



## Anticancer properties of tocotrienols: a review of cellular mechanisms and molecular targets

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**REVIEW ARTICLE****Anticancer properties of tocotrienols: a review of cellular mechanisms and molecular targets****Marina Montagnani Marelli<sup>1#</sup>, Monica Marzagalli<sup>1#,2</sup>, Fabrizio Fontana<sup>1</sup>, Michela Raimondi<sup>1</sup>, Roberta Manuela Moretti<sup>1</sup> and Patrizia Limonta<sup>1\*</sup>**<sup>1</sup>Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milano, Italy

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- **cell proliferation/apoptosis**
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## ABSTRACT

Vitamin E is composed of two groups of compounds:  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -tocopherols (TPs), and the corresponding unsaturated tocotrienols (TTs). TTs are found in natural sources such as red palm oil, annatto seeds and rice bran. In the last decades, TTs (specifically,  $\gamma$ -TT and  $\delta$ -TT) have gained interest due to their health benefits in chronic diseases, based on their antioxidant, neuroprotective, cholesterol-lowering, anti-inflammatory activities. Several *in vitro* and *in vivo* studies pointed out that TTs also exert a significant antitumor activity in a wide range of cancer cells. Specifically, TTs were shown to exert antiproliferative/proapoptotic effects and to reduce the metastatic/angiogenic properties of different cancer cells; moreover, these compounds were reported to specifically target the subpopulation of cancer stem cells, known to be deeply involved in the development of resistance to standard therapies. Interestingly, recent studies pointed out that TTs exert a synergistic antitumor effect on cancer cells when given in combination with either standard antitumor agents (i.e., chemotherapeutics, statins, 'targeted' therapies) or natural compounds with anticancer activity (i.e., sesamin, EGCG, resveratrol, ferulic acid). Based on these observations, different TT synthetic derivatives and formulations were recently developed and demonstrated to improve TT water solubility and to reduce TT metabolism in cancer cells, thus increasing their biological activity. These promising results, together with the safety of TT administration in healthy subjects, suggest that these compounds might represent a new chemopreventive/anticancer treatment (i.e., in combination with standard therapies) strategy. Clinical trials aimed at confirming this antitumor activity of TTs are needed.

## 1 | INTRODUCTION

Nutraceuticals are chemicals naturally found in foods (functional foods) or in dietary supplements that have general health benefits; they include vitamins, polyphenols,  $\omega$ -3 fatty acids, probiotics, aminoacids and soy derivatives (Rautiainen et al., 2016; Santini et al., 2017; Sauer and Plauth, 2017; Schwingshackl et al., 2017).

Vitamin E was first discovered as a fat-soluble vitamin associated with antioxidant properties and involved in the control of the reproductive functions (Evans and Bishop, 1922). It exists in eight hydrophobic compounds ('tococromanols'), named tocopherols (TPs) and tocotrienols (TTs). The term 'tocopherol' derives from the Greek language (i.e., tocos: child birth; pheros: to bear; ol: alcohol). On the other hand, the term 'tocotrienol' (i.e., TPs isoforms with three double bonds in the isoprenoid side chain) was first proposed by Bunyan and coworkers in 1961 (Bunyan et al., 1961).

TPs and TTs are divided into two groups:  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  TPs and the corresponding isomers  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  TTs. Their chemical structure is composed of a chromanol ring which is linked to a isoprenoid side chain at the C2 position; this chain is saturated in TPs and unsaturated in TTs (with three double bonds at positions 3', 7' and 11'). The unsaturated isoprenoid side chain of TTs may be responsible for the better distribution of these isomers in the cell membranes and their high penetration into tissues with saturated fatty layers (Peh et al., 2016; Suzuki et al., 1993). The four isoforms of both TPs and TTs differ dependently on the degree and position of methyl groups: the  $\alpha$  and  $\beta$  isomers are trimethylated, while the  $\gamma$  isomers are dimethylated and the  $\delta$  are monomethylated on the chromanol ring. The structure of the four TTs isoforms is shown in Figure 1.

Vitamin E members are absorbed in the small intestine and bile salts are necessary for this absorption. The presence of the  $\alpha$ -tocopherol transport protein ( $\alpha$ -TTP) in liver cells is responsible for the packaging of these compounds (mainly  $\alpha$ -tocopherol) into lipoproteins and the subsequent transportation to body tissues through the blood (Hosomi et al., 1997). Tissue uptake for both TPs and TTs may then occur with the involvement of lipoprotein lipases or by receptor-mediated lipoprotein endocytosis (Ahsan et al., 2014). Based on the observation that TTs have a low affinity for  $\alpha$ -TTP and undergo a rapid catabolism in the liver, it has been questioned for many years whether orally administered TTs can reach the different tissues (Birringer et al., 2002; Cardenas and Ghosh, 2013; Hosomi et al., 1997; Peh et al., 2016; Traber, 2007). Moreover, TPs have been reported to interfere with TTs cellular uptake both *in vitro* (Shibata et al., 2010) and *in vivo* (Ikeda et al., 2003). However, pre-clinical observations reported effective health benefits and safety after their oral administration, suggesting the bioavailability of these compounds (Khan et al., 2010; Khanna et al., 2005). Moreover, the bioavailability of TTs has been reported in healthy humans, supporting that these compounds may reach their target tissues through alternative pathways despite their low affinity for  $\alpha$ -TTP (Fu et al., 2014; Qureshi et al., 2016). This clearly supports the existence of specific mechanisms for the absorption and transport of these vitamin E isoforms.

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3 Since 1980s and 1990s, TTs attracted a great attention for their health benefits in preventing or treating  
4 chronic diseases, such as cardiovascular and neurodegenerative diseases and osteoporosis (Abdul-Majeed et  
5 al., 2013; Abdul-Majeed et al., 2015; Chin and Ima-Nirwana, 2015; Kanchi et al., 2017; Khanna et al., 2006;  
6 Parker et al., 1993; Pathak et al., 2016; Sen et al., 2004). In addition, TTs have attracted great interest for  
7 their anticancer effects (Ahsan et al., 2014; Cardenas and Ghosh, 2013; Chin et al., 2016; Henderson et al.,  
8 2012; Peh et al., 2016).

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11 This review provides the state of the art on TTs anticancer properties, based on the experimental,  
12 preclinical and clinical evidence so far available. The molecular mechanisms of the antitumor activity of  
13 these compounds, as well as their effectiveness in combination treatments are discussed. The potential  
14 increased antitumor effects of new synthetic TTs derivatives or novel formulations is also addressed.  
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## 21 | 2 | NATURAL SOURCES OF TOCOTRIENOLS

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24 TTs were first isolated from the latex of the rubber plant *Hevea brasiliensis* (Willd. ex A.Juss.)  
25 Müll.Arg. (Whittle et al., 1966); later, it became consistently clear that TTs are present in different plant  
26 sources, particularly in palm oil, annatto (*Bixa orellana* L.) seeds and rice bran (Ahsan et al., 2015; Shahidi  
27 and de Camargo, 2016).  
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30 Among all edible oils, red palm oil represents the richest source of tocotrienols, particularly  $\gamma$ -TT (about  
31 60% of total tocotrienols) (Ng et al., 2004). In palm oil, tocotrienols and tocopherols represent 70% and 30%  
32 of vitamin E derivatives (Tocotrienol Rich Fraction, TRF), respectively.  
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35 *Bixa orellana* is a small tree originating from the tropical region of the Americas. This tree is  
36 mainly known as the source of annatto, a natural orange-red condiment that can be obtained from its  
37 seeds. The annatto seeds are widely used in traditional dishes in Central and South America, in  
38 Mexico, and in the Caribbean; annatto extracts are also used as an industrial colorant for foods to  
39 add color (yellow or orange color) to many products such as cheese, butter, popcorn and cakes.  
40 Interestingly, annatto (*Bixa orellana*) seeds are the only vegetable source of TTs with virtually no  
41 tocopherols present. More importantly,  $\delta$ -TT (140-147 mg/100 gr dry seeds) accounts for almost  
42 90% of TTs in these seeds, with  $\gamma$ -TT accounting for only 10 % of total TTs. No  $\alpha$ -TT can be found  
43 in annatto seed extracts (Raddatz-Mota et al., 2017).  
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50 Another source of TTs is rice bran, containing about 41% of  $\alpha$ -TT and 59% of  $\gamma$ -TT; no or very low levels  
51 of  $\delta$ -TT can be found in this oil (Ahsan et al., 2015; Goufo and Trindade, 2014; Krager et al., 2015; Min et  
52 al., 2011). Additional sources of TTs include wheat germ, halzenuts, olive oil, grape fruit, flax seed oil and  
53 sunflower oil (Ahsan et al., 2015; Shahidi and de Camargo, 2016).  
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3 Tocotrienol-rich fraction (TRF), as well as the percentage of the different TT isoforms present in the most  
4 relevant food sources, are summarized in Table 1.  
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### 8 | **3 | ANTICANCER PROPERTIES OF TOCOTRIENOLS: *IN VITRO* AND *IN*** 9 10 ***VIVO* STUDIES**

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13 In 1986, it was reported that dietary consumption of palm oil reduce the development of mammary tumors  
14 (induced by carcinogens) in rats (Sylvester et al., 1986). These results were later confirmed by *in vitro*  
15 studies reporting that palm oil-derived TRF (tocotrienol-rich fraction) exerts an antiproliferative effect on  
16 mammary tumor cells (McIntyre et al., 2000; Shah et al., 2003).  
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19 Since then, several studies were reported pointing out that TTs, specifically  $\gamma$ - and  $\delta$ -TT, are endowed with a  
20 significant anticancer activity against different tumors (Aggarwal et al., 2010; Cardenas and Ghosh, 2013;  
21 Chin et al., 2016; Henderson et al., 2012; Malavolta et al., 2016; Meganathan and Fu, 2016; Peh et al., 2016).  
22 In addition to their antioxidant and antiinflammatory properties, the anticancer effects of these compounds  
23 were also shown to be related to their interaction with different intracellular signaling pathways involved in  
24 the mechanisms of proliferation, apoptosis, angiogenesis and metastasis (Galli and Azzi, 2010;  
25 Kannappan et al., 2012; Miyazawa et al., 2008; Nesaretnam, 2008; Sailo et al., 2018; Shanmugam et al.,  
26 2017; Sylvester et al., 2014; Zingg, 2015).  
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#### 33 | **3.1 | Antiproliferative Activity**

34 TTs were shown to exert antiproliferative effects on a wide range of tumor cells, through modulation of the  
35 activity of different intracellular signaling pathways. Most of these studies were performed on human breast  
36 cancer cells (Sylvester et al., 2014).  $\gamma$ -TT was found to reduce the expression of proteins involved in cell  
37 cycle progression, such as cyclin D1 and the cyclin-dependent kinases (CDK) CDK4, CDK2 and CDK6 in  
38 mammary cancer cells (Hsieh et al., 2010; Samant et al., 2010). At the same time, the vitamin E derivative  
39 was shown to increase the expression of CDK inhibitors and to reduce the phosphorylation of the Rb  
40 (retinoblastoma) protein (Hsieh et al., 2010; Samant et al., 2010). The antiproliferative effects of TTs were  
41 also analyzed in prostate cancer cells. It was reported that a TRF preparation exerts a significant growth  
42 inhibition on prostate cancer cells (but not in normal epithelial cells), through G1 arrest (Srivastava and  
43 Gupta, 2006). More specifically, TTs were shown to suppress proliferation and induced apoptosis in prostate  
44 cancer cells by affecting the expression/activity of different targets, such as NF- $\kappa$ B (nuclear factor-kappa B),  
45 PI3K (phosphoinositide-3 kinase)/Akt, STAT (signal transducer and activator of transcription), TGF $\beta$   
46 (transforming growth factor  $\beta$ ) receptor, cyclins, as well as the cell cycle inhibitors p27 and p21 (Barve et al.,  
47 2010; Campbell et al., 2011; Sugahara et al., 2015; Yap et al., 2008). Interestingly, Huang and coworkers  
48 (Huang et al., 2017) reported that a tocotrienol mixture inhibits the growth of the human prostate VCaP cell  
49 line, in a dose-dependent manner. TTs ( $\delta$ -TT being more effective than  $\gamma$ -TT) exert this anticancer effect by  
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3 increasing the expression of p21 and p27; this effect was associated with increased H3K9 acetylation levels  
4 at the proximal promoter regions of both CDI inhibitors and with reduced expression of HDACs (histone  
5 deacetylases). Thus, TTs can suppress tumor growth by blocking the cell cycle at the G1/S transition phase,  
6 at least partially, through epigenetic mechanisms.  
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8 Antiproliferative effects of TTs mediated by cell cycle regulation were reported for other cancer cell types  
9 such as pancreatic (Hodul et al., 2013; Hussein and Mo, 2009; Kunnumakkara et al., 2010), cervical (HeLa)  
10 (Wu and Ng, 2010), lung (Ji et al., 2012a), colon (Shibata et al., 2015) and bladder (Ye et al., 2015) cancer  
11 cells.  
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13 The family of EGF receptors, which is composed of four types of receptors (ErbB1/HER1, ErbB2/HER2,  
14 ErbB3/HER3, ErbB4/HER4) is known to be deeply involved in the control of cell proliferation (Appert-  
15 Collin et al., 2015) through different intracellular signaling pathways, such as the PI3K/Akt/mTOR, MAPK  
16 (mitogen-activated protein kinase), and JAK (Janus kinase)/STAT signaling cascades (Hynes and Lane,  
17 2005; Laurent-Puig et al., 2009; Yarden and Sliwkowski, 2001). For this reason, ErbB proteins are now  
18 considered effective molecular targets in anticancer therapy (Arteaga and Engelman, 2014; Filippi et al.,  
19 2017).  
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21 In mammary cancer cells,  $\gamma$ -TT was reported to inhibit EGF-dependent activation of mitogenic pathways  
22 by reducing the ErbB/HER receptor autophosphorylation, thus suppressing the activity of the PI3K/Akt  
23 signaling pathway and the transcriptional activity of the nuclear factor NF- $\kappa$ B (Shah et al., 2003). In line  
24 with these observations,  $\gamma$ -TT was found to significantly decrease human breast cancer cell proliferation by  
25 reducing both the PI3K/Akt/mTOR and the Ras/Raf/MEK/ERK signaling pathways; this results in the  
26 decrease of c-Myc levels due to its ubiquitination and degradation (Parajuli et al., 2015a). Interestingly,  $\gamma$ -TT  
27 also suppressed the activity of the PI3K/Akt/mTOR pathway, responsible for a rewiring of the breast cancer  
28 cell metabolism, through a decrease of the aerobic glycolysis (Parajuli et al., 2015b). TT treatments were  
29 found to reduce the development of mammary tumors in ErbB2 transgenic mice and to induce apoptosis and  
30 senescence-like growth arrest of cancer cells (Pierpaoli et al., 2013).  
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32 Similar observations were reported in pancreatic (Shin-Kang et al., 2011) and in hepatocellular cancer  
33 cells (Burdeos et al., 2016).  
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35 TTs were shown to reduce cell proliferation also by affecting the post-translational modification of proteins  
36 involved in the mitogenic signaling pathways. In particular, TT affect isoprenylation of these proteins based  
37 on their ability to inhibit HMG-CoA (hydroxymethylglutaryl-coenzyme A) reductase activity by post-  
38 transcriptional downregulation and metabolic degradation. HMG-CoA reductase is the rate-limiting enzyme  
39 in cholesterol synthesis in the mevalonate pathway. This pathway produces different farnesyl and  
40 geranylgeranyl intermediates known to be involved in the post-translational modifications of small  
41 G proteins (i.e. Ras) and of  $\alpha\beta\gamma$ -G protein subunits, thus allowing their anchorage (i.e., activation)  
42 to the plasma membrane.  $\delta$ -TT reduced the proliferation of breast and pancreatic cancer cells  
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3 through the down regulation of HMG-CoA reductase activity (Hussein and Mo, 2009; Khallouki et  
4 al., 2015).

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6 The antiproliferative activity of TTs was further supported by preclinical studies in nude mice  
7 (Kunnumakkara et al., 2010; Manu et al., 2012; Selvaduray et al., 2010; Sylvester et al., 1986; Yap et al.,  
8 2010) (Aggarwal et al., 2013; Huang et al., 2017; Montagnani Marelli et al., 2016; Zhang et al., 2015).

### 11 12 **3.2 | Proapoptotic Activity**

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14 TTs were reported to induce apoptosis in different cancer cells, by triggering both the extrinsic and intrinsic  
15 apoptosis pathways. Pure vitamin E-derived TTs as well as a TRF preparation were found to induce the  
16 intrinsic apoptosis in human breast cancer cells (Loganathan et al., 2013; Takahashi and Loo, 2004; Viola et  
17 al., 2013). In colon carcinoma RKO cells, a TRF preparation induced mitochondrial apoptosis through  
18 activation of p53, followed by a significant increase of the Bax/Bcl-2 ratio, associated with downstream  
19 activation of caspase-9 and caspase-3 (Agarwal et al., 2004). The intrinsic apoptosis pathway was also  
20 shown to mediate the anticancer activity of TTs in hematological (Inoue et al., 2011), pancreatic (Wang et  
21 al., 2015), and neuroblastoma (Tan et al., 2016) cell lines.

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26 TTs were shown to trigger the extrinsic apoptotic pathway. In mammary tumors, TTs decreased the levels  
27 of FLIP (FLICE-inhibitory protein), an apoptosis inhibitory protein that inhibits caspase-8, although  
28 this occurred without the involvement of surface death receptors (Shah and Sylvester, 2004; Sylvester and  
29 Ayoub, 2013).

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32 Interestingly, both the extrinsic and the intrinsic apoptosis pathways can be activated by TTs. For  
33 instance,  $\gamma$ -TT induces apoptosis in human T-cell lymphoma through mitochondrial ROS  
34 production and calcium release, changes in the Bax/Bcl-2 ratio and loss of mitochondrial membrane  
35 potential; it also upregulates surface expression of Fas and FasL, thus triggering caspase-8  
36 activation (Wilankar et al., 2011). Moreover, it was shown that  $\gamma$ -TT sensitizes colon cancer cells to  
37 the proapoptotic activity of TRAIL (a member of the tumor necrosis factor superfamily) and  
38 induces the expression of the TRAIL death receptors DR-4 and DR-5. This effect was mediated by  
39 the expression of p53 and Bax, proteins of the intrinsic apoptosis pathway (Kannappan et al., 2010).

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45 In breast cancer cells, it has been proposed that TTs pro-apoptotic activity involves estrogen  
46 receptor beta (ERbeta) signaling (Comitato et al., 2010). This study shows that, in MCF-7 breast  
47 cancer cells expressing both ERalpha and ERbeta, treatments with tocotrienol rich fraction from  
48 palm oil (PTRF) or purified  $\gamma$ -TT, increase ERbeta nuclear translocation and significantly inhibits  
49 ERalpha expression and complete disappearing of the protein from the nucleus. Moreover, PTRF  
50 treatment induces ER-dependent genes expression (macrophage inhibitory cytokine-1, early growth  
51 response-1 and Cathepsin D) and this is inhibited by the ER inhibitor, ICI 182.780, and induces  
52 DNA fragmentation (Comitato et al., 2010).



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4 A signaling pathway involved in the apoptosis process is also the so called endoplasmic  
5 reticulum (ER) stress. The ER stress is a cellular process that is triggered by different conditions  
6 leading to an imbalance in intracellular homeostasis. Different physiological and pathological  
7 conditions can induce ER stress, severely impairing protein folding; on the other hand, ER stress  
8 can also be induced by several compounds of synthetic or natural origins (Foufelle and Fromenty,  
9 2016; Schonthal, 2013; Wang and Kaufman, 2016). Initially, cells react to ER stress with the so  
10 called unfolded protein response (UPR), a defensive process, known to be aimed at restoring  
11 homeostasis, through the enhancement of the protein folding capacity (Halperin et al., 2014).  
12 However, in conditions of severe stress, misfolded proteins accumulate in the ER and this triggers a  
13 number of prodeath programs (Schonthal, 2013). Double-stranded RNA-dependent protein kinase  
14 PKR-like ER kinase (PERK), inositol-requiring enzyme 1 $\alpha$  (IRE1 $\alpha$ ) and activating transcription  
15 factor 6 (ATF6) are the most important proteins known to act as stress sensors in the ER (Parmar  
16 and Schroder, 2012). In physiological conditions, these proteins are associated (i.e., inactivated)  
17 with the chaperone BiP (immunoglobulin-heavy-chain-binding protein, also known as GRP78)  
18 protein. However, in conditions of severe ER stress, BiP dissociates from the sensors, leading to  
19 their activation; each of these sensors is coupled with a specific cytosolic pathway and each  
20 pathway converges to apoptosis (Hiramatsu et al., 2015; Maurel et al., 2015). In particular, the  
21 ATF4 transcription factor pathway, activated by the PERK/eIF2 $\alpha$  (eukaryotic translational initiation  
22 factor2 $\alpha$ ), stimulates the expression of the proapoptotic protein CHOP (C/EBP homologous protein,  
23 also called GADD153). IRE1 $\alpha$  leads to downstream activation of the JNK (c-Jun N-terminal  
24 kinase)/p38 MAPK, CHOP and caspase-4 pathways (Hiramatsu et al., 2015; Maurel et al., 2015;  
25 Schonthal, 2012).

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40 Based on these data, pharmacological targeting of ER stress is now considered an effective  
41 therapeutic strategy to treat tumors (Maurel et al., 2015; Schonthal, 2012; Schonthal, 2013).  
42 Different natural compounds were shown to induce ER stress-mediated death in cancer cells  
43 (Pereira et al., 2015). In mouse mammary tumor cells,  $\gamma$ -TT induced apoptosis through the  
44 activation of the PERK/eIF2 $\alpha$ /ATF4/CHOP pathway and of caspase-4 (Wali et al., 2009a). In  
45 breast cancer cells,  $\gamma$ -TT was shown to increase the expression of CHOP, leading to the  
46 upregulation of the death receptor DR5 through the JNK and p38 MAPK kinases (Park et al., 2010).  
47 The IRE1 $\alpha$  pathway was also shown to be activated after  $\gamma$ -TT treatment in breast cancer cells  
48 (Patacsil et al., 2012). By means of *in vitro* and *in vivo* studies, we reported that  $\delta$ -TT exerts a  
49 proapoptotic effect in human melanoma cells, while sparing normal melanocytes. In melanoma  
50 cells,  $\delta$ -TT exerted its antitumor activity through the PERK/p-eIF2 $\alpha$ /ATF4/CHOP, IRE1 $\alpha$  and  
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3 caspase-4 ER stress-related branches (Montagnani Marelli et al., 2016). Similar results were  
4 reported in cervical cancer cells (Comitato et al., 2016).  
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6 It is now well established that autophagy may mediate the apoptotic activity of drug-induced ER  
7 stress pathways in cancer cells. In mouse and human mammary tumor cells,  $\gamma$ -TT triggered  
8 autophagy through: increased conversion of the microtubule associated protein 1A/1B-light chain 3  
9 from LC3B-I (its cytosolic form) to LC3B-II (its lipidated form) and increased beclin-1 levels  
10 (Tiwari et al., 2014). In addition, in breast cancer cells,  $\gamma$ -TT was found to induce apoptosis by  
11 triggering both the ER stress and the early phase (LC3B-II, Beclin-1) and late phase (cathepsin-D,  
12 LAMP-1) autophagy pathways (Tiwari et al., 2015a). These observations demonstrate that both ER  
13 stress and autophagy are concurrently activated by TTs and together mediate their effects in  
14 inducing apoptosis of cancer cells. However, further studies are required to definitely confirm the  
15 role of autophagy (prodeath vs. prosurvival) in the antitumor activity of TTs (Tran et al., 2015).  
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### 24 **3.3 | Antimetastatic Activity**

25 Metastasis involves dissemination of tumor cells from the primary tumor to distant organs and subsequent  
26 growth in the new tissue microenvironment. Invasion of the extracellular matrix, formation of new blood  
27 vessels from a preexisting vasculature (angiogenesis) and colonization of distant organs are deeply involved  
28 in the metastatic process. Recent evidence demonstrates that several plant-derived dietary agents  
29 (nutraceuticals), including TTs, can exert their antitumor activity also by targeting these processes, possibly  
30 due to their antiinflammatory properties (De Silva et al., 2016; Gupta et al., 2010; Weng and Yen, 2012).  $\gamma$ -  
31 TT was reported to suppress the invasive ability of prostate cancer cells.  $\gamma$ -TT also induced up-regulation of  
32 E-cadherin (involved in the cell-cell adhesion mechanisms) (Yap et al., 2008) and decreased the  
33 expression of the matrix metalloproteinase MMP-9 in pancreatic cancer cells both *in vitro* and *in*  
34 *vivo* (Kunnumakkara et al., 2010).  $\gamma$ -TT significantly reduced gastric adenocarcinoma cell migration  
35 and matrigel invasion, by down-regulation of the matrix metalloproteinases MMP-2 and MMP-9  
36 and up-regulation of TIMP-1 (tissue inhibitors of metalloproteinase-1) and TIMP-2 (Liu et al.,  
37 2010). A similar antimetastatic activity was observed in melanoma (Chang et al., 2009), lung  
38 (NSCLC) (Ji et al., 2012b), and gastric cancer cells (Manu et al., 2012).  
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48 The epithelial-to-mesenchymal transition (EMT) is well recognized as a typical feature of cancer  
49 progression and a potential target of novel therapies. TTs inhibit the EMT process in breast cancer  
50 cells through inhibition of HGF (hepatocyte growth factor)-dependent activation of Met (the HGF  
51 receptor) (Sylvester, 2014) and activation of the canonical Wnt signaling pathway (Ahmed et al.,  
52 2016). The antimetastatic activity of TTs was also reported for the  $\delta$ -TT isoform. In particular,  
53 Husain and coworkers (Husain et al., 2017) demonstrated that, in pancreatic ductal adenocarcinoma  
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(PDAC) cells *in vitro* and tumors *in vivo*,  $\delta$ -TT significantly inhibits migration, invasion, and the expression of several biomarkers of EMT.

### 3.4 | Antiangiogenic Activity

An additional anticancer mechanism of TTs is related to their antiangiogenic properties. Angiogenesis is the process of new capillary sprouting (neovascularization) from pre-existing blood vessels, responsible for the delivery of oxygen and nutrients to the tumor microenvironment. Tumor cells synthesize and secrete angiogenesis-related proteins, such as VEGF, FGF and EGF that are the responsible, together with their receptors, for the expression of angiogenic genes in endothelial cells and for the ability of these cells to form new vessels. The VEGF/VEGFR axis is the major factor responsible for neoangiogenesis in tumors; thus, it is well recognized as a key molecular target for anticancer agents, both standard therapeutics and dietary food components (Gupta et al., 2010; Shanmugam et al., 2017).

TTs were shown to reduce the angiogenic pathways in both tumor and endothelial cells. Palm tocotrienols downregulated the expression of VEGF in murine mammary cancer cells (Selvaduray et al., 2010). Similar results were obtained in preclinical studies showing that TRF significantly reduces serum VEGF levels in mice bearing mammary tumor xenografts (Selvaduray et al., 2012; Weng-Yew et al., 2009).

A major driver to tumor angiogenesis is hypoxia: low oxygen levels lead to activation of hypoxia-inducible factors (HIFs), a family of transcription factors responsible for the regulation of genes involved in glycolysis and angiogenesis. TTs were reported to decrease hypoxia-induced VEGF secretion in liver hepatocellular and colorectal adenocarcinoma cancer cells; in this study,  $\delta$ -TT inhibited hypoxia-induced HIF-1 $\alpha$  production, thus leading to a suppression of VEGF and IL-8 expression (Shibata et al., 2008a).  $\gamma$ -TT was shown to reduce HIF-1 $\alpha$  accumulation and VEGF paracrine secretion in human gastric adenocarcinoma cells induced by cobalt(II) chloride, an hypoxia mimic, via ERK signaling pathway (Bi et al., 2010).

TTs exert their antiangiogenic activity also by directly targeting endothelial cells. The proliferation of HUVEC (human umbilical vein cells) cells was reported to be reduced by TRF treatment (Weng-Yew et al., 2009); TTs counteracted the VEGF- and FGF-induced HUVEC cell proliferation, with an order of potency of  $\delta$ -> $\beta$ -> $\gamma$ -> $\alpha$ -TT. These compounds also inhibited new blood vessel formation in *in vivo* angiogenic models (Nakagawa et al., 2007; Siveen et al., 2014). Moreover,  $\gamma$ -TT inhibited VEGF-induced autophosphorylation of VEGFR-2 in HUVEC cells through abrogation of the Akt/mTOR signaling pathway (Siveen et al., 2014). Furthermore, in endothelial cells, TTs significantly reduced the expression of two pro-angiogenic cytokines, IL-8 and IL-6, with  $\delta$ -TT being more effective than TRF or  $\gamma$ -TT (Selvaduray et al., 2012).

The direct interaction between TTs and the proangiogenic activity of cancer cells was also addressed.  $\delta$ -TT significantly inhibited colon cancer cell-induced tube formation, migration, and adhesion of HUVEC cells (Shibata et al., 2008b);  $\delta$ -TT also suppressed VEGFR expression and signaling in HUVEC cells ultimately leading to caspase activation. *In vivo* experiments further confirmed the anti-angiogenic activity of the

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3 vitamin E isomer (Shibata et al., 2009). Similar results were reported by Li and coworkers (Li et al., 2011)  
4 showing that  $\gamma$ -TT inhibits the angiogenesis process of HUVEC cells induced by the conditioned medium of  
5 gastric adenocarcinoma cells; this effect is mediated by downregulation of VEGFR-2 expressed on these  
6 cells.  
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9 These data support the notion that TTs might be considered an effective strategy to interfere with tumor  
10 progression based on their antimetastatic/antiangiogenic properties.  
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### 12 13 **3.5 | Targeting Cancer Stem Cells**

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15 Tumors are a mixture of malignant stem cells (cancer stem cells, CSCs) and their differentiated daughter  
16 cells. According to the hierarchical model of tumor progression, CSC is a tumor cell that has the capacity for  
17 self-renewal, the ability to generate all heterogeneous tumor cell lineages, giving rise to the bulk of the tumor  
18 mass and to recapitulate continuous tumor growth (Clarke et al., 2006). CSCs are identified on their ability to  
19 generate tumor spheres when cultured in suspension conditions, to give rise to the heterogeneous original  
20 tumor when inoculated in nude mice, to possess high invasive behavior, and to express specific surface  
21 markers (Nagare et al., 2017). It is now well accepted that CSCs play a major role in the development of  
22 resistance to standard cancer therapies, thereby contributing to disease relapse after an initial response  
23 (Abbaszadegan et al., 2017; Eun et al., 2017). So far, different therapeutic approaches specifically targeting  
24 the CSCs subpopulation have been developed for different tumors (Agliao et al., 2017; Ahmed et al., 2017).  
25 Interestingly, natural compounds previously shown to possess anticancer activity were also reported to  
26 specifically target CSCs (Chen et al., 2017; McCubrey et al., 2017; Siddappa et al., 2017; Siveen et al., 2017;  
27 Torquato et al., 2017).  
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29  
30 Luk and coworkers (Luk et al., 2011) reported that  $\gamma$ -TT downregulates the expression of prostate  
31 CSCs markers (CD133/CD44) in castration-resistant prostate cancer cells (PC-3 and DU145) and  
32 hamper the spheroid formation ability of these cells. In addition, pretreatment of PC-3 cells with  $\gamma$ -  
33 TT was found to suppress the tumor initiation ability of the cells when inoculated in nude mice.  
34 More importantly, CD133-enriched PC-3 cells, highly resistant to docetaxel treatment, were as  
35 sensitive to  $\gamma$ -TT treatment as the CD133-depleted population. In line with these data, Lee and  
36 coworkers (Lee et al., 2013) demonstrated that prostate cancer (PCa) patients receiving androgen-  
37 deprivation therapy display an increased PCa stem/progenitor cell population; similarly, treatment  
38 of PCa cells with antiandrogens induces an increase of the stem/progenitor cell subpopulation.  
39 These data demonstrate that the standard antiandrogen therapy in PCa might result in an undesired  
40 expansion of stem/progenitor cell population, explaining why this therapy fails in most PCa  
41 patients. Using different human PCa cell lines and mouse models, these authors concluded that  
42 targeting PCa stem/progenitor cells with  $\gamma$ -TT results in a significant suppression of the tumors in  
43 the castration-resistant stage.  
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3 TTs were also found to specifically target the CSCs subpopulation in breast cancer. In particular,  
4 it was shown that chemoresistant breast cancer cells are enriched in CSCs and express elevated  
5 levels of STAT-3 signaling mediators, which contribute to CSCs enrichment. Treatment of these  
6 cells with  $\gamma$ -TT, either alone or in combination with simvastatin, efficiently eliminated enriched  
7 CSCs and suppressed expression of STAT-3 signaling mediators. Data demonstrate that  $\gamma$ -TT and  
8 simvastatin, alone or in combination, are able to eliminate CSCs in drug resistant breast cancer cells  
9 (Gopalan et al., 2013). Similar results were reported in triple negative breast (Xiong et al., 2016)  
10 and in colon and cervical cancer cells (Gu et al., 2015). The vitamin E-derived  $\delta$ -TT isoform was  
11 shown to selectively inhibit PDAC stem-like cells. In these cells,  $\delta$ -TT inhibited the viability,  
12 survival, self-renewal, and expression of Oct4 and Sox2 transcription factors. Furthermore, in an  
13 orthotopic xenograft model of human PDAC stem-like cells,  $\delta$ -TT significantly delayed the growth  
14 and metastases of gemcitabine-resistant PDAC human stem-like cells (Husain et al., 2017).  
15 More recently, we reported that a subpopulation of autofluorescent cells expressing the ABCG2  
16 stem cell marker is present in human melanospheres;  $\delta$ -TT specifically target this CSCs  
17 subpopulation (Marzagalli et al., 2018).  
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28 Altogether, since TTs have been shown to be safe and to reach bioactive levels in humans, these  
29 data suggest that these compounds may represent effective agents in targeting CSCs; this may  
30 account for their anticancer and chemosensitizing effects reported in different studies.

31 The molecular mechanisms of the anticancer activity of TTs are summarized in Figure 2.  
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#### 37 | **4 | ORIGIN OF TOCOTRIENOL INTRACELLULAR SIGNALING IN CANCER CELLS**

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40 As discussed above, TTs exert their anticancer activity by triggering different intracellular signaling  
41 pathways; however, the precise origin of these signals is still unknown. Vitamin E signaling was proposed to  
42 originate within the different lipid environments of the cell, both at the plasma and organelle membranes,  
43 where it is delivered to specific subcellular targets (Galli and Azzi, 2010; Saito et al., 2009).  
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45 The trafficking and subcellular localization of vitamin E,  $\alpha$ -TP in particular, was shown to be regulated by  
46 cytosolic proteins that bind with their hydrophobic domains the vitamin derivative. These proteins, Sec14p-  
47 like proteins, are prototype components of the cell vitamin E regulation system that may play also a key role  
48 in the signaling of this vitamin and other lipids (Zingg et al., 2008). However, these observations were  
49 reported for TPs but not for TTs, responsible for the vitamin E anticancer activity.  
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53 As underlined above, ErbB proteins are a family of tyrosine kinase receptors. Based on their key role in  
54 the mechanisms of tumor growth and development, these receptors are considered effective molecular targets  
55 in anticancer therapy (Arteaga and Engelman, 2014; Filippi et al., 2017).  
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Lipid rafts are specialized rigid microdomains located within the plasma membrane that are enriched with cholesterol and sphingolipids. They are also associated with specific proteins, such as caveolins, flotillins, palmitoylated proteins, and nonreceptor tyrosine kinases; based on their composition, they are resistant to detergent solubilization and can be easily isolated from the surrounding plasma membrane (Chamberlain, 2004; Pike, 2009). Lipid rafts are deeply involved in ErbB activation and intracellular signal transduction. The anticancer effects of TTs,  $\gamma$ -TT in particular, were shown to be associated with the suppression of HER2 signaling (Shah and Sylvester, 2005; Tiwari et al., 2014); based on this observation, Alawin and coworkers (Alawin et al., 2016) investigated the effects of  $\gamma$ -TT on HER2 activation within the lipid raft microdomains in HER2-positive breast cancer cells. Treatment with  $\gamma$ -TT significantly inhibited cancer cell growth, through a decreased HER2 dimerization and phosphorylation. Both phosphorylated HER2 and  $\gamma$ -TT were found to accumulate exclusively within the lipid raft microdomains. Cotreatment of the cells with a compound that disrupts lipid raft integrity (hydroxypropyl- $\beta$ -cyclodextrin, HP $\beta$ CD) significantly reduced  $\gamma$ -TT cytotoxicity as well as its accumulation in the lipid raft microdomains. These results demonstrate that  $\gamma$ -TT accumulates in lipid raft microdomains directly leading to their disruption (thus interfering with growth factor receptor dimerization/activation) to exert its cytotoxic effects in breast cancer cells (Alawin et al., 2016). More recently,  $\gamma$ -TT was reported to suppress the activation of HER3 and HER4 growth factor receptors in lipid rafts microdomains in breast cancer cells through downregulation of the release of heregulin-containing exosomes (Alawin et al., 2017).

TTs were also shown to exert their anticancer effects through their direct binding to specific molecular targets. In particular, these compounds directly bind to Src and HMG-CoA reductase, thus inhibiting their role in tumor development (Aggarwal et al., 2010; Upadhyay and Misra, 2009).

## **5 | SYNERGISTIC ANTICANCER PROPERTIES OF TOCOTRIENOLS WITH STANDARD TREATMENTS OR NATURAL COMPOUNDS**

### **5.1 | Chemotherapeutic Drugs**

The major burden of standard cancer therapies is represented by the development of drug resistance and by the serious side effects often associated with these treatments. TTs were shown to possess anticancer activity against a wide range of tumors cells. Moreover, the bioavailability and safety of these compounds were demonstrated in healthy subjects (Fu et al., 2014; Qureshi et al., 2016) and in pancreatic cancer patients (Springett et al., 2015). Based on these observations, several studies were performed to investigate whether TTs might exert a synergistic antitumor activity in cancer cells when given in combination with anticancer compounds (i.e., standard chemotherapeutic agents, dietary components), with the aim to increase their efficacy in killing these cells (Eitsuka et al., 2016b).

In non-small lung cancer cells,  $\delta$ -TT was demonstrated to synergize with cisplatin in inducing the suppression of cell viability, migration and invasiveness (Ji et al., 2012b). Manu and coworkers (Manu et al.,

2012) reported that  $\gamma$ -TT, in addition to its antiproliferative/antimetastatic activity on gastric cancer cells, chemosensitizes these cells to the antitumor activity of capecitabine, both *in vitro* and *in vivo*, in nude mice bearing gastric cancer cell xenografts. More recently, similar observations were reported for  $\gamma$ -TT in colorectal tumor cells (Prasad et al., 2016).

As discussed above, the ErbB receptor family is deeply involved in the mechanisms underlying tumor growth and progression. Erlotinib and gefitinib are well known inhibitors (tyrosine kinase inhibitors, TKI) of ErbB1 based on their ability to compete with ATP for binding to the intracellular catalytic domain of this receptor, thus inhibiting its activation (Yuan et al., 2014). Unfortunately, inactivation of ErbB1 leads to heterodimerization (i.e., activation) of other ErbB receptors, thus allowing tumor cells to escape from TKI anticancer activity. For this reason, combination treatments have been considered an interesting and effective therapeutic strategy to overcome the development of cancer cell resistance to TKIs. TTs were shown to exert their antitumor activity, at least partially, through inhibition of the ErbB receptor activation and their associated signaling pathways. It was reported that treatment of +SA mammary tumor cells with  $\gamma$ -TT synergistically increases the anticancer/proapoptotic activity of both erlotinib and gefitinib; this effect was mediated by a decrease in the expression levels of ErbB2-4 receptors and in their downstream Akt and STAT signaling (Bachawal et al., 2010). However, further studies would be needed to definitely assess the efficacy of combination treatments based on both natural TTs and tyrosine kinase inhibitors.

HMG-CoA reductase is the rate-limiting enzyme in cholesterol synthesis in the mevalonate pathway (Goldstein and Brown, 1990). As discussed above, this pathway produces different intermediates that are involved in the post-translational modifications of proteins, such as small G proteins (i.e., Ras) and  $\alpha\beta\gamma$ -G protein subunits, thus allowing their anchorage to the plasma membrane and subsequent activation. Downregulation of HMG-CoA reductase inhibits the activation of these proteins, thus interfering with cancer cell proliferation, apoptosis and metastasis. Thus, statins (lovastatin, simvastatin, atorvastatin, mevastatin), through their ability to competitively inhibit HMG-CoA reductase and to induce its post-transcriptional downregulation and metabolic degradation, were shown to suppress the growth of a wide range of cancer cells by inducing cell cycle arrest and apoptosis (Demierre et al., 2005). However, their use is limited due to their severe side effects (muscle pain and damage), ultimately leading to rhabdomyolysis that can cause liver damage, kidney failure and death (Thibault et al., 1996). Moreover, chronic use of statins often leads to a compensatory upregulation of this enzyme (Wali et al., 2009b).  $\delta$ -TT is known to inhibit cancer cell growth by interfering with the HMG-CoA reductase activity (Hussein and Mo, 2009; Khallouki et al., 2015). Based on these observations, cotreatment with statins and TTs has been thought to induce a synergistic/additive anticancer activity. In prostate cancer cells,  $\gamma$ -TT was reported to potentiate the anticancer activity of lovastatin (Mo and Elson, 2004). A combination treatment with TTs and lovastatin synergistically inhibited the growth of murine melanoma, human prostate cancer and human lung adenocarcinoma cell xenografts in nude mice (McAnally et al., 2007). Wali and coworkers reported that a combination of  $\gamma$ -TT and a statin (simvastatin, mevastatin or lovastatin) synergistically decrease the

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3 proliferation of +SA mouse mammary epithelial cells, through the suppression of the MAPK, PI3K/Akt,  
4 JNK and p38 MAPK pathways (Wali and Sylvester, 2007). A synergistic antitumor activity of  $\gamma$ -TT and  
5 statins, atorvastatin and lovastatin, was also demonstrated in colon cancer and melanoma cells, respectively  
6 (Fernandes et al., 2010; Yang et al., 2010).  
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9 More recently, it has been reported that a concurrent delivery of a TRF preparation and simvastatin by lipid  
10 nanoemulsions significantly potentiates their antitumor activity against human breast cancer cells, both  
11 estrogen-dependent and estrogen-independent (Alayoubi et al., 2013). Interestingly, Gopalan and coworkers  
12 demonstrated that a combination of  $\gamma$ -TT and simvastatin is able to eliminate the cancer stem cell  
13 subpopulation in drug resistant human breast cancer cells (Gopalan et al., 2013). A synergistic effect of  
14 combined treatment with  $\gamma$ -TT and statins has been reported also in malignant mesothelioma cells (Tuerdi et  
15 al., 2013).  
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19 Synergistic antitumor effects were shown in +SA mouse mammary epithelial cells after a combination  
20 treatment with  $\gamma$ -TT and celecoxib, a non-steroidal anti-inflammatory drug that specifically inhibits  
21 cyclooxygenase-2 (COX-2). The synergistic antiproliferative activity of the two compounds was found to  
22 reduce COX-2, Akt and NF- $\kappa$ B levels and to decrease PGE2 synthesis, through suppression of HERB2-4  
23 tyrosine kinase receptor levels (Shirode and Sylvester, 2011).  
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## 28 **5.2 | Natural Compounds**

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30 TTs were widely shown to exert a synergistic anticancer activity also when given in combination with  
31 different natural dietary compounds (Eitsuka et al., 2016b).  
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33 Sesamin is a lignan that can be found in sesame seeds and flax; interestingly, it has been reported to inhibit  
34 metabolic degradation of TTs, thus improving their bioavailability (Sontag and Parker, 2002). Akl and  
35 coworkers (Akl et al., 2013; Akl et al., 2012) found that cotreatment of mammary tumor cells with  
36  $\gamma$ -TT and sesamin synergistically inhibit cell proliferation by arresting the cell cycle progression in the G1/S  
37 transition phase and by interfering with ErbB receptor activation and its downstream signaling pathways  
38 (MAPK, PI3K/Akt, JAK/STAT and NF- $\kappa$ B).  
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42 Epigallocatechin gallate (EGCG) is a plant-derived flavonoid, belonging to the flavanol subclass,  
43 mainly found in green tea (Manach et al., 2004). EGCG is well known for its cancer preventing  
44 activity through its ability to interfere with the EGFR intracellular signaling pathways (Shimizu et  
45 al., 2008), to induce ER stress, and to modulate gene expression, both by means of a direct effect on  
46 transcription factors or by indirect epigenetic mechanisms (Naponelli et al., 2017). Hsieh and Mu  
47 (Hsieh and Wu, 2008) investigated the effects of a combination treatment with  $\gamma$ -TT and EGCG on  
48 breast cancer cell growth. They found that the two natural compounds synergistically decrease cell  
49 proliferation by reducing cell cycle- and apoptosis-related proteins. This combination treatment also  
50 upregulated the expression of Nrf2 (nuclear factor erythroid 2-related factor 2), a transcription  
51 factor that regulates the transcription of different cytoprotective (antioxidant) genes, such as NQ01  
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(NAD(P)H quinone dehydrogenase 1) (Dinkova-Kostova and Talalay, 2010). Similar results were observed by cotreating cancer cells with  $\gamma$ -TT and resveratrol, a stilbene mainly found in grapes and red wine, that is associated with anticancer activity due to its ability to downregulate the expression of HMG-CoA reductase and to potentiate the antiproliferative effects of statins (Cho et al., 2008; Wong et al., 2011).

Rice bran is a source of different bioactive compounds, such as TTs and ferulic acid. The bioavailability of both TTs and ferulic acid after oral administration was clearly demonstrated (Khan et al., 2010; Khanna et al., 2005; Zhao et al., 2003). It was shown that ferulic acid can potentiate the growth inhibitory effects of  $\delta$ -TT in a wide range of cancer cells, including prostate, breast and pancreatic cancer cells. This synergistic effect was attributed by an increased intracellular level of the TT due to a suppression of its metabolism induced by ferulic acid (Eitsuka et al., 2014). More recently, Eitsuka and coworkers (Eitsuka et al., 2016a) reported that a combination treatment with  $\delta$ -TT and ferulic acid synergistically inhibits cellular telomerase activity. In particular, cotreatment with the two compounds downregulated the expression of telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase, demonstrating that the activity of this enzyme is regulated at the transcriptional level.

Additional natural compounds that have been shown to possess a synergistic anticancer activity when coadministered with TTs include: 6-gingerol, inducing colorectal cancer cell apoptosis in combination with  $\gamma$ -TT (Yusof et al., 2015); oridonin, synergistically triggering apoptosis with  $\gamma$ TT in mammary cancer cells through induction of autophagy (Tiwari et al., 2015b); geranylgeraniol, exerting a significant antitumor activity on castration-resistant DU145 prostate cancer cells when coadministered with  $\delta$ -TT, by potentiating the suppression of HMG-CoA reductase induced by the vitamin E derivative (Yeganehjoo et al., 2017). However, while the safety of TTs in humans has been demonstrated, the safety of some of these compounds still has to be defined based on their ability to affect the functions of different tissues/organs. For instance, geranylgeraniol was reported to stimulate testosterone production in testis-derived tumor cells (Ho et al., 2016) and to counteract the antitumor activity of statins (pitavastatin) in drug-resistant ovarian cancer cells (de Wolf et al., 2017).

Taken together, these observations suggest that properly formulated TT-combination treatments, with both standard anticancer or natural compounds, might represent an novel strategy in cancer preventive or therapeutic interventions (i.e., in combination with standard therapies), avoiding development of drug resistance and reducing toxic effects of standard treatments (Table 2).

## 6 | NOVEL SYNTHETIC DERIVATIVES AND FORMULATIONS OF TOCOTRIENOLS

During the last few years, several semisynthetic derivatives of tocotrienols were prepared, with the aim to improve their water solubility and to reduce their metabolism in cancer cells, thus increasing their biological

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3 activity. As underlined by Behery and coworkers, the structure of tocotrienols can be divided into three  
4 domains. Domain I is the phytol side chain, responsible for the docking of TTs into the lipophilic bilayer of  
5 the membranes; domain II is the chroman ring, which is usually not modified in synthetic TT derivatives;  
6 and domain III, the phenolic OH group, responsible for the antioxidant activity of TTs (Behery et al., 2013).  
7 During the last decade, this third domain has been modified by esterification, etherification or  
8 carbamoylation leading to the synthesis of several tocotrienol analogs demonstrated to possess an improved  
9 anticancer activity (Behery et al., 2010; Elnagar et al., 2010). In particular, esterification of TTs converts  
10 them into redox-silent compounds demonstrated to undergo a slow hydrolytic process (and subsequent  
11 release of the native compounds), and characterized by higher chemical stability and bioactivity (Behery et  
12 al., 2010; Elnagar et al., 2010; Neuzil et al., 2007). Similar observations were reported by Gagic et al.,  
13 reporting an improved stability of aminoacid esters of  $\gamma$ -TT in human plasma (Gagic et al., 2016).  
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19 As discussed above, TTs have a lower affinity for  $\alpha$ -TTP and undergo liver metabolism and biliary  
20 excretion, raising the question whether orally administered TTs can reach the different tissues. This low  
21 affinity for  $\alpha$ -TTP seems to be related to the rigidity of the tail structure (domain I) that is higher in TTs than  
22 in TPs (due to the presence of double bonds). Based on this observation, TT derivatives, the tocoflexols,  
23 were developed with a more flexible tail that might be responsible for a higher affinity for  $\alpha$ -TTP and,  
24 consequently, for an improved distribution to the different target tissues. Preliminary data demonstrate that,  
25 even after the modification of the side chain, these compounds are able to maintain the antioxidant properties  
26 of TTs (Compadre et al., 2014).  
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31 By structural modification of the domain II (by electrophilic substitution reactions), several redox-silent TT  
32 (particularly  $\gamma$ - and  $\delta$ -TT) oxazine derivatives could be obtained (Ananthula et al., 2014a; Ananthula et al.,  
33 2014b; Behery et al., 2013). These compounds were reported to decrease the growth of breast cancer cells,  
34 both *in vitro* and *in vivo* (Behery et al., 2013). In mammary cancer cells, a semisynthetic  $\delta$ -TT oxazine  
35 derivative was shown to counteract the overexpression of HIF-1 $\alpha$  consequent to artificially-induced hypoxic  
36 conditions; a corresponding decrease of the Akt/mTOR signaling pathway, the major regulator of HIF-1 $\alpha$   
37 synthesis, was also observed (Ananthula et al., 2014b). Interestingly, oxazine derivatives of  $\gamma$ - and  $\delta$ -TT were  
38 also prepared as lipid nanoemulsions. After intratumor injection in breast cancer xenografts, these TT  
39 formulations were found to significantly reduce tumor growth and this antitumor activity was associated with  
40 alterations of the expression of different cell cycle-regulatory proteins (Ananthula et al., 2014a).  
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47 To improve the aqueous solubility of TTs, Abu-Fayyad and Nazzal chemically conjugated these  
48 compounds with terminally methylated poly ethylene glycols (mPEG) with molecular weights of  
49 approximately 350 (mPEG350) and 1000 (mPEG1000), using a succinate molecule as the linker to the 6-OH  
50 group on the chroman ring (domain III). Among these ester conjugates,  $\gamma$ -TTPGS1000 and  $\delta$ -TTPGS1000  
51 were shown to be the most effective in exerting a cytotoxic activity on breast and pancreatic cancer cells,  
52 being less toxic on non-tumorigenic cells (Abu-Fayyad and Nazzal, 2017b). On the other hand, the presence  
53 of the mPEG molecule reduced the antitumor effects of TTs, possibly due to the conjugation of mPEG to the  
54 6-OH group, known to be crucial for their activity. Based on this observation, these authors developed novel  
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3 conjugates in which the mPEG moiety is linked to carbon-5 of the chroman ring via an amide bond or via  
4 hydrazone linkage. They reported that the amide derivative exerts a greater cytotoxic activity than the  
5 hydrazone conjugate on breast and pancreatic cancer cells. More importantly, the  $\gamma$ -TT amide conjugate was  
6 significantly more active than the ester conjugates (Abu-Fayyad and Nazzal, 2017c), supporting the  
7 hypothesis that a free OH group is crucial for TTs to exert their antitumor activity. Promising results were  
8 also reported in pancreatic cancer cells with a novel gemcitabine- $\gamma$ -TT conjugate entrapped into  
9 nanoemulsions (Abu-Fayyad and Nazzal, 2017a).

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11 To increase the systemic bioavailability of TTs, different formulations were developed. Nano-emulsified  
12 TT formulations were found to possess a significant anticancer activity *in vitro* and were proposed as a  
13 potential topical application of TTs against skin carcinomas (Pham et al., 2016); these formulations were also  
14 reported to exert a radioprotective effect *in vivo* after oral administration in mice exposed to total body  
15 gamma radiation (Ledet et al., 2016). A potentiation of the antitumor activity of both TTs and simvaSTATin  
16 against mammary cancer cells was observed after concurrent delivery of the two drugs by lipid  
17 nanoemulsions (Alayoubi et al., 2013). Enhanced solubility and oral bioavailability of TTs (specifically  $\gamma$ -  
18 and  $\delta$ -TT) were also observed when using a self-emulsifying drug delivery system (Alqahtani et al., 2014).

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20 Recently, tumor-targeted (transferrin-bearing) vesicles entrapping either the TRF extracted from palm oil  
21 or the individual TTs were developed. The rationale of this formulation is that transferrin receptors are  
22 frequently expressed in cancer cells (Calzolari et al., 2007) and can thus represent an effective target for the  
23 delivery of therapeutic drugs into cancer cells. It was reported that transferrin-bearing vesicles entrapping  
24  $\alpha$ -TT are highly effective in reducing the growth of human epidermoid carcinoma cancer cells and of murine  
25 melanoma cells; when intravenously administered in nude mice, these vesicles induced a significant  
26 suppression of both tumor xenografts, without signs of toxicity (Karim et al., 2017).

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28 Based on these promising results, the development of novel TT derivatives/formulations will likely  
29 improve the biological activity of these compounds further supporting their potential role as novel  
30 chemopreventive/treatment strategies against cancer.  
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## 43 | **7 | CLINICAL STUDIES OF THE ANTICANCER ACTIVITY OF TOCOTRIENOLS**

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45 Despite the high number of *in vitro* and *in vivo* (preclinical) studies supporting a significant effect  
46 of TTs in counteracting cancer development and progression, the clinical data so far available are  
47 still scanty. The first clinical trial was performed by Nesaretnam and coworkers (Nesaretnam et al.,  
48 2010) in breast cancer patients. A double-blinded, placebo-controlled pilot trial to test the  
49 effectiveness of adjuvant tocotrienol therapy in combination with tamoxifen was conducted for 5  
50 years in women with early breast cancer. Breast cancer patients with either Stage I or II estrogen  
51 receptor positive breast cancer were assigned to two groups: placebo plus tamoxifen (control group)  
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3 or tocotrienol rich fraction (TRF, 400 mg/day) plus tamoxifen (intervention group), for 5 years. The  
4 five-year breast cancer-specific survival was 98.3% in the intervention group and 95% in the  
5 control group; moreover, the five-year disease-free survival was 86.7% and 83.3% in the two  
6 groups, respectively. The mortality risk was 60% lower in the TRF group *versus* controls; however,  
7 this finding was not statistically significant and the authors concluded that no association seems to  
8 exist between adjuvant TT therapy and breast cancer-specific survival in women with early breast  
9 cancer.  
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11 It is generally believed that this unexpected negative result was possibly due to the small sample  
12 size of the study.  
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14 On the other hand, 25 patients with pancreatic ductal neoplasia were enrolled in a phase I dose  
15 escalation study and treated with different doses of  $\delta$ -TT (from 200 to 3200 mg/day) for two weeks  
16 prior to surgery. The treatment was well tolerated at all the doses of  $\delta$ -TT. At the end of the  
17 treatment, apoptosis markers (active caspase-3) was assessed in neoplastic cells. It was found that,  
18 in cancer tissues from treated patients, the levels of the active form (i.e., cleaved) of caspase-3 were  
19 significantly increased with respect to tumor control tissues. The higher percentage of caspase-3  
20 positive cells was found in tissues of patients treated with dose levels of 200-600 mg of  $\delta$ -TT. In  
21 these patients,  $\delta$ -TT was well tolerated and reached bioactive levels in blood. Thus, this vitamin E  
22 isoform significantly induces apoptosis in pancreatic ductal neoplasia tissues (Springett et al.,  
23 2015).  
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34 Based on the promising results from *in vitro* and *in vivo* studies, clinical trials investigating the  
35 chemopreventive/antitumor efficacy of TTs in cancer patients to further assess the efficacy of these  
36 compounds as novel treatment strategies are currently ongoing (see ClinicalTrials.gov).  
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## 42 **8 | CONCLUSION AND FUTURE DIRECTION**

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45 Vitamin E derived TTs (but not TPs) were reported to be associated with significant health benefits  
46 in different chronic diseases, such as neurodegenerative and cardiovascular diseases. TTs  
47 (specifically  $\gamma$ - and  $\delta$ -TT) were also shown to possess antitumor activity by suppressing cancer cell  
48 proliferation, and this was initially attributed to their antioxidant and antiinflammatory properties.  
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50 On the other hand, *in vitro* and *in vivo* studies clearly pointed out that TTs exert their anticancer  
51 (antiproliferative/proapoptotic, antimetastatic and antiangiogenic) activity also by targeting  
52 different intracellular pathways, such as: cell cycle- and apoptosis-related proteins, growth factor  
53 receptors signaling cascades, the ER stress-autophagy pathway, EMT transition, VEGF secretion  
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3 from tumor cells and activity on endothelial cells, etc. Interestingly, TTs were also reported to  
4 reduce cancer cell growth by interfering with the post-translational modification and metabolic  
5 degradation of HMG-CoA reductase, the key enzyme involved in cholesterol synthesis. The  
6 intermediates of this pathway are responsible for the post-translational modifications (i.e.,  
7 activation) of proteins, such as Ras and  $\alpha\beta\gamma$ -G protein subunits, thus allowing them to anchor to the  
8 membrane and to trigger the intracellular mechanisms leading to cell proliferation. Downregulation  
9 of HMG-CoA reductase hamper these pathways, leading to cell death.  
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14 On the other hand, despite these consistent observations, the precise origin of the antitumor activity  
15 of TTs is still unclear. In addition to their ability to directly bind to and inactivate specific molecular  
16 targets (such as HMG-CoA reductase and Src), it has been suggested that these compounds, based  
17 on their hydrophobic structure, might accumulate within membrane lipid rafts microdomains,  
18 leading to the disruption of their integrity, thus interfering with growth factor HER2 receptor  
19 dimerization, phosphorylation and downstream signaling.  
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24 In addition to their anticancer effects, TTs were widely reported to exert a synergistic/additive  
25 activity with both standard anticancer drugs and natural compounds with antitumor activity, *in vitro*  
26 and *in vivo*. For instance, TTs were shown to potentiate the anticancer activity of statins in different  
27 cancer cell lines.  
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31 Taken together, these promising results recently led to the design and synthesis of different novel  
32 TT derivatives/formulations with the aim to improve the solubility and bioavailability, and therefore  
33 the biological activity, of these compounds.  
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36 In conclusion, results from several *in vitro* and *in vivo* studies strongly support the notion that  
37 vitamin E derived TTs (but not TPs) exert a significant anticancer activity in a wide range of human  
38 cancer cell lines. However, the clinical observations so far available are still scanty; thus, clinical  
39 trials investigating the potential effectiveness of these compounds as novel  
40 chemopreventive/treatment strategies (i.e., in combination with standard therapies) in tumors are  
41 urgently needed.  
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For Peer Review

**REFERENCES**

- Abbaszadegan MR, Bagheri V, Razavi MS, Momtazi AA, Sahebkar A, Gholamin M. 2017. Isolation, identification, and characterization of cancer stem cells: A review. *J Cell Physiol* 232(8):2008-2018.
- Abdul-Majeed S, Mohamed N, Soelaiman IN. 2013. A review on the use of statins and tocotrienols, individually or in combination for the treatment of osteoporosis. *Curr Drug Targets* 14(13):1579-1590.
- Abdul-Majeed S, Mohamed N, Soelaiman IN. 2015. The use of delta-tocotrienol and lovastatin for anti-osteoporotic therapy. *Life Sci* 125:42-48.
- Abu-Fayyad A, Nazzal S. 2017a. Gemcitabine-vitamin E conjugates: Synthesis, characterization, entrapment into nanoemulsions, and in-vitro deamination and antitumor activity. *Int J Pharm* 528(1-2):463-470.
- Abu-Fayyad A, Nazzal S. 2017b. Synthesis, characterization, and in-vitro antitumor activity of the polyethylene glycol (350 and 1000) succinate derivatives of the tocopherol and tocotrienol isomers of Vitamin E. *Int J Pharm* 519(1-2):145-156.
- Abu-Fayyad A, Nazzal S. 2017c. Synthesis, physiochemical characterization, and in vitro antitumor activity of the amide and pH cleavable hydrazone conjugates of gamma-tocotrienol isomer of vitamin E with methoxy-poly(ethylene) glycol. *Int J Pharm* 529(1-2):75-86.
- Agarwal MK, Agarwal ML, Athar M, Gupta S. 2004. Tocotrienol-rich fraction of palm oil activates p53, modulates Bax/Bcl2 ratio and induces apoptosis independent of cell cycle association. *Cell Cycle* 3(2):205-211.
- Aggarwal B, Prasad S, Sung B, Krishnan S, Guha S. 2013. Prevention and treatment of colorectal cancer by natural agents from mother nature. *Curr Colorectal Cancer Rep* 9(1):37-56.
- Aggarwal BB, Sundaram C, Prasad S, Kannappan R. 2010. Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. *Biochem Pharmacol* 80(11):1613-1631.
- Agliano A, Calvo A, Box C. 2017. The challenge of targeting cancer stem cells to halt metastasis. *Semin Cancer Biol* 44:25-42.
- Ahmed M, Chaudhari K, Babaei-Jadidi R, Dekker LV, Shams Nateri A. 2017. Concise rReview: emerging drugs targeting epithelial cancer stem-like cells. *Stem Cells* 35(4):839-850.
- Ahmed RA, Alawin OA, Sylvester PW. 2016. gamma-Tocotrienol reversal of epithelial-to-mesenchymal transition in human breast cancer cells is associated with inhibition of canonical Wnt signalling. *Cell Prolif* 49(4):460-470.

- 1  
2  
3 Ahsan H, Ahad A, Iqbal J, Siddiqui WA. 2014. Pharmacological potential of tocotrienols: a review.  
4 Nutr Metab (Lond) 11(1):52.  
5  
6 Ahsan H, Ahad A, Siddiqui WA. 2015. A review of characterization of tocotrienols from plant oils  
7 and foods. J Chem Biol 8(2):45-59.  
8  
9 Akl MR, Ayoub NM, Abuasal BS, Kaddoumi A, Sylvester PW. 2013. Sesamin synergistically  
10 potentiates the anticancer effects of gamma-tocotrienol in mammary cancer cell lines.  
11 Fitoterapia 84:347-359.  
12  
13 Akl MR, Ayoub NM, Sylvester PW. 2012. Mechanisms mediating the synergistic anticancer effects  
14 of combined gamma-tocotrienol and sesamin treatment. Planta Med 78(16):1731-1739.  
15  
16 Alawin OA, Ahmed RA, Dronamraju V, Briski KP, Sylvester PW. 2017. gamma-Tocotrienol-  
17 induced disruption of lipid rafts in human breast cancer cells is associated with a reduction  
18 in exosome heregulin content. J Nutr Biochem 48:83-93.  
19  
20 Alawin OA, Ahmed RA, Ibrahim BA, Briski KP, Sylvester PW. 2016. Antiproliferative effects of  
21 gamma-tocotrienol are associated with lipid raft disruption in HER2-positive human breast  
22 cancer cells. J Nutr Biochem 27:266-277.  
23  
24 Alayoubi AY, Anderson JF, Satyanarayananjois SD, Sylvester PW, Nazzal S. 2013. Concurrent  
25 delivery of tocotrienols and simvastatin by lipid nanoemulsions potentiates their antitumor  
26 activity against human mammary adenocarcinoma cells. Eur J Pharm Sci 48(3):385-392.  
27  
28 Alqahtani S, Alayoubi A, Nazzal S, Sylvester PW, Kaddoumi A. 2014. Enhanced solubility and  
29 oral bioavailability of gamma-tocotrienol using a self-emulsifying drug delivery system  
30 (SEDDS). Lipids 49(8):819-829.  
31  
32 Ananthula S, Parajuli P, Behery FA, Alayoubi AY, El Sayed KA, Nazzal S, Sylvester PW. 2014a.  
33 Oxazine derivatives of gamma- and delta-tocotrienol display enhanced anticancer activity in  
34 vivo. Anticancer Res 34(6):2715-2726.  
35  
36 Ananthula S, Parajuli P, Behery FA, Alayoubi AY, Nazzal S, El Sayed K, Sylvester PW. 2014b.  
37 delta-Tocotrienol oxazine derivative antagonizes mammary tumor cell compensatory  
38 response to CoCl<sub>2</sub>-induced hypoxia. Biomed Res Int 2014:285752.  
39  
40 Appert-Collin A, Hubert P, Cremel G, Bennasroune A. 2015. Role of ErbB receptors in cancer cell  
41 migration and invasion. Front Pharmacol 6:283.  
42  
43 Arteaga CL, Engelman JA. 2014. ERBB receptors: from oncogene discovery to basic science to  
44 mechanism-based cancer therapeutics. Cancer Cell 25(3):282-303.  
45  
46 Bachawal SV, Wali VB, Sylvester PW. 2010. Combined gamma-tocotrienol and erlotinib/gefitinib  
47 treatment suppresses Stat and Akt signaling in murine mammary tumor cells. Anticancer  
48 Res 30(2):429-437.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3 Barve A, Khor TO, Reuhl K, Reddy B, Newmark H, Kong AN. 2010. Mixed tocotrienols inhibit  
4 prostate carcinogenesis in TRAMP mice. *Nutr Cancer* 62(6):789-794.
- 5  
6 Behery FA, Akl MR, Ananthula S, Parajuli P, Sylvester PW, El Sayed KA. 2013. Optimization of  
7 tocotrienols as antiproliferative and antimigratory leads. *Eur J Med Chem* 59:329-341.
- 8  
9 Behery FA, Elnagar AY, Akl MR, Wali VB, Abuasal B, Kaddoumi A, Sylvester PW, El Sayed KA.  
10 2010. Redox-silent tocotrienol esters as breast cancer proliferation and migration inhibitors.  
11 *Bioorg Med Chem* 18(22):8066-8075.
- 12  
13  
14 Bi S, Liu JR, Li Y, Wang Q, Liu HK, Yan YG, Chen BQ, Sun WG. 2010. gamma-Tocotrienol  
15 modulates the paracrine secretion of VEGF induced by cobalt(II) chloride via ERK  
16 signaling pathway in gastric adenocarcinoma SGC-7901 cell line. *Toxicology* 274(1-3):27-  
17 33.
- 18  
19  
20 Birringer M, Pfluger P, Kluth D, Landes N, Brigelius-Flohe R. 2002. Identities and differences in  
21 the metabolism of tocotrienols and tocopherols in HepG2 cells. *J Nutr* 132(10):3113-3118.
- 22  
23  
24 Bunyan J, Mc HD, Green J, Marcinkiewicz S. 1961. Biological potencies of epsilon- and zeta-1-  
25 tocopherol and 5-methyltol. *Br J Nutr* 15:253-257.
- 26  
27 Burdeos GC, Ito J, Eitsuka T, Nakagawa K, Kimura F, Miyazawa T. 2016. delta and gamma  
28 tocotrienols suppress human hepatocellular carcinoma cell proliferation via regulation of  
29 Ras-Raf-MEK-ERK pathway-associated upstream signaling. *Food Funct* 7(10):4170-4174.
- 30  
31  
32 Calzolari A, Oliviero I, Deaglio S, Mariani G, Biffoni M, Sposi NM, Malavasi F, Peschle C, Testa  
33 U. 2007. Transferrin receptor 2 is frequently expressed in human cancer cell lines. *Blood*  
34 *Cells Mol Dis* 39(1):82-91.
- 35  
36  
37 Campbell SE, Rudder B, Phillips RB, Whaley SG, Stimmel JB, Leesnitzer LM, Lightner J, Dessus-  
38 Babus S, Duffourc M, Stone WL, Menter DG, Newman RA, Yang P, Aggarwal BB,  
39 Krishnan K. 2011. gamma-Tocotrienol induces growth arrest through a novel pathway with  
40 TGFbeta2 in prostate cancer. *Free Radic Biol Med* 50(10):1344-1354.
- 41  
42  
43 Cardenas E, Ghosh R. 2013. Vitamin E: a dark horse at the crossroad of cancer management.  
44 *Biochem Pharmacol* 86(7):845-852.
- 45  
46 Chamberlain LH. 2004. Detergents as tools for the purification and classification of lipid rafts.  
47 *FEBS Lett* 559(1-3):1-5.
- 48  
49  
50 Chang PN, Yap WN, Lee DT, Ling MT, Wong YC, Yap YL. 2009. Evidence of gamma-tocotrienol  
51 as an apoptosis-inducing, invasion-suppressing, and chemotherapy drug-sensitizing agent in  
52 human melanoma cells. *Nutr Cancer* 61(3):357-366.
- 53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Chen Y, Wang XQ, Zhang Q, Zhu JY, Li Y, Xie CF, Li XT, Wu JS, Geng SS, Zhong CY, Han HY.  
4 2017. (-)-Epigallocatechin-3-gallate inhibits colorectal cancer stem cells by suppressing  
5 Wnt/beta-catenin pathway. *Nutrients* 9(6).
- 6  
7 Chin KY, Ima-Nirwana S. 2015. The biological effects of tocotrienol on bone: a review on evidence  
8 from rodent models. *Drug Des Devel Ther* 9:2049-2061.
- 9  
10 Chin KY, Pang KL, Soelaiman IN. 2016. Tocotrienol and its role in chronic diseases. *Adv Exp Med*  
11 *Biol* 928:97-130.
- 12  
13 Cho IJ, Ahn JY, Kim S, Choi MS, Ha TY. 2008. Resveratrol attenuates the expression of HMG-  
14 CoA reductase mRNA in hamsters. *Biochem Biophys Res Commun* 367(1):190-194.
- 15  
16 Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL, Wahl  
17 GM. 2006. Cancer stem cells--perspectives on current status and future directions: AACR  
18 Workshop on cancer stem cells. *Cancer Res* 66(19):9339-9344.
- 19  
20 Comitato R, Leoni G, Canali R, Ambra R, Nesaretnam K, Virgili F. 2010. Tocotrienols activity in  
21 MCF-7 breast cancer cells: involvement of ERbeta signal transduction. *Mol Nutr Food Res*  
22 *54(5):669-678.*
- 23  
24 Comitato R, Guantario B, Leoni G, Nesaretnam K, Ronci MB, Canali R, Virgili F. 2016.  
25 Tocotrienols induce endoplasmic reticulum stress and apoptosis in cervical cancer cells.  
26 *Genes Nutr* 11:32.
- 27  
28 Compadre CM, Singh A, Thakkar S, Zheng G, Breen PJ, Ghosh S, Kiaei M, Boerma M, Varughese  
29 KI, Hauer-Jensen M. 2014. Molecular dynamics guided design of tocoflexol: a new  
30 radioprotectant tocotrienol with enhanced bioavailability. *Drug Dev Res* 75(1):10-22.
- 31  
32 De Silva L, Chuah LH, Meganathan P, Fu JY. 2016. Tocotrienol and cancer metastasis. *Biofactors*  
33 *42(2):149-162.*
- 34  
35 de Wolf E, Abdullah MI, Jones SM, Menezes K, Moss DM, Drijfhout FP, Hart SR, Hoskins C,  
36 Stronach EA, Richardson A. 2017. Dietary geranylgeraniol can limit the activity of  
37 pitavastatin as a potential treatment for drug-resistant ovarian cancer. *Sci Rep* 7(1):5410.
- 38  
39 Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. 2005. Statins and cancer prevention.  
40 *Nat Rev Cancer* 5(12):930-942.
- 41  
42 Dinkova-Kostova AT, Talalay P. 2010. NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a  
43 multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. *Arch*  
44 *Biochem Biophys* 501(1):116-123.
- 45  
46 Eitsuka T, Tatewaki N, Nishida H, Kurata T, Nakagawa K, Miyazawa T. 2014. Synergistic  
47 inhibition of cancer cell proliferation with a combination of delta-tocotrienol and ferulic  
48 acid. *Biochem Biophys Res Commun* 453(3):606-611.
- 49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Eitsuka T, Tatewaki N, Nishida H, Nakagawa K, Miyazawa T. 2016a. A combination of delta-  
4  $\gamma$ -Tocotrienol and ferulic acid synergistically inhibits telomerase activity in DLD-1 human  
5 colorectal adenocarcinoma cells. *J Nutr Sci Vitaminol (Tokyo)* 62(5):281-287.  
6  
7 Eitsuka T, Tatewaki N, Nishida H, Nakagawa K, Miyazawa T. 2016b. Synergistic anticancer effect  
8 of tocotrienol combined with chemotherapeutic agents or dietary components: A Review. *Int*  
9 *J Mol Sci* 17(10).  
10  
11 Elnagar AY, Wali VB, Sylvester PW, El Sayed KA. 2010. Design and preliminary structure-activity  
12 relationship of redox-silent semisynthetic tocotrienol analogues as inhibitors for breast  
13 cancer proliferation and invasion. *Bioorg Med Chem* 18(2):755-768.  
14  
15 Eun K, Ham SW, Kim H. 2017. Cancer stem cell heterogeneity: origin and new perspectives on  
16 CSC targeting. *BMB Rep* 50(3):117-125.  
17  
18 Evans HM, Bishop KS. 1922. On the existence of a hitherto unrecognized dietary factor essential  
19 for reproduction. *Science* 56(1458):650-651.  
20  
21 Fernandes NV, Guntipalli PK, Mo HB. 2010. d-delta-Tocotrienol-mediated cell cycle arrest and  
22 apoptosis in human melanoma cells. *Anticancer Research* 30(12):4937-4944.  
23  
24 Filippi A, Ciolac OA, Ganea C, Mocanu MM. 2017. ErbB proteins as molecular target of dietary  
25 phytochemicals in malignant diseases. *J Oncol* 2017:1532534.  
26  
27 Foufelle F, Fromenty B. 2016. Role of endoplasmic reticulum stress in drug-induced toxicity.  
28 *Pharmacol Res Perspect* 4(1):e00211.  
29  
30 Fu JY, Che HL, Tan DM, Teng KT. 2014. Bioavailability of tocotrienols: evidence in human  
31 studies. *Nutr Metab (Lond)* 11(1):5.  
32  
33 Gagic Z, Ivkovic B, Srdic-Rajic T, Vucicevic J, Nikolic K, Agbaba D. 2016. Synthesis of the  
34 vitamin E amino acid esters with an enhanced anticancer activity and in silico screening for  
35 new antineoplastic drugs. *Eur J Pharm Sci* 88:59-69.  
36  
37 Galli F, Azzi A. 2010. Present trends in vitamin E research. *Biofactors* 36(1):33-42.  
38  
39 Goldstein JL, Brown MS. 1990. Regulation of the mevalonate pathway. *Nature* 343(6257):425-430.  
40  
41 Gopalan A, Yu W, Sanders BG, Kline K. 2013. Eliminating drug resistant breast cancer stem-like  
42 cells with combination of simvastatin and gamma-tocotrienol. *Cancer Lett* 328(2):285-296.  
43  
44 Goufo P, Trindade H. 2014. Rice antioxidants: phenolic acids, flavonoids, anthocyanins,  
45 proanthocyanidins, tocopherols, tocotrienols, gamma-oryzanol, and phytic acid. *Food Sci*  
46 *Nutr* 2(2):75-104.  
47  
48 Gu W, Prasad I, Yu M, Zhang F, Ling P, Xiao Y, Yu C. 2015. Gamma tocotrienol targets  
49 tyrosine phosphatase SHP2 in mammospheres resulting in cell death through RAS/ERK  
50 pathway. *BMC Cancer* 15:609.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Gupta SC, Kim JH, Prasad S, Aggarwal BB. 2010. Regulation of survival, proliferation, invasion,  
4 angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways  
5 by nutraceuticals. *Cancer Metastasis Rev* 29(3):405-434.  
6  
7 Halperin L, Jung J, Michalak M. 2014. The many functions of the endoplasmic reticulum  
8 chaperones and folding enzymes. *IUBMB Life* 66(5):318-326.  
9  
10 Henderson AJ, Ollila CA, Kumar A, Borresen EC, Raina K, Agarwal R, Ryan EP. 2012.  
11 Chemopreventive properties of dietary rice bran: current status and future prospects. *Adv*  
12 *Nutr* 3(5):643-653.  
13  
14 Hiramatsu N, Chiang WC, Kurt TD, Sigurdson CJ, Lin JH. 2015. Multiple mechanisms of unfolded  
15 protein response-induced cell death. *Am J Pathol* 185(7):1800-1808.  
16  
17 Ho HJ, Shirakawa H, Yoshida R, Ito A, Maeda M, Goto T, Komai M. 2016. Geranylgeraniol  
18 enhances testosterone production via the cAMP/protein kinase A pathway in testis-derived  
19 I-10 tumor cells. *Biosci Biotechnol Biochem* 80(4):791-797.  
20  
21 Hodul PJ, Dong Y, Husain K, Pimiento JM, Chen J, Zhang A, Francois R, Pledger WJ, Coppola D,  
22 Sebti SM, Chen DT, Malafa MP. 2013. Vitamin E delta-tocotrienol induces p27(Kip1)-  
23 dependent cell-cycle arrest in pancreatic cancer cells via an E2F-1-dependent mechanism.  
24 *PLoS One* 8(2):e52526.  
25  
26 Hosomi A, Arita M, Sato Y, Kiyose C, Ueda T, Igarashi O, Arai H, Inoue K. 1997. Affinity for  
27 alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E  
28 analogs. *FEBS Lett* 409(1):105-108.  
29  
30 Hsieh TC, Elangovan S, Wu JM. 2010. gamma-Tocotrienol controls proliferation, modulates  
31 expression of cell cycle regulatory proteins and up-regulates quinone reductase NQO2 in  
32 MCF-7 breast cancer cells. *Anticancer Res* 30(7):2869-2874.  
33  
34 Hsieh TC, Wu JM. 2008. Suppression of cell proliferation and gene expression by combinatorial  
35 synergy of EGCG, resveratrol and gamma-tocotrienol in estrogen receptor-positive MCF-7  
36 breast cancer cells. *Int J Oncol* 33(4):851-859.  
37  
38 Huang Y, Wu R, Su ZY, Guo Y, Zheng X, Yang CS, Kong AN. 2017. A naturally occurring  
39 mixture of tocotrienols inhibits the growth of human prostate tumor, associated with  
40 epigenetic modifications of cyclin-dependent kinase inhibitors p21 and p27. *J Nutr Biochem*  
41 40:155-163.  
42  
43 Husain K, Centeno BA, Coppola D, Trevino J, Sebti SM, Malafa MP. 2017. delta-Tocotrienol, a  
44 natural form of vitamin E, inhibits pancreatic cancer stem-like cells and prevents pancreatic  
45 cancer metastasis. *Oncotarget* 8(19):31554-31567.  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Hussein D, Mo H. 2009. d-delta-Tocotrienol-mediated suppression of the proliferation of human  
4 PANC-1, MIA PaCa-2, and BxPC-3 pancreatic carcinoma cells. *Pancreas* 38(4):e124-136.
- 5  
6 Hynes NE, Lane HA. 2005. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat*  
7  
8 *Rev Cancer* 5(5):341-354.
- 9  
10 Ikeda S, Tohyama T, Yoshimura H, Hamamura K, Abe K, Yamashita K. 2003. Dietary alpha-  
11  
12 tocopherol decreases alpha-tocotrienol but not gamma-tocotrienol concentration in rats. *J*  
13 *Nutr* 133(2):428-434.
- 14  
15 Inoue A, Takitani K, Koh M, Kawakami C, Kuno T, Tamai H. 2011. Induction of apoptosis by  
16  
17 gamma-tocotrienol in human cancer cell lines and leukemic blasts from patients:  
18  
19 dependency on Bid, cytochrome c, and caspase pathway. *Nutr Cancer* 63(5):763-770.
- 20  
21 Ji X, Wang Z, Geamanu A, Goja A, Sarkar FH, Gupta SV. 2012a. Delta-tocotrienol suppresses  
22  
23 Notch-1 pathway by upregulating miR-34a in nonsmall cell lung cancer cells. *Int J Cancer*  
24  
25 131(11):2668-2677.
- 26  
27 Ji X, Wang Z, Sarkar FH, Gupta SV. 2012b. Delta-tocotrienol augments cisplatin-induced  
28  
29 suppression of non-small cell lung cancer cells via inhibition of the Notch-1 pathway.  
30  
31 *Anticancer Res* 32(7):2647-2655.
- 32  
33 Kanchi MM, Shanmugam MK, Rane G, Sethi G, Kumar AP. 2017. Tocotrienols: the unsaturated  
34  
35 sidekick shifting new paradigms in vitamin E therapeutics. *Drug Discov Today*  
36  
37 22(12):1765-1781..
- 38  
39 Kannappan R, Gupta SC, Kim JH, Aggarwal BB. 2012. Tocotrienols fight cancer by targeting  
40  
41 multiple cell signaling pathways. *Genes Nutr* 7(1):43-52.
- 42  
43 Kannappan R, Ravindran J, Prasad S, Sung B, Yadav VR, Reuter S, Chaturvedi MM, Aggarwal  
44  
45 BB. 2010. Gamma-tocotrienol promotes TRAIL-induced apoptosis through reactive oxygen  
46  
47 species/extracellular signal-regulated kinase/p53-mediated upregulation of death receptors.  
48  
49 *Mol Cancer Ther* 9(8):2196-2207.
- 50  
51 Karim R, Somani S, Al Robaian M, Mullin M, Amor R, McConnell G, Dufes C. 2017. Tumor  
52  
53 regression after intravenous administration of targeted vesicles entrapping the vitamin E  
54  
55 alpha-tocotrienol. *J Control Release* 246:79-87.
- 56  
57 Khallouki F, de Medina P, Caze-Subra S, Bystricky K, Balaguer P, Poirot M, Silvente-Poirot S.  
58  
59 2015. Molecular and biochemical analysis of the estrogenic and proliferative properties of  
60  
61 Vitamin E compounds. *Front Oncol* 5:287.
- 62  
63 Khan MR, Siddiqui S, Parveen K, Javed S, Diwakar S, Siddiqui WA. 2010. Nephroprotective  
64  
65 action of tocotrienol-rich fraction (TRF) from palm oil against potassium dichromate (K<sub>2</sub>  
66  
67 Cr<sub>2</sub>O<sub>7</sub>)-induced acute renal injury in rats. *Chem Biol Interact* 186(2):228-238.

- 1  
2  
3 Khanna S, Patel V, Rink C, Roy S, Sen CK. 2005. Delivery of orally supplemented alpha-  
4 tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice. *Free Radic*  
5 *Biol Med* 39(10):1310-1319.  
6  
7 Khanna S, Roy S, Parinandi NL, Maurer M, Sen CK. 2006. Characterization of the potent  
8 neuroprotective properties of the natural vitamin E alpha-tocotrienol. *J Neurochem*  
9 98(5):1474-1486.  
10  
11 Krager KJ, Pineda EN, Kharade SV, Kordsmeier M, Howard L, Breen PJ, Compadre CM, Hauer-  
12 Jensen M, Aykin-Burns N. 2015. Tocotrienol-rich fraction from rice bran demonstrates  
13 potent radiation protection activity. *Evid Based Complement Alternat Med* 2015:148791.  
14  
15 Kunnumakkara AB, Sung B, Ravindran J, Diagaradjane P, Deorukhkar A, Dey S, Koca C, Yadav  
16 VR, Tong Z, Gelovani JG, Guha S, Krishnan S, Aggarwal BB. 2010. {Gamma}-tocotrienol  
17 inhibits pancreatic tumors and sensitizes them to gemcitabine treatment by modulating the  
18 inflammatory microenvironment. *Cancer Res* 70(21):8695-8705.  
19  
20 Laurent-Puig P, Lievre A, Blons H. 2009. Mutations and response to epidermal growth factor  
21 receptor inhibitors. *Clin Cancer Res* 15(4):1133-1139.  
22  
23 Ledet GA, Biswas S, Kumar VP, Graves RA, Mitchner DM, Parker TM, Bostanian LA, Ghosh SP,  
24 Mandal TK. 2016. Development of Orally Administered gamma-tocotrienol (GT3)  
25 nanoemulsion for radioprotection. *Int J Mol Sci* 18(1).  
26  
27 Lee SO, Ma Z, Yeh CR, Luo J, Lin TH, Lai KP, Yamashita S, Liang L, Tian J, Li L, Jiang Q,  
28 Huang CK, Niu Y, Yeh S, Chang C. 2013. New therapy targeting differential androgen  
29 receptor signaling in prostate cancer stem/progenitor vs. non-stem/progenitor cells. *J Mol*  
30 *Cell Biol* 5(1):14-26.  
31  
32 Li Y, Sun WG, Liu HK, Qi GY, Wang Q, Sun XR, Chen BQ, Liu JR. 2011. gamma-Tocotrienol  
33 inhibits angiogenesis of human umbilical vein endothelial cell induced by cancer cell. *J Nutr*  
34 *Biochem* 22(12):1127-1136.  
35  
36 Liu HK, Wang Q, Li Y, Sun WG, Liu JR, Yang YM, Xu WL, Sun XR, Chen BQ. 2010. Inhibitory  
37 effects of gamma-tocotrienol on invasion and metastasis of human gastric adenocarcinoma  
38 SGC-7901 cells. *J Nutr Biochem* 21(3):206-213.  
39  
40 Loganathan R, Selvaduray KR, Nesaretnam K, Radhakrishnan AK. 2013. Tocotrienols promote  
41 apoptosis in human breast cancer cells by inducing poly(ADP-ribose) polymerase cleavage  
42 and inhibiting nuclear factor kappa-B activity. *Cell Prolif* 46(2):203-213.  
43  
44 Luk SU, Yap WN, Chiu YT, Lee DT, Ma S, Lee TK, Vasireddy RS, Wong YC, Ching YP, Nelson  
45 C, Yap YL, Ling MT. 2011. Gamma-tocotrienol as an effective agent in targeting prostate  
46 cancer stem cell-like population. *Int J Cancer* 128(9):2182-2191.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57

- 1  
2  
3 Malavolta M, Pierpaoli E, Giacconi R, Costarelli L, Piacenza F, Basso A, Cardelli M, Provinciali  
4 M. 2016. Pleiotropic effects of tocotrienols and qQuercetin on cellular senescence:  
5 Introducing the perspective of senolytic effects of phytochemicals. *Curr Drug Targets*  
6 17(4):447-459.  
7  
8  
9 Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. 2004. Polyphenols: food sources and  
10 bioavailability. *Am J Clin Nutr* 79(5):727-747.  
11  
12 Manu KA, Shanmugam MK, Ramachandran L, Li F, Fong CW, Kumar AP, Tan P, Sethi G. 2012.  
13 First evidence that gamma-tocotrienol inhibits the growth of human gastric cancer and  
14 chemosensitizes it to capecitabine in a xenograft mouse model through the modulation of  
15 NF-kappaB pathway. *Clin Cancer Res* 18(8):2220-2229.  
16  
17  
18 Maurel M, McGrath EP, Mnich K, Healy S, Chevet E, Samali A. 2015. Controlling the unfolded  
19 protein response-mediated life and death decisions in cancer. *Semin Cancer Biol* 33:57-66.  
20  
21 McAnally JA, Gupta J, Sodhani S, Bravo L, Mo H. 2007. Tocotrienols potentiate lovastatin-  
22 mediated growth suppression in vitro and in vivo. *Exp Biol Med (Maywood)* 232(4):523-  
23 531.  
24  
25  
26 McCubrey JA, Lertpiriyapong K, Steelman LS, Abrams SL, Yang LV, Murata RM, Rosalen PL,  
27 Scalisi A, Neri LM, Cocco L, Ratti S, Martelli AM, Laidler P, Dulinska-Litewka J, Rakus  
28 D, Gizak A, Lombardi P, Nicoletti F, Candido S, Libra M, Montalto G, Cervello M. 2017.  
29 Effects of resveratrol, curcumin, berberine and other nutraceuticals on aging, cancer  
30 development, cancer stem cells and microRNAs. *Aging (Albany NY)* 9(6):1477-1536.  
31  
32  
33 McIntyre BS, Briski KP, Gapor A, Sylvester PW. 2000. Antiproliferative and apoptotic effects of  
34 tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial  
35 cells. *Proc Soc Exp Biol Med* 224(4):292-301.  
36  
37  
38 Meganathan P, Fu JY. 2016. Biological properties of tocotrienols: Evidence in human studies. *Int J*  
39 *Mol Sci* 17(11).  
40  
41  
42 Min B, McClung AM, Chen MH. 2011. Phytochemicals and antioxidant capacities in rice brans of  
43 different color. *J Food Sci* 76(1):C117-126.  
44  
45  
46 Miyazawa T, Shibata A, Nakagawa K, Tsuzuki T. 2008. Anti-angiogenic function of tocotrienol.  
47 *Asia Pac J Clin Nutr* 17 Suppl 1:253-256.  
48  
49  
50 Mo H, Elson CE. 2004. Studies of the isoprenoid-mediated inhibition of mevalonate synthesis  
51 applied to cancer chemotherapy and chemoprevention. *Exp Biol Med (Maywood)*  
52 229(7):567-585.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Montagnani Marelli M, Marzagalli M, Moretti RM, Beretta G, Casati L, Comitato R, Gravina GL,  
4 Festuccia C, Limonta P. 2016. Vitamin E delta-tocotrienol triggers endoplasmic reticulum  
5 stress-mediated apoptosis in human melanoma cells. *Sci Rep* 6:30502.
- 6  
7 Nagare RP, Sneha S, Priya SK, Ganesan TS. 2017. Cancer stem cells - Are surface markers alone  
8 sufficient? *Curr Stem Cell Res Ther* 12(1):37-44.
- 9  
10 Nakagawa K, Shibata A, Yamashita S, Tsuzuki T, Kariya J, Oikawa S, Miyazawa T. 2007. In vivo  
11 angiogenesis is suppressed by unsaturated vitamin E, tocotrienol. *J Nutr* 137(8):1938-1943.
- 12  
13 Naponelli V, Ramazzina I, Lenzi C, Bettuzzi S, Rizzi F. 2017. Green tea catechins for prostate  
14 cancer prevention: Present achievements and future challenges. *Antioxidants (Basel)* 6(2).
- 15  
16 Nesaretnam K. 2008. Multitargeted therapy of cancer by tocotrienols. *Cancer Lett* 269(2):388-395.
- 17  
18 Nesaretnam K, Selvaduray KR, Abdul Razak G, Veerasenan SD, Gomez PA. 2010. Effectiveness  
19 of tocotrienol-rich fraction combined with tamoxifen in the management of women with  
20 early breast cancer: a pilot clinical trial. *Breast Cancer Res* 12(5):R81.
- 21  
22 Neuzil J, Tomasetti M, Zhao Y, Dong LF, Birringer M, Wang XF, Low P, Wu K, Salvatore BA,  
23 Ralph SJ. 2007. Vitamin E analogs, a novel group of "mitocans," as anticancer agents: the  
24 importance of being redox-silent. *Mol Pharmacol* 71(5):1185-1199.
- 25  
26 Ng MH, Choo YM, Ma AN, Chuah CH, Hashim MA. 2004. Separation of vitamin E (tocopherol,  
27 tocotrienol, and tocomonoenol) in palm oil. *Lipids* 39(10):1031-1035.
- 28  
29 Parajuli P, Tiwari RV, Sylvester PW. 2015a. Anti-proliferative effects of gamma-tocotrienol are  
30 associated with suppression of c-Myc expression in mammary tumour cells. *Cell Prolif*  
31 48(4):421-435.
- 32  
33 Parajuli P, Tiwari RV, Sylvester PW. 2015b. Anticancer effects of gamma-tocotrienol are  
34 associated with a suppression in aerobic glycolysis. *Biol Pharm Bull* 38(9):1352-1360.
- 35  
36 Park SK, Sanders BG, Kline K. 2010. Tocotrienols induce apoptosis in breast cancer cell lines via  
37 an endoplasmic reticulum stress-dependent increase in extrinsic death receptor signaling.  
38 *Breast Cancer Res Treat* 124(2):361-375.
- 39  
40 Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ. 1993. Tocotrienols regulate cholesterol  
41 production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-  
42 methylglutaryl-coenzyme A reductase. *J Biol Chem* 268(15):11230-11238.
- 43  
44 Parmar VM, Schroder M. 2012. Sensing endoplasmic reticulum stress. *Adv Exp Med Biol* 738:153-  
45 168.
- 46  
47 Patacsil D, Tran AT, Cho YS, Suy S, Saenz F, Malyukova I, Ressom H, Collins SP, Clarke R,  
48 Kumar D. 2012. Gamma-tocotrienol induced apoptosis is associated with unfolded protein  
49 response in human breast cancer cells. *J Nutr Biochem* 23(1):93-100.
- 50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3 Pathak R, Bachri A, Ghosh SP, Koturbash I, Boerma M, Binz RK, Sawyer JR, Hauer-Jensen M.  
4 2016. The Vitamin E analog gamma-tocotrienol (GT3) suppresses radiation-induced  
5 cytogenetic damage. *Pharm Res* 33(9):2117-2125.  
6  
7 Peh HY, Tan WS, Liao W, Wong WS. 2016. Vitamin E therapy beyond cancer: Tocopherol versus  
8 tocotrienol. *Pharmacol Ther* 162:152-169.  
9  
10 Pereira DM, Valentao P, Correia-da-Silva G, Teixeira N, Andrade PB. 2015. Translating  
11 endoplasmic reticulum biology into the clinic: a role for ER-targeted natural products? *Nat*  
12 *Prod Rep* 32(5):705-722.  
13  
14 Pham J, Nayel A, Hoang C, Elbayoumi T. 2016. Enhanced effectiveness of tocotrienol-based nano-  
15 emulsified system for topical delivery against skin carcinomas. *Drug Deliv* 23(5):1514-  
16 1524.  
17  
18 Pierpaoli E, Viola V, Barucca A, Orlando F, Galli F, Provinciali M. 2013. Effect of annatto-  
19 tocotrienols supplementation on the development of mammary tumors in HER-2/neu  
20 transgenic mice. *Carcinogenesis* 34(6):1352-1360.  
21  
22 Pike LJ. 2009. The challenge of lipid rafts. *J Lipid Res* 50 Suppl:S323-328.  
23  
24 Prasad S, Gupta SC, Tyagi AK, Aggarwal BB. 2016. gamma-Tocotrienol suppresses growth and  
25 sensitises human colorectal tumours to capecitabine in a nude mouse xenograft model by  
26 down-regulating multiple molecules. *Br J Cancer* 115(7):814-824.  
27  
28 Qureshi AA, Khan DA, Silswal N, Saleem S, Qureshi N. 2016. Evaluation of pharmacokinetics,  
29 and bioavailability of higher doses of tocotrienols in healthy fed humans. *J Clin Exp*  
30 *Cardiol* 7(4).  
31  
32 Raddatz-Mota D, Perez-Flores LJ, Carrari F, Mendoza-Espinoza JA, de Leon-Sanchez FD, Pinzon-  
33 Lopez LL, Godoy-Hernandez G, Rivera-Cabrera F. 2017. Achiote (*Bixa orellana* L.): a  
34 natural source of pigment and vitamin E. *J Food Sci Technol* 54(6):1729-1741.  
35  
36 Rautiainen S, Manson JE, Lichtenstein AH, Sesso HD. 2016. Dietary supplements and disease  
37 prevention - a global overview. *Nat Rev Endocrinol* 12(7):407-420.  
38  
39 Sailo BL, Banik K, Padmavathi G, Javadi M, Bordoloi D, Kunnumakkara AB. 2018. Tocotrienols:  
40 The promising analogues of vitamin E for cancer therapeutics. *Pharmacol Res*.  
41  
42 Saito Y, Fukuhara A, Nishio K, Hayakawa M, Ogawa Y, Sakamoto H, Fujii K, Yoshida Y, Niki E.  
43 2009. Characterization of cellular uptake and distribution of coenzyme Q10 and vitamin E in  
44 PC12 cells. *J Nutr Biochem* 20(5):350-357.  
45  
46 Samant GV, Wali VB, Sylvester PW. 2010. Anti-proliferative effects of gamma-tocotrienol on  
47 mammary tumour cells are associated with suppression of cell cycle progression. *Cell Prolif*  
48 43(1):77-83.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Santini A, Tenore GC, Novellino E. 2017. Nutraceuticals: A paradigm of proactive medicine. *Eur J*  
4 *Pharm Sci* 96:53-61.
- 5  
6 Sauer S, Plauth A. 2017. Health-beneficial nutraceuticals-myth or reality? *Appl Microbiol*  
7 *Biotechnol* 101(3):951-961.
- 8  
9 Schonthal AH. 2012. Endoplasmic reticulum stress: its role in disease and novel prospects for  
10 therapy. *Scientifica (Cairo)* 2012:857516.
- 11  
12 Schonthal AH. 2013. Pharmacological targeting of endoplasmic reticulum stress signaling in  
13 cancer. *Biochem Pharmacol* 85(5):653-666.
- 14  
15 Schwingshackl L, Boeing H, Stelmach-Mardas M, Gottschald M, Dietrich S, Hoffmann G,  
16 Chaimani A. 2017. Dietary supplements and risk of cause-specific death, cardiovascular  
17 disease, and cancer: A systematic review and meta-analysis of primary prevention Trials.  
18 *Adv Nutr* 8(1):27-39.
- 19  
20 Selvaduray KR, Radhakrishnan AK, Kutty MK, Nesaretnam K. 2010. Palm tocotrienols inhibit  
21 proliferation of murine mammary cancer cells and induce expression of interleukin-24  
22 mRNA. *J Interferon Cytokine Res* 30(12):909-916.
- 23  
24 Selvaduray KR, Radhakrishnan AK, Kutty MK, Nesaretnam K. 2012. Palm tocotrienols decrease  
25 levels of pro-angiogenic markers in human umbilical vein endothelial cells (HUVEC) and  
26 murine mammary cancer cells. *Genes Nutr* 7(1):53-61.
- 27  
28 Sen CK, Khanna S, Roy S. 2004. Tocotrienol: the natural vitamin E to defend the nervous system?  
29 *Ann N Y Acad Sci* 1031:127-142.
- 30  
31 Shah S, Gapor A, Sylvester PW. 2003. Role of caspase-8 activation in mediating vitamin E-induced  
32 apoptosis in murine mammary cancer cells. *Nutr Cancer* 45(2):236-246.
- 33  
34 Shah S, Sylvester PW. 2004. Tocotrienol-induced caspase-8 activation is unrelated to death receptor  
35 apoptotic signaling in neoplastic mammary epithelial cells. *Exp Biol Med (Maywood)*  
36 229(8):745-755.
- 37  
38 Shah SJ, Sylvester PW. 2005. Gamma-tocotrienol inhibits neoplastic mammary epithelial cell  
39 proliferation by decreasing Akt and nuclear factor kappaB activity. *Exp Biol Med*  
40 (Maywood) 230(4):235-241.
- 41  
42 Shahidi F, de Camargo AC. 2016. Tocopherols and tocotrienols in common and emerging dietary  
43 sources: Occurrence, applications, and health benefits. *Int J Mol Sci* 17(10).
- 44  
45 Shanmugam MK, Warriar S, Kumar AP, Sethi G, Arfuso F. 2017. Potential role of natural  
46 compounds as anti-angiogenic agents in cancer. *Curr Vasc Pharmacol* 15:503-519.
- 47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

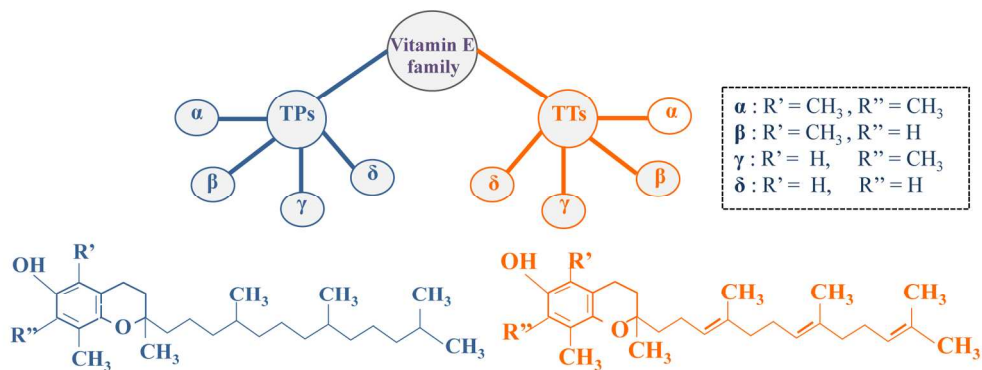
- 1  
2  
3 Shibata A, Nakagawa K, Sookwong P, Tsuduki T, Asai A, Miyazawa T. 2010. alpha-Tocopherol  
4 attenuates the cytotoxic effect of delta-tocotrienol in human colorectal adenocarcinoma  
5 cells. *Biochem Biophys Res Commun* 397(2):214-219.  
6  
7 Shibata A, Nakagawa K, Sookwong P, Tsuduki T, Oikawa S, Miyazawa T. 2009. delta-Tocotrienol  
8 suppresses VEGF induced angiogenesis whereas alpha-tocopherol does not. *J Agric Food*  
9 *Chem* 57(18):8696-8704.  
10  
11 Shibata A, Nakagawa K, Sookwong P, Tsuduki T, Tomita S, Shirakawa H, Komai M, Miyazawa T.  
12 2008a. Tocotrienol inhibits secretion of angiogenic factors from human colorectal  
13 adenocarcinoma cells by suppressing hypoxia-inducible factor-1alpha. *J Nutr* 138(11):2136-  
14 2142.  
15  
16 Shibata A, Nakagawa K, Sookwong P, Tsuzuki T, Oikawa S, Miyazawa T. 2008b. Tumor anti-  
17 angiogenic effect and mechanism of action of delta-tocotrienol. *Biochem Pharmacol*  
18 76(3):330-339.  
19  
20 Shibata A, Nakagawa K, Tsuduki T, Miyazawa T. 2015. delta-Tocotrienol treatment is more  
21 effective against hypoxic tumor cells than normoxic cells: potential implications for cancer  
22 therapy. *J Nutr Biochem* 26(8):832-840.  
23  
24 Shimizu M, Shirakami Y, Moriwaki H. 2008. Targeting receptor tyrosine kinases for  
25 chemoprevention by green tea catechin, EGCG. *Int J Mol Sci* 9(6):1034-1049.  
26  
27 Shin-Kang S, Ramsauer VP, Lightner J, Chakraborty K, Stone W, Campbell S, Reddy SA,  
28 Krishnan K. 2011. Tocotrienols inhibit AKT and ERK activation and suppress pancreatic  
29 cancer cell proliferation by suppressing the ErbB2 pathway. *Free Radic Biol Med*  
30 51(6):1164-1174.  
31  
32 Shirode AB, Sylvester PW. 2011. Mechanisms mediating the synergistic anticancer effects of  
33 combined gamma-tocotrienol and celecoxib treatment. *J Bioanal Biomed* 3:1-7.  
34  
35 Siddappa G, Kulsum S, Ravindra DR, Kumar VV, Raju N, Raghavan N, Sudheendra HV, Sharma  
36 A, Sunny SP, Jacob T, Kuruvilla BT, Benny M, Antony B, Seshadri M, Lakshminarayan P,  
37 Hicks W, Jr., Suresh A, Kuriakose MA. 2017. Curcumin and metformin-mediated  
38 chemoprevention of oral cancer is associated with inhibition of cancer stem cells. *Mol*  
39 *Carcinog* 56:2446-2460.  
40  
41 Siveen KS, Ahn KS, Ong TH, Shanmugam MK, Li F, Yap WN, Kumar AP, Fong CW, Tergaonkar  
42 V, Hui KM, Sethi G. 2014. Y-tocotrienol inhibits angiogenesis-dependent growth of human  
43 hepatocellular carcinoma through abrogation of AKT/mTOR pathway in an orthotopic  
44 mouse model. *Oncotarget* 5(7):1897-1911.  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Siveen KS, Uddin S, Mohammad RM. 2017. Targeting acute myeloid leukemia stem cell signaling  
4 by natural products. *Mol Cancer* 16(1):13.  
5  
6 Sontag TJ, Parker RS. 2002. Cytochrome P450 omega-hydroxylase pathway of tocopherol  
7 catabolism. Novel mechanism of regulation of vitamin E status. *J Biol Chem*  
8 277(28):25290-25296.  
9  
10 Springett GM, Husain K, Neuger A, Centeno B, Chen DT, Hutchinson TZ, Lush RM, Sebti S,  
11 Malafa MP. 2015. A phase I safety, pharmacokinetic, and pharmacodynamic presurgical  
12 trial of vitamin E delta-tocotrienol in patients with pancreatic ductal neoplasia.  
13 *EBioMedicine* 2(12):1987-1995.  
14  
15 Srivastava JK, Gupta S. 2006. Tocotrienol-rich fraction of palm oil induces cell cycle arrest and  
16 apoptosis selectively in human prostate cancer cells. *Biochem Biophys Res Commun*  
17 346(2):447-453.  
18  
19 Sugahara R, Sato A, Uchida A, Shiozawa S, Sato C, Virgona N, Yano T. 2015. Anatto tocotrienol  
20 induces a cytotoxic effect on human prostate cancer PC3 cells via the simultaneous  
21 inhibition of Src and Stat3. *J Nutr Sci Vitaminol (Tokyo)* 61(6):497-501.  
22  
23 Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE, Packer L. 1993. Structural  
24 and dynamic membrane properties of alpha-tocopherol and alpha-tocotrienol: implication to  
25 the molecular mechanism of their antioxidant potency. *Biochemistry* 32(40):10692-10699.  
26  
27 Sylvester PW. 2014. Targeting met mediated epithelial-mesenchymal transition in the treatment of  
28 breast cancer. *Clin Transl Med* 3(1):30.  
29  
30 Sylvester PW, Akl MR, Malaviya A, Parajuli P, Ananthula S, Tiwari RV, Ayoub NM. 2014.  
31 Potential role of tocotrienols in the treatment and prevention of breast cancer. *Biofactors*  
32 40(1):49-58.  
33  
34 Sylvester PW, Ayoub NM. 2013. Tocotrienols target PI3K/Akt signaling in anti-breast cancer  
35 therapy. *Anticancer Agents Med Chem* 13(7):1039-1047.  
36  
37 Sylvester PW, Ip C, Ip MM. 1986. Effects of high dietary fat on the growth and development of  
38 ovarian-independent carcinogen-induced mammary tumors in rats. *Cancer Res* 46(2):763-  
39 769.  
40  
41 Takahashi K, Loo G. 2004. Disruption of mitochondria during tocotrienol-induced apoptosis in  
42 MDA-MB-231 human breast cancer cells. *Biochem Pharmacol* 67(2):315-324.  
43  
44 Tan JK, Then SM, Mazlan M, Raja Abdul Rahman RN, Jamal R, Wan Ngah WZ. 2016. Gamma-  
45 tocotrienol acts as a BH3 mimetic to induce apoptosis in neuroblastoma SH-SY5Y cells. *J*  
46 *Nutr Biochem* 31:28-37.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Thibault A, Samid D, Tompkins AC, Figg WD, Cooper MR, Hohl RJ, Trepel J, Liang B, Patronas  
4 N, Venzon DJ, Reed E, Myers CE. 1996. Phase I study of lovastatin, an inhibitor of the  
5 mevalonate pathway, in patients with cancer. *Clin Cancer Res* 2(3):483-491.  
6  
7 Tiwari RV, Parajuli P, Sylvester PW. 2014. gamma-Tocotrienol-induced autophagy in malignant  
8 mammary cancer cells. *Exp Biol Med (Maywood)* 239(1):33-44.  
9  
10 Tiwari RV, Parajuli P, Sylvester PW. 2015a. gamma-Tocotrienol-induced endoplasmic reticulum  
11 stress and autophagy act concurrently to promote breast cancer cell death. *Biochem Cell*  
12 *Biol* 93(4):306-320.  
13  
14 Tiwari RV, Parajuli P, Sylvester PW. 2015b. Synergistic anticancer effects of combined gamma-  
15 tocotrienol and oridonin treatment is associated with the induction of autophagy. *Mol Cell*  
16 *Biochem* 408(1-2):123-137.  
17  
18 Torquato HF, Goettert MI, Justo GZ, Paredes-Gamero EJ. 2017. Anti-cancer phytochemicals  
19 targeting cancer stem cells. *Curr Genomics* 18(2):156-174.  
20  
21 Traber MG. 2007. Vitamin E regulatory mechanisms. *Annu Rev Nutr* 27:347-362.  
22  
23 Tran AT, Ramalinga M, Kadir H, Clarke R, Kumar D. 2015. Autophagy inhibitor 3-methyladenine  
24 potentiates apoptosis induced by dietary tocotrienols in breast cancer cells. *Eur J Nutr*  
25 54(2):265-272.  
26  
27 Tuerdi G, Ichinomiya S, Sato H, Siddig S, Suwa E, Iwata H, Yano T, Ueno K. 2013. Synergistic  
28 effect of combined treatment with gamma-tocotrienol and statin on human malignant  
29 mesothelioma cells. *Cancer Lett* 339(1):116-127.  
30  
31 Upadhyay J, Misra K. 2009. Towards the interaction mechanism of tocopherols and tocotrienols  
32 (vitamin E) with selected metabolizing enzymes. *Bioinformation* 3(8):326-331.  
33  
34 Viola V, Cifollilli S, Legnaioli S, Piroddi M, Betti M, Mazzini F, Pierpaoli E, Provinciali M, Galli  
35 F. 2013. Mitochondrial-dependent anticancer activity of delta-tocotrienol and its synthetic  
36 derivatives in HER-2/neu overexpressing breast adenocarcinoma cells. *Biofactors*  
37 39(4):485-493.  
38  
39 Wali VB, Bachawal SV, Sylvester PW. 2009a. Endoplasmic reticulum stress mediates gamma-  
40 tocotrienol-induced apoptosis in mammary tumor cells. *Apoptosis* 14(11):1366-1377.  
41  
42 Wali VB, Bachawal SV, Sylvester PW. 2009b. Suppression in mevalonate synthesis mediates  
43 antitumor effects of combined statin and gamma-tocotrienol treatment. *Lipids* 44(10):925-  
44 934.  
45  
46 Wali VB, Sylvester PW. 2007. Synergistic antiproliferative effects of gamma-tocotrienol and statin  
47 treatment on mammary tumor cells. *Lipids* 42(12):1113-1123.  
48  
49  
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2  
3 Wang C, Husain K, Zhang A, Centeno BA, Chen DT, Tong Z, Sebti SM, Malafa MP. 2015. EGR-  
4 1/Bax pathway plays a role in vitamin E delta-tocotrienol-induced apoptosis in pancreatic  
5 cancer cells. *J Nutr Biochem* 26(8):797-807.  
6  
7 Wang M, Kaufman RJ. 2016. Protein misfolding in the endoplasmic reticulum as a conduit to  
8 human disease. *Nature* 529(7586):326-335.  
9  
10 Weng-Yew W, Selvaduray KR, Ming CH, Nesaretnam K. 2009. Suppression of tumor growth by  
11 palm tocotrienols via the attenuation of angiogenesis. *Nutr Cancer* 61(3):367-373.  
12  
13 Weng CJ, Yen GC. 2012. Chemopreventive effects of dietary phytochemicals against cancer  
14 invasion and metastasis: phenolic acids, monophenol, polyphenol, and their derivatives.  
15 *Cancer Treat Rev* 38(1):76-87.  
16  
17 Whittle KJ, Dunphy PJ, Pennock JF. 1966. The isolation and properties of delta-tocotrienol from  
18 Hevea latex. *Biochem J* 100(1):138-145.  
19  
20 Wilankar C, Khan NM, Checker R, Sharma D, Patwardhan R, Gota V, Sandur SK, Devasagayam  
21 TP. 2011. gamma-Tocotrienol induces apoptosis in human T cell lymphoma through  
22 activation of both intrinsic and extrinsic pathways. *Curr Pharm Des* 17(21):2176-2189.  
23  
24 Wong DH, Villanueva JA, Cress AB, Sokalska A, Ortega I, Duleba AJ. 2011. Resveratrol inhibits  
25 the mevalonate pathway and potentiates the antiproliferative effects of simvastatin in rat  
26 theca-interstitial cells. *Fertil Steril* 96(5):1252-1258.  
27  
28 Wu SJ, Ng LT. 2010. Tocotrienols inhibited growth and induced apoptosis in human HeLa cells  
29 through the cell cycle signaling pathway. *Integr Cancer Ther* 9(1):66-72.  
30  
31 Xiong A, Yu W, Liu Y, Sanders BG, Kline K. 2016. Elimination of ALDH+ breast tumor initiating  
32 cells by docosahexanoic acid and/or gamma tocotrienol through SHP-1 inhibition of Stat3  
33 signaling. *Mol Carcinog* 55(5):420-430.  
34  
35 Yang Z, Xiao H, Jin H, Koo PT, Tsang DJ, Yang CS. 2010. Synergistic actions of atorvastatin with  
36 gamma-tocotrienol and celecoxib against human colon cancer HT29 and HCT116 cells. *Int J*  
37 *Cancer* 126(4):852-863.  
38  
39 Yap WN, Chang PN, Han HY, Lee DT, Ling MT, Wong YC, Yap YL. 2008. Gamma-tocotrienol  
40 suppresses prostate cancer cell proliferation and invasion through multiple-signalling  
41 pathways. *Br J Cancer* 99(11):1832-1841.  
42  
43 Yap WN, Zaiden N, Luk SY, Lee DT, Ling MT, Wong YC, Yap YL. 2010. In vivo evidence of  
44 gamma-tocotrienol as a chemosensitizer in the treatment of hormone-refractory prostate  
45 cancer. *Pharmacology* 85(4):248-258.  
46  
47 Yarden Y, Sliwkowski MX. 2001. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*  
48 2(2):127-137.  
49  
50  
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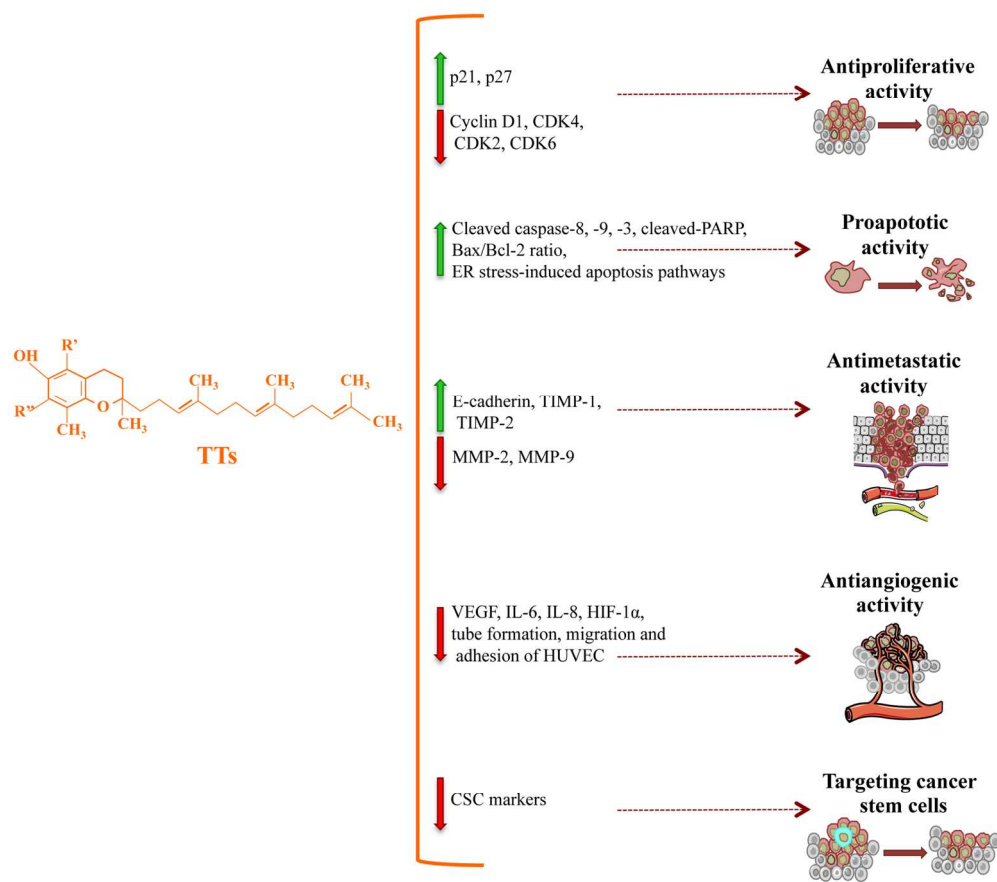
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2  
3 Ye C, Zhao W, Li M, Zhuang J, Yan X, Lu Q, Chang C, Huang X, Zhou J, Xie B, Zhang Z, Yao X,  
4 Yan J, Guo H. 2015. delta-tocotrienol induces human bladder cancer cell growth arrest,  
5 apoptosis and chemosensitization through inhibition of STAT3 pathway. *PLoS One*  
6 10(4):e0122712.  
7  
8  
9 Yeganehjoo H, DeBose-Boyd R, McFarlin BK, Mo H. 2017. Synergistic impact of d-delta-  
10 tTocotrienol and geranylgeraniol on the growth and HMG CoA reductase of human DU145  
11 prostate cCarcinoma cells. *Nutr Cancer* 69(4):682-691.  
12  
13 Yuan Y, Li XF, Chen JQ, Dong CX, Weng SS, Huang JJ. 2014. Critical appraisal of the role of  
14 gefitinib in the management of locally advanced or metastatic non-small cell lung cancer.  
15 *Onco Targets Ther* 7:841-852.  
16  
17 Yusof KM, Makpol S, Jamal R, Harun R, Mokhtar N, Ngah WZ. 2015. gamma-Tocotrienol and 6-  
18 gingerol in combination synergistically induce cytotoxicity and apoptosis in HT-29 and  
19 SW837 human colorectal cancer cells. *Molecules* 20(6):10280-10297.  
20  
21 Zhang JS, Zhang SJ, Li Q, Liu YH, He N, Zhang J, Zhou PH, Li M, Guan T, Liu JR. 2015.  
22 Tocotrienol-rich fraction (TRF) suppresses the growth of human colon cancer xenografts in  
23 Balb/C nude mice by the Wnt pathway. *PLoS One* 10(3):e0122175.  
24  
25 Zhao Z, Egashira Y, Sanada H. 2003. Ferulic acid sugar esters are recovered in rat plasma and urine  
26 mainly as the sulfoglucuronide of ferulic acid. *J Nutr* 133(5):1355-1361.  
27  
28 Zingg JM. 2015. Vitamin E: A Role in Signal Transduction. *Annu Rev Nutr* 35:135-173.  
29  
30 Zingg JM, Kempna P, Paris M, Reiter E, Villacorta L, Cipollone R, Munteanu A, De Pascale C,  
31 Menini S, Cueff A, Arock M, Azzi A, Ricciarelli R. 2008. Characterization of three human  
32 sec14p-like proteins: alpha-tocopherol transport activity and expression pattern in tissues.  
33 *Biochimie* 90(11-12):1703-1715.  
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20 FIGURE 1 Chemical structure of the vitamin E derived tocopherols (TPs) and tocotrienols (TTs).

21 163x64mm (300 x 300 DPI)





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FIGURE 2 Molecular mechanisms of the antitumor activity of TTs. In a wide range of cancer cells, TTs were reported to: counteract cell proliferation while triggering apoptosis, exert an antimetastatic and antiangiogenic activity, specifically target the aggressive cancer stem cell subpopulation. Bax: bcl-2-like protein, Bcl-2: B cell lymphoma-2, CDK: cyclin dependent kinase, CSC: cancer stem cells, ER: endoplasmic reticulum, HIF-1 $\alpha$ : hypoxia inducible factor-1 $\alpha$ , IL: interleukin, MMP: matrix metalloproteinase, PARP: poly (ADP-ribose) polymerase, TIMP: tissue inhibitor of metalloproteinase, VEGF: vascular-endothelial growth factor.

160x142mm (300 x 300 DPI)

**TABLE 1** Presence of tocotrienols in different plant sources

Source	TRF	$\alpha$ -TT	$\gamma$ -TT	$\delta$ -TT
Palm oil	738 mg/l	28%	59%	13%
Annatto seeds	160 mg/100 gr	-	10%	90%
Rice bran	585 mg/l	41%	59%	-
Wheat germ	26 mg/l	100%	-	-

TRF: tocotrienol rich fraction; TT: tocotrienol.

Adapted from Ahsan et al., 2015.

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**TABLE 2** Effects of tocotrienol combination treatments on different types of cancer cells

	<b>Compound</b>	<b>Tumor cell types</b>	<b>Effects</b>	<b>References</b>
<b>Anticancer drugs</b>				
	Cisplatin	Non-small lung cancer cells	↓ Cell viability, migration and invasiveness	Ji et al.(2012)
	Capecitabine	Gastric cancer cells, colorectal cancer cells	↓ Cell proliferation, cell cycle-related proteins, NFκB, VEGF and MMP-9 expression	Manu et al. (2012), and Prasad et al. (2016)
	Erlotinib, Gefitinib	Malignant mammary epithelial cells	↓ ErbB2-4 expression, Akt and STAT pathways. Overcoming resistance to TKIs, apoptosis induction	Bachawal et al. (2010)
	Celecoxib	Malignant mammary epithelial cells	↓ Tumor cell growth, Akt/NFκB pathway, COX-2 ErbB2-4 and PGE2 expression	Shirode et al. (2011)
<b>Natural compounds</b>				
	Sesamin	Mammary tumor cells	↓ Cell proliferation, cell cycle progression, ErbB receptor activation, MAPK, PI3K/Akt, JAK/STAT, NFκB pathways. ↑ TT bioavailability	Sontag et al. (2002), and Akl et al. (2012, 2013)

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3	EGCG	Breast cancer cells	↓Cell proliferation. ↑Nrf2, NQO1 expression (antioxidant activity)	Hsieh et al. (2008), and Dinkova- Kostova et al. (2010)
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9	Resveratrol	Breast cancer cells	↓Cell proliferation, HMG-CoA reductase expression	Cho et al. (2008), and Wong et al. (2011)
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14	Ferulic acid	Prostate cancer cells, breast cancer cells, pancreatic cancer cells	↓Telomerase activity, TERT expression. ↑TT intracellular levels	Eitsuka et al. (2014, 2016)
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21	6-gingerol	Colorectal cancer cells	↑Apoptosis	Yusof et al. (2015)
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24	Oridonin	Mammary cancer cells	↑Autophagy	Tiwari et al. (2015)
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28 COX-2 = cyclooxygenase 2, HMG-CoA = 5-hydroxy-3-methylglutaryl-coenzyme A, JNK = c-Jun  
29 N-terminal kinase, MAPK = mitogen activated protein kinase, MMP-9 = matrix metalloproteinase-  
30 9, NF-κB = nuclear factor-kappa B, NQO1 = NAD(P)H quinone dehydrogenase 1, Nrf2 = nuclear  
31 factor E2-related factor 2, PGE2 = prostaglandin 2, PI3K = phosphoinositide-3 kinase, STAT =  
32 signal transducer and activator of transcription, TERT = telomerase reverse transcriptase, TKIs =  
33 tyrosine kinase inhibitors.  
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