68], while the other compares mTORi with placebo, without using other types of treatment [71].

Administration-related parameters are also essential to address because they can compromise the patient adherence to a treatment [72]. In two articles, both with Temsirolimus as intervention, the administration of the drugs is intravenous, and in the remaining eight the administration is oral. The most advantageous **frequency** of administration is present in one article, where the mTOR inhibitor is taken only once while the standard therapy once a week [62], in six articles the frequency of medication is higher for mTOR inhibitors compared to standard therapies [64] [65] [66] [67] [68] [71], in one article the mTOR inhibitor Vistusertib has a lower frequency, twice daily on two days per week, when compared to standard therapy, daily [69], and finally an article demonstrates that the mTOR inhibitor Sapanisertib has a lower frequency, weekly, when compared to its combination with TAK-117 and the mTOR inhibitor Everolimus, three times a week and daily respectively [70]. The shortest **duration** of treatment is verified in one article, 3.0-3.5 cycles [70], the longest is 444 cycles for the mTOR inhibitor and 370 cycles for

standard therapy [66], the remaining articles present durations of 8.4 months cycles [62], 48 weeks [69], 54 weeks [71] and 21 months [67]. Three articles do not refer the exact duration, only the information that each cycle is 28 days [63] [65] [68], and finally one article does not provide information regarding the duration [64].

These results demonstrate that the oral administration is used in the majority of the randomized controlled trials, representing an advantage since the literature shows that most cancer patients prefer to take a tablet instead of an intravenous drug, thus incresing the patient adherence to the treatment [72]. The increase in the frequency of administration when using mTOR inhibitors is a disadvantage since the literature relates the increase in frequency with the decrease in adherence to treatment [72].

The heterogeneity of data between articles demonstrates that in the future it would be relevant to carry out more clinical trials with similar characteristics in terms of population, type of cancer, intervention, mTOR inhibitor, and comparator. This would allow us to perform the analysis of homogeneous data using statistical techniques to obtain a quantitative estimate of the overall effects of mTORi in cancer treatment.

Table 3 - *Treatment allocated in each randomized controlled trial.*

Authors, year [Reference]	mTORi (Drug)	Experimental Groups	Sample size (N)	Administration-related parameters		
				Dose	Frequency	Duration
Mascarenhas L, et al. 2019 [62]	Temsirolimus	Bevacizumab + Vinorelbine + Cyclophosphamide	44	15 mg/kg +25mg/m²+ 1.200mg/m² (IV)	Once + weekly	8.4 months
		Temsirolimus + Vinorelbine + Cyclophosphamide	42	15mg/m²+25mg/m²+1.200mg/m² (IV)	Weekly	
Seiwert T et al., 2020 [63]	Temsirolimus	Temsirolimus + Cetuximab	40	25mg + 250mg/m² (IV)	Weekly	Cycles of 28days until disease progression or intercurrent illness
		Temsirolimus	40	25mg (IV)		
El Dika I et al., 2020 [64]	Everolimus	Everolimus	9	7.5mg (oral)	Daily	-
		Letrozole + Leuprolide	9	2.5mg +7.5 mg (oral)	Weekly	
		Everolimus + Letrozole + Leuprolide	10	7.5mg + 2.5mg + 7.5mg (oral)	Daily + Weekly	
Fan Y et al., 2021 [65]	Everolimus	Everolimus + Letrozole	101	10mg + 2.5mg (oral)	Daily	28-days cycles
		Letrozole	98	2.5mg (oral)		
Slomovitz B et al., 2022 [66]	Everolimus	Everolimus + Letrozole	37	10mg + 2.5mg (oral)	Daily	444 cycles (28-day cycles)
		Tamoxifen + Medroxyprogesterone (hormonal therapy)	37	20mg+200mg (oral)	Twice a day + every 2 weeks	370 cycles (28-day cycles)
Kulke M et al., 2022 [67]	Everolimus	Everolimus	75	10 mg (oral)	Daily	21 months
		Everolimus + Bevacizumab	75	10mg+10mg/kg (oral)	Daily + every 2 weeks	
Sweeney C et al., 2022 [68]	Samotolisib	Samotolisib + Enzalutamide	65	200mg + 160mg (oral)	Twice daily + daily	28-day treatment cycle
		Placebo + Enzalutamide	64	200mg + 160mg (oral)	Daily	

Table 3 – *Treatment allocated in each randomized controlled trial (**Cont.**)*

Authors, year [Reference]	mTORi (Drug)	Experimental Groups	Sample size (N)	Administration-related parameters		
				Dose	Frequency	Duration
Heudel P et al., 2022 [69]	Vistusertib	Vistusertib + Anastrozole	49	125mg + 1mg (oral)	Twice daily on 2 days per week and daily	48 weeks
		Anastrozole	24	1mg (oral)	Daily	
Choueiri TK et al., 2022 [70]	Everolimus and Sapanisertib	Everolimus	32	10 mg (oral)	Daily	3.5 cycles (28-day cycles)
		Sapanisertib	32	30 mg (oral)	Weekly	3.0 cycles (28-day cycles)
		Sapanisertib + TAK-117	31	4mg +200mg (oral)	3 days per week	3.0 cycles (28-day cycles)
Ryan CW et al., 2023 [71]	Everolimus	Everolimus	755	10 mg (oral)	Daily	54 weeks
		Placebo	744	-		

Legend: IV – intravenous; TAK-117 – Selective PI3Kα isoform inhibitor.

6.4 Efficacy Outcomes

To evaluate the efficacy of mTOR inhibitors as a treatment, certain outcomes were selected to extract information, including overall survival, overall response rate, partial response, stable disease, and progression-free survival. Other outcomes obtained and considered relevant for evaluating efficacy were also mentioned in an extra column in **Table 4**, including event-free survival, complete response, clinical benefit, clinical benefit rate, progressive disease, recurrence-free survival and stable response. The results of each article present a 95% confidence interval (CI).

The **overall survival (OS)** outcome was one of the reported outcomes in eight of the ten articles. The lowest value obtained when using mTOR inhibitors was 176 days (equivalent to 5.7 months) [63] and the highest was 42.5 months [67]. In two articles [62] [66] OS values were higher for the mTOR inhibitor arm combined with standard therapy compared to standard therapy, showing 24 month-OS of 39.2% and 29.6%, respectively [62], and median time-OS of 31.3 months and 16.6 months, respectively [66]. In two articles both inhibitor treatment alone or in combination presented similar values of median time-OS of 176 and 177 days [63], and 42.1 and 42.5 months [67], respectively. In one article, standard therapy presented a higher median time-OS value (14 months), followed by the mTOR inhibitor alone (12.5 months) and then the combined treatment (10.6 months) [64]. The first mTOR inhibitor, Everolimus, presented the highest value, with an increase of 6.2 and 4.3 months compared to Sapanisertib and Sapanisertib plus TAK-117 groups, respectively [70]. Unfortunately, two articles did not report the OS outcome, due to the lack of data at the established date of publication [65] [71].

The **overall response rate (ORR)** was analysed in five of the ten articles. In three articles the inhibitor presents higher values [62] [65] [69], the mTOR inhibitor combined with standard therapy percentage is higher than mTORi alone [63], difference of 10%, and in other article the Everolimus inhibitor has a percentage of 16.7%, while Sapanisertib alone has 0% that can be related with the dosage and frequency of administration, and Sapanisertib plus TAK-117 7.1% [70].

The **partial response (PR)** was analysed in five of the ten articles. In two articles, the combined inhibitor showed increased PR values when comparison to standard therapy [65] [69]. For another article, the combined mTORi presented a percentage four times higher than mTORi alone, 10% and 2.5%, respectively [63]. The values are the same for the mTOR inhibitor and for the placebo in one article, both 4.6% [68]. Finally, the values in an article are the same as those obtained for the overall response rate, Everolimus 16.7%, Sapanisertib 0% and Sapanisertib plus TAK-117 7.1% [70].

Stable disease (SD) was one of the reported outcomes of four of the ten articles. In an article, the combined mTORi showed a higher SD rate (22.7%) compared to standard therapy alone (8.2%) [65]. Also, in one other article, the mTORi alone had a higher SD rate (75%) compared to combined mTORi and standard therapy (61%). Consequently, Everolimus, alone and combined with standard therapy, appears to contribute to the stability of the disease [67]. In one article, mTORi presents the highest and most promising SD rate of 55% [64]. In one article, the rates are all equal to or greater than 50%, with the highest rate for the inhibitor Sapanisertib (61.5%) [70].

Progression-free survival (PFS) was analysed in eight of the ten articles. In three articles, the higher median time-PFS for mTORi combined with standard therapy, 19.4, 6.4 and 5.2 months, respectively [65] [66] [69], in one article the values are the same for mTORi alone and combined, both 105 days [63], while in an article the combined inhibitor has a higher value than the isolated inhibitor, 16.7 and 14 months [67]. In two articles the values of the three regimens are very similar [64] [70], and in other article the mTORi combined with standard therapy value is higher than the placebo, 3.8 and 2.8 months, respectively [68].

In a non-consistently approach, some other efficacy outcomes were assessed. In one article [62], it was also found that **event-free survival** was superior in combined mTORi, this difference was more pronounced at 12 months, and progressive disease at 6 months was superior in standard therapy. In an article [63], none of the participants showed a complete response in the isolated inhibitor, but around 40% had a progressive disease with mTORi alone, while the stable response was similar for the intervention and control groups. An article presents similar values in **complete response** for combined mTORi and standard therapy, but the clinical benefit rate is higher (72.7%) in combined mTORi, and **progressive disease** is higher in standard therapy [65]. In other, the number of participants who obtained a response was the same for both regimens, the **clinical benefit rate** was slightly higher in the combined mTORi [66], as in other, progressive disease was very similar for both regimens, with a difference of 1% [67]. Another article also evaluated complete response, but the percentages of combined mTORi and standard therapy were 2% and 0% respectively [69]. In other, none of the participants had a complete response to treatments, the clinical benefit rate was similar for the three regimens, but the number of deaths during treatment with Everolimus was higher, 12.5% [70]. As in another, **recurrence-free survival** was evaluated, with similar results for mTORi and placebo, 67% and 63% [71]. This approximation of values may be related to the high risk of bias in deviations from the intervention, since for this outcome, most participants did not comply with the surveillance schedule of the study.

Through these results, it is possible to state that the use of mTOR inhibitors in combination with standard therapies is more effective in treating cancer compared to the inhibitor alone or standard therapy. The mTORi Everolimus combined with standard therapy is the one that appears to have the best results, in terms of overall survival, overall response rate, partial response, stable disease and progression-free survival. These results reflect what is described in the literature, in which this inhibitor has high prolonged efficacy in the treatment of different types of cancer, such as HR+/HER2− breast cancer and neuroendocrine prostate cancer (NEPC) [5] [73] [74].

The inhibitor with the most modest results is Samotolisib, since it's use as treatment combined with standard therapy has the same effect as when compared with placebo combined, even though other clinical trials demonstrate high efficacy in the use of this inhibitor in the treatment of cancer [5]. This difference in results may be related to the fact that only one of the ten articles analysed in this systematic review studied the effect of Samotolisib, making this result less robust.

Inhibitors Temsirolimus and Vistusertib each combined with standard therapy and Sapanisertib combined with a PI3K inhibitor had good results in the efficacy outcomes, but since they're studied in a smaller number of articles, compared to Everolimus, the results, as consequence, are less solid. These results are confirmed in other articles since Temsirolimus, Vistusertib and Sapanisertib are referred as promising drugs in anticancer efficacy, acting in cell cycle, apoptosis and autophagy [5] [42] [75-77].

These results are expected since the literature suggests that mTOR inhibitors in combination are more effective than their use alone [78] [79], showing improvements in outcomes such as progression-free survival, overall response and disease control rate [79].

The fact that some articles fail to verify improvements in the parameters assessed when using mTORi, may be related to the type of cancer, since some types of cancer are already known to present alterations in the mTOR signalling pathway and others do not present that correlation.

None of the articles reported the outcome time to progression, which would be an interesting result to determining whether these types of treatment can have positive effects on the disease in the short or long term. Additionally, other outcomes of efficacy should be more consistently addressed in all RCT reporting, including complete response, duration of response, progressive disease, number of metastatic sites, tumour proportion score, and death rate [80] [81].

6.5 Biomarkers and mTOR Evaluation for Efficacy Assessment

Data including biomarkers assessment were also collected and presented in **Table 4**. One article analyses specific biomarkers, AR-v7 and PTEN, before and after the intervention under study, having verified, interestingly, that the 50% decrease in PSA as well as the decrease in androgen receptor splice variant (AR-v7), a biomarker associated with disease progression, was greater in participants allocated to placebo combined with standard therapy. No loss of the PTEN tumor suppressor gene occurred in 50% of participants for both regimens [68]. This report allows a better understanding of treatment results monitoring, along with the efficacy and safety outcomes.

The analysis and report of specific biomarkers in RCT is crucial to clarify the efficacy of the treatment and could contribute for a more personalized treatment of the disease [82].

In cancer treatment research, it would be an added value to study biomarkers such as AKT, S6K1, 4EBP1 and eIF4e, which can assess the activation state of the mTOR signalling pathway, and therefore evaluate if the inhibitors are having a positive effect in the treatment. The analysis of the pro-inflammatory cytokine, IFγ, with polymorphisms verified in several case-control studies regarding breast and cervical cancer, leukocyte count, serum amyloid A (SAA), prostaglandin E2 (PGE-2) and nuclear factor kappa B (NF-κB) levels analysis would help to assess the cancer-related inflammation, capable of promoting tumor growth. The study of genetic biomarkers would also be very important, since genetic variations, such as mutations, amplifications, deletions, losses and substitutions, are present in genes with activity in the mTOR signalling pathway in different types of cancer. For example, analyse the PIK3CA gene, since it's mutation alters the PI3K/AKT/mTOR signalling pathway, the PTEN gene, since mutations compromise the regulation of the cell cycle and the TSC1-TSC2 genes, which allows communication between the PI3K/AKT signalling pathway and mTOR, would be important, thus allows, through genetic profiling, to target more effectively the type of mTOR inhibitor for each patient [83] [84] [85] .

It would also be important to analyse specific predictive biomarkers of the cancer in study to better verify the patients with good responses to the treatment, allowing a more personalized treatment of the disease. For example, studying HER2 for breast cancer, microsatellite instability (MSI) for endometrial cancer, chromogranin A (CgA) for pancreatic neuroendocrine tumors, programmed cell death ligand-1 (PD-L1) for renal cell cancer and protein kinase cAMP-activated catalytic subunit alpha (PRKACA) for fibrolamellar carcinoma.[4] [17] [82] [86-91].

None of the articles included analysis of the status of mTOR and its evaluation, both important parameters to verify whether participants present altered mTOR expression, contributing to the relevance of using this type of treatment in these cases, as well for subsequent verification of its efficacy. It is possible that in some cases this analysis was not carried out since deregulation of the mTOR signalling pathway is common in the types of cancer studied by the articles or was carried out *a priori* and was not included in the article, but this information is not mentioned [64].

Several studies in cell lines and in animal models, that explore the anti-tumor effect of mTORi, frequently analyse the expression of mTOR. The effect of Temsirolimus in malignant pleural mesothelioma human cell lines where, using the Western blot technique, reported a decrease in the expression of the downstream proteins of the mTOR signalling pathway, as well as a decrease in mTOR expression through immunohistochemistry [92]. Also, Everolimus induced decreased expressions of mTOR, PI3K and AKT, assessed through immunohistology, on breast cell lines implanted in mice [93]. The combination of histology techniques for the mTOR expression analysis with the assessment of outcomes related to survival and disease progression results in more complete data, thus it would be relevant to analyse mTOR before and after treatment in human clinical trials [92] [93].

Table 4 - *Efficacy assessment outcomes in each randomized controlled trial*

Authors, year [Reference]	Experimental Groups	OS (% or median value)	ORR	PR	SD	PFS (median value)	Biomarkers	mTOR evaluation	Other Outcomes Assessed
Mascarenhas L, et al. 2019 [62]	Bevacizumab + Vinorelbine + Cyclophosphamide	6months:84.1% 12months:59.1% 24months:29.6%	28%	-	-	-	-	-	**EFS**: 6months:54.6% 12months:18.2% 24months:6.8% **PD at 6 weeks:** 28%
	Temsirolimus + Vinorelbine + Cyclophosphamide	6months:90.5% 12months:78.4% 24months:39.2%	47%						**EFS**: 6months:69.1% 12months:40.5% 24months:19.1% **PD at 6 weeks:** 11%
Seiwert T et al., 2020 [63]	Temsirolimus + Cetuximab	177 days	12.5%	10%	-	105 days	-	-	CR: 2.5%; SR: 52.5%; PD: 25%
	Temsirolimus	176 days	2.5%	2.5%		105 days			CR: 0%; SR: 50%; PD: 40%
El Dika I et al., 2020 [64]	Everolimus	12.5 months	-	-	55%	2.6 months	-	-	-
	Letrozole + Leuprolide	14 months			37%	2.7 months			
	Everolimus + Letrozole + Leuprolide	10.6 months			11%	2.4 months			
Fan Y et al., 2021 [65]	Everolimus + Letrozole	Not reached	50%	48.5%	22.7%	19.4 months	-	-	CR: 1.5%; CBR: 72.7%; PD: 27.3%
	Letrozole		39.3%	37.7%	8.2%	12.9 months			CR: 1.6%; CBR: 47.5%; PD: 52.5%
Slomovitz B et al., 2022 [66]	Everolimus + Letrozole	31.3 months	-	-	-	6.4 months	-	-	Response: 21.6%; CBR: 78.4%
	Tamoxifen + Medroxyprogesterone (hormonal therapy)	16.6 months				3.6 months			Response: 25%; CBR: 69.4%
Kulke M et al., 2022 [67]	Everolimus	42.1 months	-	-	75%	14 months	-	-	PD: 8%
	Everolimus + Bevacizumab	42.5 months			61%	16.7 months			PD: 7%
Sweeney C et al., 2022 [68]	Samotolisib + Enzalutamide	-	-	4.6%	-	3.8 months	AR-v7 and PTEN (plasma samples using qualitative reporting; IHC in soft tumor tissues)	-	PSA: 50% decline in 13 patients; Ar-v7 negative: 78.5%; No PTEN loss: 50%
	Placebo + Enzalutamide			4.6%		2.8 months			PSA: 50% decline in 16 patients; Ar-v7 negative: 84.4%; No PTEN loss: 50%

Table 4 – *Efficacy assessment outcomes in each randomized controlled trial (**Cont.**)*

Authors, year [Reference]	Experimental Groups	OS (% or median value)	ORR	PR	SD	PFS (median value)	Biomarkers	mTOR evaluation	Other Outcomes Assessed
Heudel P et al., 2022 [69]	Vistusertib + Anastrozole	-	24.5%	22.4%	-	5.2 months	-	-	CR: 2%
	Anastrozole		17.4%	17.4%		1.9 months			CR: 0%
Choueiri T et al., 2022 [70]	Everolimus	22.4 months	16.7%	16.7%	50%	3.8 months	-	-	CR: 0%; CBR: 66.7%; Death: 12.5%
	Sapanisertib	16.2 months	0%	0%	61.50%	3.6 months			CR: 0%; CBR: 61.5%; Death: 9.4%
	Sapanisertib + TAK-117	18.1 months	7.1%	7.1%	53.60%	3.1 months			CR: 0%; CBR: 60.7%; Death: 6.5%
Ryan CW et al., 2023 [71]	Everolimus	Not reached	-	-	-	-	-	-	RFS (5 years): 67%
	Placebo								RFS (5 years): 63%

Legend: *CBR (clinical benefit rate), CR (complete response), EFS (event-free survival, ORR (overall response rate), OS (overall survival), PD (progressive disease), PFS (progression-free survival), PR (partial response), RFS (recurrence-free survival), SD (stable disease), and SR (stable response).*

6.6 Safety Outcomes

Safety was also one of the outcomes analysed, **Table 5**, since treatment-related adverse events (AE) are one of the major concerns when using mTORi.

Its analysis was carried out through the adverse events outcome, which was divided into three topics: severity, signs and symptoms. **Appendix B** presents the definition of the degrees of severity of adverse events.

The degree of **severity** of adverse events was analysed in all articles. In three articles, grade 3 or higher events presented higher percentages in treatments with combined mTORi than with standard therapy, 28.6%, 45.8% and 50% respectively [62] [65] [69]. The fact that one article presents such a distinct difference between the two regimes may be related to the high risk of bias detected in the randomization process [69]. In another article, the percentages of grade 3 or higher events in inhibitors alone or in combination are similar [63], in other, the events are higher when using mTORi combined with standard therapy [67]. Although these two articles study 1st generation mTORi's, the fact that in one the dose is 25mg weekly [63] while in the other it is 10mg daily [67], may lead to these differences in results, demonstrating that in these cases it may be more advantageous to take a lower dose inhibitor but a daily. The duration of treatment may also play a major role in these differences, and in this case, it is not possible to compare them, since one of them does not mention the exact time [63]. One of the articles is a bit vague on this parameter, making it not possible to draw conclusions with confidence [64]. One article presents high percentages of grade 3 or higher events but similar between regimens, indicating that the inhibitor combined with standard therapy will cause the same number of side effects as the standard therapy alone [68]. In another article, the regimen with Sapanisertib combined with a PI3K inhibitor presents a slightly higher value than the other regimens [70]. The last article, as expected, presents several participants with higher grade 3 or higher events to the mTORi regimen [71]. Finally, the fact that the first article [62] is the one with the lowest percentages in this parameter may be related to the use of mTORi in combination, as well as the dose and frequency.

Signs and symptoms were also analysed in all articles, the most common being nausea, vomiting, fatigue, mucositis, lack of appetite, diarrhea, leukopenia and anemia.

In general, the presence of grade 3 or higher adverse events was more noted in participants treated with mTORi combined, being the Everolimus and the Vistusertib the inhibitors with higher percentages of AE when compared to standard therapy, placebo

and alone. Being Temsirolimus and Samotolisib, each one alone, to cause the smaller number of adverse events when compared with the other mTOR inhibitors.

The literature describes the adverse events as the principal limitation of the mTOR inhibitors [5] , with Everolimus showing toxicity in the treatment of metastatic castration-resistant prostate cancer [94], Temsirolimus, Vistusertib and Sapanisertib manageable toxicity in gastric cancer, squamous non-small-cell lung cancer, and acute lymphocytic leukaemia [95] [96] [97], respectively, as well as Samotolisib who is associated with less preoccupant adverse events such as nausea and vomiting [5]. The symtoms of these drugs are very similar to the ones described in **Table 5**. Indicating that the study of these inhibitors as well as their development must continue to overcome this limitation [5].

Degree of severity and symptoms, in one article [66], are only determined for participants allocated to the mTOR inhibitor, making it impossible to compare the regimens, possibly it may have been carried out but not included in the article.

The topic of **systems,** such as for example digestive, endocrine or respiratory system, was not reported in any of the articles, which would be very important to analyse the systems most affected by treatments, thus helping future studies to overcome or minimize the safety obstacle by focusing on the systems mostly affected.

Table 5 – *Safety outcomes evaluated in each randomized controlled trial*

Authors, year [Reference]	Experimental Groups	Severity	Signs and Symptoms
Mascarenhas L, et al. 2019 [62]	Bevacizumab + Vinorelbine + Cyclophosphamide	Grade 3 or higher (11.4%)	Hypertension, bleeding and wound infection
	Temsirolimus + Vinorelbine + Cyclophosphamide	Grade 3 or higher (28.6%)	Hypertriglyceridemia, mucositis, pneumonitis and liver enzyme elevation
Seiwert TY et al., 2020 [63]	Temsirolimus + cetuximab	Grade 3 or higher (70%)	Leukopenia, electrolyte abnormalities, and fatigue
	Temsirolimus	Grade 3 or higher (77.5%)	
El Dika I et al., 2020 [64]	Everolimus	Grade 3 (≥10%)	Nausea, vomiting, elevated AST, ALT and alkaline phosphatase
	Letrozole + leuprolide		
	Everolimus + letrozole + leuprolide		
Fan Y et al., 2021 [65]	Everolimus + letrozole	Grade 3 or higher (45.8%)	Stomatitis, hypertriglyceridemia, elevated ALT and AST, infection, hypercholesterolemia, rash, leukopenia and neutropenia, diarrhea, headache, anemia, pneumonitis, and fatigue
	Letrozole	Grade 3 or higher (19.7%)	
Slomovitz BM et al., 2022 [66]	Everolimus + Letrozole	Grade 3 or higher (46%)	Anemia, mucositis, high cholesterol, hyperglycemia, pneumonitis and thromboembolic event
	Tamoxifen + medroxyprogesterone (hormonal therapy)	-	-
Kulke MH et al., 2022 [67]	Everolimus	Grade 3 or 4 (57%)	Hyperglycemia
	Everolimus + bevacizumab	Grade 3 or 4 (85%)	Hypertension, proteinuria and hyperglycemia
Sweeney CJ et al., 2022 [68]	Samotolisib + enzalutamide	Grade 3 or higher (53.8%)	Fatigue, nausea, diarrhea, decreased appetite, anemia and constipation
	Placebo + enzalutamide	Grade 3 or higher (51.6%)	
Heudel P et al., 2022 [69]	Vistusertib + anastrozole	Grade 3 or 4 (50%)	Nausea, fatigue, vomiting, diarrhea, arthralgia, decrease in lymphocytes and hyperglycemia
	Anastrozole	Grade 3 or 4 (8%)	
Choueiri TK et al., 2022 [70]	Everolimus	Grade 3 or higher (68.8%)	Asthenia, decreased appetite, diarrhea, stomatitis, dyspnea, cough
	Sapanisertib	Grade 3 or higher (65.6%)	Nausea, vomiting, asthenia, constipation and pruritus
	Sapanisertib + TAK-117	Grade 3 or higher (74.2%)	Nausea, vomiting, fatigue diarrhea and decreased appetite
Ryan CW et al., 2023 [71]	Everolimus	Grade 3 or higher (46%)	Mucositis, hypertriglyceridaemia, hyperglycaemia
	Placebo	Grade 3 or higher (11%)	Fatigue, hypertriglyceridaemia

Legend: *ALT (alanine aminotransferase) and AST (aspartate transaminase)*

6.7 Risk of Bias Assessment

The risk of bias was performed for each article, where the following plot was obtained (Figure 10).

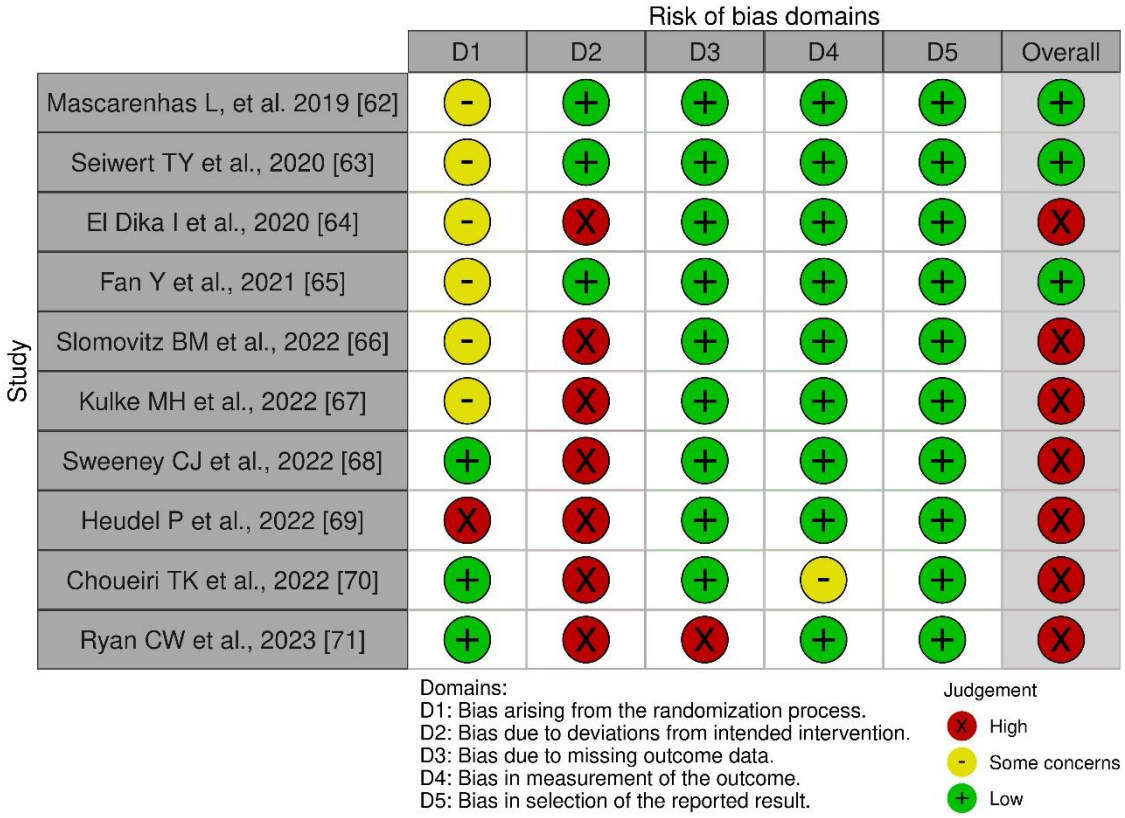

Figure 10 - *Risk of bias assessment of each included randomized clinical trial using RoB 2 tool [61].*

It is possible to verify that there are some reservations regarding the randomization process in the first six articles, and a high risk of bias in one article [69]. Seven articles present a high risk of bias in the deviations of the intended intervention [64] [66] [67] [68] [69] [70] [71]. There is also a high risk of bias in the lack of outcome in one article [71]. Regarding the measurement of the outcome, there are some concerns in one article [70]. Finally, the risks of bias for the selection of reported results are low for all articles. In general, only three articles present a low risk of bias, while the remaining articles present a high risk of bias [62] [63] [65].

One methodological limitation of this systematic review includes the presence of only one reviewer. According to PRISMA Guidelines, screening, data collection and risk of bias assessment should be performed by two independent and blinded reviewers, with a third reviewer to resolve discrepancies [59]. In addition, the inclusion of heterogeneous

tumors, as well as different types of mTORi, makes the comparison multifaceted and more complexed, being necessary to focus on a specific type of cancer and inhibitor. The collection of quantitative information related to statistical relevance could contribute to a more reliable and robust evaluation of efficacy and safety of mTORi.

These results thus allow a synthesis of information on the efficacy and safety of mTORi in the treatment of cancer to the detriment of standard therapies, through studies of high importance, providing reliable results and reflecting the state of current knowledge on current evidence of intervention studies in clinical practice for the treatment of malignancies. Also, it is possible to emphasizes the lack of information about specific aspects such as biomarkers and mTOR evaluation.

7. Conclusions

The present systematic review aimed to verify whether mTOR inhibitors would be effective for the clinical treatment of cancer patients when compared to placebo or standard therapy, evaluating their efficacy and safety through randomized controlled trials.

Through the analysis of the ten articles, it was possible to observe that the articles studied adult cancer patients with Rhabdomyosarcoma, head and neck squamous cell carcinoma, fibrolamellar carcinoma, breast cancer, endometrial cancer, pancreatic neuroendocrine tumors, prostate cancer or renal cell carcinoma.

Improvements in most efficacy outcomes, in terms of overall survival, overall response rate, partial response, stable disease and progression-free survival, for mTOR inhibitors combined with standard therapy when compared to standard therapy alone or mTORi alone was observed. Everolimus combined with standard therapy had the best results, and Samotolisib demonstrated to be the inhibitor with less effect in the treatment of cancer.

mTOR inhibitors, despite showing positive results in treatment of the disease, can lead to a greater number of serious adverse events compared to current therapies. Therefore, safety is the major limitation of mTORi clinical use.

This study contributes to the synthesis of information, based on studies with a high level of evidence (RCT), regarding mTORi in various types of cancer, including respective doses, frequency of administration and duration of treatment, to the identification of useful outcomes in determining efficacy, overall survival, overall response rate, partial response, stable disease, progression-free survival, time to progression, event-free survival, complete response, clinical benefit, progressive disease, recurrence-free survival and stable response, as well as for safety, through the evaluation of adverse events (severity, signs and symptoms).

The presence of overall high risk of bias in 70% of the articles, indicates that some results may be biased due to the lack of control in the randomization process, deviations in the intended intervention, as well as the lack of outcome data. In the future, the improvement of these domains would be relevant while carrying out randomized controlled trials and reporting its results.

8. Limitations and Future Perspectives

The lack of data in terms of biomarkers assessment, only presented in one article, was a limitation of this systematic review since their analysis in RCT is crucial to clarify the efficacy of the treatment, then contributing for a more personalized treatment of the disease.

Despite RCT importance to address mTORi efficacy, it is known that the survival of patients treated with chemotherapy or targeted therapy in real-world practice is nearly one-quarter than for patients included in RCT. The performance status of patients, earlier discontinuation and fewer successive lines of treatment could help to explain this discrepancy [98]. As a future perspective, studies comparing the mTORi efficacy and safety in RCT with the real-world practice results of effectiveness are necessary. Nevertheless, preclinical and clinical studies have an undisputable role in cancer research and will continue to contribute to the development and improvement of mTORi, aiming to obtaining significant effects in cancer treatment and increasing its safety.

Future studies should aim to apply a meta-analysis of the results, with homogenous and standardized data in terms of the disease, mTORi treatment-related parameters, and assessed outcomes.

Moreover, a cost analysis of mTORi could contribute to highlight the most efficient solutions to real-world clinical practice.

9. References

1. Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, Lazar V, Kurzrock R. Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. *J Clin Oncol*. 2015; 33(32):3817-25. doi: 10.1200/JCO.2015.61.5997.

2. Ward RA, Fawell S, Floc'h N, Flemington V, McKerrecher D, Smith PD. Challenges and Opportunities in Cancer Drug Resistance. *Chem Rev*. 2021; 121(6):3297-3351. doi: 10.1021/acs.chemrev.0c00383.

3. Peng Y, Wang Y, Zhou C, Mei W, Zeng C. PI3K/Akt/mTOR Pathway and Its Role in Cancer Therapeutics: Are We Making Headway? *Front. Oncol*. 2022; 12:819128. doi: 10.3389/fonc.2022.819128.

4. Albanell J, Dalmases A, Rovira A, Rojo F. mTOR signalling in human cancer. *Clin Transl Oncol*. 2007. doi: 10.1007/s12094-007-0092-6.

5. Panwar V, Singh A, Bhatt M. et al. Multifaceted role of mTOR (mammalian target of rapamycin) signalling pathway in human health and disease. *Sig Transduct Target Ther.* 2023; 8, 375. doi: 10.1038/s41392-023-01608-.

6. World Health Organization. Data visualization: estimated number of deaths from 2022 to 2045 – World Cancer Tomorrow [Internet]. 2024. [cited 2024 June 27]. Available from: https://gco.iarc.fr/tomorrow/en/dataviz/isotype?types=1&single_unit=500000

7. Xiaona Liu, Jiahao Yi, Tina Li, Jianguo Wen, Kexin Huang, Jiajia Liu, Grant Wang, Pora Kim, Qianqian Song, Xiaobo Zhou, DRMref: comprehensive reference map of drug resistance mechanisms in human cancer, *Nucleic Acids Research*.2014;52,(D1):D1253–D1264. doi: 10.1093/nar/gkad1087.

8. Eslami M, Memarsadeghi O, Davarpanah A, Arti A, Nayernia K, Behnam B. Overcoming Chemotherapy Resistance in Metastatic Cancer: A Comprehensive Review. *Biomedicines.* 2024;12,183. doi: 10.3390/biomedicines12010183.

9. Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the Definition of Cancer. *Mol Cancer Res*. 2023 ;21(11):1142-1147. doi: 10.1158/1541-7786.MCR-23-0411.

10. Sarkar S, Horn G, Moulton K, Oza A, Byler S, Kokolus S, Longacre M. Cancer development, progression, and therapy: an epigenetic overview. *Int J Mol Sci*. 2013;14(10):21087-113. doi: 10.3390/ijms141021087.

11. Siddiqui IA, Sanna V, Ahmad N, Sechi M, Mukhtar H. Resveratrol nanoformulation for cancer prevention and therapy. *Ann N Y Acad Sci*. 2015;1348(1):20-31. doi: 10.1111/nyas.12811.

12. de Visser KE, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. *Cancer Cell*. 2023 ;41(3):374-403. doi: 10.1016/j.ccell.2023.02.016.

13. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-74. doi: 10.1016/j.cell.2011.02.013.

14. Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. *Immunity*. 2019 ;51(1):27-41. doi: 10.1016/j.immuni.2019.06.025.

15. Hanahan D. Hallmarks of Cancer: New Dimensions. *Cancer Discov*. 2022; 12 (1): 31–46. doi :10.1158/2159-8290.CD-21-1059

16. Jayesh R. Cancer: A Comprehensive Review. *International Journal of Research in Pharmacy and Chemistry*. 2023; 1. 42-49.

17. Das S, Dey MK, Devireddy R, Gartia MR. Biomarkers in Cancer Detection, Diagnosis, and Prognosis. *Sensors*. 2024; 24(1):37. doi: 10.3390/s24010037

18. Hammarsten P, Josefsson A, Thysell E. et al. Immunoreactivity for prostate specific antigen and Ki67 differentiates subgroups of prostate cancer related to outcome. *Mod Pathol.* 2019; 32, 1310–1319. doi: 10.1038/s41379-019-0260-6

19. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harbor perspectives in biology.* 2010; 2(1), a001008. doi: 10.1101/cshperspect.a001008

20. Marant Micallef C, Shield K. D, Baldi I, Charbotel B, Fervers B, Gilg Soit Ilg A, Guénel P, et al. Occupational exposures and cancer: a review of agents and relative risk estimates. *Occupational and environmental medicine*. 2018 ;75(8), 604–614. doi: 10.1136/oemed-2017-104858

21. Teng Y, Yu Y, Li S, Huang Y, Xu D, Tao X, Fan Y. Ultraviolet Radiation and Basal Cell Carcinoma: An Environmental Perspective. *Frontiers in public health*. 2021 ;9, 666528. doi: 10.3389/fpubh.2021.666528

22. Arem H, Loftfield E. Cancer Epidemiology: A Survey of Modifiable Risk Factors for Prevention and Survivorship. *American journal of lifestyle medicine*. 2017; 12(3), 200–210. doi: 10.1177/1559827617700600

23. World Health Organization. Data visualization tools for exploring the global cancer burden in 2022 - W*orld*. Cancer Today [Internet]. 2022. [cited 2024 June 26]. Available from: https://gco.iarc.fr/today/en/dataviz/pie?mode=population&group_populations=0

24. World Health Organization. Data visualization tools for exploring the global cancer burden in 2022 – Europe Cancer Today [Internet]. 2022. [cited 2024 June 26]. Available in: https://gco.iarc.fr/today/en/dataviz/pie?mode=population&group_populations=0&populations=100_112_191_196_203_208_233_246_250_276_300_348_352_372_380_40_428_440_442_470_498_499_528_56_578_616_620_642_643_688_70_703_705_724_752_756_8_804_807_826

25. World Health Organization. Data visualization tools for exploring the global cancer burden in 2022 – Portugal Cancer Today [Internet]. 2022. [cited 2024 June 26]. Available from:https://gco.iarc.fr/today/en/dataviz/pie?mode=cancer&group_populations=1&populations=620

26. Schwartz SM. Epidemiology of Cancer. *Clin Chem*. 2024 ;70(1):140-149. doi: 10.1093/clinchem/hvad202.

27. Chiu MI, Katz H, Berlin V. RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. Proc Natl Acad Sci U S A. 1994; 91:12574–12578. doi: 10.1073/pnas.91.26.12574

28. Pópulo H, Lopes JM, Soares P. The mTOR signalling pathway in human cancer. *Int J Mol Sci*. 2012;13(2):1886-1918. doi: 10.3390/ijms13021886.

29. Schmelzle T, Hall MN.TOR, a central controller of cell growth. *Cell*. 2000; 103:253–262. doi: 10.1016/s0092-8674(00)00117-3

30. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev*. 2004; 18:1926– 1945. doi:10.1101/gad.1212704

31. Zaytseva YY, Valentino JD, Gulhati P, Evers BM. mTOR inhibitors in cancer therapy. *Cancer Lett*. 2012;319(1):1-7. doi: 10.1016/j.canlet.2012.01.005.

32. Moschetta M, Reale A, Marasco C, Vacca A, Carratù MR. Therapeutic targeting of the mTOR-signalling pathway in cancer: benefits and limitations. *Br J Pharmacol*. 2014;171(16):3801-13. doi: 10.1111/bph.12749.

33. Marques-Ramos A, Cervantes R. Expression of mTOR in normal and pathological conditions. *Mol Cancer.* 2023;22,112. doi: 10.1186/s12943-023-01820-z

34. Karagianni F, Pavlidis A, Malakou LS, Piperi C, Papadavid E. Predominant Role of mTOR Signaling in Skin Diseases with Therapeutic Potential. *International Journal of Molecular Sciences*. 2022; 23(3):1693. doi: 0.3390/ijms23031693

35. Liu Grace Y, David M Sabatini. mTOR at the nexus of nutrition, growth, ageing and disease. Nature reviews. *Molecular cell biology.* 2020; 21,4: 183-203. doi:10.1038/s41580-019-0199-y

36. Felix Daniel A, Gutiérrez-Gutiérrez Óscar, Espada Lilia, Thems Anne, González-Estévez Cristina. It is not all about regeneration: Planarians striking power to stand starvation. *Seminars in Cell & Developmental Biology*. 2018; 87:169-181. doi:10.1016/j.semcdb.2018.04.010.

37. Wullschleger S, Loewith R, Hall MN. TOR signalling in growth and metabolism. *Cell*. 2006;124(3):471-84. doi: 10.1016/j.cell.2006.01.016.

38. Chun Y, Kim J. AMPK-mTOR Signaling and Cellular Adaptations in Hypoxia. *International journal of molecular sciences*. 2021; 22(18), 9765. doi: 10.3390/ijms22189765

39. Mao B, Zhang Q, Ma L, Zhao D-S, Zhao P, Yan P. Overview of Research into mTOR Inhibitors. *Molecules*. 2022; 27(16):5295. doi: 10.3390/molecules27165295

40. Ali E.S, Mitra K, Akter S. et al. Recent advances and limitations of mTOR inhibitors in the treatment of cancer. *Cancer Cell Int.* 2022; 22, 284. doi: 10.1186/s12935-022-02706-8.

41. Zhang YJ, Duan Y, Zheng XF. Targeting the mTOR kinase domain: the second generation of mTOR inhibitors. *Drug Discov Today*. 2011;16(7-8):325-31. doi: 10.1016/j.drudis.2011.02.008.

42. Chen Y, Zhou X. Research progress of mTOR inhibitors. *Eur J Med Chem*. 2020;15,208:112820. doi: 10.1016/j.ejmech.2020.112820.

43. Formisano L, Napolitano F, Rosa R, D'Amato V, Servetto A, Marciano R, De Placido P, Bianco C, Bianco R. Mechanisms of resistance to mTOR inhibitors. *Critical reviews in oncology/hematology*. 2020; 147, 102886. doi: 10.1016/j.critrevonc.2020.102886.

44. Soefje S. A, Karnad A, Brenner A. J. Common toxicities of mammalian target of rapamycin inhibitors. *Targeted oncology*. 2011; 6(2), 125–129. doi: 10.1007/s11523-011-0174-9.

45. Nguyen L. S, Vautier M, Allenbach Y. et al. Sirolimus and mTOR Inhibitors: A Review of Side Effects and Specific Management in Solid Organ Transplantation. *Drug Saf.* 2019; 42, 813–825. doi: 10.1007/s40264-019-00810-9.

46. Informed. Everolimus (Afinitor) [Internet]. 2024. [cited 2024 July 10] Available from: https://www.ema.europa.eu/pt/documents/product-information/afinitor-epar-product-information_pt.pdf

47. Informed. Temsirolimus (Torisel) [Internet]. 2024. [cited 2024 July 10]. Available from: https://www.ema.europa.eu/pt/documents/product-information/torisel-epar-product-information_pt.pdf

48. Informed. Alpelisib (Pikray) [Internet]. 2024. [cited 2024 July 10]. Available from: https://www.ema.europa.eu/pt/documents/product-information/piqray-epar-product-information_pt.pdf

49. FDA. FDA approves alpelisib for PIK3CA-related overgrowth spectrum [Internet]. 2022. [cited 2024 July 10]. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-alpelisib-pik3ca-related-overgrowth-spectrum

50. Tilsed C. M, Fisher S. A, Nowak A. K, Lake R. A, Lesterhuis W. J. Cancer chemotherapy: insights into cellular and tumor microenvironmental mechanisms of action. *Frontiers in oncology*. 2022; 12, 960317. doi: 10.3389/fonc.2022.960317.

51. Amjad MT, Chidharla A, Kasi A. Cancer Chemotherapy. [Updated 2023 Feb 27]. In: StatPearls [Internet]. *Treasure Island (FL): StatPearls Publishing*. 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK564367/

52. Levin K. A. Study design VII. Randomised controlled trials. *Evidence-based dentistry*. 2007; 8(1), 22–23. doi: 10.1038/sj.ebd.6400473.

53. Goldstein C. E, Weijer C, Brehaut J. C, Fergusson D. A, Grimshaw J. M, Horn A. R, Taljaard M. Ethical issues in pragmatic randomized controlled trials: a review of the recent literature identifies gaps in ethical argumentation. *BMC medical ethics*. 2018; 19(1), 14. doi: 10.1186/s12910-018-0253-x.

54. Soerjomataram I, Bray F. Planning for tomorrow: global cancer incidence and the role of prevention 2020–2070. *Nat Rev Clin Oncol*. 2021;18, 663–672. doi:10.1038/s41571-021-00514-z

55. Carrera PM, Kantarjian HM. Blinder VS. The financial burden and distress of patients with cancer: Understanding and stepping-up action on the financial toxicity of cancer treatment. *CA Cancer J Clin*. 2018;68(2):153-165. doi: 10.3322/caac.21443.

56. Zafar SY, Peppercorn JM, Schrag D, et al. The financial toxicity of cancer treatment: a pilot study assessing out-of-pocket expenses and the insured cancer patient's experience. Oncologist. 2013;18:381–390. doi:10.1634/theoncologist.2012-0279

57. Hofmarcher T, Lindgren P, Wilking N, Jönsson B. The cost of cancer in Europe 2018. *European journal of cancer*. 2020; 129, 41–49. doi: 10.1016/j.ejca.2020.01.011

58. Glaviano A, Foo ASC, Lam HY, Yap KCH, Jacot W, Jones RH, Eng H, Nair MG, Makvandi P, Geoerger B, Kulke MH, Baird RD, Prabhu JS, Carbone D, Pecoraro C, Teh DBL, Sethi G, Cavalieri V, Lin KH, Javidi-Sharifi NR, Toska E, Davids MS, Brown JR, Diana P, Stebbing J, Fruman DA, Kumar AP. PI3K/AKT/mTOR signalling transduction pathway and targeted therapies in cancer. *Mol Cancer*. 2023 ;22(1):138. doi: 10.1186/s12943-023-01827-6.

59. Page M. J, McKenzie J. E, Bossuyt P M, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. thebmj. 2021.

60. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng H-Y, Corbett MS, Eldridge SM, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF, Higgins JPT. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019; 366: l4898.

61. McGuinness, LA, Higgins, JPT. Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. *Res Syn Meth*. 2020; 1- 7. doi:10.1002/jrsm.1411

62. Mascarenhas L, Chi YY, Hingorani P, Anderson JR, Lyden ER, Rodeberg DA, Indelicato DJ, Kao SC, Dasgupta R, Spunt SL, Meyer WH, Hawkins DS. Randomized Phase II Trial of Bevacizumab or Temsirolimus in Combination With Chemotherapy for First Relapse Rhabdomyosarcoma: A Report From the Children's Oncology Group. *J Clin Oncol*. 2019;37(31):2866-2874. doi: 10.1200/JCO.19.00576.

63. Seiwert TY, Kochanny S, Wood K, Worden FP, Adkins D, Wade JL, Sleckman BG, Anderson D, Brisson RJ, Karrison T, Stadler WM, Vokes EE. A randomized phase 2 study of temsirolimus and cetuximab versus temsirolimus alone in recurrent/metastatic, cetuximab-resistant head and

neck cancer: The MAESTRO study. *Cancer*. 2020;126(14):3237-3243. doi: 10.1002/cncr.32929.

64. El Dika I, Mayer RJ, Venook AP, Capanu M, LaQuaglia MP, Kobos R, O'Neill AF, Chou JF, Ly M, Ang C, O'Reilly EM, Gordan JD, Abou-Alfa GK. A Multicenter Randomized Three-Arm Phase II Study of (1) Everolimus, (2) Estrogen Deprivation Therapy (EDT) with Leuprolide + Letrozole, and (3) Everolimus + EDT in Patients with Unresectable Fibrolamellar Carcinoma. *Oncologist*. 2020;25(11):925-e1603. doi: 10.1634/theoncologist.2020-0367.

65. Fan Y, Sun T, Shao Z, Zhang Q, Ouyang Q, Tong Z, Wang S, Luo Y, Teng Y, Wang X, Wang S, Liu Q, Feng J, Shen K, Song Y, Wang J, Ma F, Li Q, Zhang P, Xu B. Effectiveness of Adding Everolimus to the First-line Treatment of Advanced Breast Cancer in Premenopausal Women Who Experienced Disease Progression While Receiving Selective Estrogen Receptor Modulators: A Phase 2 Randomized Clinical Trial. *JAMA Oncol*. 2021;7(10):e213428. doi: 10.1001/jamaoncol.2021.3428.

66. Slomovitz BM, Filiaci VL, Walker JL, Taub MC, Finkelstein KA, Moroney JW, Fleury AC, Muller CY, Holman LL, Copeland LJ, Miller DS, Coleman RL. A randomized phase II trial of everolimus and letrozole or hormonal therapy in women with advanced, persistent or recurrent endometrial carcinoma: A GOG Foundation study. *Gynecol Oncol*. 2022;164(3):481-491. doi: 10.1016/j.ygyno.2021.12.031.

67. Kulke MH, Ou FS, Niedzwiecki D, Huebner L, Kunz P, Kennecke HF, Wolin EM, Chan JA, O'Reilly EM, Meyerhardt JA, Venook A. Everolimus with or without bevacizumab in advanced pNET: CALGB 80701 (Alliance). *Endocr Relat Cancer*. 2022;29(6):335-344. doi: 10.1530/ERC-21-0239.

68. Sweeney CJ, Percent IJ, Babu S, Cultrera JL, Mehlhaff BA, Goodman OB, Morris DS, Schnadig ID, Albany C, Shore ND, Sieber PR, Guba SC, Zhang W, Wacheck V, Donoho GP, Szpurka AM, Callies S, Lin BK, Bendell JC. Phase Ib/II Study of Enzalutamide with Samotolisib (LY3023414) or Placebo in Patients with Metastatic Castration-Resistant Prostate Cancer.

Clin Cancer Res. 2022 ;28(11):2237-2247. doi: 10.1158/1078-0432.CCR-21-2326.

69. Heudel P, Frenel JS, Dalban C, Bazan F, Joly F, Arnaud A, Abdeddaim C, Chevalier-Place A, Augereau P, Pautier P, Chakiba C, You B, Lancry-Lecomte L, Garin G, Marcel V, Diaz JJ, Treilleux I, Pérol D, Fabbro M, Ray-Coquard I. Safety and Efficacy of the mTOR Inhibitor, Vistusertib, Combined With Anastrozole in Patients With Hormone Receptor-Positive Recurrent or Metastatic Endometrial Cancer: The VICTORIA Multicenter, Open-label, Phase 1/2 Randomized Clinical Trial. *JAMA Oncol*. 2022;8(7):1001-1009. doi: 10.1001/jamaoncol.2022.1047.

70. Choueiri TK, Porta C, Suárez C, Hainsworth J, Voog E, Duran I, Reeves J, Czaykowski P, Castellano D, Chen J, Sedarati F, Powles T. Randomized Phase II Trial of Sapanisertib ± TAK-117 vs. Everolimus in Patients With Advanced Renal Cell Carcinoma After VEGF-Targeted Therapy. *Oncologist*. 2022;27(12):1048-1057. doi: 10.1093/oncolo/oyac192..

71. Ryan CW, Tangen CM, Heath EI, Stein MN, Meng MV, Alva AS, Pal SK, Puzanov I, Clark JI, Choueiri TK, Agarwal N, Uzzo RG, Haas NB, Synold TW, Plets M, Vaishampayan UN, Shuch BM, Thompson IM Jr, Lara PN Jr. Adjuvant everolimus after surgery for renal cell carcinoma (EVEREST): a double-blind, placebo-controlled, randomised, phase 3 trial. *Lancet*. 2023;402(10407):1043-1051. doi: 10.1016/S0140-6736(23)00913-3.

72. Baryakova TH, Pogostin BH, Langer R, McHugh KJ. Overcoming barriers to patient adherence: the case for developing innovative drug delivery systems. *Nat Rev Drug Discov*. 2023;22(5):387-409. doi: 10.1038/s41573-023-00670-0.

73. François-Martin H, Lardy-Cléaud A, Pistilli B, Levy C, Diéras V, Frenel J. S, Guiu S, Mouret-Reynier M. A, Mailliez A, Eymard J. C, Petit T, Ung M, Desmoulins I, Augereau P, Bachelot T, Uwer L, Debled M, Ferrero J. M, Clatot F, Goncalves A, Cottu P. Long-Term Results with Everolimus in Advanced Hormone Receptor Positive Breast Cancer in a Multicenter National Real-World Observational Study. *Cancers*. 2023; 15(4), 1191. doi: 10.3390/cancers15041191

74. Tatsuya Shimomura et al. Treatment outcome of everolimus against neuroendocrine prostate cancer (NEPC). *JCO.* 2018;36, 365-365. doi:10.1200/JCO.2018.36.6

75. Sun JG, Nie P, Herdewijn P, Li XJ. Exploring the synthetic approaches and clinical prowess of established macrocyclic pharmaceuticals*. Eur J Med Chem.* 2024;264:116051. doi: 10.1016/j.ejmech.2023.116051.

76. Tronconi F, Nero C, Giudice E, Salutari V, Musacchio L, Ricci C, Carbone MV, Ghizzoni V, Perri MT, Camarda F, Gentile M, Berardi R, Scambia G, Lorusso D. Advanced and recurrent endometrial cancer: State of the art and future perspectives. *Crit Rev Oncol Hematol*. 2022; 180:103851. doi: 10.1016/j.critrevonc.2022.

77. Occhiuzzi MA, Lico G, Ioele G, De Luca M, Garofalo A, Grande F. Recent advances in PI3K/PKB/mTOR inhibitors as new anticancer agents. *Eur J Med Chem*. 2023; 246:114971. doi: 10.1016/j.ejmech.2022.114971.

78. Hua H, Kong Q, Zhang H, Wang J, Luo T, Jiang Y. Targeting mTOR for cancer therapy. *J Hematol Oncol*. 2019;12(1):71. doi: 10.1186/s13045-019-0754-1.

79. Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. *Cell Biosci*. 2020;10:31. doi: 10.1186/s13578-020-00396-1.

80. Banna GL, Cantale O, Muthuramalingam S, Cave J, Comins C, Cortellini A, Addeo A, Signori A, McKenzie H, Escriu C, Barone G, Chan S, Hicks A, Bainbridge H, Pinato DJ, Ottensmeier C, Gomes F. Efficacy outcomes and prognostic factors from real-world patients with advanced non-small-cell lung cancer treated with first-line chemoimmunotherapy: The Spinnaker retrospective study. *Int Immunopharmacol*. 2022;110:108985. doi: 10.1016/j.intimp.2022.108985.

81. de Castro G Jr, Kudaba I, Wu YL, Lopes G, Kowalski DM, Turna HZ, Caglevic C, Zhang L, Karaszewska B, Laktionov KK, Srimuninnimit V, Bondarenko I, Kubota K, Mukherjee R, Lin J, Souza F, Mok TSK, Cho BC. Five-Year Outcomes With Pembrolizumab Versus Chemotherapy as First-Line Therapy in Patients With Non-Small-Cell Lung Cancer and

Programmed Death Ligand-1 Tumor Proportion Score ≥ 1% in the KEYNOTE-042 Study. *J Clin Oncol*. 2023 ;41(11):1986-1991. doi: 10.1200/JCO.21.02885.

82. Owonikoko TK, Khuri FR. Targeting the PI3K/AKT/mTOR pathway: biomarkers of success and tribulation. *Am Soc Clin Oncol Educ Book*. 2013;10.1200/EdBook_AM.2013.33.e395.doi:10.14694/EdBook_AM.2013.33.e395.

83. Menzel A, Samouda H, Dohet F, Loap S, Ellulu M. S, Bohn T. Common and Novel Markers for Measuring Inflammation and Oxidative Stress Ex Vivo in Research and Clinical Practice-Which to Use Regarding Disease Outcomes?. *Antioxidants*. 2021; 10(3), 414. doi: 10.3390/antiox10030414.

84. Finetti F, Travelli C, Ercoli J, Colombo G, Buoso E, Trabalzini L. Prostaglandin E2 and Cancer: Insight into Tumor Progression and Immunity. *Biology*. 2020; 9(12), 434. doi:10.3390/biology9120434

85. Zou Z, Tao T, Li H, Zhu X. mTOR signaling pathway and mTOR inhibitors in cancer: progress and challenges. *Cell Biosci*. 2020;10:31. doi: 10.1186/s13578-020-00396-1.

86. Novelli G, Ciccacci C, Borgiani P, Papaluca Amati M, Abadie E. Genetic tests and genomic biomarkers: regulation, qualification and validation. *Clin Cases Miner Bone Metab.* 2008;5(2):149-54.

87. Asati V, Bharti SK, Mahapatra DK, Asati V, Budhwani AK. Triggering PIK3CA Mutations in PI3K/Akt/mTOR Axis: Exploration of Newer Inhibitors and Rational Preventive Strategies. *Curr Pharm Des*. 2016;22(39):6039-6054. doi: 10.2174/1381612822666160614000053.

88. Rosset C, Netto CBO, Ashton-Prolla P. TSC1 and TSC2 gene mutations and their implications for treatment in Tuberous Sclerosis Complex: a review. *Genet Mol Biol.* 2017;40(1):69-79. doi: 10.1590/1678-4685-GMB-2015-0321.

89. Alexander ES, Ziv E. Neuroendocrine Tumors: Genomics and Molecular Biomarkers with a Focus on Metastatic Disease. *Cancers*. 2023; 15(8):2249. doi: 10.3390/cancers15082249

90. Renée M. Saliby et al. Update on Biomarkers in Renal Cell Carcinoma. *Am Soc Clin Oncol Educ Book* 44, e430734(2024). doi:10.1200/EDBK_430734

91. NIH - National Cancer Institute. Fibrolamellar Carcinoma (FLC/FLHCC) [Internet]. 2019. [cited 2024 June 27]. Available from: https://www.cancer.gov/pediatric-adult-rare-tumor/rare-tumors/rare-digestive-system-tumors/fibrolamellar-hepatocellular-carcinoma

92. Moriya M, Yamada T, Tamura M, Ishikawa D, Hoda MA, Matsumoto I, Klepetko W, Oda M, Yano S, Watanabe G. Antitumor effect and antiangiogenic potential of the mTOR inhibitor temsirolimus against malignant pleural mesothelioma. *Oncol Rep*. 2014;31(3):1109-15. doi: 10.3892/or.2013.2948.

93. Du L, Li X, Zhen L, Chen W, Mu L, Zhang Y, Song A. Everolimus inhibits breast cancer cell growth through PI3K/AKT/mTOR signalling pathway. *Mol Med Rep*. 2018;17(5):7163-7169. doi: 10.3892/mmr.2018.8769.

94. George DJ, Halabi S, Healy P, Jonasch D, Anand M, Rasmussen J, Wood SY, Spritzer C, Madden JF, Armstrong AJ. Phase 2 clinical trial of TORC1 inhibition with everolimus in men with metastatic castration-resistant prostate cancer. *Urol Oncol*. 2020; 38(3):79.e15-79.e22. doi: 10.1016/j.urolonc.2019.08.015.

95. Baghery Saghchy Khorasani A, Pourbagheri-Sigaroodi A, Pirsalehi A, Safaroghli-Azar A, Zali MR, Bashash D. The PI3K/Akt/mTOR signalling pathway in gastric cancer; from oncogenic variations to the possibilities for pharmacologic interventions. *Eur J Pharmacol*. 2021; 898:173983. doi: 10.1016/j.ejphar.2021.

96. Basu B, Krebs M. G, Sundar R, Wilson R. H, Spicer J, Jones R. et al. Vistusertib (dual m-TORC1/2 inhibitor) in combination with paclitaxel in patients with high-grade serous ovarian and squamous non-small-cell lung cancer. Annals of oncology: official journal of the *European Society for Medical Oncology*. 2018; 29(9), 1918–1925. doi:10.1093/annonc/mdy245

97. Al-Kali A, Aldoss I, Atherton PJ, Strand CA, Shah B, Webster J, Bhatnagar B, Flatten KS, Peterson KL, Schneider PA, Buhrow SA, Kong J, Reid JM, Adjei AA, Kaufmann SH. A phase 2 and pharmacological study of sapanisertib in patients with relapsed and/or refractory acute lymphoblastic leukemia. *Cancer Med.* 2023;12(23):21229-21239. doi: 10.1002/cam4.6701.

98. Cramer-van der Welle CM, Peters BJM, Schramel FMNH, Klungel OH, Groen HJM, van de Garde EMW; Santeon NSCLC Study Group; Santeon NSCLC study group. Systematic evaluation of the efficacy-effectiveness gap of systemic treatments in metastatic nonsmall cell lung cancer. *Eur Respir J*. 2018;52(6):1801100. doi: 10.1183/13993003.01100-2018.

99. NIH – National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE). 2017. [cited 2024 November 10] Available From: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_8.5x11.pdf

10. Appendices

10.1 Appendix A - Search strategy for each database

For each database was developed a search strategy as follows:

Database	Expression of investigation	Import of articles
PubMed	(''neoplasms''[MeSH Terms] OR Neoplasm*[tiab] OR Neoplasia*[tiab] OR Cancer*[tiab] OR Tumor*[tiab] OR Malignanc*[tiab] OR carcinoma [MeSH Terms] OR carcinoma[tiab]) AND (''mtor inhibitors''[MeSH Terms] OR ''mtor inhibit*'' OR rapalogs) AND (''clinical studies as topic''[MeSH Terms] OR ''clinical stud*''[tiab] OR ''clinical trial*''[tiab])	May 6, 2024
SCOPUS	(TITLE-ABS (neoplasm* OR neoplasia* OR tumor* OR cancer* OR malignanc* OR carcinoma)) AND (TITLE-ABS (“mtor inhibit*” OR rapalogs)) AND (TITLE-ABS (''clinical trial*'' OR “controlled trial*” OR “clinical stud*”))	May 6, 2024
Web of Science	((TI=(neoplasm* OR neoplasia* OR tumor* OR cancer* OR malignanc* OR carcinoma)) OR (AB=(neoplasm* OR neoplasia* OR tumor* OR cancer* OR malignanc* OR carcinoma))) AND ((TI=(“mtor inhibit*” OR rapalogs)) OR (AB=(“mtor inhibit*” OR rapalogs))) AND ((TI=(''clinical trial*'' OR “controlled trial*” OR “clinical stud*”)) OR (AB=(''clinical trial*'' OR “controlled trial*” OR “clinical stud*”)))	May 6, 2024

10.2 Appendix B – Degrees of Severity of Adverse Events

Degrees of severity of adverse events based on the NCI Common Terminology Criteria for Adverse Events (CTCAE) [99]:

Degree	Definition
1	Mild symptoms or asymptomatic
2	Moderate symptoms
3	Severe or medically significant symptoms, not immediately life-threatening
4	Life-threatening symptoms
5	Death