Universidade de Lisboa

Faculdade de Farmácia

Anticancer Properties of Astaxanthin

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Monografia orientada pelo Professor Doutor Nuno G. Oliveira, Categoria Professor Catedrático

Mestrado Integrado em Ciências Farmacêuticas

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Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

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To my friends, who have shared in the highs and lows of the past five years, it has been an honor and a pleasure to face all of this with you. Thank you.

Last, but certainly not least, I would like to give Professor Nuno Oliveira a word of thanks, for all his effort and concern over the past few months.

Declaração de Código de Conduta

Declaro ter desenvolvido e elaborado o presente trabalho em consonância com o Código de Conduta e de Boas Práticas da Universidade de Lisboa. Mais concretamente, afirmo não ter incorrido em qualquer das variedades de fraude académica, que aqui declaro conhecer, e que atendi à exigida referenciação de frases, extratos, imagens e outras formas de trabalho intelectual, assumindo na íntegra as responsabilidades da autoria.

Resumo

O cancro é uma das causas de morte mais prevalentes em países desenvolvidos, colocando um peso significativo nos seus sistemas de saúde e tendo um grande impacto na qualidade de vida da população. As estratégias de terapêutica atuais incluem ressecção cirúrgica, quimioterapia e radioterapia, entre outras. Embora todas estas opções melhorem a taxa de sobrevida do doente, frequentemente apresentam desvantagens, tais como efeitos secundários que afetem a capacidade de o doente realizar as suas atividades de vida diárias, toxicidade, ou falências secundárias.

A astaxantina (ASTX) é um carotenoide xantófilo frequentemente encontrado em algas e animais aquáticos, frequentemente usado como corante em peixes e comida para animais. Vários estudos demonstraram que, além dos seus efeitos benéficos ao nível da saúde da pele, ocular, cardiovascular e neurológica, demonstrou resultados promissores no tratamento do cancro. A ASTX apresenta vários mecanismos anticancerígenos, incluindo efeito anti-proliferativo, anti-apoptótico e anti-invasivo. Doentes suplementados com ASTX não reportaram efeitos secundários, o que a torna ainda mais apelativa como terapêutica para o cancro. Esta dissertação tem como objetivo fazer uma revisão dos potenciais usos da ASTX no combate ao cancro e ilustrar os seus principais mecanismos de ação.

Palavras-chave: fatores de risco para cancro; terapêutica para cancro; astaxantina; carotenoides; antioxidantes

Abstract

Cancer is one of the leading causes of death in developed countries, placing a significant strain on a country's health system and greatly impacting the population's quality of life. Current treatment strategies include surgical resection, chemotherapy and radiation therapy, among other. While all of them increase patient survival rates, they often have their own drawbacks, such as severe side effects that impact the patient's ability to perform their daily activities, toxicity, or development of therapy resistance.

Astaxanthin (ASTX) is a xanthophyll carotenoid widely found in algae and aquatic animals that is frequently used as food colorant in fish and animal feed.

Several studies have shown that ASTX is a potent antioxidant and anti-inflammatory, which piqued interest as a therapeutic agent for several diseases, including cancer. In addition to the benefits in the skin and eye health and a protective effect against cardiovascular and neurological diseases, ASTX shows promising results as an anticancer agent. It displays several anticancer mechanisms of action, including anti-proliferation, anti-apoptosis, and anti-invasion. Patients taking ASTX supplementation also did not report side effects, further increasing its value as an anticancer agent. The aim of this study is to review the potential uses of ASTX in cancer, as well as illustrate its main mechanisms of action.

Keywords: cancer risk factors; cancer therapy; astaxanthin; carotenoids; antioxidant.

List of Abbreviations

ASTX – Astaxanthin

CRC - Colorectal cancer

CVD – Cardiovascular disease

FAP - Familial adenomatous polyposis coli

GWAS - Genome wide association studies

HDI – Human Development Index

HER2 -Human epidermal growth factor receptor 2

HNPCC - Hereditary nonpolyposis colorectal cancer

IBD - Inflammatory bowel disease

LC – Lung cancer

MAPK - Mitogen-activated protein kinase

MMP - Matrix metalloproteinase

NCD – Noncommunicable diseases

NF-κB - Nuclear factor-κB

NSCLC - Non-small cell lung cancer

PAH - Polycyclic aromatic hydrocarbons

PC - Prostate cancer

PI3K - phospatidylinositide 3-kinases

ROS - Radical oxygen species

SCLC - Small cell lung cancer

SOD - superoxide dismutase

TNF-α - Tumor necrosis factor-α

TSNA - Tobaccospecific N-nitrosamines

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1. Introduction

Noncommunicable diseases (NCD) account for most premature deaths in our society. It is estimated that more than three-quarters of premature deaths in persons aged 30-70 are a result of NCD (1).

Cancer is one of the leading causes od premature death in over 120 countries (2). In a world with continuous advances in modern medicine and quality of life, and their resulting reduction of mortality from other sources, it is expected that the health burden of cancer will continue to grow (3). On an individual level, this can result in increased societal and economic burden that can vary across cancer types, geography and gender (4); on a national level, the increased prevalence of cancer is accompanied by an additional burden on the country's health systems and becomes an important factor in policymaking, with many countries implementing cancer-related plans (5).

Cancer therapy is a fundamental area within medical research, with many therapeutic approaches being currently in use and many new ones in constant evolution (6,7). Carotenoids are a group of molecules that have been shown to have a potential protective effect against cancer due to their antioxidant properties and, as such, have been met with growing interest. Among these, astaxanthin (ASTX) has been of particular interest in recent years (8). This dissertation aims to review the available literature on the uses of ASTX as a potential anticancer agent.

2. Cancer

Epidemiology

Cancer and cardiovascular disease (CVD) are the leading causes of premature death in 127 countries, with cancer being more prevalent than CVD in 57 countries and CVD being more prevalent than cancer in 70 countries. In 50 other countries, mainly in sub-Saharan Africa, both CVD and cancer rank in the top three causes of death, and finally, in six countries, neither CVD nor cancer rank in the top three causes of death (2). Figure 1 illustrates this distribution on a global scale.

There appears to be an overlap between a country's Human Development Index (HDI) and its leading cause of death (Figure 2). Cancer leads the statistics in most countries with a very high HDI, whereas CVD is most prevalent in countries with a medium to high HDI. In countries with a low HDI, communicable diseases tend to rank higher than noncommunicable ones (3).

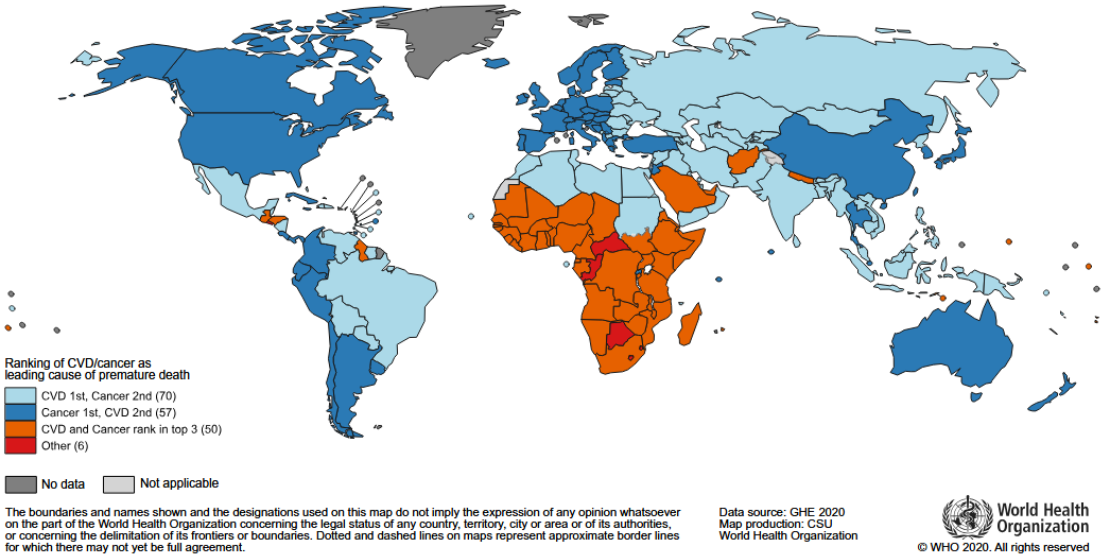

Figure 1. Global map of leading NCD causes of death in 183 countries. Source: World Health Organization, retrieved from Bray *et al*.

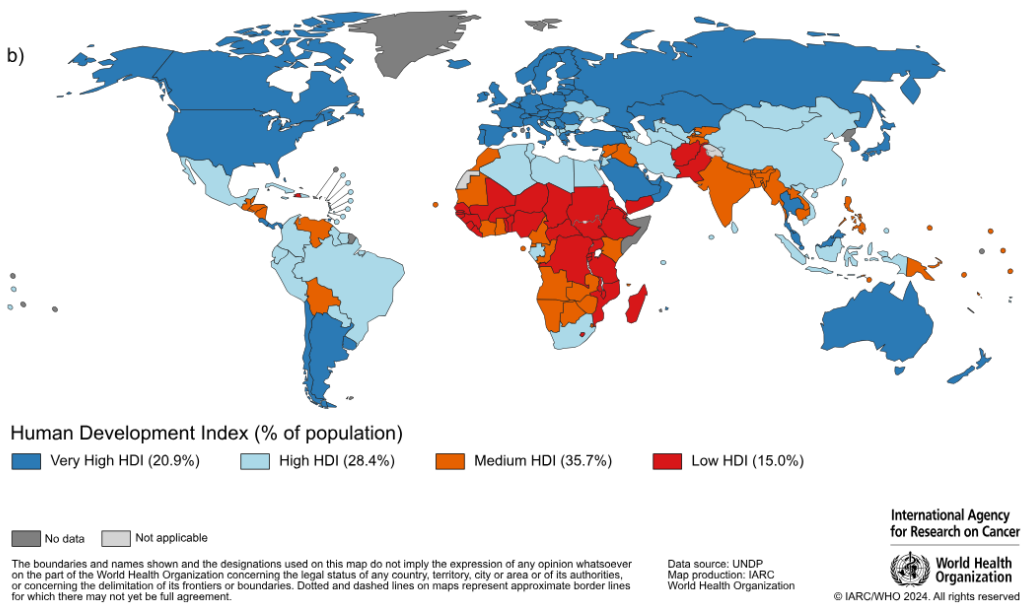

Figure 2. Human Development Index distribution on a global scale. Source: United Nations Procurement Division/United Nations Development Program. Adapted from GLOBOCAN 2022.

In a society with continuous advances in modern medicine and quality of life, and their resulting reduction of mortality from other sources, it is expected that the health burden of cancer will continue to grow (3). On an individual level, this can result in increased societal and economic burden that can vary across cancer types, geography and gender (4); on a national level, the increased prevalence of cancer is accompanied by an additional burden on the country's health systems and becomes an important factor in policymaking, with many countries implementing cancer-related plans (5).

Incidence and mortality

There were an estimated 20.0 million new cases and 9.7 million deaths for both sexes combined in 2022 (9). Figure 3 shows the distribution of new cases and number of deaths for both sexes, as well as separated by sex, for the most common types of cancer in 2022. The top ten most diagnosed types of cancer account for over 60% of all new cancer cases and cancer-related deaths.

Across both sexes, the most common types of cancer were lung (12.4%), female breast (11.6%), colorectal (9.6%), prostate (7.3%), and stomach (4.9%); lung and colorectal

cancer accounted for the highest number of deaths (18.7% and 9.3%, respectively). In males, prostate cancer moved to the second highest incidence, with lung cancer maintaining the top spot in both incidence and mortality. For females, breast cancer was the most diagnosed type of cancer and the leading cause of cancer-related death, followed by lung cancer on both metrics.

Potential new preventive and therapeutic strategies can have a great impact in cancer incidence and mortality. This is especially the case for the types of cancer where incidence and mortality are highest. Thus, it is important to take a closer look at these types of cancer.

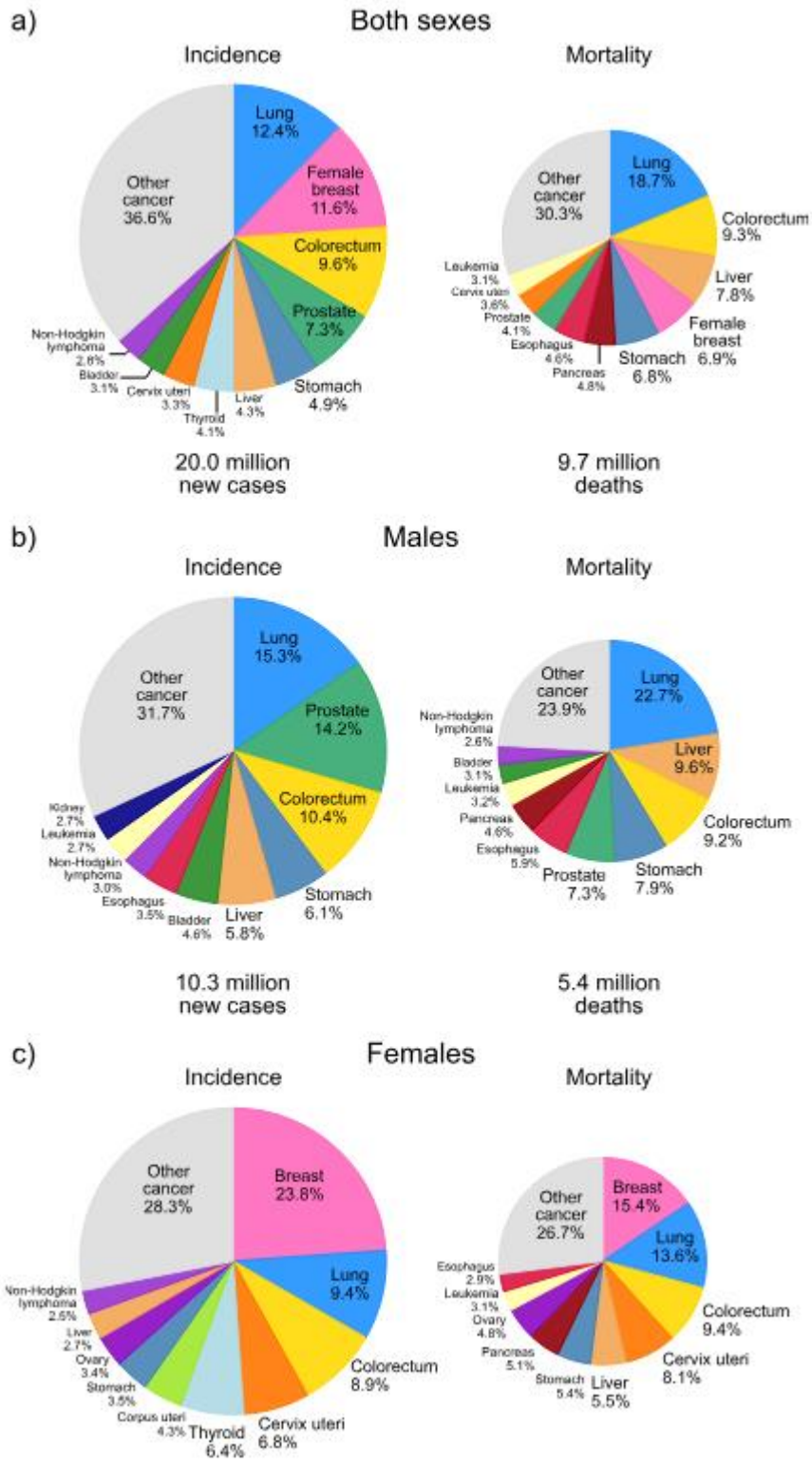

Figure 3. Distribution of total number of cases and deaths for the most common types of cancer in 2022 for (A) both sexes, (B) men, and (C) women. Nonmelanoma skin cancers (excluding basal cell carcinoma) are included in the "Other" category. Source: GLOBOCAN 2022.

2.1 Lung Cancer

2.1.1 Epidemiology

Lung cancer (LC) has the second highest incidence worldwide in both men and women (10). Additionally, it is the leading cause of cancer-related deaths worldwide, accounting for 18.7% of all cancer deaths in 2022. Five-year relative survival for all stages of LC has been trending up but is still relatively low compared to other cancers (22% as of 2022) in large part due to late diagnosis (11). Recent implementation of screening guidelines for early detection, as well as higher emphasis on reducing risk factors, such as tobacco use, have led to a significant decrease in LC mortality (12).

LC incidence is affected by demographic factors such as gender, age, ethnicity, education, occupation, income, and geography. Most of these factors can be correlated with tobacco use, which is the main risk factor for developing LC.

In developed countries, smoking habits are more prevalent compared to less-developed countries (13,14). Historically, more men than women smoke tobacco, which contributed to a higher LC incidence in men; however, there has been an increase in women's smoking habits as well as a lower rate of smoking cessation when compared to men. This has caused LC incidence and mortality in women to still be on the rise, whereas for men it has stabilized (15).

Level of education also plays a major role in adopting risky behaviours such as smoking. Individuals with a lower education level are much more likely to use tobacco regularly and less likely to quit. This is reflected in LC incidence, which is almost three times higher in individuals with less than a high school education when compared to college graduates (13).

There is a correlation between socioeconomic status and the incidence of LC, suggesting that tobacco use is only one of several risk factors associated with this type of cancer, including (but not limited to) occupational exposure and housing accommodations (16,17).

Older age is associated with cancer development due to biologic factors, such as DNA damage over time and shortening telomeres (18). Accordingly, LC has a higher incidence in older individuals, with approximately 90 percent of cases occurring in individuals over the age of 55 (13).

2.1.2. Risk factors

In addition to being a significant risk factor for many other health conditions, the use of tobacco cigarettes is the single most impactful risk factor in the development of LC; as many as 90% of LC cases can be attributed to smoking. Because there is long time gap between smoking initiation and development of LC, there was initially a poor understanding of the real potential health hazards of cigarette smoking (19). By the time the relationship between smoking and LC was understood, cigarette smoking was already a widespread practice in society. It was only in the second half of the 20th century that intensive efforts were made to decrease tobacco consumption.

While nicotine, the addictive component in tobacco, is not carcinogenic, tobacco smoke contains several substances classified as carcinogenic to animals and/or humans, the most significant ones being polycyclic aromatic hydrocarbons (PAH), nitrates, and tobacco-specific N-nitrosamines (TSNA) (20). Mechanisms of carcinogenesis from tobacco smoke include formation of DNA adducts by carcinogens and their metabolites as well as free radical damage (21).

When it comes to the potential hazards of smoking, it is crucial to mention that it is not only the smoker that is at risk; exposure to secondhand smoke also poses a significant risk of developing LC and other diseases (22). It is therefore important to encourage individuals to quit smoking and adhere to healthier lifestyle choices.

Other environmental factors that can be predictors of LC development are exposure to carcinogenic substances in daily life. This exposure can be a consequence of poor living conditions, environmental pollution, or occupational.

Exposure to radon is also an important cause of LC incidence, for both smokers or non-smokers (23), being usually considered the primary cause of lung cancer in non-smokers. Although exposure to radon is probably more common in an occupational setting (such as mine workers), it can also accumulate in basements and lower levels of buildings depending on soil composition and ventilation, among other variables (24).

Occupational exposure to asbestos can also be linked to LC in smokers and non-smokers, although it is especially harmful to smokers due to the synergistic effect between asbestos and cigarette smoke (25,26).

Presence of hazardous contaminants in the atmosphere, such as PAH, have also been associated with LC incidence and mortality (27,28). Because of the increased

carcinogenic risk these particulate matter entail, efforts have been made at the regulatory level to reduce air pollution (29).

Most of the risk factors presented above can be avoided or heavily mitigated through lifestyle changes such as smoking cessation and work/living environment changes. However, several authors reported a genetic component to the onset of LC: individuals with a family history of LC had as much as 70% higher risk of developing LC regardless of smoking habits (30,31).

Finally, some genetic factors can also be ascribed. In fact, genome wide association studies (GWAS) have associated chromosome regions 3q28, 5p15, 15q25-26 and 6q21 with increased risk for LC (32,33). Additionally, mutations in GTP-ase Kirsten rat sarcoma virus (K-ras) account for 10-30 percent of lung adenocarcinomas (34). Mutations of the epidermal growth factor receptor (EGFR) are frequent in non-small cell lung cancer, making this receptor a good target for therapy, with several EGFR-inhibitors being approved for use worldwide (34).

2.1.3. Classification

There are two main types of LC: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (35). NSCLC accounts for the majority (approximately 85%) of all LC cases, whereas SCLC accounts for a smaller fraction of cases but is much more aggressive (35). NSCLC can be further classified in three main subtypes: adenocarcinoma, squamous cell carcinoma, and large-cell lung carcinoma (36). Adenocarcinoma represents the majority of NSCLC, accounting for approximately 38% percent of cases. In general, adenocarcinoma is located at the periphery of the lung, originating in epithelial cells capable of secreting mucus. Squamous cell carcinoma accounts for nearly 20% of all LC (37). It usually presents in a central location, most frequently in a main or lobar bronchus.

Large-cell carcinoma is defined as an undifferentiated NSCLC carcinoma, without (immuno)histological evidence of squamous cell, glandular or small-cell differentiation (38). It can present in any region of the lung and accounts for a smaller fraction of LC cases (approximately 3%).

As stated previously, SCLC has a lower incidence than NSCLC and is typically associated with cigarette smoking. Small cell carcinomas are clinically very aggressive, and patients usually have a poor prognosis that is a consequence of late diagnosis (39).

2.1.4. Treatment

The treatment approach for NSCLC is heavily dependent on the stage of the cancer, histology, genetic alterations, and the patient's condition (12). Typical options include surgery, radiotherapy, chemotherapy, and immunotherapy, either alone or in combination. Surgical resection is recommended in medically stable patients with early stages of NSCLC. For these patients, adjuvant chemotherapy is sometimes recommended, but there is a relatively high recurrence and toxicity rates.

For patients with more advanced NSCLC, surgery is not recommended; instead, these patients undergo chemoradiotherapy, followed by immunotherapy (40). Due to late diagnosis of the disease and subsequent late therapy initiation, these patients tend to have worse outcomes. A compounding factor that leads to worse clinical outcomes is the inherent resistance to chemo- and radiotherapy displayed in this type of cancer (41).

Targeted therapy has improved clinical outcomes in many patients with advanced stage NSCLC. Several tyrosine kinase inhibitors (TKI) have already been approved for the treatment of several subtypes of NSCLC patients. There are several potentially useful targets for TKIs in NSCLC patients, such as EGFR, anaplastic lymphoma kinase (ALK), and ROS1, among others. These are mutated in some NSCLC patients and lead to the progression of the cancer. Thus, it is increasingly important to perform molecular testing to determine if the patient displays any mutations in these targets and would therefore benefit from targeted therapy (12). However, patients who use TKIs tend to develop resistance to the therapy over time, due to the occurrence of secondary mutations (42).

Immunotherapy has also been a great focus of research in recent years and has demonstrated remarkable benefits in terms of survival in NSCLC patients without targetable genetic alterations, either in monotherapy or in combination. The main target in immunotherapy is the checkpoint pathways. Several compounds have demonstrated efficacy in inhibiting programmed cell-death protein-1 and its ligand (PD-1 and PD-L1, respectively); these are pembrolizumab, nivolumab, and atezolizumab. Ipilimumab acts an inhibitor at the CTLA-4 level (12).

2.2 Female Breast Cancer

2.2.1 Epidemiology

Breast cancer in women is the leading factor of cancer incidence worldwide. It accounts for approximately one quarter of cancer cases and one sixth of cancer-related deaths in women (Figure 3).

Breast cancer incidence is considerably higher for women in countries with very high or high HDI than those in countries with medium or low HDI (54.1 vs. 30.8 per 100,000 women); however, mortality is significantly higher for women in countries with medium or low HDI (11.3 vs. 15.3 per 100,000 women), as depicted in Figure 4 (9).

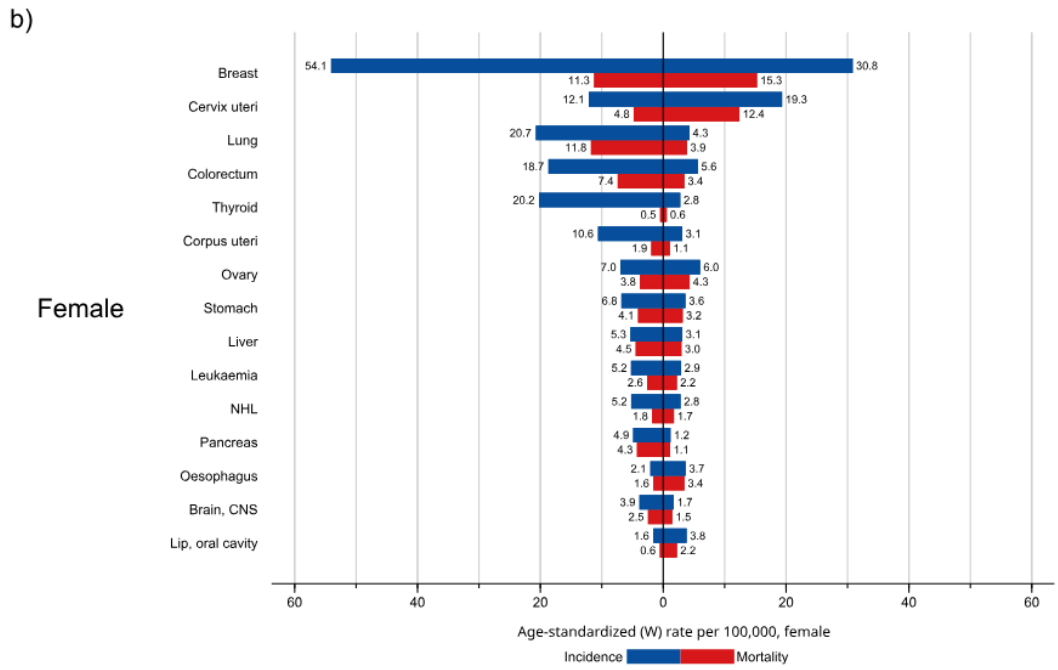

Figure 4. Age-standardized incidence and mortality of different cancer types in females in countries with high or very high High Development Index (left) vs. medium or low High Development Index (right). Source: GLOBOCAN 2022.

2.2.2. Risk factors

Risk factors for breast cancer include genetic factors, environmental factors, and lifestyle factors. These include older age, earlier menarche, advanced maternal age,

dense breasts, family history of breast cancer, history of hormone therapy, obesity, smoking, alcohol consumption, physical inactivity, and late menopause (43,44).

All these factors are also more prevalent in high HDI countries than in lower HDI ones, which helps explain the higher incidence of breast cancer in those countries. Another important factor is that screening medical exams, such as mammograms, are much more common in higher-income countries than in lower-income countries, resulting in a higher number of diagnoses (45).

Because most of the risk factors listed above are not modifiable, the focus of breast cancer control is mainly on population-wide screening and early diagnosis and treatment (46). If present, the modifiable risk factors should also be addressed to reduce the risk of breast cancer, such as increasing physical activity, reducing excess body weight, reduced alcohol intake, and promotion of breastfeeding (43).

2.2.3. Treatment

There are several treatment patterns in breast cancer cases, depending on how advanced the disease is. In early-stage (I or II) patients, breast conserving surgery and adjuvant radiation therapy is a frequent approach; for stage III patients, it is instead more common to undergo mastectomy followed by chemotherapy. Finally, most women diagnosed with stage IV breast cancer received only radiation or chemotherapy (47).

There are many factors that affect long-term survival in breast cancer patients, such as tumor stage, grade, estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (HER2) status, with triple-negative cancers generally responding only to cytotoxic chemotherapy (43).

Other therapeutic strategies such as immunotherapy are becoming increasingly used in early-stage and metastatic breast cancer patients, with good results in triple-negative cancer types (48). Breast cancer typically has a good prognosis and survival rate, especially when early diagnosed. As of 2017, the year-year survival rate was 90% (49,50), reaching nearly 100% for stage I patients but much lower for stage IV patients, at approximately 28% (51).

2.3 Colorectal Cancer

2.3.1. Epidemiology

Among all types of cancer, colorectal cancer (CRC) ranks third in global incidence and second in mortality (9). However, its incidence has been trending upwards and some authors predict that, by the year 2035, deaths by colon and rectum cancer will increase by 71.5% and 60%, respectively (52).

Like all types of cancer, CRC incidence is highest in developed countries, but countries with a medium HDI have reported a significant increase in cases and number of deaths (53,54). As with other types of cancer, lifestyle habits more prevalent in developed countries, as well as increased quality of life and reduced mortality from other causes, are determining factors for the higher incidence of CRC in developed countries (55,56).

Prognosis and five-year survival rates are highly dependent on detection stage: patients whose cancer is diagnosed at an early stage have approximately 90% five-year survival rate, whereas those who are diagnosed later have only approximately 13% five-year survival rate (53,57).

2.3.2. Risk factors

There are many factors that influence the likelihood of developing CRC. They can be divided in three major groups: personal and family medical history, lifestyle, and others (58). Figure 5 illustrates the most significant risk factors for CRC.

A family history of CRC is correlated with an increased risk of an individual developing CRC, suggesting a genetic predisposition for the disease. Furthermore, the increase in likelihood of developing CRC based on family history is also influenced by several other factors, such as the generational distance to the relatives affected by CRC (higher risk with first-degree relatives); the age at which the relative developed CRC (higher risk with lower age); the number of relatives that developed CRC (higher risk with more affected relatives); co-occurrence of other cancers; and personal history of cancer (59,60).

Additionally, there is an increased risk of developing CRC in individuals with hereditary nonpolyposis colorectal cancer (HNPCC), and familial adenomatous polyposis coli (FAP) (61).

Inflammatory bowel disease (IBD) is the third-highest risk condition for the development of CRC. It is a chronic and incurable disease which results in the development of uncontrolled inflammation of the bowel (62). Because inflammation promotes tumor growth and progression, individuals with IBD are more likely to develop CRC than healthy individuals. The increased risk ratio depends on the duration, extent, and severity of the disease (63). Other conditions are also important risk factors, such as history of colon polyps, diabetes mellitus, and cholecystectomy (57).

Lifestyle habits that increase the risk of CRC include inadequate diet (high in red and processed meat; low in fiber, fruits and vegetables; low in calcium, vitamin D and dairy products), physical inactivity, excess weight, cigarette smoking and alcohol consumption. These are the habits that can be consciously altered as primary prevention.

Gut microbiota also appears to play a key role in many pathological processes, including carcinogenesis. Our microbiome is involved in several physiological processes, such as nutrient absorption and xenobiotic metabolism and elimination, as well as participating in the maintenance of the intestinal barrier integrity, protecting against pathogens, and playing a fundamental role in immunomodulation.

When the gut microbiome is compromised, toxic bacterial metabolites can cause DNA damage, stimulate an immune response leading to inflammation, and compromise the intestinal barrier function, all of which are favourable to the initiation and progression of CRC (64,65).

Age is also one of the most significant factors in predicting an individual's risk for developing CRC. Most CRC cases occur in individuals above 50 years of age (59), with individuals over 65 years of age having nearly three times greater risk of developing CRC compared to individuals between the ages of 50 and 64, and approximately 30 times greater risk of developing CRC than individuals aged 25-49 (66).

Men appear to be at an increased risk for CRC than women, as well as having a worse prognosis and higher mortality. Women, on the other hand, tend to have their diagnosis at a later stage of disease (67).

Finally, lower socioeconomic status is correlated with higher risk of CRC, which may be due to reduced access to healthcare, unhealthy dietary habits, sedentary lifestyle, and higher prevalence of smoking habits in this population (68).

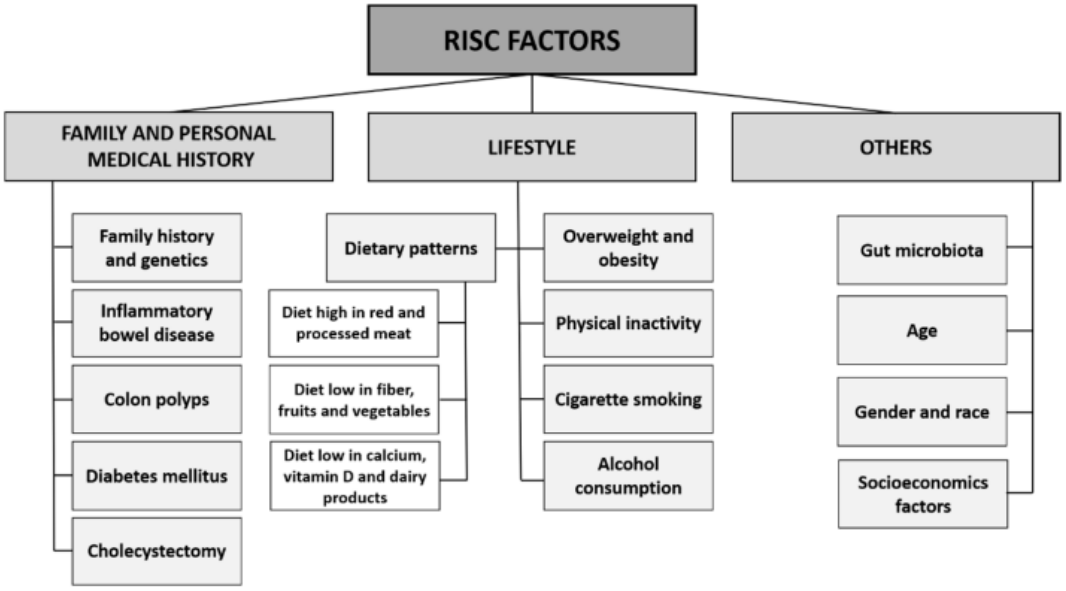

Figure 5. Risk factors associated with colorectal cancer. Retrieved from Sawicki *et al*. (57)

2.3.3. Treatment

In terms of treatment options, CRC can be classified in two major groups: resectable and non-resectable. In most cases where surgical removal is possible, it is the preferred method of treatment. However, when surgery is not an option, then there are several possible approaches to treating CRC patients, which can be used independently or in combination (69).

These include radiation therapy, chemotherapy, and immunotherapy. Each of them has their own drawbacks, such as non-specific cytotoxicity, which can itself lead to complications (70). The efficacy of treatment can be increased by combining different therapeutic strategies, but even patients who undergo combinational therapies tend to relapse into acquired multidrug resistance CRC (71).

There has been significant research into immunotherapy as a promising candidate for CRC treatment. It has been used, with promising results, in other types of cancer and has therefore been established as a prominent therapeutic strategy for a variety of solid tumors (72). In the case of CRC, several potential targets have been identified, as illustrated in Figure 6.

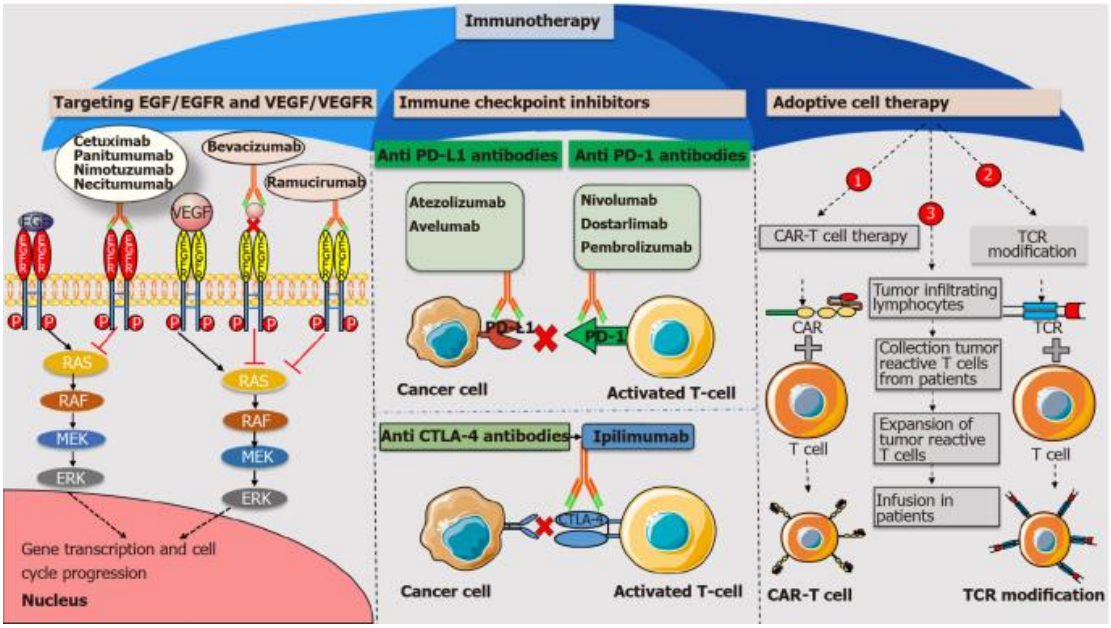

Figure 6. Potential immunotherapeutic approaches against CRC. EGF/EGFR – epidermal growth factor/EGF receptor; VEGF/VEGFR – vascular EGF/VEGF receptor; PD-L1 – programmed cell death ligand 1; PD-1 programmed cell death protein 1. (69)

2.4 Prostate Cancer

2.4.1. Epidemiology

Prostate cancer (PC) is the second most diagnosed cancer in males and one of the most frequently diagnosed types of cancer overall, with nearly 1.5 million new cases approximately 400,000 deaths registered in 2022 (9).

There are significant disparities in incidence and mortality between countries that can be attributed to HDI, healthcare infrastructure, and ease of access to screening/diagnostic procedures and treatment (73).

Five-year survival rate for PC is typically very high when diagnosed and treated early – around 97%. On the other hand, patients diagnosed at a later stage or presenting with metastasis, the five-year survival rate drops to 30% (10).

2.4.2. Risk factors

There is a long list of risk factors for PC, including genetic factors, environmental factors, and lifestyle factors (73).

Nonmodifiable risk factors include age, height, family and personal medical history, and genetic predisposition. Higher age is one of the most important risk factors for developing PC, with individuals over 70 years of age having nearly 5 times higher risk of PC than those between 60-69 years old. Family history of cancer (PC or otherwise) is another extremely important risk factor for PC. Height has also been associated with an increased risk of developing PC, with this type of cancer being more frequently diagnosed in taller individuals.

Among the modifiable risk factors, statistics report that having a vegetarian diet appears to result in a lower risk of PC compared to a diet including meat (74). Additionally, inflammatory and hyperinsulinemic diets were also associated with higher risk of PC (75). Other dietary factors may also be risk factors for PC.

Surprisingly, sedentary behaviour, cigarette smoking or alcohol consumption does not appear to increase the risk for PC. Individuals with poor physical activity habits and smokers appear to have worse outcomes, whereas alcohol consumption does not appear to affect PC-specific mortality (76,77).

Other health conditions also affected the likelihood of developing PC. For example, infertile men were at higher risk than fertile men (78). Prostate size correlated inversely with PC risk. Certain autoimmune diseases, such as Sjorgen's syndrome was associated with an overall increased PC risk (73).

Other diseases were evaluated on whether they affected the risk of developing PC, but results were either contradictory, circumstantial, or could be attributed to other factors unrelated to the disease.

3. Astaxanthin

3.1. Overview

ASTX (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a xanthophyll carotenoid widely used in aquaculture, being used to provide a characteristic reddish-orange coloration to certain foodstuffs (79). Although there are natural sources of ASTX, such as the green algae, *Haematococcus pluvialis*, and the red yeast, *Phaffia rhodozyma*, these only account for a very small fraction of ASTX on the market, with most of the compound being synthetic (79). As of 2021, *H. pluvialis* is the only approved source of ASTX for human consumption (80).

ASTX is a very potent antioxidant, displaying other interesting properties, namely anti-inflammatory, anti-diabetic, anticancer and anti-lipid peroxidation activity, as well as contributing to a decline in cardiovascular disease risk (81). Due to these potential benefits, the interest in this carotenoid has significantly increased in recent years. While its use in nutritional supplements and nutraceuticals is clearly rising, its potential use as a pharmaceutical active ingredient has not been fully explored. As an antioxidant, ASTX is 100-500 times more potent than α-tocopherol (82), clearly a much more widely recognized antioxidant chemical.

3.2. Chemistry

The chemical structure of carotenoids is derived from lycopene. Carotenoids can be divided in two major groups based on their structure: carotenes, which have only carbon and hydrogen in their structure, and xanthophylls, the oxygenated derivatives. Oxygen can be present in OH groups, carbonyl groups, or both.

ASTX is a xanthophyll with two chiral centers (in C3 and C3') and three possible configurational isomers (Figure 7), with the (3S,3'S) isomer being the most abundant in nature and synthetic ASTX being a racemic mixture (83).

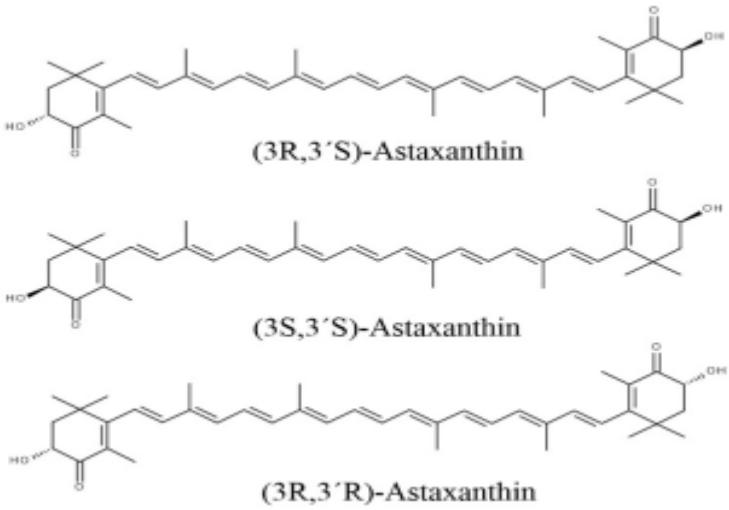

Figure 7. Stereoisomers of ASTX. Retrieved from A. Donoso *et al*. (81)

ASTX contains several functional groups in its structure, including a long polyene system, hydroxyl groups, and keto groups. Each of these serves its purpose: the polyene system is crucial for ASTX's antioxidant properties. The shared electrons from the conjugated double bonds can be donated to free radicals in other to stabilize them, blocking radical chain reactions (78). Additionally, radical chain reactions can be further reduced due to a radical trapping site at the C3 hydrogen (84).

Because ASTX has both hydrophilic and lipophilic properties, it can be simultaneously exposed to both the interior and the exterior of the cell (Figure 8), allowing it to scavenge radicals from both environments. This is a unique advantage over other antioxidants, which can only be exposed to one environment or the other (85).

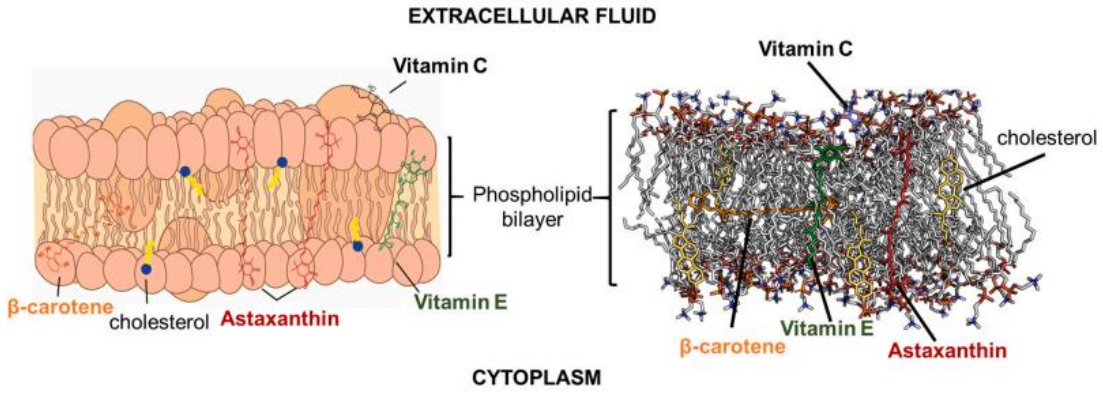

Figure 8. Schematic (left) and tridimensional (right) representations of common antioxidants location at the cell membrane. Retrieved from Doloso *et al*. (83)

3.3. Pharmacokinetics

Similarly to other xanthophylls, ASTX mixes with bile acid after ingestion, forming micelles in the intestine. These micelles are partially absorbed by intestinal mucosal cells and subsequently incorporated into chylomicrons to be delivered to the liver. The chylomicrons containing ASTX are digested by lipoprotein lipase within systemic circulation, with the ASTX molecules being incorporated into very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL), to be transported to the tissues (86).

4. Biological activities and health benefits

In recent years, many studies have been conducted to demonstrate the wide range of potential mechanisms by which ASTX can produce health benefits. Several authors reported ASTX to not only be a potent antioxidant, but also to display photoprotective, anti-inflammatory, and anti-apoptotic effects (84). The combination of these effects resulted in benefits on several levels, including the eye, skin, brain, cardiovascular system, and immune system (Figure 9).

4.1. Skin health

The skin is the body's largest organ and is in constant contact with the external environment. For this reason, it is a prime target for premature aging due to oxidative stress, namely from environmental factors such as pollution and ultraviolet radiation (84). Skin health is mostly affected by the generation of reactive oxygen species due to UV radiation exposure and oxidative metabolism, leading to premature aging ("photo-aging") (87). Other oxidant events are also associated with skin aging, namely DNA damage, inflammatory response, lowered production of antioxidants, and induction of matrix metalloproteinases that degrade important components of the extracellular matrix, such as collagen and elastin (88) and are involved in angiogenesis, promoting cancer cell growth and migration. ASTX was shown in clinical trials to increase skin elasticity, reduce dryness and lower redness (84).

4.2. Eye health

Oxidative damage is a major factor in the pathophysiology of several ocular diseases, including age-related macular degeneration, cataract, uveitis, retinopathy of prematurity, corneal inflammation, and keratitis (84).

Studies suggest that ASTX has a protective effect by accumulating in the epithelial cells of the eye, preventing UVB-induced oxidative stress (84). In addition to this, clinical trials showed that ASTX had a positive effect on retinal blood flow, visual acuity, uveitis, and other parameters (89).

4.3 Cardiovascular health

Pathophysiology of CVD is often driven by ROS, namely in conditions such as atherosclerosis, myocardial infarction, and others. Given how commonplace CVD are in current society – being the leading cause of morbidity and morbidity worldwide (90), it is of particular interest to promote research on possible new therapeutic approaches.

Other antioxidants, such as ascorbic acid and vitamin E, as well as multiple polyphenols and carotenoids, have already been shown to have a positive impact on prevention and treatment of CVD (91). Importantly, clinical trials showed that ASTX supplementation resulted in lower lipid peroxidation, lower LDL levels, and improved several oxidative stress biomarkers (84).

4.4 Neuroprotective properties

The brain is particularly vulnerable to the damages caused by oxidative stress, due to its high oxygen demand, abundance of lipid cells susceptible to peroxidation, and limited capacity for cellular regeneration (92).

Neurodegenerative diseases (e.g. Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis) are characterized by progressive neuronal loss. Age is the biggest risk factor for these diseases, but oxidative stress also plays a major role in their physiopathology, since it can lead to cellular damage, impairment of DNA repair mechanisms, and mitochondrial dysfunction (92). Therefore, antioxidants have become one of many new points of focus for the development of new therapeutic approaches for these conditions.

Clinical trials in animal and human models have shown ASTX to be a promising candidate and therapeutic option, especially in Alzheimer's disease, dementia, and cognitive decline (93), due to its antioxidant, anti-inflammatory, and anti-apoptotic effects.

4.5 Immune response

While the effects of ASTX on the immune response have not yet been extensively researched, multiple trials showed that ASTX supplementation resulted in increased NK-cell cytotoxic activity, increased T- and B-cell subpopulation, and accurate response to bacterial colonization (84).

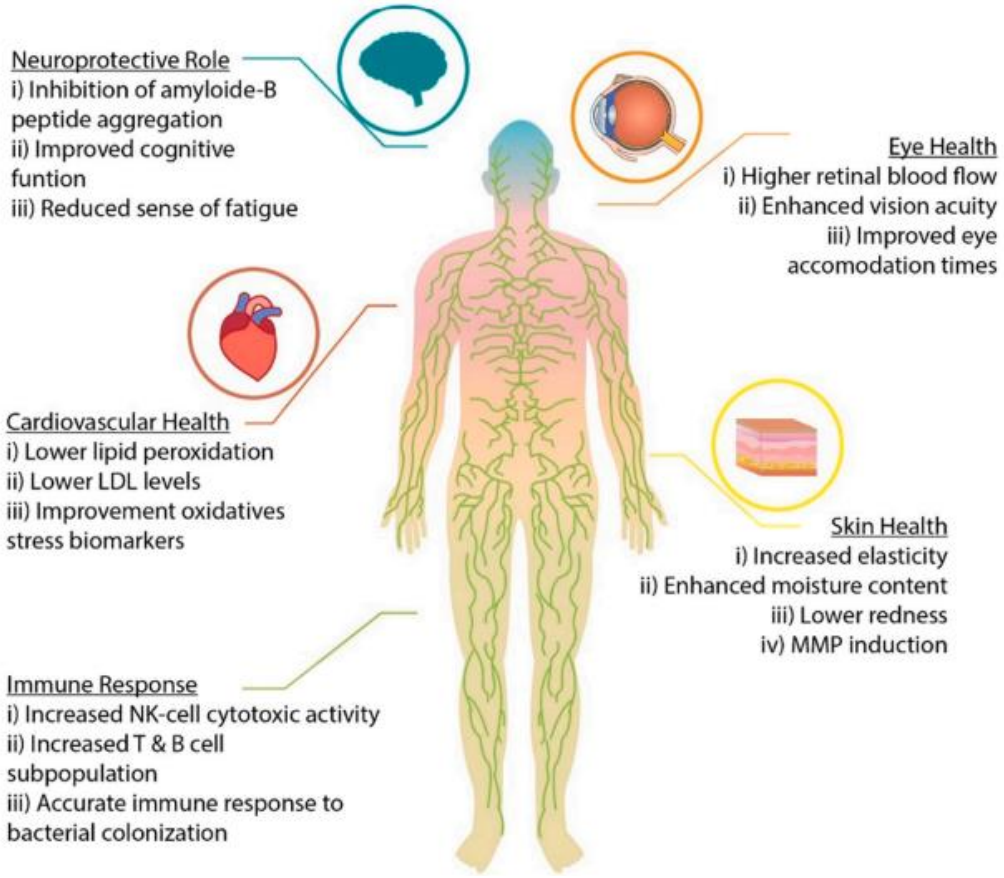

Figure 9. Summary of therapeutic effects of ASTX supplementation as reported in clinical trials. Retrieved from Doloso *et al.* (84)

5. Anticancer effects

5.1. Antiproliferative effects

Cancer cells are characterized by their continuous, rapid proliferation. Proliferation promotes invasion, migration, and adhesion to target tissues. Cell proliferation typically depends on signal transmission by growth factors and adhesion proteins and is usually regulated by signaling pathways such as mitogen-activated protein kinase (MAPK) and phospatidylinositide 3-kinases (PI3K) cascades (94,95).

Some authors reported that ASTX seemed to have a concentration-dependent anti-proliferative effect on different cancer cells, including human hepatoma, rat breast cancer, and mouse lung cancer. Furthermore, this effect was not observed or less observed in normal cells, suggesting a focused targeting of cancer cells (96). The anti-

proliferative effect of ASTX was compared to other carotenoids and was found to be superior to β-carotene, capsanthin, and bixin on K562 leukaemia cells (97).

5.2. Induction of apoptosis

Apoptosis is the process of programmed cell death, which occurs in a controlled and regulated manner in a normal physiological state (98). However, in cancer cells, the apoptotic pathway is typically inhibited due to various processes, such as overexpression of antiapoptotic proteins and/or under-expression of proapoptotic proteins, leading to chemotherapy resistance (99). Thus, an increase in apoptotic activity is a promising therapeutic option in cancer patients, resulting in reduced tumor volume and ultimately increasing life expectancy. Song *et al.* reported the appearance of a hypodiploid peak indicative of apoptosis in flow cytometry of cells treated with ASTX. Additionally, ASTX induced changes in mitochondria morphology, transmembrane potential and respiratory chain and regulated apoptotic proteins (96). Other authors reported that ASTX induced mitochondrial apoptosis in a hamster model oral cancer via down-regulation of anti-apoptotic proteins (100).

5.3 Antioxidant-based mechanisms

Reactive oxygen species (ROS) can contribute to cancer initiation, progression and spreading, due to their potential to act as secondary messengers and activate and maintain specific signalling pathways (101). Oxidative stress can be inhibited by antioxidants such as ASTX. This compound has been demonstrated to reduce intracellular O_2^- by restoring the antioxidant activity of superoxide dismutase (SOD) and catalase (CAT), reversing lipopolysaccharide-induced toxicity and ROS production (102).

5.4 Anti-inflammatory effects

The role of inflammation in the onset of cancer has long since been described. During the inflammatory process, there is an increase of pro-inflammatory cytokines (e.g. IL-

6, IL-1) and tumor necrosis factor-α (TNF-α). The interaction of this generalized pro-inflammatory status with other factors such as the individual's genetic makeup and environment can potentially trigger the onset of cancer (103). Some authors reported that ASTX could play a significant part in cancer prevention, being capable of inhibiting ROS-induced activation of nuclear factor-κB (NF-κB) transcription factor, which results in suppression of inflammatory cytokine production (104). This suggests that ASTX could potentially be a good therapeutic alternative in cancer prevention and treatment. The inflammatory process is present in all stages of cancer progression, which can make it a key target in emerging cancer treatments.

5.5 Invasion and Migration

These two processes are paramount in the development of cancer (105). Malignant cells break away from the primary tumor, attach to proteins in the extracellular matrix and degrade them, and then escape to other parts of the body via direct extension, bloodstream or lymphatic system (106). Matrix metalloproteinases (MMPs) are vital in this process and are overexpressed in cancer cells. ASTX was reported to result in lower mRNA and protein expressions of MMP-2 and MMP-9 (107), suggesting that ASTX can decrease cancer cell invasion and metastasis.

5.6 Gap Junctional Intracellular Communication (GJIC)

GJIC refers to intercellular channels that allow for the diffusion of small hydrophilic molecules between the cytoplasm of neighbour cells, resulting in metabolic and electrical coordination (108). In pathological situations such as cancer, this process is compromised; however, authors reported that ASTX treatment improved GJIC via up-regulated expression of Cx43 protein, which may ultimately result in inhibition of cancer cells and reduction of tumor growth (109,110).

6. Conclusion

Despite all the advances in medicine, especially in highly developed countries, cancer still poses a serious burden which has ramifications in many aspects of society, including the health, financial and political sectors. On an individual level, cancer patients also have an added socioeconomic burden to deal with on top of the disease itself, due to the reduced quality of life, treatment courses that often have many unpleasant side effects, and cost of treatment.

Depending on cancer type and disease progression, it is quite possible for patients to develop a resistance to the treatment (e.g. chemotherapy) and therefore for their treatment to be ineffective, leading to accelerated progression of the disease.

It is crucial to keep looking for new therapeutic strategies, either with added benefits or reduced adverse side effects compared to existing ones. ASTX is a bioactive compound that shows promise in this area due to its multiple protective effects and multiple possible molecular targets. Current studies suggest that it can potentially be effective against several of the more prevalent cancer types; however, the mechanisms by which this occurs are not yet fully understood. Further studies should be done to clarify this issue. Moreover, a significant number of the studies carried out to understand the effects of ASTX on cancer cells were done using traditional *in vitro* models, which does not translate accurately to what happens *in vivo*. Additional advanced in vitro cell-based methodologies as well as well design animal studies are needed to fully assess the beneficial effects of ASTX in cancer.

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