Research Article

A Phase I Safety, Pharmacokinetic, and Pharmacodynamic Presurgical Trial of Vitamin E δ-tocotrienol in Patients with Pancreatic Ductal Neoplasia

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ABSTRACT

Background: Vitamin E δ-tocotrienol (VEDT), a natural vitamin E from plants, has shown anti-neoplastic and chemoprevention activity in preclinical models of pancreatic cancer. Here, we investigated VEDT in patients with pancreatic ductal neoplasia in a window-of-opportunity preoperative clinical trial to assess its safety, tolerability, pharmacokinetics, and apoptotic activity.
Methods: Patients received oral VEDT at escalating doses (from 200 to 3200 mg) daily for 13 days before surgery and one dose on the day of surgery. Dose escalation followed a three-plus-three trial design. Our primary endpoints were safety, VEDT pharmacokinetics, and monitoring of VEDT-induced neoplastic cell apoptosis (ClinicalTrials.gov number NCT00985777).
Findings: In 25 treated patients, no dose-limiting toxicity was encountered; thus no maximum-tolerated dose was reached. One patient had a drug-related adverse event (diarrhea) at a 3200-mg daily dose level. The effective half-life of VEDT was ~4 h. VEDT concentrations in plasma and exposure profiles were quite variable but reached levels that are bioactive in preclinical models. Biological activity, defined as significant induction of apoptosis in neoplastic cells as measured by increased cleaved caspase-3 levels, was seen in the majority of patients at the 400-mg to 1600-mg daily dose levels.
Interpretation: VEDT from 200 to 1600 mg daily taken orally for 2 weeks before pancreatic surgery was well tolerated, reached bioactive levels in blood, and significantly induced apoptosis in the neoplastic cells of patients with pancreatic ductal neoplasia. These promising results warrant further clinical investigation of VEDT for chemoprevention and/or therapy of pancreatic cancer.

1. Introduction

Pancreatic ductal adenocarcinoma (PDA) is a highly lethal form of pancreatic malignancy (Hidalgo, 2010; Siegel et al., 2013, 2015). Late detection, when cure is rare, underscores the significant need to develop novel early detection, prevention, and treatment strategies for PDA. No chemoprevention agent exists for pancreatic cancer, and less than 5 potential pancreatic cancer chemoprevention agents have so far entered early-phase clinical trials (Stan et al., 2010).

The cancer preventive activity of vitamin E has been under intense investigation for decades (Ling et al., 2012). Among the eight forms of vitamin E (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol), alpha-tocopherol has been studied in two large epidemiological chemoprevention trials with disappointing results. In the Women's Health Study trial, women who received alpha-tocopherol every other day had the same chance of developing lung, breast, or colon cancer as those given the placebo (Lee et al., 2005). Similarly, in the selenium, vitamin E, and prostate cancer chemoprevention trial (completed in 2008), alpha-tocopherol consumption, alone or in combination with selenium, was found to have no significant effect in reducing prostate cancer incidence (Lippman et al., 2009). These negative results could be because the type of vitamin E used was not bioactive for cancer prevention. In contrast to the mostly inconsistent results with alpha-tocopherols, tocotrienols (Fig. 1) have shown positive chemopreventive effects in preclinical animal carcinogenesis models (colon, prostate, mammary, and lung) (Gould et al., 1991; Iqbal et al., 2004; Ngah et al., 1991;

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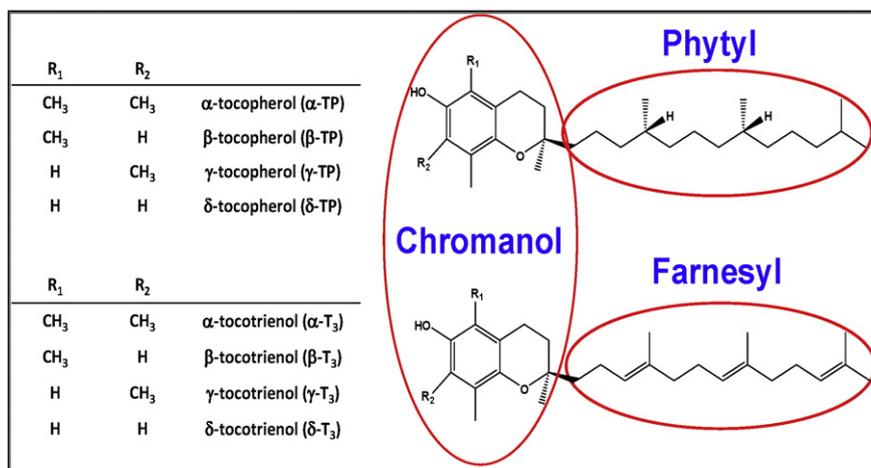E-mail address: Mokenge.malafa@moffitt.org (M.P. Malafa).

Fig. 1. Chemical structures of tocopherols and tocotrienols are similar in that they both contain an aromatic ring chromanol ring to which the free radical scavenging hydroxyl moiety is attached. The main difference between tocopherols and tocotrienols is in the saturation of the aliphatic side chain attached to the chromanol ring. The aliphatic side chain is saturated in tocopherols and unsaturated in tocotrienols. The 4 isomers (alpha, beta, gamma, delta) are named for specific methyl group substitutions at positions 5, 7, and 8 of the chromanol ring.

Rahmat et al., 1993; Sundram et al., 1989; Wada et al., 2005). However, evidence of anticancer activity in humans is lacking.

Recently, we demonstrated that vitamin E δ-tocotrienol (VEDT) prevents pancreatic carcinogenesis in the K-ras transgenic mouse model (Husain et al., 2013a). This form has shown apoptogenic activity previously (Birringer et al., 2003). Also, using the more aggressive K-ras-p53 transgenic mouse model of pancreatic carcinogenesis, we showed that VEDT prolonged mouse survival by preventing pancreatic cancer growth and metastasis (Husain et al., 2013b). We also showed that VEDT's anticancer activity in pancreatic neoplasia was associated with selective induction of apoptosis in pancreatic transformed and malignant epithelial cells but not in normal immortalized human pancreatic ductal epithelial cells (Hodul et al., 2013; Husain et al., 2011).

Here, we aimed to translate our preclinical findings in a phase I dose-escalation trial of VEDT in pancreatic cancer patients, with the primary objective of determining the VEDT dose that exhibits biologic activity in humans. In particular, we sought to identify the dose that selectively induces apoptosis in malignant pancreatic tissue and to characterize VEDT pharmacokinetic properties and its safety and tolerability. The systematic measurement of pharmacodynamic endpoints in trials of signaling agents in pancreatic cancer is hampered by the limited quantity of patient tissue that can be obtained with fine-needle aspiration biopsies. Thus, we employed a presurgical trial design in which patients with pancreatic ductal neoplasia who had indications for surgical resection of their tumors consumed VEDT for a brief period of time immediately preceding the day of surgery. Finally, the patient's entire tumor was resected along with adjacent non-malignant pancreatic tissue, thus yielding abundant tissue for pharmacodynamic analyses.

2. Methods

This study was a single-center, open-label, dose escalation phase I trial (ClinicalTrials.gov NCT00985777) in patients with presumptive premalignant (intraductal papillary mucinous neoplasm or mucinous cystic neoplasm of the pancreas) or malignant (pancreatic carcinoma) neoplasms of the exocrine pancreas having indications for curative surgical resection. Presumptive premalignancy was detected by computed tomography or endoscopic ultrasonography. All patients provided written informed consent on a protocol approved by the University of South Florida Institutional Review Board.

2.1. Patient Population

Inclusion criteria included age ≥ 18 years, Eastern Cooperative Oncology Group (ECOG) performance status of <2, serum creatinine level < 1.5 mg/dL or calculated creatinine clearance > 60 mL/min, total bilirubin < 2.0 mg/dL, alanine aminotransferase and aspartate aminotransferase < 2.5 times the upper limit of normal, absolute neutrophil count > 1000 mm^3, and platelet count > 50,000/mm^3. Patients with borderline resectable PDA were not eligible. Exclusion criteria included having had radiation therapy, chemotherapy, or investigational therapy or had major surgery within 30 days before the first dose of study drug. Patients with active infection or fever >38.5 °C within 3 days of first dose of study drug or with uncontrolled intercurrent illness were ineligible. An uncontrolled intercurrent illness is one that develops in a patient between clinical assessment visits and then becomes uncontrolled (that is, medical therapy has become ineffective).

2.2. Treatment and Dose Escalation

We gave patients the study drug orally as a single agent twice daily for 13 consecutive days before surgery and one dose on the day of surgery (day 14). The formulation consisted of 99% pure VEDT encapsulated into soft gels, where the soft gel comprises pharmaceutical-grade medium-chain triglyceride. BED was defined as the maximum-tolerated dose (MTD) of VEDT that demonstrates significant induction of apoptosis in the neoplastic cells from resected tumor samples of our treated patients. Because this micronutrient might not exhibit dose-limiting toxicity (DLT) beyond its BED, dose escalation was stopped at 3200 mg daily if MTD was not reached. Using the body-surface area normalization method from mice to human, we estimated 3200 mg daily to be 5.6 times the predicted BED. If biological effect was seen at lower doses, we planned to continue to dose escalate to MTD to investigate dose-related increases in biological effect.

Patients were treated in cohorts of three starting at our initial dose level (100 mg twice daily). Subsequent cohorts were escalated to 200, 300, 400, 800, and 1600 mg twice daily. Patients were instructed to take VEDT with a full glass of water (~8 oz) after eating a light breakfast except on day 14 when VEDT was taken with water only. On day 14, patients had standard pancreatectomy. We did not allow intra-patient dose escalation.

2.3. Toxicity Evaluation

We graded adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4·0. The safety analysis included all patients who received at least one dose of VEDT. Adverse events were recorded at each visit and, if related to VEDT, were followed to resolution. Laboratory assessments, including hematology, were done pretreatment and on days 1, 8, and 14.

2.4. Pharmacokinetics

Blood samples were collected on 1) day 1 pre-dose and post-dose at 30 min and at 1, 2, 4, 6, and 8 h; 2) day 2 pre-dose; 3) day 8 pre-dose and post-dose at 4 h; 4) day 13 pre-dose and post-dose at 30 min and at 1, 2, 4, 6, and 8 h; and 5) day 14 post-dose.

VEDT concentrations were determined in plasma and tissue by liquid chromatography. Our methods were validated according to ICH/FDA guidelines. Calibration and quality control (QC) samples were made by adding known amounts of VEDT to plasma or tissue. All calibration, QC, and unknown patient samples were prepared as follows: VEDT was extracted from plasma using protein precipitation with a mixture of acetonitrile and tetrahydrofuran, followed by taking the extracts to solid-supported liquid extraction plates. Elution from plates was carried out by hexane. Tissue was treated with a combination of ethanol, water, and hexane, and extraction was aided by homogenization, with hexane layer then captured. Plasma and tissue extracts in hexane were evaporated to dryness. Concentrated samples were reconstituted with 110 μL hexane to ready them for injection.

Samples were injected into an Agilent Technologies (Palo Alto, CA) 1100/1200 liquid chromatography system coupled to a fluorescence detector. A Zorbax (Agilent) pure silica, normal-phase chromatographic column was used for separation. The mobile phase was hexane:isopropanol (99:1), and flow rate was set to 0.65 mL/min. The fluorescence detector was set at an excitation wavelength of 296 nm and emission wavelength of 330 nm to capture chromatographic peaks of interest.

Chromatographic peaks were integrated by Agilent Technologies Chemstation software (version B.03.02). Linear regression was used to form the calibration curve from calibration standards; QCs were checked against the regression line, and unknowns were plotted for back calculation of the patients' raw concentrations. The calibration of VEDT is linear in plasma from 5 to 1000 ng/mL and from 5 to 1000 ng/100 mg of tissue. Inter- and intra-assay variabilities were <11%, with a relative mean error of <9.4%.

Raw concentration data from HPLC assays were used to formulate pharmacokinetic data for VEDT. The area under the curve (AUC) for the dosing interval (AUC_{0-T}), AUC to infinity, maximum concentration (Cmax) and time to maximum concentration (Tmax), volume of distribution, and clearance were determined for VEDT on day 1. Non-compartmental methods were applied to determine pharmacokinetic parameters, and segmented AUC between samples was determined by the trapezoid rule.

2.5. Pharmacodynamics

The apoptotic effect of VEDT in pancreatic tumors was assessed by caspase-3 activation in the neoplastic cells of treated patients, determined by immunohistochemistry. Histology slides stained against antibodies to caspase-3 were scanned using the Aperio™ (Vista, CA) ScanScope XT with a 200×/0.75NA objective lens at a rate of 3 min/slide via Basler tri-linear-array. Immunopositivity of caspase-3 was quantitatively scored using commercial algorithms from the Positive Pixel Count v9 Aperio Toolbox®, which used scored staining intensities for pixels as follows: 3+, strong; 2+, moderate; 1+, weak; 0, negative. Staining intensities were thresholded by the 0–255 8-bit dynamic range value (220, 175, and 100, respectively). This algorithm was optimized by a board-certified pathologist (BAC) and applied to specific regions identified by the pathologist. Twenty-five total fields of view were counted to calculate the number of caspase-3-positive cells. On average, 3051 cells were counted for neoplastic cells and 485 for non-neoplastic cells. We used only cells that had a 3+ or 2+ staining intensity as caspase-3-positive cells to maintain a high threshold for scoring apoptosis induction.

To establish the threshold for significant induction of apoptosis in pancreatic neoplastic tissue, we examined archival surgical tumor specimens from 20 patients without VEDT treatment. These 20 patients who did not receive VEDT comprised our control group, with their pancreatic neoplasms resected by the same surgeon who performed resections of the treated patients over the same period as treated patients. Results show the following frequency distribution of the percentage of caspase-3-positive cells in the untreated neoplastic tissue: 0th percentile: 0.3%; 50th percentile: 2.1%; 90th percentile: 7.6%; 100th percentile: 11.6% (mean 3.3% and SD 3.3). The majority of VEDT untreated tumor samples had caspase-3-positive cells <2.1%. We used a conservative threshold of the 90th percentile, 7.6%, as the threshold to determine the biological response. This high threshold is likely to minimize the false-positive rate.

2.6. Statistical Analyses

Patient demographics, adverse events, and clinical laboratory evaluations were categorized descriptively. Quantitative laboratory measurements such as pharmacokinetic parameters were summarized as means and SD. For evaluation of caspase-3 expression, complete permutation test was used to test any group differences for each two-group comparison, because data were skewed with many zeros and the sample size was in a manageable scale (n < 50).

3. Results

3.1. Patient Characteristics

Between September 2009 and May 2012, 19 patients were enrolled, with 18 treated. One patient was withdrawn before treatment due to the development of duodenal obstructive symptoms. An additional seven patients were treated at the expansion dose (800 mg daily). Patient characteristics are summarized in Table 1.

3.2. Safety and Tolerability

VEDT was well tolerated at all dose levels. No drug-related toxicities were seen at 200–1600 mg daily. At 3200 mg daily, one patient had two episodes of drug-related grade 1 diarrhea (Table 2). Ten patients (including three dose-escalation (800 mg) patients) from the expansion cohort were used to evaluate safety and efficacy (biological effect response). Two metrics were evaluated: serious adverse event (SAE) rate and adverse event (AE) rate. We considered a dose to be tolerable if the SAE rate was <30% and the AE rate was <50%. The lower bounds (8% and 20%, respectively) of the 95% CI for the 30% SAE and the 50% AE toxicity rates were used for determination.

Table 1
Patient characteristics (N = 25).

	No.	Percentage
Age, years		
Mean	65.3	
Range	49–84	
Sex		
Male	16	64
Female	9	36
Ethnicity		
White	21	84
Black	3	12
Unknown	1	4
Histologic type		
Ductal adenocarcinoma	10	40
Intraductal papillary mucinous	7	28
Invasive mucinous carcinoma	1	4
Mucinous cystic neoplasm	2	16
Pancreatic intraepithelial neoplasia	3	8
Squamoid cyst	1	4
Serous cystadenoma	1	4

Table 2
Treatment-related adverse events (grades I to IV) in all patients.

	200 mg (n = 3)	400 mg (n = 3)	600 mg (n = 3)	800 mg (n = 3)	Expansion Cohort, 800 mg (n = 7)	1600 mg (n = 3)	3200 mg (n = 3)
Fatigue	0	0	0	0	0	0	0
Weight loss	0	0	0	0	0	0	0
Bruising	0	0	0	0	0	0	0
Pruritus	0	0	0	0	0	0	0
Rash	0	0	0	0	0	0	0
Wound complication	0	0	0	0	0	0	0
Anorexia	0	0	0	0	0	0	0
Constipation	0	0	0	0	0	0	0
Dehydration	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	2
Abdominal bloating	0	0	0	0	0	0	0
Flatulence	0	0	0	0	0	0	0
Other GI	0	0	0	0	0	0	0
Nausea	0	0	0	0	0	0	0
Vomiting	0	0	0	0	0	0	0
UTI	0	0	0	0	0	0	0
Hyperglycemia	0	0	0	0	0	0	0
Hypoglycemia	0	0	0	0	0	0	0
Mental status change	0	0	0	0	0	0	0
Pain	0	0	0	0	0	0	

There were 0 SAEs and 0 AEs in the 10 patients who received 800 mg; these rates were lower than the lower bounds (8% and 20%) of the 95% CI for the 30% and 50% rates, respectively. In addition, in our evaluated patients (n = 25), only one drug-related AE was noted in one patient at the 1600-mg dose (4% AE rate, which is again lower than the lower bounds). We concluded that VEDT was statistically confirmed to be safe and tolerable at all of the doses tested with 95% confidence.

3.3. Pharmacokinetics

Pharmacokinetic analyses were conducted in the first 18 patients. We found substantial interpatient variability in pharmacokinetic parameters at all dose levels (Table 3). The possibility that this could be related to variability in the composition of the meal consumed by patients was considered. Therefore, the meal with the morning VEDT dose was standardized. Nevertheless, interpatient variability continued to be observed. Beyond 400 mg daily, there was no clear dose-proportionate relationship to Cmax and AUC (Fig. 2A). For all patients treated, Tmax was 5.6 ± 2.1 h and half-life was 3.8 ± 1.8 h (Table 3). For patients treated at ≥400 mg daily, mean Cmax was 2111 ± 1940 ng/mL or 5.32 ± 4.89 μM, a concentration level comparable to that seen in patients dosed with mixed tocotrienols (Yap et al., 2001). It is also in the concentration range seen in mouse plasma dosed orally with VEDT (Husain et al., 2013a). This plasma concentration level of VEDT has been demonstrated to have antitumor activity in preclinical models (Husain et al., 2009). Furthermore, the AUC observed in humans is consistent with the AUC observed with the bioactive dosing in mice (Husain et al., 2009) and in cell culture (Fig. 2B).

3.4. Biological Activity

First, we compared the levels of cleaved caspase 3 in control samples from untreated patients with samples from the patients treated in the six dose-escalation cohorts. We expected to see increased caspase-3-positive cells in tumor tissues from treated but not from untreated patients, and also likely not in normal tissues from treated patients. Before analyses, samples from the dose-escalation patient cohorts were grouped into "treated normal" and "treated dysplastic or malignant" tissues. As shown in pairwise comparisons in Fig. 3, control tumor tissues from untreated patients had comparable percentages of caspase-3-positive cells to normal tissues from treated patients (adjusted P = 0.725). In contrast, control tumor tissues from untreated patients had a statistically significant difference (lower) compared with dysplastic or malignant tissues from treated patients (adjusted P = 0.005). Moreover, the dysplastic or malignant tissues had a higher percentage of caspase-3-positive cells than the normal tissues from the same treated patients (adjusted P = 0.011). These results suggest that caspase-3 is a good biomarker to evaluate the biological effect of VEDT.

Next, we investigated the biological effect response in the six dose-escalation cohorts, using the threshold of 7.6% to evaluate each patient (n = 16 patients). As shown in Fig. 4, the percentage of caspase-3-positive cells increased for patients given 200–600 mg and then decreased for patients given 800–3200 mg daily. The biological response rate had a similar pattern, starting with 50% with 200 mg, then increasing 100% with 400 and 600 mg, and then decreasing to 66% with 800 mg and 1600 mg and to 33% with 3200 mg.

Because we wanted to identify a high dose level with a high biological effect response rate (>50%), we considered the 800- and 1600-mg
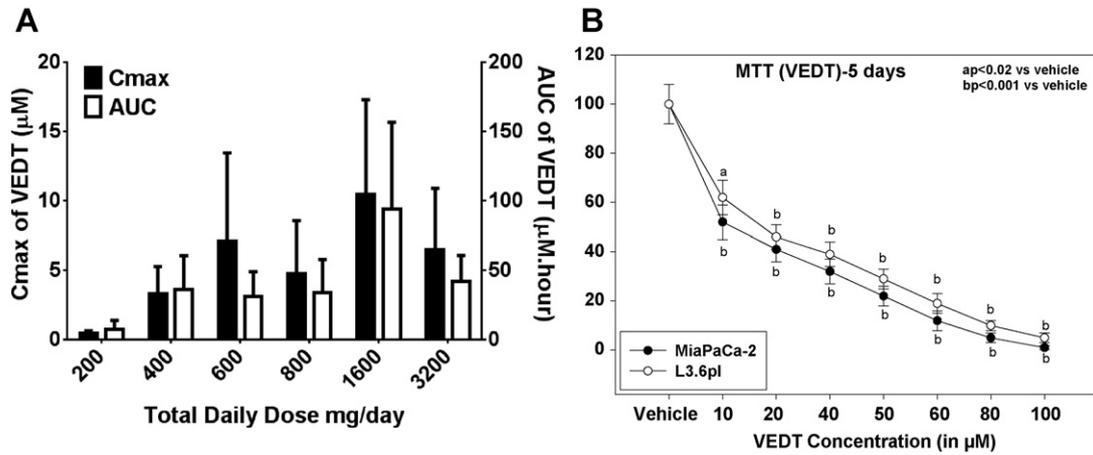
Table 3
Pharmacokinetic parameters for VEDT by dose level.

Dose level (mg daily)	No.	Cmax (ng/mL)		AUC$_{0-12}$ (ng × h/mL)		AUC$_{0-\infty}$ (ng × h/mL)		t$_{1/2}$ (hours)		Clearance (L/h)		Vd (L)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
200	3	178.1	67.4	3157.4	1750.1	3024.1	2461.9	2.0	0.5	49.5	40.3	235.9	192.2
400	3	1299.5	790.8	13,486.9	9499.0	14,300.9	9785.2	5.5	0.5	38.2	24.0	317.0	218.2
600	2	2818.5	2511.1	10,707.7	9111.8	12,292.7	7150.2	2.9	1.5	29.4	17.1	141.1	136.2
800	3	1882.1	1505.8	12,503.2	9898.8	13,436.1	9473.4	2.9	2.0	39.5	21.2	195.9	208.3
1600	3	4141.8	2717.2	27,424.6	16,631.3	37,340.8	24,810.6	3.9	1.9	40.1	42.4	151.1	74.5
3200	3	2585.1	1733.6	13,291.6	5198.5	16,692.6	7434.3	5.4	1.6	106.4	47.4	890.5	614.6

AUC = area under the serum concentration curve; Cmax = peak serum concentration; t$_{1/2}$ = half-life; Vd = volume of distribution.

Fig. 2. VEDT reaches bioactive levels in human serum. **a:** Large variability in pharmacokinetic parameters is shown, with peak VEDT serum levels (Cmax) of up to 18 μM and AUC up to 170 μM. **b:** MTT assay of VEDT treatment of MiaPaCa-2 and L3.6 pl human PDAC cells in vitro demonstrates IC_{50} of 5 and 10 μM with 5-day treatment.

dose levels for the expansion cohort to investigate BED. We elected to start with the 800-mg daily dose.

Ten patients (including three patients in the dose-escalation cohort) were used to evaluate biological effect response. A biological effect response rate > 50% would be considered a BED. The upper bound (79.9%) 95% CI for a 50% response rate was used for determination. As shown in Fig. 5, the 800-mg dose yielded an 80% (8/10) biological effect response rate, which was higher than the upper bound (79.9%) of the 95% CI for a 50% response rate. These results indicate that 800 mg could be selected as a BED.

Finally, we compared control samples from untreated patients to samples from treated patients from the dose-escalation plus expansion cohorts. As shown in pairwise comparisons (Fig. 6), untreated control tumor tissues had comparable percentage of caspase-3-positive cells to the 'treated normal' tissues (adjusted P = 0.985), with a statistically significant difference (lower) versus treated dysplastic or malignant

tissues (adjusted P = 0.044). Moreover, the treated dysplastic or treated malignant tissues had a higher percentage of caspase-3-positive cells than treated normal tissues (adjusted P = 0.044). Both treated dysplastic and treated malignant tissues had a similar higher percentage of caspase-3-positive cells (P = 0.985). We therefore concluded that the 800-mg dose was statistically confirmed to be a BED with 95% confidence and therefore stopped further accrual.

4. Discussion

Our study confirmed the safety and tolerability of highly pure VEDT (99%) at doses up to five times greater than those given to patients in previous studies, which used a mixture of tocotrienols (Rahmat et al., 1993; Qureshi et al., 1995, 2002; Zaiden et al., 2010). The relatively short time to Tmax and half-life of pure VEDT are similar to results reported with lower doses of mixed tocotrienols (Qureshi et al., 1995,
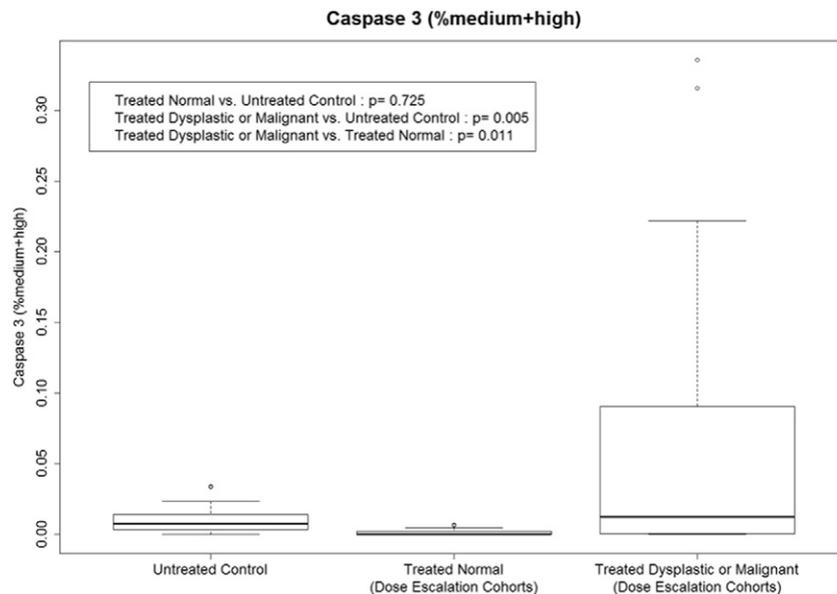

Fig. 3. Apoptosis in untreated control pancreatic ductal malignant cells compared with VEDT-treated pancreatic ductal normal and neoplastic (dysplastic or malignant) cells. The apoptotic index, as measured by % cells staining positive for caspase-3 in untreated control malignant cells in patients (n = 20) with PDAC operated on by the same surgeon who operated on the VEDT-treated patients on the same period of time was low. Moreover, the apoptotic index of these malignant cells was not statistically different from the apoptotic index of the normal ductal epithelial cells in VEDT-treated patients (P = 0.725). In contrast, the apoptotic index of the VEDT-treated neoplastic cells (Dysplastic and Malignant) were significantly higher than those of the untreated control malignant cells (P = 0.005). Moreover, the apoptotic index of the VEDT-treated neoplastic cells was also significantly higher than the VEDT-treated normal epithelial cells (P = 0.011). These findings strongly suggest selective induction of apoptosis in neoplastic cells by VEDT treatment.
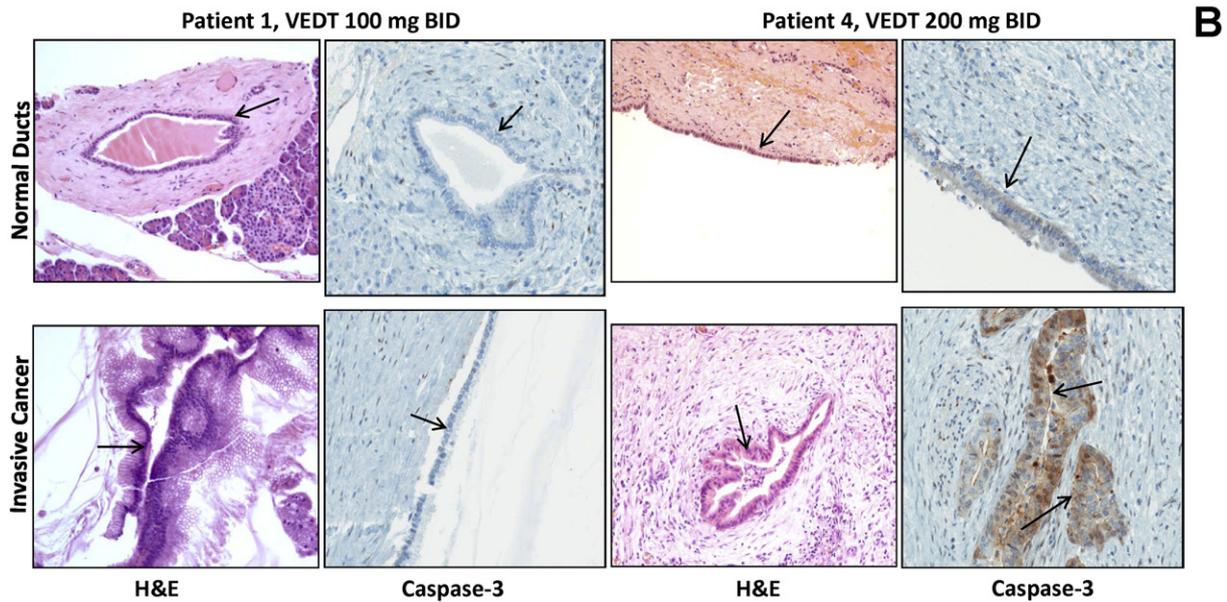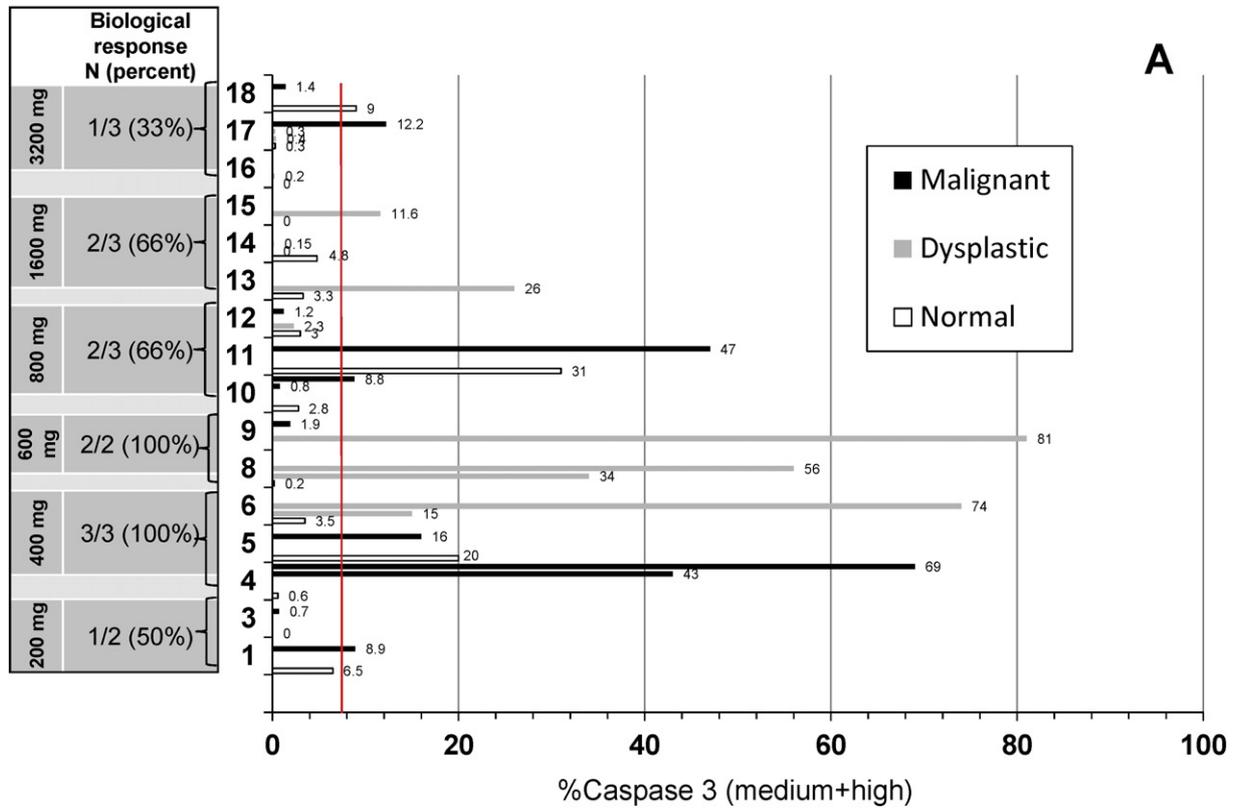
Fig. 4. Biologic evidence of tumor response. **a:** Waterfall plot of the best biologic response to VEDT at different doses in patients at the escalation doses. Red line represents statistically calculated threshold of significant induction of apoptosis (7.6%). **b:** Immunohistochemistry sections from pancreatectomy specimens stained with hematoxylin and eosin (×20 magnification), as well as for cleaved caspase-3 (×20 magnification), highlighting biologic responses in representative patients. Both invasive mucinous adenocarcinoma and adjacent normal specimens are shown at two VEDT dose levels: 100 mg (Patient 1) and 200 mg (Patient 4). Arrows in normal specimens indicate normal duct or normal duct cells negative for caspase-3. For Patient 1, arrows in invasive specimens indicate mucinous adenocarcinoma (H&E slide) or carcinomatous epithelium (caspase-3 slide). For Patient 4, arrows in invasive specimens indicate malignant gland (H&E slide) or cells undergoing apoptosis based on caspase-3 expression (caspase-3 slide).

2002; Zaiden et al., 2010). The fact that levels in the plasma did not correlate well with administered dose suggests that there are factors affecting the metabolism and circulation of this nutrient that remain unknown. Nevertheless, the concentrations obtained in plasma in this study are comparable to those obtained in mice treated with VEDT in which tumor growth was delayed and apoptosis was induced (Husain et al., 2009). The finding that pancreatic tumors from several patients treated with VEDT displayed much higher percentages of apoptotic
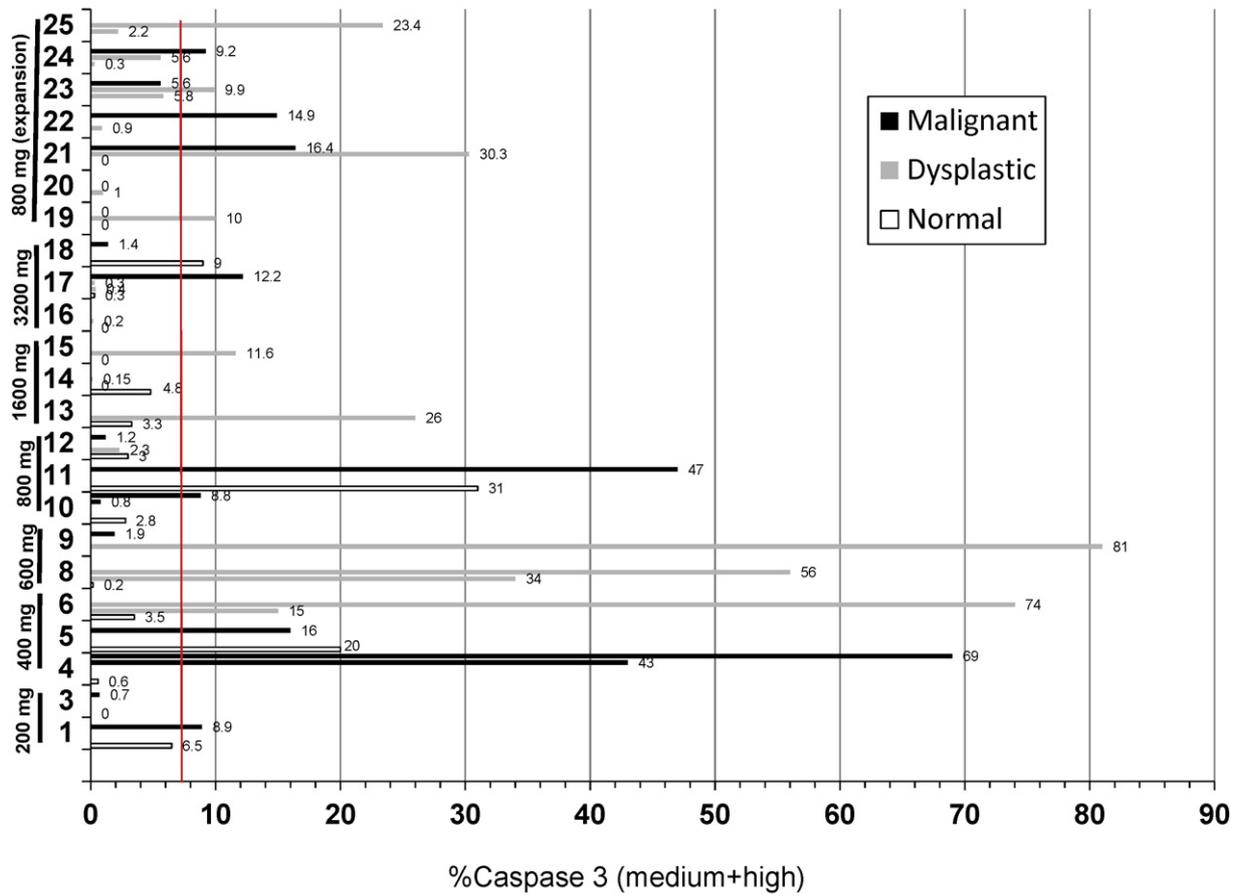
Fig. 5. Biologic response in the expansion cohort. Apoptotic index as measured by % cells staining positive for caspase-3 was used to assess biologic response in the expansion cohort. Ten patients (including three patients in the dose-escalation cohort) were treated at the 800-mg dose level; 80% (8/10) patients had significant induction of apoptosis in their dysplastic or malignant tissue. Red line indicates threshold of biological response (7.6%).

tumor cells than those from untreated patients suggests that plasma levels obtained may be sufficient to have the desired biological effect. The challenge now is to understand why some patients treated at a higher dose failed to show a higher percentage of apoptotic tumor cells. This could be due to tumor genetic heterogeneity, altered susceptibility to the biological effect of VEDT, or intrinsic variability among
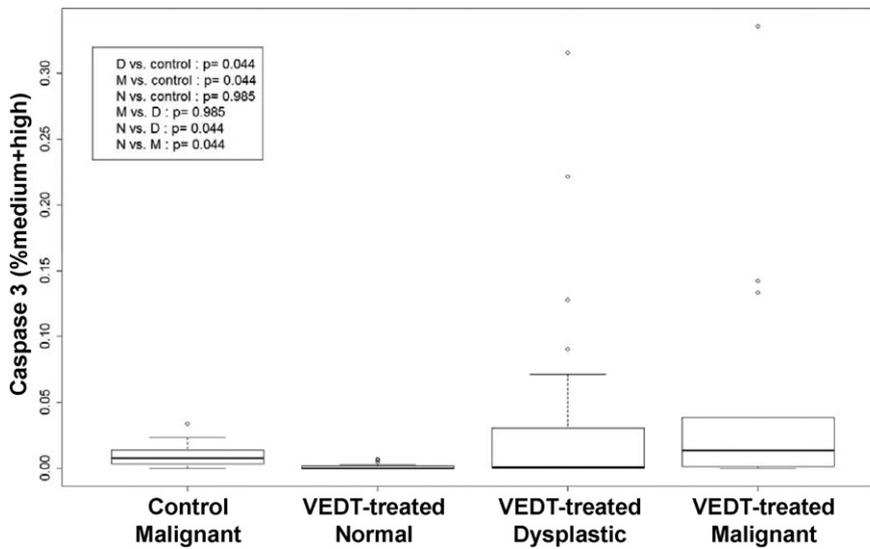

Fig. 6. Pairwise comparison of apoptotic index in untreated control versus VEDT-treated patient in both the dose-escalation and expansion cohorts. Untreated control tumor tissues had comparable percentage of caspase-3-positive cells to the "treated normal" tissues (adjusted P = 0.985), with a statistically significant difference (lower) versus treated dysplastic or malignant tissues (adjusted P = 0.044). Treated dysplastic or treated malignant tissues had a higher percentage of caspase-3-positive cells than treated normal tissues (adjusted P = 0.044). Both treated dysplastic and treated malignant tissues had a similar higher percentage of caspase-3-positive cells (P = 0.985).

individuals in the metabolism and clearance of the drug at high doses. Higher concentrations could also modulate other cellular targets, which counteract the VEDT pro-apoptotic effects.

Regarding dose, our selection was designed to ensure the greatest margin of safety and to focus on identifying the biologic effective dose as defined by biological activity in tumor samples. Our starting dose (100 mg twice a day) was based on the rationale described in the Food and Drug Administration (FDA) guidance "General Guide for Starting Dose Selection for a Cytotoxic Agent in Cancer Patients" (available at www.fda.gov/cder/cancer/docs/doseflow.pdf) as well as on the no-observed-adverse-effect level (NOAEL) determined in a published repeat-dose toxicity study in rats (Nakamura et al., 2001). In this 13-week toxicity study, the NOAELs for VEDT were concluded to be 0.19% in the diet (120 mg/kg body weight/day for male rats and 130 mg/kg body weight/day for female rats). The human equivalent doses based on equivalent relative body surface areas of 1:6:2 will yield 19.4 mg/kg/day dosed daily for up to 13 weeks. Therefore, for a 65-kg subject the NOAEL dose of VEDT will be 1261 mg/day.

In mice, VEDT dosed at 100 mg/kg/day once daily (via oral gavage) resulted in significant inhibition of tumor growth and induction of apoptosis in the neoplastic cells. Using the recommended body surface area (BSA) normalization method for allometric dose translations when starting new clinical studies (Reagan-Shaw et al., 2008), the formula below was used: *Human Equivalent Dose* (mg/kg) = *Animal dose* (mg/kg) × *Animal Km* / *Human Km* (where *Km* values based on body surface area for a mouse is 3 and an adult human is 37 (60 kg)). Converting a dose of VEDT (100 mg/kg) given to a mouse into the human equivalent dose based on body surface area is: $HED =$ 100 (mg/kg) × 3 (mouse Km) / 37 ($Human$ Km); The $HED =$ 8.11 mg/kg. A 70 kg subject would therefore require 567 mg/day. Dose escalation to a maximum of 3200 mg/day will be 5.6 times the predicted bioactive dose from the preclinical studies.

The ability of VEDT to induce apoptosis in pre-malignant lesions (dysplastic tissue) but not in adjacent normal tissue has particular promise for pancreatic cancer prevention. Pancreatic carcinogenesis is a multistep process that involves the accumulation of a set of genetic changes that convert normal cells first to premalignant cells and then to malignant cells (Iacobuzio-Donahue, 2012; Iacobuzio-Donahue et al., 2012). In a recent study, an average of 63 mutations per cancer was altered in 24 human PDAs (Jones et al., 2008). The most widely accepted explanation for the accumulation of multiple mutations in the same cell is the model of clonal evolution, when cells with one crucial mutation expand clonally (Aparicio and Caldas, 2013). By increasing in number through mitosis and suppression of apoptosis, cells with the first mutation become more likely to develop a second mutation, which predisposes to a third mutation and so on. The continuing clonal expansion, selection, and heterogeneity allow accumulation of multiple mutations in the same cell and the ultimate generation of malignant clones (Iacobuzio-Donahue, 2012). Therefore, reducing or eliminating the number of premalignant cells could be an effective strategy to reduce pancreatic cancer risk (Wu and Lippman, 2011). One intriguing finding demonstrated consistently by us and others is the selective killing effect of tocotrienols against cancer cells over normal cells (Hodul et al., 2013; Husain et al., 2011; Srivastava and Gupta, 2006; Yap et al., 2008, 2010). Tocotrienol was found to induce apoptosis in prostate and breast cancer cells but not in nonmalignant breast and prostate epithelial cells (Srivastava and Gupta, 2006; Yap et al., 2008, 2010). Because no effective chemoprevention therapy for the medical treatment of pre-malignant pancreatic lesions is available, patients face the choice of potentially life-changing pancreatectomy versus observation with the risk of transformation to invasive adenocarcinoma and metastasis. In addition, no effective surveillance strategies are available to identify transformation before metastasis. The ability of VEDT to induce apoptosis in the neoplastic cells of intraductal papillary mucinous neoplasm and pancreatic intraepithelial neoplasia lesions with minimal toxicity establishes this micronutrient as a candidate chemoprevention agent

for the medical treatment of patients with these lesions who opt to not have surgery. The tolerability of VEDT with longer-term administration of high doses will be the subject of future investigation.

The survival benefit of our intervention is unknown. This was a proof-of-concept biologic endpoint trial, where the goal was to demonstrate induction of apoptosis in patient tumors after a 2-week exposure to VEDT in the preoperative setting. Enhancement of survival was not an anticipated or stated endpoint. Its effect on survival outcomes is a subject for a phase II efficacy trial.

In conclusion, this first-in-human phase I study of VEDT was designed to establish the BED of VEDT for future investigations of the clinical activity of VEDT. VEDT is distinguished from other tocotrienols by its consistently superior anticancer activity in preclinical models. Although there are no approved agents for pancreatic cancer prevention, the dismal natural history of pancreatic cancer and increasing recognition of high-risk individuals warrants the investigation of promising agents like VEDT from preclinical studies (Husain et al., 2013a). Here, we have shown that VEDT use in untreated patients with pancreatic cancer and high-risk premalignant tumors was associated with selective induction of apoptosis in neoplastic cells without toxicity. These results, as well as efficacy data of VEDT in preclinical studies, encourage our further exploration of VEDT as an alternative treatment for patients at risk of pancreatic cancer. Additional considerations of VEDT include its use in treatment of early-stage disease to prevent or delay relapse, as well as combining VEDT with chemotherapy and other targeted agents.

Contributors

All authors were responsible for the study design, data collection, data analysis, data interpretation, and writing of the report. All authors approved the final version of this manuscript and agreed to the submission. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. GMS wrote the first draft of the report, which was subsequently amended and reviewed by all coauthors, and all authors were involved in the decision to submit the report for publication.

Role of the Funding Source

Potential Conflicts of Interest

Drs. Malafa and Sebti are named as inventors on US Patent "Delta-Tocotrienol Treatment and Prevention of Pancreatic Cancer (June 26, 2007; OTML docket number 06A069) but do not have financial interest in the companies that have licensed this patent.

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References

Aparicio, S., Caldas, C., 2013. The implications of clonal genome evolution for cancer medicine. N. Engl. J. Med. 368 (9), 842–851.
Birringer, M., EyTina, J.H., Salvatore, B.A., Neuzil, J., 2003. Vitamin E analogues as inducers of apoptosis: structure–function relation. Br. J. Cancer 88 (12), 1948–1955.

Gould, M.N., Haag, J.D., Kennan, W.S., Tanner, M.A., Elson, C.E., 1991. A comparison of tocopherol and tocotrienol for the chemoprevention of chemically induced rat mammary tumors. Am. J. Clin. Nutr. 53 (4 Suppl.), 1068S–1070S.

Hidalgo, M., 2010. Pancreatic cancer. N. Engl. J. Med. 362 (17), 1605–1617.

Hodul PJ, Dong Y, Husain K, et al. Vitamin E delta-tocotrienol induces p27(Kip1)-dependent cell-cycle arrest in pancreatic cancer cells via an E2F-1-dependent mechanism. PLoS One 2013; 8(2): e52526.

Husain, K., Francois, R.A., Hutchinson, S.Z., et al., 2009. Vitamin E delta-tocotrienol levels in tumor and pancreatic tissue of mice after oral administration. Pharmacology 83 (3), 157–163.

Husain, K., Francois, R.A., Yamauchi, T., Perez, M., Sebti, S.M., Malafa, M.P., 2011. Vitamin E delta-tocotrienol augments the antitumor activity of gemcitabine and suppresses constitutive NF-kappaB activation in pancreatic cancer. Mol. Cancer Ther. 10 (12), 2363–2372.

Husain, K., Centeno, B.A., Chen, D.T., et al., 2013a. Prolonged survival and delayed progression of pancreatic intraepithelial neoplasia in LSL-KrasG12D/+;Pdx-1-Cre mice by vitamin E delta-tocotrienol. Carcinogenesis 34 (4), 858–863.

Husain, K., Centeno, B.A., Chen, D.T., Hingorani, S.R., Sebti, S.M., Malafa, M.P., 2013b. Vitamin E delta-tocotrienol prolongs survival in the LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1-Cre (KPC) transgenic mouse model of pancreatic cancer. Cancer Prev. Res. (Phila.) 6 (10), 1074–1083.

Iacobuzio-Donahue, C.A., 2012. Genetic evolution of pancreatic cancer: lessons learnt from the Pancreatic cancer genome sequencing project. Gut 61 (7), 1085–1094.

Iacobuzio-Donahue, C.A., Velculescu, V.E., Wolfgang, C.L., Hruban, R.H., 2012. Genetic basis of pancreas cancer development and progression: insights from whole-exome and whole-genome sequencing. Clin. Cancer Res. 18 (16), 4257–4265.

Iqbal, J., Minhajuddin, M., Beg, Z.H., 2004. Suppression of diethylnitrosamine and 2-acetylaminofluorene-induced hepatocarcinogenesis in rats by tocotrienol-rich fraction isolated from rice bran oil. Eur. J. Cancer Prev. 13 (6), 515–520.

Jones, S., Zhang, X., Parsons, D.W., et al., 2008. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321 (5897), 1801–1806.

Lee, I.M., Cook, N.R., Gaziano, J.M., et al., 2005. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. JAMA 294 (1), 56–65.

Ling, M.T., Luk, S.U., Al-Ejeh, F., Khanna, K.K., 2012. Tocotrienol as a potential anticancer agent. Carcinogenesis 33 (2), 233–239.

Lippman, S.M., Klein, E.A., Goodman, P.J., et al., 2009. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 301 (1), 39–51.

Nakamura, H., Furukawa, F., Nishikawa, A., et al., 2001. Oral toxicity of a tocotrienol preparation in rats. Food Chem. Toxicol. 39 (8), 799–805.

Ngah, W.Z., Jarien, Z., San, M.M., et al., 1991. Effect of tocotrienols on hepatocarcinogenesis induced by 2-acetylaminofluorene in rats. Am. J. Clin. Nutr. 53 (4 Suppl.), 1076S–1081S.

Qureshi, A.A., Bradlow, B.A., Brace, L., et al., 1995. Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids 30 (12), 1171–1177.

Qureshi, A.A., Sami, S.A., Salser, W.A., Khan, F.A., 2002. Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. Atherosclerosis 161 (1), 199–207.

Rahmat, A., Ngah, W.Z., Shamaan, N.A., Gapor, A., Abdul, K.K., 1993. Long-term administration of tocotrienols and tumor-marker enzyme activities during hepatocarcinogenesis in rats. Nutrition 9 (3), 229–232.

Reagan-Shaw, S., Nihal, M., Ahmad, N., 2008. Dose translation from animal to human studies revisited. FASEB J. 22 (3), 659–661.

Siegel, R., Naishadham, D., Jemal, A., 2013. Cancer Statistics, 2013. CA Cancer J. Clin. 63 (1), 11–30.

Siegel, R.L., Miller, K.D., Jemal, A., 2015. Cancer Statistics, 2015. CA Cancer J. Clin. 65 (1), 5–29.

Srivastava, J.K., Gupta, S., 2006. Tocotrienol-rich fraction of palm oil induces cell cycle arrest and apoptosis selectively in human prostate cancer cells. Biochem. Biophys. Res. Commun. 346 (2), 447–453.

Stan, S.D., Singh, S.V., Brand, R.E., 2010. Chemoprevention strategies for pancreatic cancer. Nat. Rev. Gastroenterol. Hepatol. 7 (6), 347–356.

Sundram, K., Khor, H.T., Ong, A.S., Pathmanathan, R., 1989. Effect of dietary palm oils on mammary carcinogenesis in female rats induced by 7,12-dimethylbenz(a)anthracene. Cancer Res. 49 (6), 1447–1451.

Wada, S., Satomi, Y., Murakoshi, M., Noguchi, N., Yoshikawa, T., Nishino, H., 2005. Tumor suppressive effects of tocotrienol in vivo and in vitro. Cancer Lett. 229 (2), 181–191.

Wu, X., Lippman, S.M., 2011. An intermittent approach for cancer chemoprevention. Nat. Rev. Cancer 11 (12), 879–885.

Yap, S.P., Yuen, K.H., Wong, J.W., 2001. Pharmacokinetics and bioavailability of alpha-, gamma- and delta-tocotrienols under different food status. J. Pharm. Pharmacol. 53 (1), 67–71.

Yap, W.N., Chang, P.N., Han, H.Y., et al., 2008. Gamma-tocotrienol suppresses prostate cancer cell proliferation and invasion through multiple-signalling pathways. Br. J. Cancer 99 (11), 1832–1841.

Yap, W.N., Zaiden, N., Luk, S.Y., et al., 2010. In vivo evidence of gamma-tocotrienol as a chemosensitizer in the treatment of hormone-refractory prostate cancer. Pharmacology 85 (4), 248–258.

Zaiden, N., Yap, W.N., Ong, S., et al., 2010. Gamma delta tocotrienols reduce hepatic triglyceride synthesis and VLDL secretion. J. Atheroscler. Thromb. 17 (10), 1019–1032.