American Institute of Integrative Oncology
RESEARCH & EDUCATION

Foundations of Integrative Oncology
COURSE

GRAND ROUNDS CALL
With Dr. Nalini Chilkov
April 10th, 2019
Second Wednesday of Every Month
5:30 PM Pacific / 6:30 PM Mountain / 7:30 PM Central / 8:30 PM Eastern

Clinical Pearl: Delta Tocotrienols: Potential Against Cancer
PDF SLIDES

Case Study: LABCORP REPORT: Stage IV Colorectal Cancer_Cancer Terrain
Submitted by: DR NALINI
Note: Low Hgb, Fe, (highest quartile of normal LDH)
Elevated Platelets, BUN/Creat Ratio, Vitamin B12, CEA, GAL-3, D-Dimer, Fibrinogen, CRP, ESR, Cp, Cu,

Questions & Answers
Susie Thomson: **I have a client with cervical cancer and the doctors have told her she is now in forced menopause after chemo / radio / Curie therapy; I was in doubt whether I should have her eat phytoestrogens in case this was contraindicated in cervical cancer as I thought it was a hormonal cancer ie driven by estrogen as well as contributed by HPV, she told me the doctors said in NO way was her cancer estrogen-driven and cervical cancer was only linked to HPV, I have done my own research which throws up the possibility of cervical cancer also being hormone-driven (as well as HPV) and now I am unsure whether to include phytoestrogens in her protocol, I think I should as I understand these are not the same oestrogen's as estradiol which is cancer -causing, what are your thoughts please or a definitive research paper you can direct me to?**
Dr. Chilkov's Response: Cervical CA etiology is most often HPV, not typically ER+
Dr. Stacy D'Andre: **Can you please discuss circulating tumor cells as well as insurance coverage?**
Dr. Chilkov's Response: Yes, most private insurance and Medicare cover where clinically indicated. May be repeated q 3 months. Biocept Lab Insurance copay $100.00 Cash price $350.00 Full Price $1500 *Source:* https://biocept.com/ **Understanding Liquid Biopsy** Biocept offers a simple blood test known as liquid biopsy that lets you establish approved, clinically actionable biomarker status for more personalized, responsive treatment. When cancer metastasizes or spreads, it sheds tumor cells and cancer-causing DNA fragments into the blood.

Biocept has developed the most advanced method for detecting and assessing tumor cells and cancer-causing DNA fragments in the blood.
You get the same information you would get from a tissue sample—from a simple blood test.
Biocept uses blood. You are informed about what's happening with your patients' cancer NOW—not weeks, months, or years ago.

When should you consider a liquid biopsy?

- **Not enough tissue was obtained from an initial biopsy.**
- **Cancer starts in a place that is difficult to access for surgical biopsy.**
- **Cancer recurs after the initial tumor tissue has been removed.**
- **The patient is not responding to treatment as expected.**
- **The patient wishes to avoid additional biopsies.**

Research: Oncology Association of Naturopathic Physicians: Principles of Care Guidelines

Marsden, E., Nigh, G., Birdsall, S., Wright, H., & Traub, M. (2019). **Oncology Association of Naturopathic Physicians: Principles of Care Guidelines.** *Current Oncology,26*(1). doi:10.3747/co.26.4815

ABSTRACT
Patient use of integrative oncology (the inclusion of nonconventional treatments alongside the conventional standard of care) continues to grow, with some studies showing its use in cancer patients to be as high as 91%.

Naturopathic physicians are primary care providers who use integrative therapies to deliver patient-centred care. The Oncology Association of Naturopathic Physicians (oncanp) was formed in 2004 as a specialty association for naturopathic physicians providing integrative cancer care (nd oncs). Currently, the membership encompasses more than 400 naturopathic physicians and students, 115 of whom are board-certified Fellows of the American Board of Naturopathic Oncology.

In 2016, oncanp established a committee comprising recognized experts in the field of naturopathic oncology to develop a Principles of Care (poc) guideline. The committee first undertook a review of existing standard-of-care and best-practice guidelines in the field of oncology and then adapted those concepts into a draft document. The draft document was then reviewed by naturopathic physicians, medical and radiation oncologists, naturopathic policy experts, and finally the oncanp membership at large.

The poc document presented here provides clear guidelines for nd oncs on how best to deliver patient-centred care in the areas of assessment, treatment planning, care management, interprofessional collaboration, and survivorship care.

This naturopathic oncology poc document can be a valuable resource for nd oncs and other oncology care providers to further an understanding of the naturopathic and integrative oncology care model and its potential for collaboration.

Research: Biomarkers Coagulation Assays in the Prognosis of Colorectal Cancer - Can coagulation assays serve as prognostic factors in patients with colorectal cancer?

A retrospective study finds that D-dimer and INR correlate significantly with tumor markers and disease stage in patients with colorectal cancer.

Reference

Kilic, L., Yildiz, I., Sen, F. K., Erdem, M. G., Serilmez, M., Keskin, S., . . . Tas, F. (2015). **D-dimer and international normalized ratio (INR) are correlated with tumor markers and disease stage in colorectal cancer patients.** *Cancer Biomarkers,15*(4), 405-411. doi:10.3233/cbm-150477

Overview

The aim of this study was to **evaluate the prognostic significance of coagulation tests and clarify their relationship with tumor markers and other clinical variables in colorectal cancer.**

Retrospective cohort study.

Results:

This study compared 94 patients with histologically proven colorectal cancer to healthy controls. All coagulation tests, including D-dimer, fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ration (INR), and **platelet counts, were significantly different between the patient group and control group** ($p < .001$ for all variables except PT).

Metastatic disease correlated with elevated INR (median values 1.08 vs 1.1; $p = .03$), and stage III patients had **higher D-dimer levels** than stage II patients (324 IU/mL vs 589 IU/mL; $p = .03$). Comparison with tumor markers showed that **high CA 19-9 (tumor marker) levels correlated with higher INR and that high CEA (tumor marker) levels correlated with elevated D-dimer**.

Coagulation tests did not correlate with overall survival. Analysis of factors including D-dimer, CEA, CA 19-9, state of metastasis, and age found **only CEA to be an independent prognostic factor for overall survival.**

Although coagulation assays did not predict overall survival in the current study, they did show a statistically significant correlation with tumor markers and disease stage. The authors of the study suggest that D-dimer might be an even more reliable prognostic factor than CEA or CA 19-9 in the preoperative setting for patients with colorectal cancer.

Research: Dietary iron intake and Breast Cancer Risk: Modulation by an Antioxidant Supplementation

Diallo, A., Deschasaux, M., Partula, V., Latino-Martel, P., Srour, B., Hercberg, S., . . . Touvier, M. (2016). **Dietary iron intake and breast cancer risk: Modulation by an antioxidant supplementation**. *Oncotarget,7*(48). doi:10.18632/oncotarget.12592

Experimental results suggested that iron-induced lipid peroxidation may explain the direct associations observed between red/processed meat intakes and colorectal and breast cancer risk.

However, epidemiological evidence is lacking. Thus, we **investigated the association between dietary iron intake and breast cancer risk, and its potential modulation by an antioxidant supplementation and lipid intake.**

This prospective study included **4646 women** from the SU.VI.MAX trial (daily low dose antioxidants vs.

placebo). 188 incident breast cancers were diagnosed (median follow-up=12.6y). Dietary iron intake was assessed using repeated 24h dietary records. Multivariable Cox proportional hazards models were computed.

Dietary iron intake was associated with an increased breast cancer risk (HRT3vs.T1=1.67 (1.02-2.71), P-trend=0.04).

This association was observed in the placebo group (HRT3vs. T1=2.80 (1.42-5.54), P-trend=0.003), **but not in the antioxidant-supplemented group** (P-trend=0.7, P-interaction=0.1).

Besides, in the placebo group, the increased breast cancer risk associated with dietary iron intake was more specifically observed in women with higher lipid intake (P-trend=0.046).

These findings suggest that dietary iron intake may be associated with an increased breast cancer risk, especially in women who did not receive antioxidants during the trial and who consumed more lipids. This supports the experimental results **suggesting that breast cancer risk may be increased by iron-induced lipid peroxidation.**

Research: TGFβ Controls Ovarian Cancer Cell Proliferation

Alsina-Sanchís, E., Figueras, A., Lahiguera, A., Gil-Martín, M., Pardo, B., Piulats, J., . . . Viñals, F. (2017). **TGFβ Controls Ovarian Cancer Cell Proliferation**. *International Journal of Molecular Sciences,18*(8), 1658. doi:10.3390/ijms18081658

There have been no major improvements in the overall survival of ovarian cancer patients in recent decades. Even though more accurate surgery and more effective treatments are available, the mortality rate remains high. Given the differences in origin and the heterogeneity of these tumors, research to elucidate the signaling pathways involved is required. The Transforming Growth Factor (TGFβ) family controls different cellular responses in development and cell homeostasis. Disruption of TGFβ signaling has been implicated in many cancers, including ovarian cancer. **This article considers the involvement of TGFβ in ovarian cancer progression, and reviews the various mechanisms that enable the TGFβ signaling pathway to control ovarian cancer cell proliferation.** These mechanistic explanations support the therapeutic use of TGFβ inhibitors in ovarian cancer, which are currently in the early phases of development.

TGFβ signaling plays a role in ovarian physiology as well as **acting as a tumor promoter that controls proliferation in ovarian cancer.**

Recent work by our group has demonstrated that **TGFβ positively controls ovarian cancer proliferation through the control of insulin like growth factor 1 receptor (IGF1R) expression**

Botanicals
Astragalus
Salvia Dan Shen
Ganoderma
Gan Cao
Zizyphus

References:

1. Abdul-Majeed, S., Mohamed, N., & Soelaiman, I. (2012). **Effects of Tocotrienol and Lovastatin Combination on Osteoblast and Osteoclast Activity in Estrogen-Deficient Osteoporosis.** *Evidence-Based Complementary and Alternative Medicine,2012*, 1-9. doi:10.1155/2012/960742

2. Aggarwal, B. B., Sundaram, C., Prasad, S., & Kannappan, R. (2010). **Tocotrienols, the vitamin E of the 21st century: Its potential against cancer and other chronic diseases**. *Biochemical Pharmacology,80*(11), 1613-1631. doi:10.1016/j.bcp.2010.07.043

3. Aggarwal, V., Kashyap, D., Sak, K., Tuli, H., Jain, A., Chaudhary, A., . . . Yerer, M. (2019). **Molecular Mechanisms of Action of Tocotrienols in Cancer: Recent Trends and Advancements.** *International Journal of Molecular Sciences,20*(3), 656. doi:10.3390/ijms20030656

4. Alsina-Sanchís, E., Figueras, A., Lahiguera, A., Gil-Martín, M., Pardo, B., Piulats, J., . . . Viñals, F. (2017). **TGFβ Controls Ovarian Cancer Cell Proliferation**. *International Journal of Molecular Sciences,18*(8), 1658. doi:10.3390/ijms18081658

5. Chin, K., & Tay, S. (2018). **A Review on the Relationship between Tocotrienol and Alzheimer Disease**. *Nutrients,10*(7), 881. doi:10.3390/nu10070881

6. Diallo, A., Deschasaux, M., Partula, V., Latino-Martel, P., Srour, B., Hercberg, S., . . . Touvier, M. (2016). **Dietary iron intake and breast cancer risk: Modulation by an antioxidant supplementation**. *Oncotarget,7*(48). doi:10.18632/oncotarget.12592

7. Fontana, F., Raimondi, M., Marzagalli, M., Moretti, R. M., Marelli, M. M., & Limonta, P. (2019). **Tocotrienols and Cancer: From the State of the Art to Promising Novel Patents**. *Recent Patents on Anti-Cancer Drug Discovery,14*(1), 5-18. doi:10.2174/1574892814666190116111827

8. Jiang, Q. (2017). **Natural Forms of Vitamin E as Effective Agents for Cancer Prevention and Therapy.** *Advances in Nutrition: An International Review Journal,8*(6), 850-867. doi:10.3945/an.117.016329

9. Kilic, L., Yildiz, I., Sen, F. K., Erdem, M. G., Serilmez, M., Keskin, S., . . . Tas, F. (2015). **D-dimer and international normalized ratio (INR) are correlated with tumor markers and disease stage in colorectal cancer patients.** *Cancer Biomarkers,15*(4), 405-411. doi:10.3233/cbm-150477

10. Marsden, E., Nigh, G., Birdsall, S., Wright, H., & Traub, M. (2019). **Oncology Association of Naturopathic Physicians: Principles of Care Guidelines.** *Current Oncology,26*(1). doi:10.3747/co.26.4815

11. Meganathan, P., & Fu, J. (2016). **Biological Properties of Tocotrienols: Evidence in Human Studies.** *International Journal of Molecular Sciences,17*(11), 1682. doi:10.3390/ijms17111682

12. Melo, A., Monteiro, L., Lima, R. M., Oliveira, D. M., Cerqueira, M. D., & El-Bachá, R. S. (2011). **Oxidative Stress in Neurodegenerative Diseases: Mechanisms and Therapeutic Perspectives**. *Oxidative Medicine and Cellular Longevity,2011*, 1-14. doi:10.1155/2011/467180

13. Shen, C., Kaur, G., Wanders, D., Sharma, S., Tomison, M. D., Ramalingam, L., . . . Dufour, J. M. (2018). **Annatto-extracted tocotrienols improve glucose homeostasis and bone properties in high-fat diet-induced type 2 diabetic mice by decreasing the inflammatory response**. *Scientific Reports,8*(1). doi:10.1038/s41598-018-29063-9

Tocotrienols
Potential Against Cancer

OVERIEW

Dr. Nalini Chilkov, Founder

American Institute of Integrative Oncology
RESEARCH & EDUCATION®

www.AIIORE.com

TOCOTRIENOLS: The Vitamin E of the 21st Century
It's potential Against Cancer and Other Chronic Diseases

- **Found in Rice Bran and Palm Oil and Annatto Seeds**
- **Four Isomers: alpha beta, gamma, delta**
- **Most potent forms are delta and gamma**
- **Separate dosing away from tocopherols is required for optimum assimilation and utilization**
- **Tocopherols may interfere with actions of tocotrienols**
- **No known adverse effects when consumed as part of a normal diet**

Biochem. Pharm. 2010 Dec 1;80(11): 1613-31. BB Aggarwal et al

TABLE 1 Presence of tocotrienols in different plant sources

Source	TRF	α-TT (%)	γ-TT (%)	δ-TT (%)
Palm oil	738 mg/l	28	59	13
Annatto seeds	160 mg/100 g	–	10	90
Rice bran	585 mg/l	41	59	–
Wheat germ	26 mg/l	100	–	–

TRF: tocotrienol rich fraction; TT: tocotrienol.

J Cell Physiol. 2019;234:1147–1164.

Tocopherols and Tocotrienols

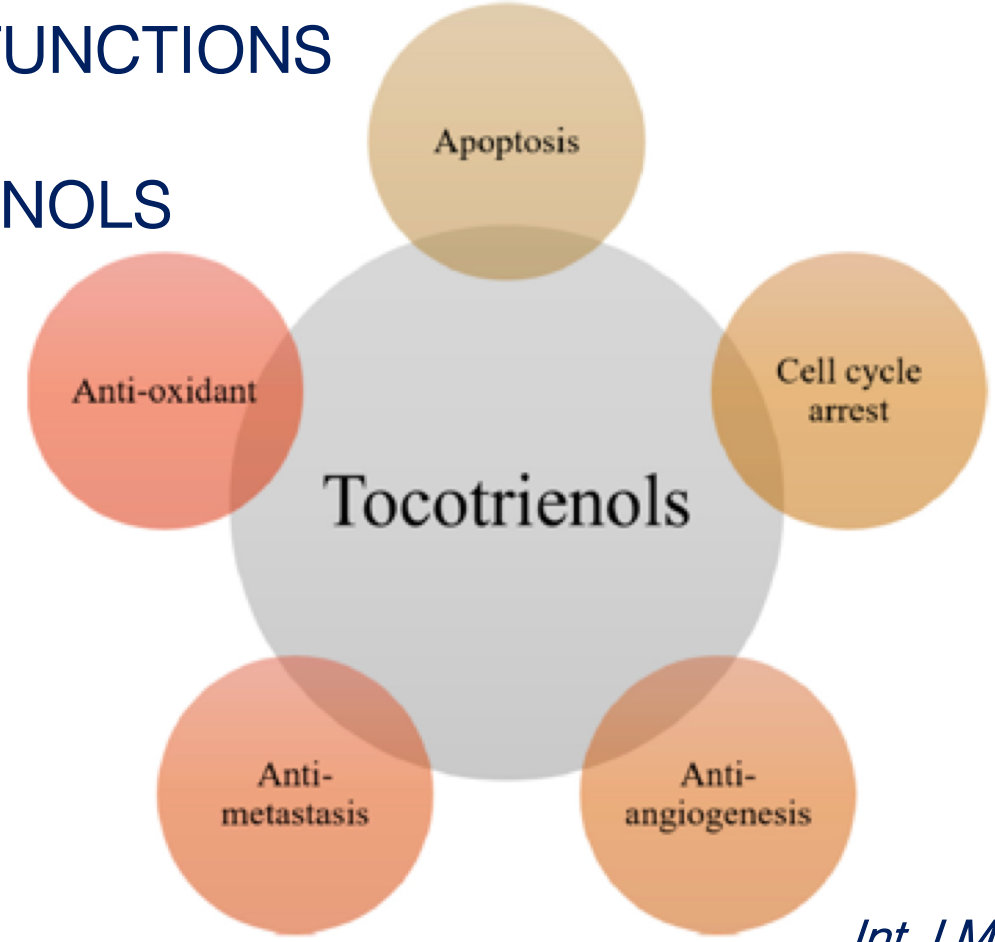
- Suppresses the growth of different malignancies, including those of breast, lung, ovary, prostate, liver, brain, colon, myeloma, and pancreas.

- Absorbed in the Small Intestine

- Bile salts are necessary for absorption

- Transport proteins in the liver are responsible for packaging these compounds into lipoproteins for transport to body tissues through the bloodstream

ANTI CANCER FUNCTIONS OF TOCOTRIENOLS



Int J Mol Sci 2019, 20, 656

APOPTOSIS

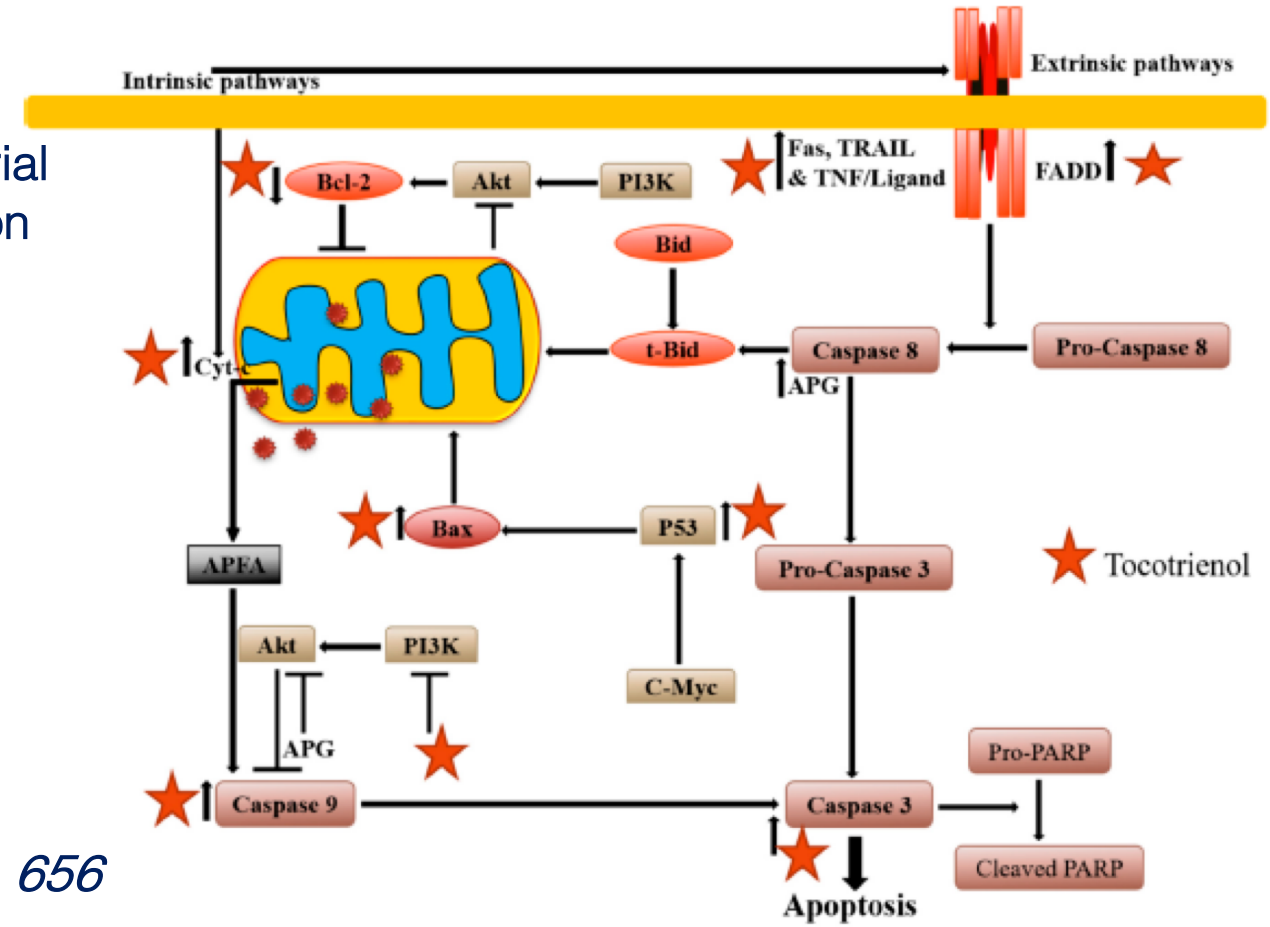
Activation of mitochondrial membrane depolarization

Cytochrome C complex release

Activation of caspase cascade

Modulation of Bcl-2/BAX expression

Int J Mol Sci 2019, 20, 656

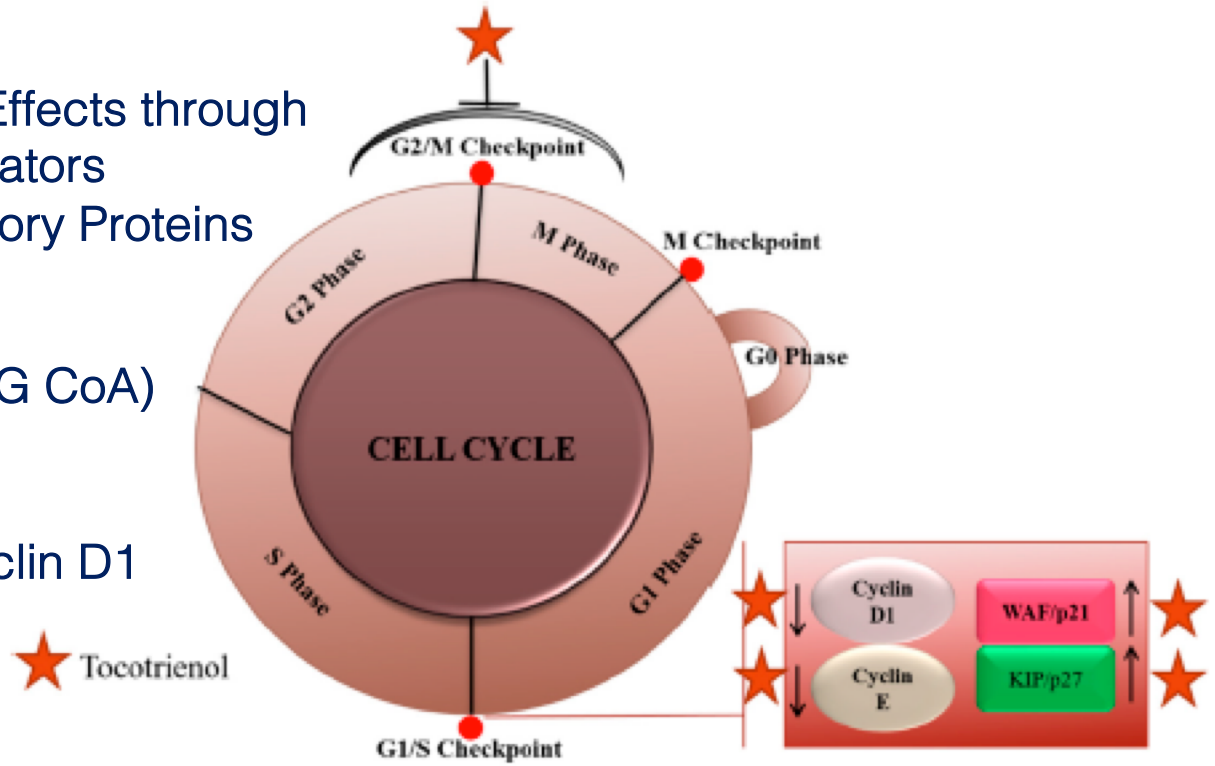


CELL CYCLE ARREST | Inhibition of Proliferation

Growth Inhibitory Effects through
CheckPoint Regulators
Cell Cycle Regulatory Proteins

Down Regulates
Coenzyme A. (HMG CoA)
reductase

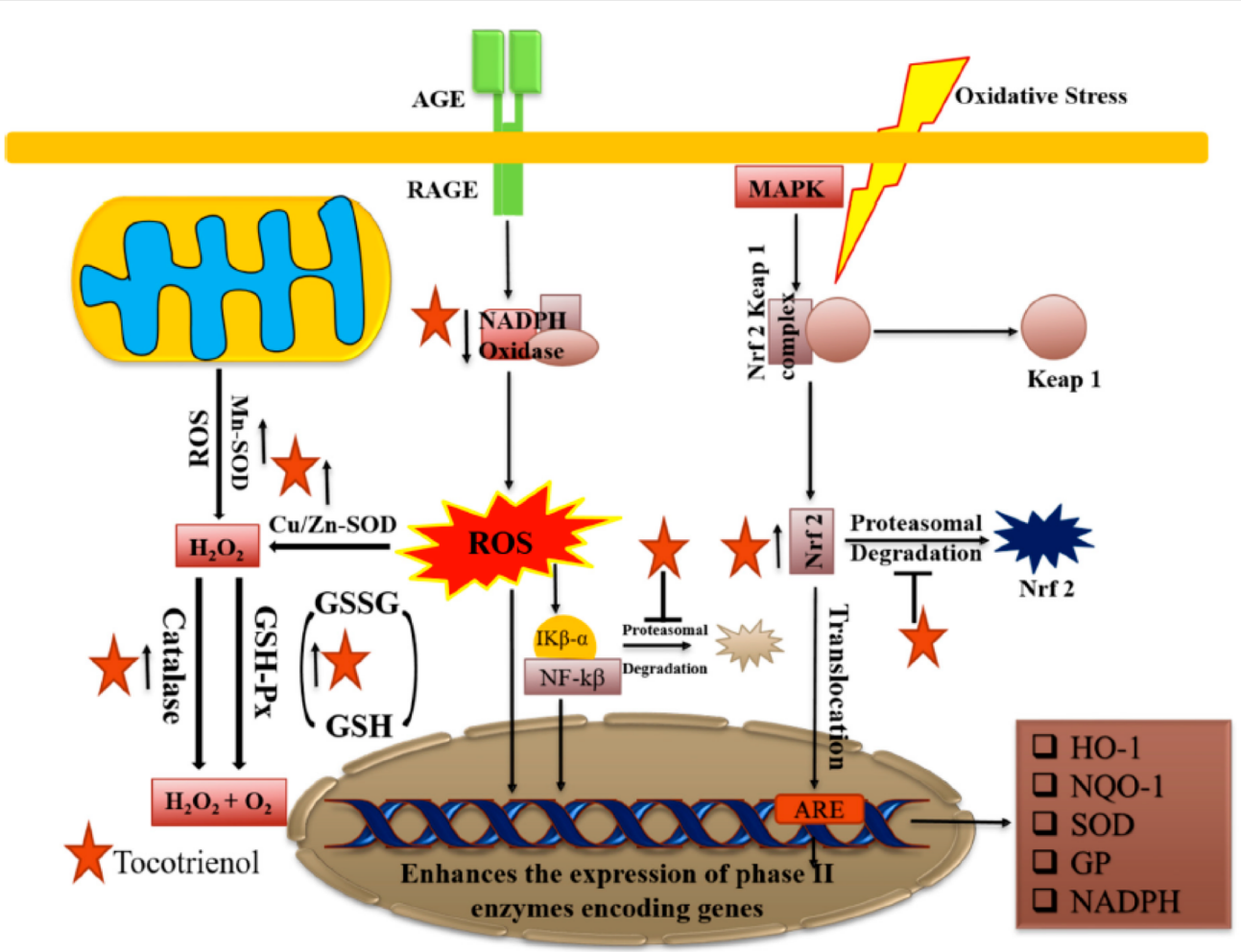
Reduces Ki67, Cyclin D1



Int J Mol Sci 2019, 20, 656

ANTI-OXIDANT

- Increase expression of antioxidant enzymes GSH, SOD, Catalase
- Block NADPH oxidase complex
- Increase nuclear translocation of Nrf2



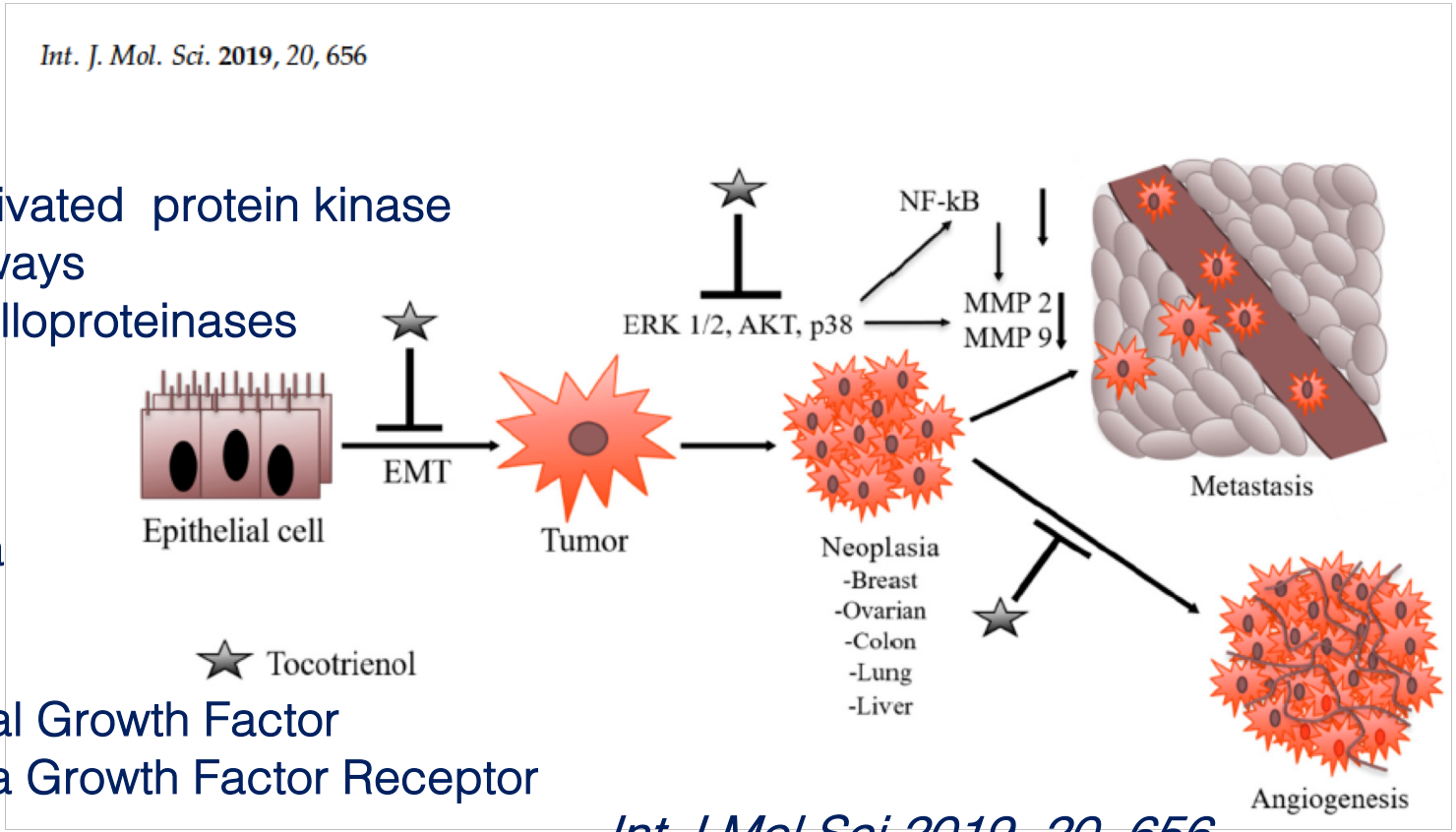
Int J Mol Sci 2019, 20, 656

METASTASIS and ANGIOGENESIS

Regulate
- MAPK Mitogen Activated protein kinase
- Akt signaling pathways
- MMPs. Matrix Metalloproteinases

Reduce activity of
- HIF-1a Hypoxia Inducible Factor-1a

- VEGF-VEGFR Vascular Endothelial Growth Factor Vascular Endothelia Growth Factor Receptor



Int J Mol Sci 2019, 20, 656

ANTI-INFLAMMATORY

Inhibit

- NFkB
- STAT3
- Src kinase
- Janus kinase (JAK)-1
- Akt –mTOR Activation
- COX-2
- PGE2
- IL-1b
- HIF-1a

TARGET CANCER STEM CELLS

Cancer Stem Cell is a tumor cell that

- has the capacity for self-renewal
- the ability to generate all heterogeneous tumor cell lineages,
- gives rise to the bulk of the tumor mass
- recapitulates continuous tumor growth
- possesses high invasive behavior
- expresses specific surface markers

- plays a major role in the development of resistance to standard cancer therapies
- contribute to disease relapse after an initial response

SYNERGISTIC ANTICANCER PROPERTIES OF TTs WITH STANDARD TREATMENTS OR NATURAL COMPOUNDS

Compound	Tumor cell types	Effects
Anticancer drugs		
Cisplatin	Nonsmall lung cancer cells	↓Cell viability, migration and invasiveness
Capecitabine	Gastric cancer cells, colorectal cancer cells	↓Cell proliferation, cell cycle-related proteins, NFκB, VEGF, and MMP-9 expression
Erlotinib, Gefitinib	Malignant mammary epithelial cells	↓ErbB2–4 expression, Akt and STAT pathways. Overcoming resistance to TKIs, apoptosis induction
Celecoxib	Malignant mammary epithelial cells	↓Tumor cell growth, Akt/NFκB pathway, COX-2 ErbB2–4 and PGE2 expression
Natural compounds		
Sesamin	Mammary tumor cells	↓Cell proliferation, cell cycle progression, ErbB receptor activation, MAPK, PI3K/Akt, JAK/STAT, NFκB pathways ↑TT bioavailability
EGCG	Breast cancer cells	↓Cell proliferation. ↑Nrf2, NQO1 expression (antioxidant activity)
Resveratrol	Breast cancer cells	↓Cell proliferation, HMG-CoA reductase expression
Ferulic acid	Prostate cancer cells, breast cancer cells, pancreatic cancer cells	↓Telomerase activity, TERT expression ↑TT intracellular levels
6-gingerol	Colorectal cancer cells	↑Apoptosis
Oridonin	Mammary cancer cells	↑Autophagy

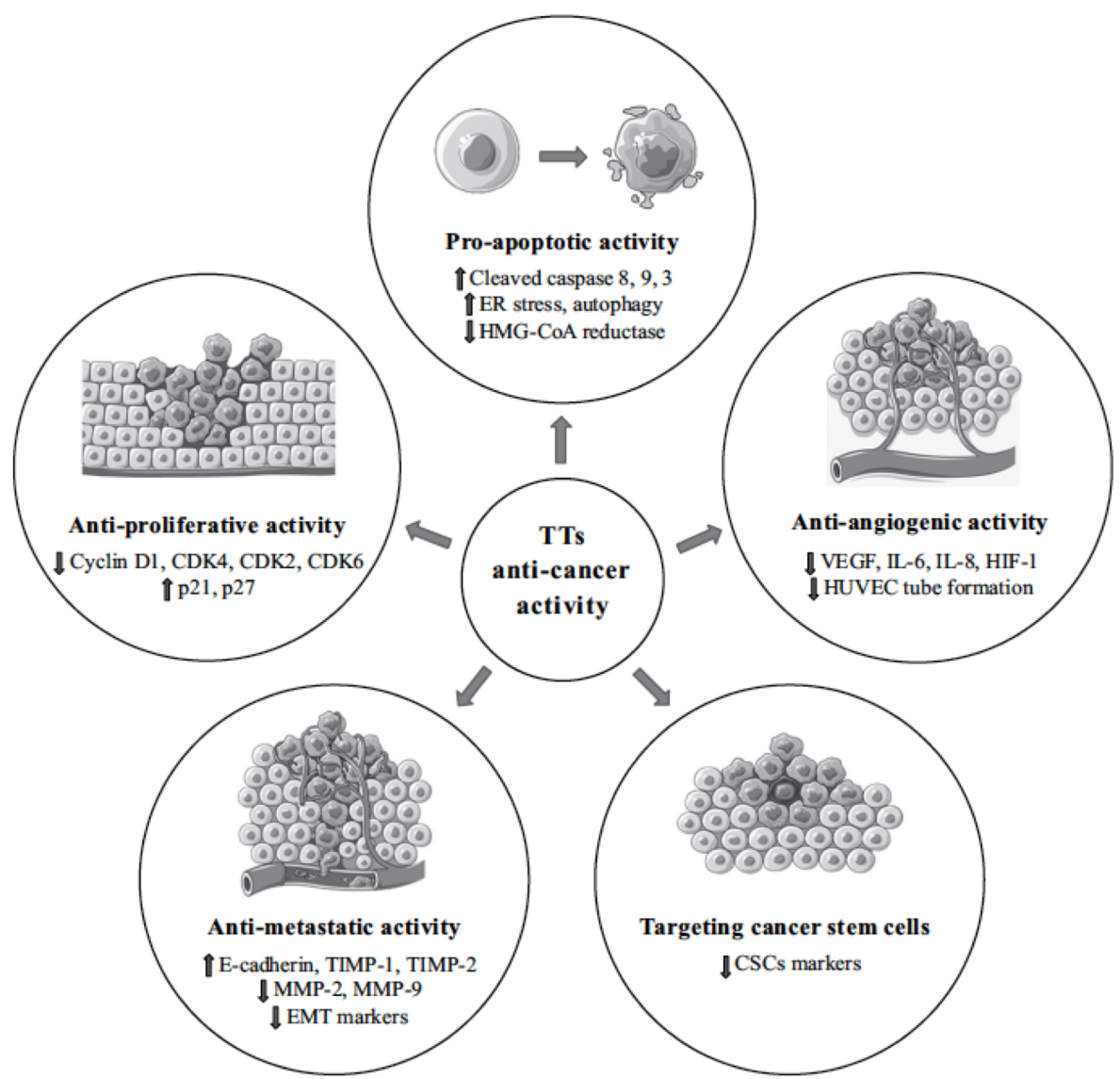
J Cell Physiol. 2019;234:1147–1164.

Tumor Type	Current Studies
Breast cancer	• Two *in vivo* studies demonstrating the anti-proliferative and anti-angiogenic effects of redox-silent TT oxazine derivatives • One clinical trial aimed at investigating the TTs adjuvant potentials when given in combination with tamoxifen
Colon cancer	• One *in vivo* study showing the synergistic anti-cancer effects of TTs and capecitabine • Two *in vivo* studies demonstrating the anti-angiogenic properties of TTs
Gastric cancer	• One *in vivo* study showing the synergistic anti-cancer effects of TTs and capecitabine
Lung cancer	• One *in vivo* study showing the synergistic anti-cancer effects of TTs and lovastatin
Pancreatic cancer	• One *in vivo* study demonstrating the pro-apoptotic properties of TTs • One *in vivo* study demonstrating the anti-invasive properties of TTs • One clinical trial conducted to test TTs efficacy and safety
Prostate cancer	• Two *in vivo* studies demonstrating the anti-proliferative properties of TTs • Two *in vivo* studies demonstrating the specific CSCs-targeting ability of TTs
Skin cancer	• One *in vivo* study demonstrating the pro-apoptotic properties of TTs • One *in vivo* study demonstrating the specific CSCs-targeting ability of TTs • One *in vivo* study demonstrating the chemopreventive properties of TTs • One *in vivo* study demonstrating the anti-cancer effects of transferrin-bearing vesicles containing TTs
Liver cancer	• One *in vivo* study demonstrating the anti-proliferative and anti-angiogenic effects of TTs

SUMMARY

- Anti-cancer
- Neuroprotective,
- Anti-inflammatory
- Anti-oxidant
- Cholesterol lowering
- Immunomodulatory
- Bone remodeling
- Glycemic control

Recent Patents on Anti-Cancer Drug Discovery, 2019, 14, 5-18



Daily Recommended Dose (Barrie Tan PhD)

Typical dose: Cancer Prevention and Support 600-900mg/day

- **Clinical studies determined that the optimal dose of tocotrienol for cholesterol and triglyceride reduction is 100mg/day**
- **The safe dose of various tocotrienols for human consumption is estimated to be 200-1,000mg/day.**
- **The supplement is best taken with a meal to increase absorption in the gut**
- **Due to possible interference, it is recommended for tocotrienols to be taken approximately six hours apart from tocopherol-containing supplements.**

PRIMARY REFERENCES | Cancer

Qing Jiang **Natural Forms of Vitamin E as Effective Agents for Cancer Prevention and Therapy**

Adv Nutr 2017;8:850–67; doi: https://doi.org/10.3945/an.117.016329.

Fabrizio Fontana, et al **Tocotrienols and Cancer: From the State of the Art to Promising Novel Patents.**

Recent Patents on Anti-Cancer Drug Discovery, 2019, 14, 5-18

Vaishali Aggarwal et al

Molecular Mechanisms of Action of Tocotrienols in Cancer: Recent Trends and Advancements

Int J Mol Sci 2019, 20, 656

Bharat B. Aggarwal, et al, **Tocotrienols, the Vitamin E of the 21st Century: It's Potential Against Cancer and Other Chronic Diseases**

Biochem Pharmacol. 2010 December 1; 80(11): 1613–1631. doi:10.1016/j.bcp.2010.07.043

Puvaneswari Meganathan and Ju-Yen Fu

Biological Properties of Tocotrienols: Evidence in Human Studies

Int. J. Mol. Sci. 2016, 17, 1682; doi:10.3390/ijms17111682

Barrie Tan, PhD

Vitamin E: Tocotrienols The Science Behind Tocotrienols

Tocotrienols_Science_White_Paper-1.12-EN-ALL 1

REFERNCES: OSTEPOROSIS | GLYCEMIC CONTROL

Saif Abdul-Majeed, et al
Effects of Tocotrienol and Lovastatin Combination on Osteoblast and Osteoclast Activity in Estrogen-Deficient Osteoporosis
Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine Volume 2012, Article ID 960742, 9 pages doi:10.1155/2012/960742

Chwan Li-Shen et al
Annatto Extracted Tocotrienols improve glucose homeostasis and bone properties in high fat diet induced Type 2 diabetic mice by decreasing inflammatory response
Nature Scientific Reports (2018) 8:11377

Tocotrienols exhibit stronger antioxidant and anti-inflammatory activities compared to alpha-tocopherol

NEURODEGENERATIVE DISEASE

Nutrients. 2018 Jul; 10(7): 881. PMID: 29987193

A Review on the Relationship between Tocotrienol and Alzheimer Disease

Kok-Yong Chin, Shu Shen Tay

Oxid Med Cell Longev. 2011;2011:467180. **Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives.** Melo A[1], Monteiro L, Lima RM, et al

J Am Coll Nutr. 2010 June ; 29(3 Suppl): 314S. **Palm Oil–Derived Natural Vitamin E α-Tocotrienol in Brain Health and Disease** Chandan K. Sen, PhD, et al

THIS INFORMATION IS PROVIDED FOR THE USE OF PHYSICIANS AND OTHER LICENSED HEALTH CARE PRACTITIONERS ONLY. THIS INFORMATION IS INTENDED FOR PHYSICIANS AND OTHER LICENSED HEALTH CARE PROVIDERS TO USE AS A BASIS FOR DETERMINING WHETHER OR NOT TO RECOMMEND SUPPORT OR PRODUCTS TO THEIR PATIENTS. THIS MEDICAL AND SCIENTIFIC INFORMATION IS NOT FOR USE BY CONSUMERS. THE DIETARY SUPPLEMENTS ARE NOT INTENDED FOR USE BY CONSUMERS AS A MEANS TO CURE, TREAT, PREVENT, DIAGNOSE, OR MITIGATE ANY DISEASE OR OTHER MEDICAL CONDITION.

ADVANCED colorectal cancer

LabCorp

Patient Report

[redacted]

Patient Details
[redacted]
Age(y/m/d): 069/01/08
Gender: F [redacted]

Specimen Details
Date collected: 03/18/2019 0936 Local
Date received: 03/18/2019
Date entered: 03/18/2019
Date reported: 03/25/2019 1608 ET

Physician Details
Ordering: [redacted]
Referring:
ID:
NPI: [redacted]

General Comments & Additional Information
Alternate Control Number: [redacted]
Total Volume: Not Provided
Alternate Patient ID: [redacted]
Fasting: Yes

Ordered Items
TSH+T4F+T3Free; CBC With Differential/Platelet; CMP14; Urinalysis, Complete; COMT Genetic Test; APOE Alzheimer's Risk; Vitamin B12 and Folate; Trans. Growth Fact. beta 1*; CEA; Galectin-3; IGF-1; Vitamin A, Serum; D-Dimer; C-Reactive Protein, Cardiac; LDH; GGT; Iron; Sedimentation Rate-Westergren; Ceruloplasmin; Copper, Serum; Fibrinogen Activity; Zinc, Plasma or Serum; Insulin; Ferritin, Serum; Selenium, Serum/Plasma; Venipuncture

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
TSH+T4F+T3Free					
TSH	1.800		uIU/mL	0.450 - 4.500	01
Triiodothyronine (T3), Free	**1.6**	**Low**	pg/mL	2.0 - 4.4	01
T4,Free(Direct)	1.34		ng/dL	0.82 - 1.77	01
CBC With Differential/Platelet					
WBC	6.5		x10E3/uL	3.4 - 10.8	01
RBC	4.29		x10E6/uL	3.77 - 5.28	01
Hemoglobin	**11.0**	**Low**	g/dL	11.1 - 15.9	01
Hematocrit	35.3		%	34.0 - 46.6	01
MCV	82		fL	79 - 97	01
MCH	**25.6**	**Low**	pg	26.6 - 33.0	01
MCHC	**31.2**	**Low**	g/dL	31.5 - 35.7	01
RDW	**17.2**	**High**	%	12.3 - 15.4	01
Platelets	**548**	**High**	x10E3/uL	150 - 379	01
Neutrophils	64		%	Not Estab.	01
Lymphs	20		%	Not Estab.	01
Monocytes	12		%	Not Estab.	01
Eos	3		%	Not Estab.	01
Basos	1		%	Not Estab.	01
Neutrophils (Absolute)	4.1		x10E3/uL	1.4 - 7.0	01
Lymphs (Absolute)	1.3		x10E3/uL	0.7 - 3.1	01
Monocytes(Absolute)	0.8		x10E3/uL	0.1 - 0.9	01
Eos (Absolute)	0.2		x10E3/uL	0.0 - 0.4	01
Baso (Absolute)	0.1		x10E3/uL	0.0 - 0.2	01
Immature Granulocytes	0		%	Not Estab.	01
Immature Grans (Abs)	0.0		x10E3/uL	0.0 - 0.1	01

NLR 3.2

[redacted]

Specimen ID [redacted]
Date collected: 03/18/2019 0936 Local

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
CMP14					
Chemistries					01
Glucose	80		mg/dL	65 - 99	01
BUN	14		mg/dL	8 - 27	01
Creatinine	**0.47**	**Low**	mg/dL	0.57 - 1.00	01
eGFR If NonAfricn Am	101		mL/min/1.73	>59	
eGFR If Africn Am	117		mL/min/1.73	>59	
BUN/Creatinine Ratio	**30**	**High**		12 - 28	
Sodium	138		mmol/L	134 - 144	01
Potassium	4.3		mmol/L	3.5 - 5.2	01
Chloride	96		mmol/L	96 - 106	01
Carbon Dioxide, Total	23		mmol/L	20 - 29	01
Calcium	9.6		mg/dL	8.7 - 10.3	01
Protein, Total	7.1		g/dL	6.0 - 8.5	01
Albumin	4.3		g/dL	3.6 - 4.8	01
Globulin, Total	2.8		g/dL	1.5 - 4.5	
A/G Ratio	1.5			1.2 - 2.2	
Bilirubin, Total	0.3		mg/dL	0.0 - 1.2	01
Alkaline Phosphatase	86		IU/L	39 - 117	01
AST (SGOT)	36		IU/L	0 - 40	01
ALT (SGPT)	18		IU/L	0 - 32	01
Urinalysis, Complete					
Urinalysis Gross Exam					01
Specific Gravity	**>=1.030**	**Abnormal**		1.005 - 1.030	01
pH	5.5			5.0 - 7.5	01
Urine-Color	Yellow			Yellow	01
Appearance	**Cloudy**	**Abnormal**		Clear	01
WBC Esterase	**2+**	**Abnormal**		Negative	01
Protein	**1+**	**Abnormal**		Negative/Trace	01
Glucose	Negative			Negative	01
Ketones	**2+**	**Abnormal**		Negative	01
Occult Blood	Negative			Negative	01
Bilirubin	Negative			Negative	01
Urobilinogen,Semi-Qn	0.2		mg/dL	0.2 - 1.0	01
Nitrite, Urine	Negative			Negative	01
Microscopic Examination	See below:				01
WBC	**>30**	**Abnormal**	/hpf	0 - 5	01
RBC	**11-30**	**Abnormal**	/hpf	0 - 2	01
Epithelial Cells (non renal)	0-10		/hpf	0 - 10	01
Mucus Threads	Present			Not Estab.	01
Bacteria	Few			None seen/Few	01

[redacted]

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
COMT Genetic Test					
Predicted Metabolic Activity	Will Follow				02
Interpretation	Will Follow				02
Additional Interpretation	Will Follow				02
COMT Genetic Test Information	Will Follow				02
Comments	Will Follow				02
References	Will Follow				02

APOE Alzheimer's Risk

Methodology: 03

Patient DNA is assayed for the APOE genotype by PCR amplification of a specific region in exon 4 of the APOE gene followed by digestion with restriction enzyme Hha I and separation of fragments by polyacrylamide gel electrophoresis. This approach allows the APOE E2, E3, and E4 alleles to be distinguished. Analytical sensitivity and specificity are >99.5%. Individuals are interpreted as having one of the following genotypes: E2/E2, E3/E3, E4/E4, E2/E3, E2/E4, E3/E4.

APO E Genotyping Result: 03

E3/E4 (one copy of the APOE4 variant)

Interpretation: 03

Positive for one copy of the APOE4 variant that is associated with increased risk for late onset Alzheimer's disease (AD). Therefore the lifetime risk for AD is increased in this individual. However, most individuals with one copy of the APOE4 variant do not develop AD.

RECOMMENDATIONS
Genetic counseling is recommended.
Due to the lack of measures to prevent the development of AD, the ACMG/NSGC guidelines do not recommend presymptomatic testing, but if it is performed, guidelines are provided (Goldman JS et al. 2011). The APOE Genotyping: Alzheimer's Risk test is not recommended for children.

NOTE: This is not a diagnostic test. Results should be interpreted along with clinical findings and other data. This test evaluates only for the APOE genotype and cannot detect genetic abnormalities elsewhere in the genome. It should be realized that there are possible sources of error including sample misidentification, rare technical errors, trace contamination of PCR reactions, and rare genetic variants that may interfere with analysis.

For inquiries or genetic consultation, please call Esoterix at 1-800-444-9111.

Comment: 03

INFORMATION ABOUT THE APOE GENOTYPE AND ALZHEIMER'S DISEASE

[redacted]

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB

Alzheimer's disease (AD) is the most common form of dementia in the elderly and currently affects more than 5 million Americans. It is a progressive neurodegenerative disorder with brain findings of plaques and neurofibrillary tangles containing beta-amyloid and tau protein respectively.

The predominant form of AD is late onset (age > 60-65), which can be familial (15-20%) or sporadic. The APOE4 variant increases the risk for late onset AD and may contribute to the pathology of the disease. This risk is increased by approximately 2 to 3-fold for individuals with one copy of the APOE4 variant and by approximately 10 to15-fold for individuals with two copies of this variant (E4/E4 genotype). The APOE2 variant has some protective effect against development of late onset AD. The lifetime risk for late onset AD is approximately 10-12% in the general population, though it is higher in women than men and doubles when there is a first degree relative with this disorder. The lifetime risk is approximately 9% for individuals negative for APOE4, and for individuals with E4/E4 may be as high as 25% for males and 45% for females. Among patients with late onset AD, the presence of APOE4 may lead to earlier development of symptoms.

However, APOE4 is neither necessary nor sufficient for the development of AD. Approximately 30-50% of patients with late onset AD do not have an APOE4 allele.

APOE4 is common, with 25% of the general population having one copy and 1% having two copies of this variant. Among patients with late onset AD, 50-70% are positive for APOE4.

The development of late onset AD is influenced by many factors other than APOE4 including age, gender, family history, level of education and history of head trauma. Midlife cardiovascular risk factors in individuals with APOE4 also increase risk for cognitive decline. A number of genetic influences in addition to APOE4 have also been reported and are under investigation.

This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

REFERENCES

Altmann A et al. Sex modifies the APOE-related risk of developing Alzheimer disease. Annal Neurol 2014;75(4):563-573

[redacted]

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB

Bird TD. Alzheimer Disease Overview. GeneReviews (internet). Pagon RA et al., editors. Seattle WA: University of Washington, Seattle, WA. Last revised 2014.

Goldman JS et al. Genetic counseling and testing for Alzheimer disease: Joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. Genet in Med 2011;13(6)597-605.

Schipper HM. Apolipoprotein E: Implications for AD neurobiology, epidemiology and risk assessment. Neurobiology of Aging 2011;32:778-790

Vitamin B12 and Folate

Vitamin B12	**>1999**	**High**	pg/mL	232 - 1245	01
Folate (Folic Acid), Serum	9.1		ng/mL	>3.0	01

Note: 01

A serum folate concentration of less than 3.1 ng/mL is considered to represent clinical deficiency.

Trans. Growth Fact. beta 1*	4372		pg/mL	867 - 6662	04

The result is reported in pg/mL. The assay range is approximately 98 to 400,000. The reference range for a healthy population is 867-6662. However it should be noted that these ranges are obtained from a limited population of apparently healthy adults and are not diagnostic thresholds.
*This test was developed and its performance characteristics determined by Viracor Eurofins. It has not been cleared or approved by the U.S. Food and Drug Administration.

CEA	**880.3**	**High**	ng/mL	0.0 - 4.7	01
			Nonsmokers	**<3.9**	
			Smokers	**<5.6**	

Roche Diagnostics Electrochemiluminescence Immunoassay (ECLIA)
Values obtained with different assay methods or kits cannot be used interchangeably. Results cannot be interpreted as absolute evidence of the presence or absence of malignant disease.

Galectin-3	17.5		ng/mL	<22.2	05

Reference range of <22.2 ng/mL applies to population without known heart disease.
Galectin-3 is NOT a marker of cardiac distress or decompensation.
Galectin-3 is a marker of fibrosis and adverse remodeling resulting in a more progressive form of chronic heart failure. The BGM Galectin-3 assay results should be

[redacted]

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB

interpreted in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure.
* Galectin-3 levels less than or equal to 17.8 ng/mL - LOWER risk of adverse outcomes including mortality or hospitalization.
* Galectin-3 levels greater than 17.8 ng/mL - HIGHER risk of adverse outcomes including mortality or hospitalization.
* Galectin-3 levels between 17.8 ng/mL and 25.9 ng/mL should be interpreted with caution because these values lie within the reference range.

Additional Considerations
- Approximately 30% of NYHA class II/III outpatient population were found to have elevated galectin-3 levels (>17.8 ng/mL). [1]
- Elevated galectin-3 is found in similar percentages of patients with systolic dysfunction and preserved ejection fraction. [1]
- Galectin-3 shows modest correlations with clinical variables, including Age (slightly higher levels in older subjects), Gender (women have slightly higher values) certain co-morbidities (diabetes and atrial fibrillation), and NYHA classification. [2]
- Once elevated galectin-3 levels are generally stable over time. [2]
- Drugs effective in the management of patients with heart failure often fail to reduce levels of galectin-3 and galectin-3 levels should not be used to guide therapy.
* Galectin-3 testing was cleared by the FDA for use in patients with chronic heart failure only. [1]

(1) BG Galectin-3 Product Label
(2) de Boer, R; Lok, D; Jaarsma, T, et al. Value of Plasma Galectin-3 levels in Heart Failure with Reduced and Preserved Ejection Fraction. Ann Medicine, 2011; 43(1):60-68.

IGF-1

Insulin-Like Growth Factor I	78		ng/mL	38 - 163	05

Vitamin A, Serum

Vitamin A	23.8		ug/dL	22.0 - 69.5	05

Reference intervals for vitamin A determined from LabCorp internal studies. Individuals with vitamin A less than 20 ug/dL are considered vitamin A deficient and those with serum concentrations less than 10 ug/dL are considered severely deficient.
This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration.

D-Dimer	**1.94**	**High**	mg/L FEU	0.00 - 0.49	01

According to the assay manufacturer's published package insert, a normal (<0.50 mg/L FEU) D-dimer result in conjunction with a non-high

[redacted]

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
clinical probability assessment, excludes deep vein thrombosis (DVT) and pulmonary embolism (PE) with high sensitivity. D-dimer values increase with age and this can make VTE exclusion of an older population difficult. To address this, the American College of Physicians, based on best available evidence and recent guidelines, recommends that clinicians use age-adjusted D-dimer thresholds in patients greater than 50 years of age with: a) a low probability of PE who do not meet all Pulmonary Embolism Rule Out Criteria, or b) in those with intermediate probability of PE. The formula for an age-adjusted D-dimer cut-off is "age/100". For example, a 60 year old patient would have an age-adjusted cut-off of 0.60 mg/L FEU and an 80 year old 0.80 mg/L FEU.					
C-Reactive Protein, Cardiac Results confirmed on dilution.	79.48	High	mg/L	0.00 - 3.00	01
			Relative Risk for Future Cardiovascular Event Low Average High	<1.00 1.00 - 3.00 >3.00	
LDH	217		IU/L	119 - 226	01
GGT	18		IU/L	0 - 60	01
Iron	25	Low	ug/dL	27 - 139	01
Sedimentation Rate-Westergren	42	High	mm/hr	0 - 40	01
Ceruloplasmin	46.8	High	mg/dL	19.0 - 39.0	05
Copper, Serum [A]	179	High	ug/dL Detection Limit = 5	72 - 166	05
Fibrinogen Activity	678	High	mg/dL	193 - 507	01
Zinc, Plasma or Serum [A]	64		ug/dL Detection Limit = 5	56 - 134	05
Insulin	1.7	Low	uIU/mL	2.6 - 24.9	01
Ferritin, Serum	70		ng/mL	15 - 150	01
Selenium, Serum/Plasma [A]	148		ug/L	91 - 198	05

Comments:

[A] This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug

[redacted]

Administration.

01	SO	LabCorp San Diego 13112 Evening Creek Dr So Ste 200, San Diego, CA 92128-4108	Dir: Jenny Galloway, MD
02	ES	Esoterix Inc 4301 Lost Hills Road, Calabasas Hills, CA 91301-5358	Dir: Samuel Pepkowitz, MD
03	UY	Esoterix Inc 8490 Upland Drive Ste 100, Englewood, CO 80112-7116	Dir: Brian F. Poirier, MD
04	NEWXW	Viracor Eurofins 1001 NW Technology Drive, Lees Summit, MO 64086-5603	Dir: Michelle Altrich, PhD
05	BN	LabCorp Burlington 1447 York Court, Burlington, NC 27215-3361	Dir: Sanjai Nagendra, MD

For inquiries, the physician may contact **Branch: 800-859-6046 Lab: 858-668-3700**

CURRENT ONCOLOGY
A Canadian Cancer Research Journal

ORIGINAL ARTICLE

Oncology Association of Naturopathic Physicians: Principles of Care Guidelines

E. Marsden ND BSc,* G. Nigh ND LAc,† S. Birdsall ND, H. Wright ND,‡ and M. Traub ND§

ABSTRACT

Patient use of integrative oncology (the inclusion of nonconventional treatments alongside the conventional standard of care) continues to grow, with some studies showing its use in cancer patients to be as high as 91%. Naturopathic physicians are primary care providers who use integrative therapies to deliver patient-centred care. The Oncology Association of Naturopathic Physicians (oncanp) was formed in 2004 as a specialty association for naturopathic physicians providing integrative cancer care (nd oncs). Currently, the membership encompasses more than 400 naturopathic physicians and students, 115 of whom are board-certified Fellows of the American Board of Naturopathic Oncology.

In 2016, oncanp established a committee comprising recognized experts in the field of naturopathic oncology to develop a Principles of Care (poc) guideline. The committee first undertook a review of existing standard-of-care and best-practice guidelines in the field of oncology and then adapted those concepts into a draft document. The draft document was then reviewed by naturopathic physicians, medical and radiation oncologists, naturopathic policy experts, and finally the oncanp membership at large.

The poc document presented here provides clear guidelines for nd oncs on how best to deliver patient-centred care in the areas of assessment, treatment planning, care management, interprofessional collaboration, and survivorship care. This naturopathic oncology poc document can be a valuable resource for nd oncs and other oncology care providers to further an understanding of the naturopathic and integrative oncology care model and its potential for collaboration.

Key Words Naturopathic medicine, integrative oncology, naturopathic oncology, principles of care

Curr Oncol. 2019 Feb;26(1):12-18 www.current-oncology.com

INTRODUCTION AND BACKGROUND

The Oncology Association of Naturopathic Physicians (oncanp) was formed in 2004 to advance the practice of naturopathic oncology, with the goal of improving survival and quality of life for patients with cancer. The profession of naturopathic oncology has grown: approximately 400 oncanp members are practicing in North America, 115 of whom are board-certified Fellows of the American Board of Naturopathic Oncology. That growth has been fueled by patient demand for integrative cancer treatment options and by an increase in published research documenting the safety and efficacy of natural and supportive therapies. Several studies and meta-analyses investigating the frequency of use of integrative therapies in cancer patients have shown that the prevalence of complementary and alternative medicine use in cancer patients ranges from 22% to 91%[1–5].

Naturopathic doctor oncology providers (nd oncs) play an important role in cancer care, seeking to educate, to help manage side effects of cancer treatment, to improve overall response, to help prevent recurrence, and to optimize health. To achieve positive patient outcomes, nd oncs use a wide range of natural therapeutics and supportive strategies based on clinical trials, long-standing traditional use, and patient preference. It is essential that patients receive guidance in the use of integrative therapeutic options from high-quality care providers.

The present Principles of Care document is not meant to be prescriptive nor to give providers instructive advice about therapeutic options. Instead, the intention is to ensure safe and effective care by giving clear guidance for the naturopathic management of oncology patients. The document summarizes the key elements that should be present in respect to

- patient assessment,
- an integrative oncology plan,
- naturopathic oncology treatments,

Correspondence to: Eric Marsden, A1–9131 Keele Street, Vaughan, Ontario L4K 0G7.
E-mail: eric.marsden@marsdencentre.com **DOI:** https://doi.org/10.3747/co.26.4815

- care coordination, and
- continuing care management.

The Principles of Care Guidelines can also serve as a means for other health care professionals and organizations to understand the naturopathic approach to patient care within the setting of a collaborative health care team.

This document was developed by oncanp's Principles of Care Committee, which consists of experts in the field of naturopathic oncology who practice in a range of clinical settings from hospitals to private clinics. The guidelines were developed after a review of existing standards and guidance documents within the fields of naturopathic medicine, oncology, and other medical specialties. The Principles of Care Committee sought commentary and feedback about the document from stakeholder groups internal to oncanp and external stakeholders in the naturopathic profession and conventional oncology. The document was then reviewed and approved by the Board of Directors of oncanp.

GUIDELINE

Comprehensive Assessment as a Patient-Centred Approach

This section outlines the elements of patient assessment that could be a part of naturopathic oncology care.

To conduct a comprehensive and timely assessment of the patient's needs for care, an nd onc will use a systematic approach that considers the whole individual, including physical, psycho-emotional, cultural, social, and spiritual dimensions. Pertinent findings will be entered into the patient's health record.

A complete and thorough assessment of the patient should be done at the initial consult, or as soon as possible afterward, and before development of a treatment plan. In situations in which a complete assessment of the patient's entire health history is not possible (for example, an acute patient presentation), providers should undertake an appropriate assessment and history so as to develop effective initial treatment and to formulate a care plan that includes a full assessment as soon as reasonably possible. Assessments should be repeated to monitor patient response to care.

These are the constituents of a patient assessment:

- An optimal health history that encompasses the following elements, recognizing that the ability to obtain certain information might be limited:
  - History and knowledge of current illness
  - Current and past conventional treatments and the patient's response
  - Prior complementary cancer treatment and response
  - Review of pertinent pathology, laboratory, and imaging studies
  - Relevant family health history
  - Allergies (medications, dietary, environmental)
  - Lifestyle choices, including diet, sleep habits, smoking, alcohol consumption, drug use, and social history
  - Environmental factors, including occupation and work environment
  - Comorbid conditions and, if relevant, their potential effect on disease trajectory and treatment
  - Identification of key members of the patient's health care team, including medical and radiation oncologists, surgeon, primary care provider, and so on
  - Consideration for the patient's values and attitudes with respect to the current health situation and how they might affect therapeutic choices

- A physical assessment that encompasses the following elements:
  - A physical examination that focuses on patient-reported symptoms, key systems, and common or known complications
  - A baseline performance status assessment using, preferably, although not exclusively, a validated tool (for example, Karnofsky performance status scale[6], Eastern Cooperative Oncology Group performance status[7], Lansky Play-Performance status[8], Palliative Performance Scale[9])
  - Height and weight and calculated body mass index
  - Vital signs

- Laboratory assessment
  - Conventional laboratory testing should be ordered (or recommended) in addition to any laboratory testing ordered by the patient's oncologist and should be used to inform diagnosis, response to treatment, and tolerability of treatment.
  - Nonstandard laboratory testing (tests that are considered experimental or not part of routine conventional oncology care and assessment) might also be used by the nd onc in the development of a whole-patient assessment. Such tests are not to be used alone to establish a diagnosis, determine prognosis, or decide on treatment.

- Symptom assessment
  - Assess and document the patient's symptom picture using medical terminology.
  - The use of validated, focused assessment tools for the oncology patient is encouraged (for example, the Edmonton Symptom Assessment System[10], Brief Pain Inventory[11], Patient-Reported Outcomes Measurement Information Systems–Fatigue Short Form[12], Generalized Anxiety Disorder 7-Item-Scale[13], the 9-item Patient Health Questionnaire[14]).
  - Symptom assessments should be repeated at appropriate frequencies so as to continually update the nd onc about the patient's symptom experience.

- Psychosocial, spiritual, and cultural assessment
  - Consider the impact and meaning of the illness for the patient, the family, and their support systems, with particular attention to current life circumstances and personal views on quality of life.
  - Consider the needs of the patient and family for information and their preferred or legal role in decision-making.

- Consider cultural, spiritual, and religious influences on the patient's view of the illness and how it affects therapeutic choices and personal ability to cope with the illness.
- Given the prevalence of undiagnosed depression in the cancer patient population, the ND onc should screen for depression and anxiety using a clinically validated assessment tool.

- Sexual health assessment
  - Consider the effect of changes in the patient's sexual health and the effects that such changes have on the patient's partner.
  - If relevant, explore the understandings on the part of the patient and the partner of possible changes to fertility status as a result of treatment.

Integrative Oncology Plan

The ND onc's comprehensive evaluation of each patient, combined with the provider's broad understanding of conventional and complementary treatment options, enables the development of a rational set of therapies appropriate to each patient. Specific recommendations might vary between practitioners, but all ND oncs should give attention to a common set of components in developing any program. In this subsection, we review key principles involved in developing a naturopathic oncology plan, including how the provider expects to involve other referring providers, social support networks, and the documentation of clinical decisions.

Coordinating Medical Care

Throughout the evaluation process, the ND onc comes to a full understanding of the health needs of the oncology patient. When referral of the patient for other services or evaluation is indicated, the ND onc initiates and coordinates the referral and acts to facilitate communication between all practitioners associated with the patient's care.

In North America, ND oncs practice within widely diverse licensure and regulatory environments. It is incumbent upon each ND onc to communicate both the scope and the limitations of the ND onc's clinical work with each patient as it pertains to the treatment program that is being recommended. Specific areas that might need to be addressed include, but are not limited to

- naturopathic oncology treatment,
- prescriptive authority,
- parenteral therapy authorization, and
- authority for ordering laboratory testing and diagnostic imaging.

Continuity of care through appropriate coordination is a critical aspect of high-quality patient care. The Care Coordination subsection provides greater detail about core principles for ensuring continuity of care.

Providing Therapeutic Options

A primary role of the ND onc is to assure that the patient makes fully informed decisions about treatment. That assurance includes providing full information about conventional oncology treatment and the inclusion of naturopathic care. Although no individual ND onc can reasonably know all relevant information about all treatment options, it is critical that the ND onc provide comprehensive information about the potential risks and benefits of the various strategies they are recommending and, where relevant, reasonable alternatives. In addition, ND oncs also enhance the patient's understanding of the risks and benefits of conventional therapies. To solidify that education, the ND onc should refer the patient to an oncologist for a complete review of their conventional treatment options if the patient has not already received an oncology consultation. When necessary and possible, the ND onc will refer to other practitioners who can contribute productively to the patient's comprehensive education and health care.

The ND onc ultimately provides each patient with a comprehensive and coherent treatment program, detailing a set of therapies based on the personalized evaluation of the patient. The treatment program considers not only the clinician's best-practice recommendations (given the body of published evidence), but also the patient's diagnosis and individual circumstances. It also incorporates the patient's personal preferences, values, and existential inclinations. The ND onc's treatment recommendations seek to balance those components in a patient-centred manner that optimizes all elements to the greatest extent possible.

Once the plan is formulated and presented, the ND onc completes and documents a full Procedures, Alternatives, Risks, and Questions (PARQ) or comparable process with the patient for the established program (see the Naturopathic Oncology Treatment subsection).

Informed Consent

Patients have the right to receive information from the doctor about the benefits of any procedure, the alternative treatment options, and the associated risks. Patients also have the right to receive answers to any questions they might have about treatment recommendations that might be complex or might carry a more significant possibility of short- or long-term risk. That process is often called a "PARQ discussion" or "PARQ conference." The PARQ conference should be documented in writing in the patient record and should consider patient preferences.

It is important that the ND onc provide a clear explanation of the logical relationships between alternative care options and health outcomes, and a summary (where possible and appropriate) of both the quality of the evidence and the strength of the recommendations.

Evaluating Safety

The ND onc is responsible for the safety of the patient with respect to the treatment recommendations. As the patient progresses through both the conventional and naturopathic treatment program, adjustments to the program are commonplace. It is the responsibility of the ND onc to assure that each change in complementary therapies is compatible with any concurrent conventional therapies. If the state of knowledge about the use of the ND onc's recommended treatment or treatments and their potential effects on the patient's conventional treatments is incomplete, the ND onc should disclose that lack of completeness to the patient.

Addressing Comorbidity

The ND onc understands that the cancer diagnosis happens within the larger context of the patient's overall health. As such, the plan is not exclusively focused on eradication of the cancer, but on re-establishing health in all bodily systems that are assessed to be dysfunctional or contributory to the patient's predilection for malignancy, ability to tolerate conventional treatment, and overall wellness.

The ND onc gives attention to all comorbidities, addresses them as indicated within the context of the treatment program, and monitors their change, as appropriate.

Involving Social Support Networks

Naturopathic doctor oncology providers recognize that supportive social networks are an integral component of each patient's overall health and contribute substantially to an optimal treatment outcome. Therefore, the ND onc, to the extent of each patient's comfort and agreement, seeks to incorporate family and friends in discussions about patient care and treatment. For mental and emotional support, ND oncs assist patients with referrals to resources in the community such as counsellors or support groups.

Involvement in Clinical Research

Clinical research is critically important to advancing knowledge in any field. Good correlation has been observed between clinical trial systems that increase clinical trial enrolment and more rapid advances in treatment and clinical outcomes. All ND oncs are encouraged to participate in research and to support patient involvement in clinical trials. In situations in which the patient of an ND onc is participating in a clinical trial, the ND onc should communicate with the clinical trial coordinator or coordinators about any proposed interventions so as to ensure that those interventions are not excluded in the study protocol.

Naturopathic Oncology Treatment

This subsection presents principles relating to the responsibilities of the ND onc for ensuring the delivery of high-quality patient care throughout the care pathway. Where legally sanctioned, ND oncs are primary care providers or specialty providers, who, through continuing patient assessment and care, can play an important role in ensuring whole-person care for the cancer patient. In other jurisdictions, ND oncs provide lifestyle-based support based on continuing patient evaluation. To provide continuing high-quality and professionally appropriate care, the principles described here should be followed.

Consent

Consent is an evolving process during the treatment of a patient. Although the initial informed consent based on a detailed PARQ discussion with the patient is important in treatment planning, provision of that consent should be understood not to preclude the need for future discussions to obtain patient consent as treatment progresses. The ND onc should continue to obtain consent as meaningful changes occur in the conventional and nonconventional treatment of the patient and in the expected effects and prognosis. Those discussions should be adequately documented in the patient's record.

If a patient decides to refuse conventional oncologic care during their care pathway, the ND onc should make an appropriate referral (as outlined in the Providing Therapeutic Options and Care Coordination subsections) for a discussion of the implications of that choice with an oncologist. The reason for treatment refusal, together with steps taken to ensure that the patient was adequately informed about the potential consequences of refusal and the ND onc's specific recommendations with respect to treatment refusal, should be well documented in the patient's record.

Treatment Documentation

A written treatment plan or set of recommendations should be included in the patient record and given to the patient. The plan should be

- rationally based on identified patient needs and realistic treatment goals.
- based on a proper assessment, including ruling out or identifying life-threatening or hidden conditions with appropriate history, examination, and testing (including referral for specialized evaluation, when appropriate) and allowing for timely ongoing reassessment.
- practical, in light of the patient's condition and situation, including the physical, psycho-emotional, social, and financial spheres.
- in the best interest of the patient and prioritized to the patient's most pressing conditions.
- logical in sequence and internally consistent.
- compatible with other therapies that the patient might be undergoing (especially conventional oncology treatments).
- cost-effective (estimates of the value-for-expenditure returned to the patient based on available evidence and clinical experience of the procedures or treatments—for example, longer life, better quality of life, or both).
- flexible so as to accommodate new developments and findings.
- experimental only with informed consent and only in areas of ND onc's expertise.

Advance Care Planning and End-of-Life Issues

During naturopathic oncology care, patients could require planning for end-of-life care. The role of the ND onc in that part of care will vary depending on the jurisdiction of practice and the associated scope of practice. Although this discussion can be very challenging for the clinician, involving not only the mechanics of care, but also the patient's social dynamics and spiritual beliefs, it has been shown that early discussion and planning for end-of-life care issues is associated with better quality of life during the patient's last months and could be associated with a better chance of the patient experiencing a "good death."

The ND onc should ensure that these essential issues relating to end-of-life care are at least discussed with the patient:

- Personal goals of treatment and a discussion of what is important to the patient
- Advance directives (for example, desire to limit conventional or nonconventional treatment together with life-sustaining treatment)

- Identification of substitute decision-makers and the granting of power of attorney
- Creation of a will
- Place of care, including authority for referral to hospice care and collaboration with hospice care

Ethical Issues in Practice Management

Management and disclosure by the ND onc of any potential, perceived, or real conflict of interest is important—for example, financial interest in products or services offered to patients. In addition, any health care provider is assumed to have a position of power in the context of a therapeutic relationship, and with that in mind, the ND onc should not enter into a therapeutic relationship with or accept a patient with whom they already have a personal relationship (as defined by the ND onc's state or provincial regulatory authority) and with whom professional boundaries might not be sustained.

Timely Delivery of Care

Timely delivery of care is critical to effective patient care management in the oncology treatment setting. The requirements for timely care will be governed, in part, by the ND onc's jurisdictional regulatory environment and corresponding scope of practice. The care management principles for timely care delivery set out here should be applied in ND onc practice as appropriate based on the individual scope of practice as set out in the regulatory and practice settings[15].

- The ND onc should review with the patient, in a timely manner, the results of ordered tests and consultations. The ND onc should explain, where appropriate, how results might affect care decisions and prognosis.
- The ND onc should arrange for any necessary follow-up care or should notify the patient of any necessary follow-up care relating to assessment findings and responses to treatment.
- The ND onc should document all contacts with a patient, including failed attempts to notify a patient about follow-up care.
- In jurisdictions and practice settings that require it, the ND onc should directly provide or arrange for continuous afterhours care to be provided through an appropriate health care provider, providers, or service with the capacity to assess and triage care needs. The ND onc will ensure handover of relevant patient information to the afterhours health care provider, providers, or service when the patient's need for afterhours care is reasonably foreseeable.
- As a part of ongoing practice-based quality assurance, the ND onc should provide a basis for measuring, evaluating, and improving provider performance and timely delivery of quality care based on the provider's jurisdictional requirements.
- For effective case management, it is essential that the ND onc provide timely and ongoing assessment of patient's subjective and objective progress with the recommended treatment. The ND onc should use laboratory and imaging results, together with the aforementioned functional assessments, to track and document in the patient record, at appropriate intervals, the patient's response.
- All assessments performed by the ND onc and other providers in the patient's care team should be considered at regular intervals and used to evolve the patient's treatment plan.

Care Coordination

Collaborative, coordinated care is a key attribute of high-quality care, and it is the responsibility of the ND onc who directly provides specialty care or supervises and manages specialty care provided by other clinicians. Studies have shown that systematic sharing of information between primary care providers in oncology settings is an important factor in the delivery of high-quality, patient-centred care; however, that sharing is often lacking[16–18]. The ND onc is in a unique role to advocate for health care collaboration, which ideally includes multiple professionals, together with family and community members, and to assist patients as they maneuver through often complex multicomponent systems of care.

Role in Collaborative Health Care for Cancer Patients

The ND onc plays an integral role in the multidisciplinary care of a cancer patient. The ND onc performs a detailed assessment and develops deep contextual knowledge about the patient and their overall health that allows the ND onc to take a key role in ensuring effective continuity of care in a collaborative team of providers. Continuity of care is rooted in a long-term patient–physician partnership in which the physician knows the patient's history from experience and can integrate new information and decisions from a whole-person perspective efficiently without extensive investigation or record review. The ND onc, together with the rest of the patient's health care team, should have the shared goal of providing the highest-quality cost-effective medical care. The ND onc should cultivate positive health care team relationships by identifying shared treatment goals and developing interprofessional trust through effective, clear communication[19,20].

During care transitions, ND oncs should ensure that their information about the patient's health status, current treatment plan, treatment adherence, and treatment progress is shared with current and subsequent providers. Examples of interprofessional communication that could be used include copies of consult notes or treatment forms, formal referral letters, and e-mail communications with respect to the patient's naturopathic oncology treatment progress, among others. Before sharing information with other health care providers in the patient's care team, the ND onc should, where appropriate and necessary, ensure that proper authorizations for release of information are obtained.

The ND onc should ensure patient privacy when transmitting information to other care providers and should use methods of communication that guarantee secure private data transmission according to jurisdiction-specific regulation—for example, the Health Insurance Portability and Accountability Act in the United States and the Personal Information Protection and Electronic Documents Act in Canada. If the patient asserts personal privilege to block

the sharing of confidential naturopathic treatment information with other providers, the ND onc should educate the patient about the health and safety risks inherent in poorly coordinated care.

Referral Recommendations

A patient meeting any of the following criteria should be referred to the appropriate specialty:

- Suspected oncology diagnosis in either a primary or recurrent setting
- Oncology diagnosis not currently under the care of the appropriate conventional oncology specialty, unless the patient refuses conventional oncology care (such a refusal should be well documented, and a second or third opinion should be strongly encouraged; see the section on refusal of standard of care in the Consent subsection within the Naturopathic Oncology Treatment subsection)
- Suspected oncologic emergency
- Any symptoms or diagnosis that the naturopathic physician does not have the knowledge, skills, or judgment to manage, or is outside the scope of practice to manage in the practice jurisdiction.

Survivorship Care Management, Screening and Surveillance, and Prevention of Recurrence

A cancer patient is a survivor from the moment of diagnosis through the rest of life. From the perspective of naturopathic oncology patient management, survivorship care begins once active conventional treatment (surgery, radiation, chemotherapy) ceases and clinical remission is achieved. Care plans should involve not only the patient, but also the patient's family, friends, and caregivers per the patient's request. In this portion of the treatment pathway, ND oncs play an important role for patients because of the provider's unique understanding of the interplay between long-term health and environmental factors, diet and lifestyle, and psychosocial and other factors.

Patients should receive a detailed summary of their individualized naturopathic cancer survivorship plan from their ND onc. Discussion of the therapeutic options outlined in the plan should be conducted in a way similar to that outlined in the Providing Therapeutic Options subsection within the Integrative Oncology Plan subsection.

A naturopathic cancer survivorship plan should include

- support for rapid and effective recovery to maximal functioning, including resolving or minimizing, wherever possible, lingering side effects of disease and treatment, including physical, psycho-emotional, and social functioning.
- interventions that reduce the risks of long-term side effects of care and provide appropriate screening to monitor potential long-term or future side effects of treatment.
- support for the prevention of recurrence, assurance of adequate surveillance for cancer recurrence, and provision of continued appropriate primary cancer screening where the ND onc is acting as the patient's primary care provider.

SUMMARY

Changing patient demographics, cultural attitudes, and increased access to health and related information are causing a massive transformation in health care today[21]. Those transformational forces have led to the burgeoning field of naturopathic oncology. Because of their training as primary care providers delivering whole-person care, in concert with expertise in integrative oncology treatment approaches, ND oncs can play an important role in the care of cancer patients. Guiding principles of care are needed if ND oncs are to be effective players in the larger care team for cancer patients.

In the present document, we have set out clear guidelines covering areas of patient assessment, treatment planning and management, care coordination, and survivorship. The guidelines were developed in a comprehensive process that brought together experts in the field, extended invitations for internal and external stakeholder review and commentary, and culminated with final dissemination, whose goal was broad adoption by ND oncs. The OncANP Principles of Care Guidelines constitute a tool not only for ensuring consistent, high-quality care delivery by ND oncs, but also for cultivating a greater understanding of naturopathic oncology practice with other oncology care professionals.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

AUTHOR AFFILIATIONS

*Marsden Centre For Excellence in Integrative Medicine, Vaughan, ON; †Immersion Health, Portland, OR, U.S.A.; ‡Goodapple Wellness, Philadelphia, PA, U.S.A.; §Hawaii Integrative Oncology, Kailua-Kona, HI, U.S.A.

REFERENCES

1. Judson PL, Abdallah R, Xiong Y, Ebbert J, Lancaster JM. Complementary and alternative medicine use in individuals presenting for care at a comprehensive cancer center. *Integr Cancer Ther* 2017;16:96–103.
2. Horneber M, Bueschel G, Dennert G, Less D, Ritter E, Zwahlen M. How many cancer patients use complementary and alternative medicine: a systematic review and metaanalysis. *Integr Cancer Ther* 2012;11:187–203.
3. John GM, Hershman DL, Falci L, Shi Z, Tsai WY, Greenlee H. Complementary and alternative medicine use among US cancer survivors. *J Cancer Surviv* 2016;10:850–64.
4. Mao JJ, Palmer CS, Healy KE, Desai K, Amsterdam J. Complementary and alternative medicine use among cancer survivors: a population-based study. *J Cancer Surviv* 2011;5:8–17.
5. Link AR, Gammon MD, Jacobson JS, *et al.* Use of self-care and practitioner-based forms of complementary and alternative medicine before and after a diagnosis of breast cancer. *Evid Based Complement Alternat Med* 2013;2013:301549.
6. Schag CC, Heinrich RL, Ganz PA. Karnofsky performance status revisited: reliability, validity, and guidelines. *J Clin Oncol* 1984;2:187–93.
7. Oken MM, Creech RH, Tormey DC, *et al.* Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982;5:649–55.
8. Lansky SB, List MA, Lansky LL, Ritter-Sterr C, Miller DR. The measurement of performance in childhood cancer patients. *Cancer* 1987;60:1651–6.

9. Anderson F, Downing GM, Hill J, Casorso L, Lerch N. Palliative performance scale (PPS): a new tool. *J Palliat Care* 1996;12:5–11.
10. Bruera E, Kuehn N, Miller MJ, Selmser P, Macmillan K. The Edmonton Symptom Assessment System (ESAS): a simple method for the assessment of palliative care patients. *J Palliat Care* 1991;7:6–9.
11. Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. *Ann Acad Med Singapore* 1994;23:129–38.
12. Macpherson CF, Wang J, DeWalt DA, Stern ED, Jacobs S, Hinds PS. Comparison of legacy fatigue measures with the PROMIS pediatric fatigue short form. *Oncol Nurs Forum* 2018;45:106–14.
13. Spitzer RL, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 2006;166:1092–7.
14. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001;16:606–13.
15. College of Physicians and Surgeons of Alberta (CPSA). Standard of Practice: Continuity of Care [Web page]. Edmonton, AB: CPSA; 2015. [Available at: http://www.cpsa.ca/standards practice/continuity-of-care; cited 11 December 2017]
16. Dicicco-Bloom B, Cunningham RS. The experience of information sharing among primary care clinicians with cancer survivors and their oncologists. *J Cancer Surviv* 2013;7:124–30.
17. Gagliardi AR, Dobrow MJ, Wright FC. How can we improve cancer care? A review of interprofessional collaboration models and their use in clinical management. *Surg Oncol* 2011;20:146–54.
18. Haggerty JL, Roberge D, Freeman GK, Beaulieu C. Experienced continuity of care when patients see multiple clinicians: a qualitative metasummary. *Ann Fam Med* 2013;11:262–71.
19. American Academy of Family Physicians (AAFP). Continuity of Care, Definition of [Web page]. Leawood, KS: AAFP; 2015. [Available online at: http://www.aafp.org/about/policies/all/definition-care.html; cited 1 December 2018]
20. Gil F, Grassi L, Travado L, Tomamichel M, Gonzalez JR on behalf of the Southern European Psycho-Oncology Study Group. Use of distress and depression thermometers to measure psychosocial morbidity among southern European cancer patients. *Support Care Cancer* 2005;13:600–6.
21. Geffen JR. Integrative oncology for the whole person: a multidimensional approach to cancer care. *Integr Cancer Ther* 2010;9:105–21.

Biomarkers:
Coagulation Assays in the Prognosis of Colorectal Cancer

Can coagulation assays serve as prognostic factors in patients with colorectal cancer?

A retrospective study finds that D-dimer and INR correlate significantly with tumor markers and disease stage in patients with colorectal cancer.

Reference

Kilic L, Yildiz I, Sen FK, et al. **D-dimer and international normalized ratio (INR) are correlated with tumor markers and disease stage in colorectal cancer patients.** *Cancer Biomark.* 2015;15(4):405-411.

Overview

The aim of this study was to **evaluate the prognostic significance of coagulation tests and clarify their relationship with tumor markers and other clinical variables in colorectal cancer.**

Study Design

Retrospective cohort study.

Results

This study compared 94 patients with histologically proven colorectal cancer to healthy controls. All coagulation tests, including D-dimer, fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ration (INR), and platelet counts, were significantly different between the patient group and control group ($p < .001$ for all variables except PT). Metastatic disease correlated with elevated INR (median values 1.08 vs 1.1; $p = .03$), and stage III patients had higher D-dimer levels than stage II patients (324 IU/mL vs 589 IU/mL; $p = .03$). Comparison with tumor markers showed that high CA 19-9 (tumor marker) levels correlated with higher INR and that high CEA (tumor marker) levels correlated with elevated D-dimer. Coagulation tests did not correlate with overall survival. Analysis of factors including D-dimer, CEA, CA 19-9, state of metastasis, and age found only CEA to be an independent prognostic factor for overall survival.

Discussion

Hypercoagulation and fibrinolysis are characteristic of malignancy, with as many as 95% of metastatic patients displaying hemostatic abnormalities. Hypercoagulation not only puts patients at increased risk for thromboembolism and death but also serves as an indication of tumor activity. Coagulation pathways are activated by tumor cells or tumor-associated inflammatory cells to drive tumor stroma formation, and both fibrin remodeling and angiogenesis play a critical role in tumor growth, invasion, and metastasis.[1]

The process of tumor-related fibrin remodeling produces fibrin degradation products, including D-dimer. In previous trials of patients with colorectal cancer, plasma levels of D-dimer were associated with larger tumors, deeper wall penetration, lymph node metastases, CEA levels, and shorter survival.[2],[3] The current study confirmed 2 of these findings: that D-dimer is associated with lymph node metastases (stage III vs stage II) and CEA levels.

Although coagulation assays did not predict overall survival in the current study, they did show a statistically significant correlation with tumor markers and disease stage. The authors of the study suggest that D-dimer might be an even more reliable prognostic factor than CEA or CA 19-9 in the preoperative setting for patients with colorectal cancer.

[1] Zacharski LR, Wojtukiewicz MZ, Costantini V, Ornstein DL, Memoli VA. **Pathways of coagulation/fibrinolysis activation in malignancy.** *Semin Thromb Hemost*. 1992;18(1): 104-116.

[2] Oya M, Akiyama Y, Yanagida T, Akao S, Ishikawa H. **Plasma D-dimer level in patients with colorectal cancer: its role as a tumor marker.** *Surg Today*. 1998;28(4):373-378.

[3] Yamamoto M, Yoshinaga K, Matsuyama A, et al. **Plasma D-dimer level as a mortality predictor in patients with advanced or recurrent colorectal cancer.** *Oncology*. 2012;83(1): 10-15.

Research Paper

Dietary iron intake and breast cancer risk: modulation by an antioxidant supplementation

Abou Diallo[1,2,3,*], Mélanie Deschasaux[1,3,*], Valentin Partula[1,3], Paule Latino-Martel[1,3], Bernard Srour[1,3], Serge Hercberg[1,2,3], Pilar Galan[1,3], Philippine Fassier[1,3], Françoise Guéraud[3,4], Fabrice H. Pierre[3,4], Mathilde Touvier[1,3]

[1]Sorbonne Paris Cité Epidemiology and Statistics Research Center (CRESS), Inserm U1153, Inra U1125, Cnam, Paris 5, 7 and 13 Universities, Nutritional Epidemiology Research Team (EREN), Bobigny, France

[2]Public Health Department, Avicenne Hospital, Bobigny, France

[3]French Network for Nutrition And Cancer Research (NACRe Network), Bobigny, France

[4]UMR 1331 Toxalim, INRA/INP/UPS, Toulouse, France

[*]These authors have contributed equally to this work

Correspondence to: *Mélanie Deschasaux,* ***email:*** *m.deschasaux@eren.smbh.univ-paris13.fr*

Keywords: *breast cancer, dietary iron, antioxidants, lipid peroxidation, prospective study*

Received: *July 07, 2016* ***Accepted:*** *September 29, 2016* ***Published:*** *October 12, 2016*

ABSTRACT

Experimental results suggested that iron-induced lipid peroxidation may explain the direct associations observed between red/processed meat intakes and colorectal and breast cancer risk. However, epidemiological evidence is lacking. Thus, we investigated the association between dietary iron intake and breast cancer risk, and its potential modulation by an antioxidant supplementation and lipid intake. This prospective study included 4646 women from the SU.VI.MAX trial (daily low-dose antioxidants vs. placebo). 188 incident breast cancers were diagnosed (median follow-up=12.6y). Dietary iron intake was assessed using repeated 24h dietary records. Multivariable Cox proportional hazards models were computed. Dietary iron intake was associated with an increased breast cancer risk ($HR_{T3vs.T1}$=1.67 (1.02-2.71), P-trend=0.04). This association was observed in the placebo group ($HR_{T3vs.T1}$=2.80 (1.42-5.54), P-trend=0.003), but not in the antioxidant-supplemented group (P-trend=0.7, P-interaction=0.1). Besides, in the placebo group, the increased breast cancer risk associated with dietary iron intake was more specifically observed in women with higher lipid intake (P-trend=0.046). These findings suggest that dietary iron intake may be associated with an increased breast cancer risk, especially in women who did not received antioxidants during the trial and who consumed more lipids. This supports the experimental results suggesting that breast cancer risk may be increased by iron-induced lipid peroxidation.

INTRODUCTION

Recently, the International Agency for Research on Cancer (IARC) classified red and processed meat consumption as "(probably) carcinogenic to humans" (Group 2A and 1 respectively) [1]. Although these conclusions were mainly based on colorectal cancer risk [1, 2], existing evidence also suggests a positive association with other cancer sites such as female breast [3–6]. Notably, we previously observed an increased breast cancer risk associated with processed meat intake in the Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) cohort [6].

These associations could be explained by several potential pro-carcinogenic compounds found in red and processed meat such as heme iron, heterocyclic amines or *N*-nitroso compounds [1]. With an experimental approach on rodent models, we recently demonstrated that among all these potential pro-carcinogens, iron, as a pro-oxidant, may be of particular importance in the promotion of colon carcinogenesis [7, 8]. Furthermore, a possible role of elevated iron intake in breast carcinogenesis

has been hypothesized [9, 10], in particular through its involvement in lipid peroxidation. Indeed, the interaction between lipids and iron in the intestinal tract may form lipid peroxidation end-products that are able to reach the systemic blood circulation and to induce oxidative stress in other sites [11, 12].

Epidemiological evidence regarding the association between iron intake and breast cancer risk is still limited and did not allow the World Cancer Research Fund/ American Institute for Cancer Research (WCRF/AICR) to draw any conclusion [13]. To our knowledge, only five prospective studies are available [14–18]. Three of them observed null results [14, 16, 18] while the other two observed a direct association between iron intake and breast cancer risk in post-menopausal women [15, 17].

Since iron may promote breast carcinogenesis in particular through lipid peroxidation, it could be hypothesized that iron intake may all the more increase cancer risk as diet has a low antioxidant potential and high lipid content.

To our knowledge, no epidemiological study has investigated a potential modification of the association between iron intake and breast cancer risk by antioxidant or lipid intakes. In a previous work from the SU.VI.MAX cohort [6], the positive association between processed meat intake and breast cancer risk observed in the overall population was no longer observed when analyses were restricted to women who received an antioxidant supplementation. This work suggested that antioxidant intake may counteract some of the deleterious effect of processed meat intake on breast carcinogenesis, such as lipid peroxidation induced by iron.

Thus, our objectives were to prospectively investigate the association between dietary iron intake and breast cancer risk, and to study whether this association was modified by the antioxidant supplementation of the SU.VI.MAX trial and by lipid intake.

RESULTS

During a median follow-up of 12.6y (52,500 person-years), 188 women developed a first primary breast cancer with a mean age at diagnosis of 55.7±7.0y.

Characteristics of participants according to tertiles of total dietary iron intake are presented in Table 1. Participants in the upper tertile tended to have a higher educational level, to be less physically active, and to have higher intakes of energy, alcohol and lipids. Mean ± SD of total dietary iron intake per subject was 10.9±3.2 g/d. Overall, women who provided at least three 24h dietary records (compared to women who did not) were slightly smaller and thinner, were more likely to take hormonal treatment for menopause and to have a family history of breast cancer and were less likely to drink alcohol or to smoke [data not tabulated].

Table 2 displays the associations between tertiles of dietary iron intake and breast cancer risk overall and according to menopausal status. Higher iron intake was associated with an increased breast cancer risk overall ($HR_{T3vs.T1}$=1.67 (1.02, 2.71), P-trend=0.04) and in post-menopausal women ($HR_{T3vs.T1}$=1.85 (1.02, 3.34), P-trend=0.04). No association was detected in analyses restricted to pre-menopausal women ($HR_{T3vs.T1}$=1.39 (0.58, 3.29), P-trend=0.4), but the number of cases was limited (59 cases/ 3190 non-cases). Similar results were observed for iron intake from processed meat (overall, $HR_{T3vs.T1}$=1.60 (1.07, 2.37), P-trend=0.02) but not from red meat (overall, $HR_{T3vs.T1}$=1.00 (0.70, 1.43), P-trend=0.9) [data not tabulated].

The association between total dietary iron intake and breast cancer risk was modulated by antioxidant intake (Table 3, P-interaction=0.1): in stratified analyses according to the intervention group of the SU.VI.MAX trial, higher iron intakes were associated with an increased breast cancer risk in the placebo group ($HR_{T3vs.T1}$=2.80 (1.42, 5.54), P-trend=0.003) but not in the group supplemented with antioxidants ($HR_{T3vs.T1}$=0.86 (0.43, 1.74), P-trend=0.7). A similar modulation was observed when analyses were restricted to post-menopausal women (placebo group: P-trend=0.03, supplemented group: P-trend=0.6, P-interaction=0.6) or to pre-menopausal women (placebo group: P-trend=0.02, supplemented group: P-trend=0.2, P-interaction=0.04).

A further exploratory stratification was performed according to the median intake of total lipids (Table 4). No association was observed in the group supplemented with antioxidants, whatever the level of lipid intake. In the placebo group, although P for interaction was not statistically significant (P-interaction=0.3), different associations were observed according to lipid intake: higher dietary intakes of total iron were positively associated with breast cancer risk in women with higher intakes of total lipids (≥ median, $HR_{T3vs.T1}$=2.57 (0.86, 7.69), P-trend=0.046) while no significant association was detected in women with lower lipid intakes (< median, $HR_{T3vs.T1}$=1.99 (0.79, 4.99), P-trend=0.1). Similar results were observed with two major long chain n-3 polyunsaturated fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA): no association in the antioxidant group, while in the placebo group, positive associations were observed in women with higher intakes (≥ median) of EPA ($HR_{T3vs.T1}$=4.38 (1.58, 12.1), P-trend=0.004) and DHA ($HR_{T3vs.T1}$=3.67 (1.30, 10.33), P-trend=0.01), but not in women with intakes < median ($HR_{T3vs.T1}$= 1.77 (0.66, 4.72), P-trend=0.3 for EPA, and $HR_{T3vs.T1}$= 2.15 (0.82, 5.66), P-trend=0.1 for DHA) (P-interactions=0.04 for EPA and 0.3 for DHA) [data not tabulated].

Results were similar when analyses were restricted to women who provided at least six (156 cases/ 3586 non-cases) or nine (116 cases/ 2737 non-cases) 24h-dietary records within the first two years of follow-up, when

Table 1: Baseline characteristics of participants according to tertiles of iron intake, SU.VI.MAX cohort, france, 1994–2007[a]

	Tertile 1 (n=1548)	Tertile 2 (n=1549)	Tertile 3 (n=1549)	P[b]
Age, y	47.2 ± 6.6	46.7 ± 6.6	47.1 ± 6.5	0.9
Children, n	2.0 ± 1.1	1.9 ± 1.2	1.9 ± 1.1	0.2
Height, cm	160.9 ± 5.7	161.9 ± 5.9	162.7 ± 5.9	0.3
Body mass index, kg/m^2	23.2 ± 3.8	23.0 ± 3.7	23.1 ± 3.8	0.8
Menopause, yes	486 (31.4)	436 (28.1)	475 (30.7)	0.1
Hormonal treatment for menopause, yes	458 (29.6)	436 (28.1)	479 (30.9)	0.2
Intervention group of the initial trial				0.4
Antioxidant supplementation group	751 (48.5)	765 (49.4)	788 (50.9)	
Placebo group	797 (51.5)	784 (50.6)	761 (49.1)	
Family history of breast cancer[c], yes	146 (9.4)	135 (8.7)	124 (8.0)	0.4
Smoking status				0.07
Never	920 (59.4)	891 (57.5)	868 (56.0)	
Former	406 (26.2)	462 (29.8)	472 (30.5)	
Current	222 (14.3)	196 (12.7)	209 (13.5)	
Physical activity				0.02
Irregular	419 (27.1)	377 (24.3)	394 (25.4)	
< 1 h/d walking or equivalent	489 (31.6)	563 (36.3)	569 (36.7)	
≥ 1 h/d walking or equivalent	640 (41.3)	609 (39.3)	586 (37.8)	
Educational level				<.0001
Primary	353 (22.8)	263 (17.0)	231 (14.9)	
Secondary	609 (39.3)	628 (40.5)	595 (38.4)	
University	586 (37.9)	658 (42.5)	723 (46.7)	
Alcohol intake, g/d	5.2 ± 6.8	10.0 ± 10.9	17.0 ± 17.1	<.0001
Energy intake, kcal/d	1392 ± 312	1777 ± 313	2088 ± 424	<.0001
Dietary iron, mg/d	7.7 ± 1.3	10.6 ± 0.7	14.4 ± 2.3	<.0001
Total lipids, g/d	63.0 ± 17.0	80.4 ± 17.9	95.7 ± 23.6	<.0001
Eicosapentaenoic acid (EPA), g/d	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	<.0001
Docosahexaenoic acid (DHA), g/d	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	<.0001

SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants.

[a] Values are means ± SDs or N (%). Cut-offs for tertiles of total dietary iron intake were 9.3 and 11.9 mg/d.

[b] P value for the comparison between tertiles of iron intake using χ^2 tests or Fisher tests (P-trend) as appropriate. All statistical tests were 2-sided.

[c] Among first-degree relatives.

Table 2: Associations between tertiles of dietary iron intake and breast cancer risk from multivariable cox proportional hazards models, SU.VI.MAX cohort, france, 1994–2007[a, b]

	N for cases/non-cases	HR (95% CI)	P-trend
All women			0.04
Tertile 1	53/1495	1.00	
Tertile 2	57/1492	1.18 (0.78, 1.79)	
Tertile 3	78/1471	1.67 (1.02, 2.71)	
Premenopausal women			0.4
Tertile 1	17/1045	1.00	
Tertile 2	19/1094	1.05 (0.51, 2.18)	
Tertile 3	23/1051	1.39 (0.58, 3.29)	
Postmenopausal women			0.04
Tertile 1	36/1143	1.00	
Tertile 2	38/1093	1.25 (0.75, 2.08)	
Tertile 3	55/1107	1.85 (1.02, 3.34)	

CI, confidence interval, HR, Hazard ratio, SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants

[a] Multivariable models were adjusted for age (timescale), energy intake without alcohol, intervention group of the initial SU.VI.MAX trial, number of 24-h dietary records, smoking status, educational level, physical activity, height, BMI, alcohol intake, family history of breast cancer, lipid intake, use of hormone replacement therapy, number of children and for premenopausal women: use of contraceptive pill, heavy period, and use of a hormonal intrauterine system.

[b] Cut-offs for tertiles of dietary iron intake were 9.3 and 11.9 mg/d.

Table 3: Associations between tertiles of dietary iron intake and breast cancer risk from multivariable cox proportional hazards models, stratified by antioxidant/placebo group of the SU.VI.MAX trial, france, 1994–2007[a, b]

	Placebo group			Antioxidant supplementation group			
	N for cases/ non- cases	HR (95% CI)	P-trend	N for cases/ non- cases	HR (95% CI)	P-trend	P-interaction[c]
All women			0.003			0.7	0.1
Tertile 1	25/772	1.00		28/723	1.00		
Tertile 2	33/751	1.83 (1.03, 3.25)		24/741	0.70 (0.38, 1.29)		
Tertile 3	42/719	2.80 (1.42, 5.54)		36/752	0.86 (0.43, 1.74)		
Premenopausal women			0.02			0.2	0.04
Tertile 1	7/545	1.00		10/500	1.00		
Tertile 2	10/549	1.83 (0.62, 5.39)		9/545	0.60 (0.21, 1.68)		
Tertile 3	16/521	3.87 (1.16, 12.86)		7/530	0.39 (0.1, 1.56)		
Postmenopausal women			0.03			0.6	0.6
Tertile 1	18/593	1.00		18/550	1.00		
Tertile 2	23/544	1.90 (0.96, 3.76)		15/549	0.74 (0.35, 1.59)		
Tertile 3	26/535	2.49 (1.08, 5.74)		29/572	1.18 (0.51, 2.73)		

CI, confidence interval, HR, Hazard ratio, SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants

[a] Multivariable models were adjusted for age (timescale), energy intake without alcohol, intervention group of the initial SU.VI.MAX trial, number of 24-h dietary records, smoking status, educational level, physical activity, height, BMI, alcohol intake, family history of breast cancer, lipid intake, use of hormone replacement therapy, number of children and for premenopausal women: use of contraceptive pill, heavy period, and use of a hormonal intrauterine system.

[b] Cut-offs for tertiles of dietary iron intake were 9.3 and 11.9 mg/d.

[c] Between dietary iron intake and supplementation group

Table 4: Associations between tertiles of dietary iron intake and breast cancer risk from multivariable cox proportional hazards models, stratified by antioxidant/placebo group of the SU.VI.MAX trial and by lipid intake, france, 1994–2007[a, b, c]

	Placebo group			Antioxidant supplementation group		
	N for cases/ non-cases	HR (95% CI)	P-trend	N for cases/ non-cases	HR (95% CI)	P-trend
Total lipid intake < median (78.5 g/d)			0.1			0.3
Tertile 1	20/632	1.00		21/602	1.00	
Tertile 2	18/338	1.58 (0.76, 3.24)		12/345	0.94 (0.41, 2.15)	
Tertile 3	12/143	1.99 (0.79, 4.99)		11/169	1.67 (0.63, 4.42)	
Total lipid intake ≥ median (78.5 g/d)			0.046			0.2
Tertile 1	5/140	1.00		7/121	1.00	
Tertile 2	15/413	1.41 (0.49, 4.06)		12/396	0.40 (0.15, 1.05)	
Tertile 3	30/576	2.57 (0.86, 7.68)		25/583	0.42 (0.15, 1.17)	

CI, confidence interval, HR, Hazard ratio, SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants

[a] Multivariable models were adjusted for age (timescale), energy intake without alcohol, intervention group of the initial SU.VI.MAX trial, number of 24-h dietary records, smoking status, educational level, physical activity, height, BMI, alcohol intake, family history of breast cancer, lipids intake, use of hormone replacement therapy, and number of children.

[b] Cut-offs for tertiles of dietary iron intake were 9.3 and 11.9 mg/d.

[c] P for interaction between dietary iron and lipid intakes: Placebo group, 0.3; Antioxidant supplementation group, 0.5.

cases diagnosed within the first two years of follow-up were excluded (163 cases/ 4458 non-cases) or when *in situ* breast cancers were excluded (165 cases/ 4458 non-cases) [data not shown].

DISCUSSION

In this prospective study, total dietary iron intake was associated with an increased risk of breast cancer. This association was no longer observed in the group supplemented with antioxidants during the SU.VI.MAX trial. In contrast, in the placebo group, a direct association was observed between total dietary iron intake and breast cancer risk, especially in women with higher intakes of total lipids (thus, with more precursors for lipid peroxidation), and notably EPA and DHA, two long chain n-3 polyunsatured fatty acids particularly prone to peroxidation because of their high number of double bonds [19].

To our knowledge, only five prospective studies were performed regarding the association between iron intake and breast cancer risk, with inconsistent results [14–18]. While three of them observed null results [14, 16, 18], our results are in line with those of two large prospective studies that observed a direct association between iron intake and postmenopausal breast cancer risk [15, 17]. Ferrucci et al. [17] observed a direct association with dietary iron intake in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, but no association was observed with total iron (dietary + supplemental). Inoue-Choi et al. [15] observed a direct association with heme iron intake in the NIH-AARP Diet and Health Study. Some prospective epidemiological studies also reported a positive association between elevated blood iron concentration and breast cancer risk [20, 21].

Our results are consistent with the hypothesis that iron intake would increase breast cancer risk, through lipid peroxidation. To our knowledge, this epidemiological study was the first to investigate a potential modulation of the association between dietary iron intake and breast cancer risk by an antioxidant supplementation and by lipid intake. However, in a previous study performed in the SU.VI.MAX cohort [6], a direct association was observed between processed meat intake (rich in heme iron) and breast cancer risk, and this association was no longer significant in the group supplemented with antioxidants. No association was detected for red meat, probably because intakes were relatively low (below 500g/ week) for most women in this study [6]. These results suggested that the antioxidants may have counteracted some of the deleterious effects of dietary iron towards breast cancer either by preventing the lipid peroxidation or by protecting the cells against the oxidative stress induced by lipid peroxidation end-products. In this way, in a recent publication on the E3N cohort, we have observed that the positive association between heme iron

intake and risk of colorectal adenoma was only observed in women with a total dietary antioxidant capacity ratio below the median cohort value [22]. These findings are in line with experimental data. Iron is considered as one of the major compounds explaining the association between red/processed meat intake and colorectal cancer risk [8]. Indeed, iron is a pro-oxidant involved in the production of reactive oxygen species that interact with all classes of macromolecules (e.g. DNA, lipid) [9]. In particular, the peroxidation of lipids leads to the formation of end-products involved in oxidative stress/damages and chronic inflammation [7, 9–11, 23]. Although these compounds may be mainly produced in the digestive tract from the interaction between oxidative compounds and lipids, they also appeared to be able to enter the systemic circulation [12] and thus to reach other organs. In particular, evidence from a case-control study reported that blood concentration of these end-products was increased in breast cancer patients [24]. Lipid peroxidation may thus be harmful to human health and could be involved in carcinogenesis, not only at the digestive tract level but also in non-digestive organs such as the breast [9, 10, 25].

Besides, experimental studies showed a protective effect of antioxidants, in particular vitamins C and E (both included in the SU.VI.MAX capsule), towards lipid peroxidation [26–28]: Vulcain et al. observed *in vitro* an inhibition of iron-induced lipid peroxidation by α-tocopherol [26]; Pierre et al. observed that when α-tocopherol was ingested in addition to cured meat, as compared to cured meat alone, there was a decrease in lipid peroxidation biomarkers in human volunteers [27]; and Klouche et al. showed that vitamins C and E inhibited the heme-induced oxidation of low-density lipoproteins [28]. Therefore, our results support the mechanistic hypotheses linking dietary iron and breast cancer through lipid peroxidation.

Strengths of our study include its prospective design with a long follow-up. The randomized control trial design of the SU.VI.MAX study allowed us to test the potential modification effect of an antioxidant supplementation at nutritional doses on the association between dietary iron and breast cancer. Dietary intakes were assessed by repeated 24-hour dietary records (mean of 9 records per subject) accounting for intra-individual variability (day-to-day and seasonal variations). The results were highly consistent with our initial hypotheses based on mechanistic data and were original regarding existing literature. Finally, a large range of confounding factors has been taken into account, thus limiting potential bias.

However, some limitations should also be acknowledged. First, no information was available regarding heme iron so that it was not possible to specifically test its association with breast cancer risk. Heme iron is supposed to be one of the major compounds explaining the association between red and processed meat and cancer [8]. However, total dietary iron intake may also be relevant when studying breast carcinogenesis [9]. Besides, as a proxy for heme iron, we investigated dietary iron from processed meat and from red meat in secondary analyses. The results (iron from processed meat: positive association, from red meat: no association) were consistent with those of the previous SU.VI.MAX study on red and processed meat intake and breast cancer risk presented above [6]. Second, we could not perform analyses on the association between other potential carcinogens from red and processed meat (heterocyclic amines or *N*-nitroso compounds) and breast cancer risk since this information was not available. Third, although the number of cases was appropriate for the main analyses reported here, it was nonetheless a limit in stratified analyses so that the results of these stratified analyses should be interpreted with caution. Finally, in SU.VI.MAX, participants received a combination of antioxidants (ascorbic acid, vitamin E, β-carotene, selenium and zinc) so that it was not possible to isolate the potential specific effect of each compound in the studied interaction.

This prospective study suggests that total dietary iron intake may be associated with an increased risk of breast cancer. For the first time, these results also suggest that this association could be modified by a supplementation with nutritional doses of antioxidants and by lipid intake, thus supporting the hypotheses raised by experimental studies that iron may increase breast cancer risk, in particular through lipid peroxidation. Indeed, dietary iron intake was associated with an increased breast cancer risk in women not supplemented with antioxidants, suggesting that antioxidants may counteract some of the deleterious effects of dietary iron on breast carcinogenesis, and in women with higher lipid intakes (i.e. with more substrates for lipid peroxidation). Although our results regarding the association between dietary iron intake and breast cancer risk were consistent with two large prospective studies [15, 17], they have to be confirmed in future large cohorts, and especially the observed interaction between iron and antioxidant intakes. If these results are confirmed and if the causality of the associations can be established, this may lead to formulate explicit public health recommendations towards the limitation of the consumption of iron-rich foods such as red and processed meat (also containing other carcinogenic compounds [1]) while promoting the consumption of antioxidant-rich foods.

MATERIALS AND METHODS

Participants

The SU.VI.MAX study was at first designed as a randomized, double-blind, placebo-controlled primary prevention trial (clinicaltrials.gov NCT00272428) aiming to assess the effect of a daily supplementation with nutritional doses of antioxidants (120 mg ascorbic acid, 30 mg vitamin E, 6 mg β-carotene, 100 µg selenium,

and 20 mg zinc) versus a placebo, on the incidence of cardiovascular diseases and cancers [29]. In 1994–1995, 13,017 individuals, among which 7876 women (35-60y), were enrolled for an 8y intervention study. Follow-up of health events lasted until September 30th, 2007. 5.2% of participants were lost to follow-up. As reported before [30], the antioxidant supplementation was not associated with breast cancer risk in this trial. A graphical presentation of the study design with data collection phases is available in Supplemental Figure S1.

Compliance with ethical standards

The SU.VI.MAX study was conducted according to the Declaration of Helsinki guidelines and was approved by the Paris-Cochin Hospital Ethics Committee for Studies with Human Subjects (CCPPRB nos.706 and 2364, respectively) and the French National Commission for Computed Data and Individual Freedom (CNIL nos. 334641 and 907094, respectively). Written informed consent was obtained from all participants.

Baseline data collection

Dietary data

Every 2 months during the trial phase (1994–2002), participants were invited to complete a 24-h dietary record via the Minitel Telematic Network, a French telephone-based terminal equivalent to an Internet prototype used widely at the beginning of the study. The records were randomly distributed between weeks and weekends and over seasons to take into account intra-individual variability. Participants assessed portion sizes with a validated picture booklet [31], and the amounts consumed from composite dishes were estimated using French recipes validated by food and nutrition professionals. The mean daily energy, alcohol, macro- and micronutrient intakes were estimated using a published French food composition table [32]. Participants were advised against taking any self-prescribed supplementation during the trial.

Other covariates

Baseline information about socio-demographics, smoking status, physical activity, and family history of breast cancer were collected by self-administered questionnaires. Anthropometric measures (height and weight) were obtained during a medical examination by the study nurses and physicians.

Case ascertainment

During the follow-up period, participants were invited to self-report health events (through a monthly questionnaire). Investigations were then conducted to obtain medical data from participants, physicians, and/or hospitals. All information was reviewed by an independent physician expert committee. All cancer cases were documented by a pathology report and were validated by histologic reports. The International Chronic Diseases Classification, 10th Revision, Clinical Modification [33] was used to classify the cancer cases. All first-incident primary breast cancers were considered as cases in this study.

Statistical analyses

From the 7876 women included in the SU.VI.MAX study, we excluded those who reported a cancer diagnosis before the start of the follow-up (N=120) and those with a chronic inflammatory disease that may impact iron metabolism (N=58, among which 28 rheumatoid arthritis, 8 ankylosing spondylitis, 6 hemorrhagic rectocolitis, 5 hemochromatosis, and 11 others). Among the remaining participants, 4646 provided at least 3 valid 24-h dietary records within the first 2 years of follow-up and thus were included in the analyses (see the flowchart in Supplemental Figure S2). Food and nutrient intakes were assessed using mean intakes calculated from all dietary records provided during the first two years of follow-up for each woman.

Baseline characteristics of participants were compared between tertiles of total dietary iron intake using χ^2 tests or Fisher tests (from ANOVA models) wherever appropriate. Hazard ratios (HR) and 95% confidence intervals (CI) obtained from Cox proportional hazards models, with age as the primary time variable, were used to characterize the association between tertiles of total dietary iron intake and the incidence of breast cancer. Participants contributed person-time until the date of diagnosis of breast cancer, the date of last completed questionnaire, the date of death, or September 30th, 2007, whichever occurred first. Participants who reported a cancer other than breast cancer during the study period were included and censored at the date of diagnosis (except those with basal cell skin carcinoma, which was not considered as cancer). We confirmed that the assumptions of proportionality were satisfied through examination of the log-log (survival) vs. log-time plots. Tests for linear trend were performed using the ordinal score on tertiles of total dietary iron intake.

Stratified analyses were performed according to menopausal status (premenopausal and postmenopausal). For these analyses, women contributed person-time until their date of menopause for premenopausal breast cancer analysis or from their date of menopause for postmenopausal breast cancer analysis.

Multivariable models were adjusted for factors constitutive to the study design [initial SU.VI.MAX trial

intervention group (antioxidant/placebo), number of dietary records (continuous)], socio-demographic variables [age (time-scale) and educational level (primary, secondary, or university)], lifestyle factors [smoking status (never, former, or current), physical activity (irregular, <1h/d or ≥1h/d walking or equivalent), and alcohol intake (continuous)], anthropometric factors [height (continuous) and BMI (continuous)], dietary factors [dietary intakes of energy without alcohol (continuous), lipid intake (continuous)] and factors indicating higher individual susceptibility to breast cancer [family history of breast cancer (yes/no), menopausal status at baseline (yes/no), use of hormonal treatment for menopause at baseline (yes/no), number of children (continuous) and, for analyses restricted to premenopausal women, contraceptive pill (yes/no), heavy period (yes/no) and hormonal intrauterine system (yes/no)]. Interactions were tested between tertiles of dietary iron intake and 1) the antioxidant supplementation of the initial SU.VI.MAX trial and 2) lipid intake. Stratified analyses were performed according to the intervention group of the initial SU.VI.MAX trial (antioxidant/placebo) and further exploratory stratified analyses were also performed according to the median intakes of lipid.

For all covariates, less than 5% of values were missing and were replaced by the respective mode value.

Since we showed in a previous work [6] that processed meat intake was associated with an increased breast cancer risk (while no association was observed for red meat intake), we performed secondary analyses on the associations between dietary iron from red and from processed meat and breast cancer risk.

Sensitivity analyses were carried out by excluding women who provided less than six or nine 24h-dietary records within the first two years of follow-up, cases diagnosed within the first two years of follow-up, or *in situ* breast cancers.

All tests were two-sided, and $P<0.05$ was considered statistically significant. SAS version 9.3 (SAS Institute Inc., Cary, NC.) was used for the analyses.

ACKNOWLEDGMENTS

The authors thank Nathalie Arnault, Younes Esseddik, Paul Flanzy, Yasmina Chelghoum, and Van Than Duong (computer scientists), and Véronique Gourlet, Fabien Szabo and Laurent Bourhis (statisticians) for their technical contribution to the SU.VI.MAX study. We also thank all the volunteers of the SU.VI.MAX cohort.

This project has been conducted in the framework of the NACRe network (French network for Nutrition And Cancer Research, www.inra.fr/nacre).

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

FUNDING

Mélanie Deschasaux and Philippine Fassier were funded by PhD grants from the Cancéropôle Ile-de-France (public funding from the Paris region). The funders had no role in the design, implementation, analysis, or interpretation of the data.

REFERENCES

1. Bouvard V, Loomis D, Guyton KZ, Grosse Y, Ghissassi FE, Benbrahim-Tallaa L, Guha N, Mattock H, Straif K. Carcinogenicity of consumption of red and processed meat. Lancet Oncol. 2015; 16: 1599-1600.
2. WCRF/AICR. Systematic Literature Review - Continuous Update Project Report: The Associations between Food, Nutrition and Physical Activity and the Risk of Colorectal Cancer. 2011. Washington, DC: AIRC.
3. Latino-Martel P, Cottet V, Druesne-Pecollo N, Pierre FH, Touillaud M, Touvier M, Vasson M, Deschasaux M, Le Merdy J, Barrandon E, Ancellin R. Alcoholic beverages, obesity, physical activity and other nutritional factors, and cancer risk: a review of the evidence. Crit Rev Oncol Hematol. 2016; 99: 323.
4. Lippi G, Mattiuzzi C, Cervellin G. Meat consumption and cancer risk: a critical review of published meta-analyses. Crit Rev Oncol Hematol. 2016; 97: 1-14.
5. Alexander DD, Morimoto LM, Mink PJ, Cushing CA. A review and meta-analysis of red and processed meat consumption and breast cancer. Nutr Res Rev. 2010; 23: 349-365.
6. Pouchieu C, Deschasaux M, Hercberg S, Druesne-Pecollo N, Latino-Martel P, Touvier M. Prospective association between red and processed meat intakes and breast cancer risk: modulation by an antioxidant supplementation in the SU.VI.MAX randomized controlled trial. Int J Epidemiol. 2014; 43: 1583-1592.
7. Bastide NM, Pierre FH, Corpet DE. Heme iron from meat and risk of colorectal cancer: a meta-analysis and a review of the mechanisms involved. Cancer Prev Res (Phila). 2011; 4: 177-184.
8. Bastide NM, Chenni F, Audebert M, Santarelli RL, Tache S, Naud N, Baradat M, Jouanin I, Surya R, Hobbs DA, Kuhnle GG, Raymond-Letron I, Gueraud F et al. A central role for heme iron in colon carcinogenesis associated with red meat intake. Cancer Res. 2015; 75: 870-879.
9. Kabat GC, Rohan TE. Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis. Cancer Causes Control. 2007; 18: 1047-1053.
10. Tappel A. Heme of consumed red meat can act as a catalyst of oxidative damage and could initiate colon, breast and prostate cancers, heart disease and other diseases. Med Hypotheses. 2007; 68: 562-564.

11. Dalleau S, Baradat M, Gueraud F, Huc L. Cell death and diseases related to oxidative stress: 4-hydroxynonenal (HNE) in the balance. Cell Death Differ. 2013; 20: 1615-1630.

12. Awada M, Soulage CO, Meynier A, Debard C, Plaisancie P, Benoit B, Picard G, Loizon E, Chauvin MA, Estienne M, Peretti N, Guichardant M, Lagarde M et al. Dietary oxidized n-3 PUFA induce oxidative stress and inflammation: role of intestinal absorption of 4-HHE and reactivity in intestinal cells. J Lipid Res. 2012; 53: 2069-2080.

13. WCRF/AICR. Systematic Literature Review - Continuous Update Project Report: The Associations between Food, Nutrition and Physical Activity and the Risk of Breast Cancer. 2010. Washington, DC: AICR.

14. Farvid MS, Cho E, Chen WY, Eliassen AH, Willett WC. Adolescent meat intake and breast cancer risk. Int J Cancer. 2015; 136: 1909-1920.

15. Inoue-Choi M, Sinha R, Gierach GL, Ward MH. Red and processed meat, nitrite, and heme iron intakes and postmenopausal breast cancer risk in the NIH-AARP Diet and Health Study. Int J Cancer. 2015.

16. Kabat GC, Cross AJ, Park Y, Schatzkin A, Hollenbeck AR, Rohan TE, Sinha R. Intakes of dietary iron and heme-iron and risk of postmenopausal breast cancer in the National Institutes of Health-AARP Diet and Health Study. Am J Clin Nutr. 2010; 92: 1478-1483.

17. Ferrucci LM, Cross AJ, Graubard BI, Brinton LA, McCarty CA, Ziegler RG, Ma X, Mayne ST, Sinha R. Intake of meat, meat mutagens, and iron and the risk of breast cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Br J Cancer. 2009; 101: 178-184.

18. Kabat GC, Miller AB, Jain M, Rohan TE. Dietary iron and heme iron intake and risk of breast cancer: a prospective cohort study. Cancer Epidemiol Biomarkers Prev. 2007; 16: 1306-1308.

19. Gueraud F, Tache S, Steghens JP, Milkovic L, Borovic-Sunjic S, Zarkovic N, Gaultier E, Naud N, Helies-Toussaint C, Pierre F, Priymenko N. Dietary polyunsaturated fatty acids and heme iron induce oxidative stress biomarkers and a cancer promoting environment in the colon of rats. Free Radic Biol Med. 2015; 83: 192-200.

20. Gaur A, Collins H, Wulaningsih W, Holmberg L, Garmo H, Hammar N, Walldius G, Jungner I, Van HM. Iron metabolism and risk of cancer in the Swedish AMORIS study. Cancer Causes Control. 2013; 24: 1393-1402.

21. Wen CP, Lee JH, Tai YP, Wen C, Wu SB, Tsai MK, Hsieh DP, Chiang HC, Hsiung CA, Hsu CY, Wu X. High serum iron is associated with increased cancer risk. Cancer Res. 2014; 74: 6589-6597.

22. Bastide N, Morois S, Cadeau C, Kangas S, Serafini M, Gusto G, Dossus L, Pierre FH, Clavel-Chapelon F, Boutron-Ruault MC. Heme iron intake, dietary antioxidant capacity, and risk of colorectal adenomas in a large cohort study of French women. Cancer Epidemiol Biomarkers Prev. 2016.

23. Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis. 2000; 21: 361-370.

24. Gonenc A, Ozkan Y, Torun M, Simsek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. J Clin Pharm Ther. 2001; 26: 141-144.

25. Kanner J. Dietary advanced lipid oxidation endproducts are risk factors to human health. Mol Nutr Food Res. 2007; 51: 1094-1101.

26. Vulcain E, Goupy P, Caris-Veyrat C, Dangles O. Inhibition of the metmyoglobin-induced peroxidation of linoleic acid by dietary antioxidants: Action in the aqueous vs. lipid phase. Free Radic Res. 2005; 39: 547-563.

27. Pierre FH, Martin OC, Santarelli RL, Tache S, Naud N, Gueraud F, Audebert M, Dupuy J, Meunier N, Attaix D, Vendeuvre JL, Mirvish SS, Kuhnle GC et al. Calcium and alpha-tocopherol suppress cured-meat promotion of chemically induced colon carcinogenesis in rats and reduce associated biomarkers in human volunteers. Am J Clin Nutr. 2013; 98: 1255-1262.

28. Klouche K, Morena M, Canaud B, Descomps B, Beraud JJ, Cristol JP. Mechanism of in vitro heme-induced LDL oxidation: effects of antioxidants. Eur J Clin Invest. 2004; 34: 619-625.

29. Hercberg S, Preziosi P, Briancon S, Galan P, Triol I, Malvy D, Roussel AM, Favier A. A primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers in a general population: the SU.VI.MAX study--design, methods, and participant characteristics. SUpplementation en VItamines et Mineraux AntioXydants. Control Clin Trials. 1998; 19: 336-351.

30. Hercberg S, Galan P, Preziosi P, Bertrais S, Mennen L, Malvy D, Roussel AM, Favier A, Briancon S. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med. 2004; 164: 2335-2342.

31. Le Moullec N, Deheeger M, Preziosi P, Montero P, Valeix P, Rolland-Cachera M, Potier de Courcy G, Christides J, Galan P, Hercberg S. Validation du manuel photo utilisé pour l'enquête alimentaire de l'étude SU.VI.MAX. [Validation of the food portion size booklet used in the SU.VI.MAX study]. (in French). Cah Nutr Diet. 1996; 31: 158-164.

32. Hercberg S. Table de composition SU.VI.MAX des aliments. [SU.VI.MAX food composition table]. (in French). Paris: Les éditions INSERM/Economica; 2005.

33. WHO. ICD-10, International classification of diseases and related health problems. 10th revision. 2010. Geneva, Switzerland: World Health Organization.

International Journal of
Molecular Sciences

MDPI

Review

TGFβ Controls Ovarian Cancer Cell Proliferation

Elisenda Alsina-Sanchís [1,2], Agnès Figueras [1,2], Alvaro Lahiguera [1,2], Marta Gil-Martín [2,3], Beatriz Pardo [2,3], Josep M. Piulats [2,3], Lola Martí [2,4], Jordi Ponce [2,4], Xavier Matias-Guiu [2,5], August Vidal [2,5,6,7], Alberto Villanueva [1,2,6] and Francesc Viñals [1,2,8,*]

1 Program Against Cancer Therapeutic Resistance (ProCURE), Institut Català d'Oncologia, Hospital Duran i Reynals, L'Hospitalet de Llobregat, 08908 Barcelona, Spain; elisenda.as@gmail.com (E.A.-S.); afigueras@iconcologia.net (A.F.); alvarolahiguerabelenguer@gmail.com (A.L.); avillanueva@iconcologia.net (A.V.)
2 Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), L'Hospitalet de Llobregat, 08908 Barcelona, Spain; mgilmartin@iconcologia.net (M.G.-M.); bpardo@iconcologia.net (B.P.); jmpiulats@iconcologia.net (J.M.P.); lmarti@csub.scs.es (L.M.); jponce@bellvitgehospital.cat (J.P.); fjmatiasguiu.lleida.ics@gencat.cat (X.M.-G.); avidal@bellvitgehospital.cat (A.V.)
3 Medical Oncology Department, Institut Català d'Oncologia, Hospital Duran i Reynals, IDIBELL, L'Hospitalet de Llobregat, 08908 Barcelona, Spain
4 Gynaecologic Department, Bellvitge University Hospital, L'Hospitalet de Llobregat, 08908 Barcelona, Spain
5 Servei d'Anatomia Patològica, Hospital Universitari de Bellvitge, CIBERONC, L'Hospitalet de Llobregat, 08908 Barcelona, Spain
6 Xenopat, Carrer de la Feixa Llarga S/N, L'Hospitalet de Llobregat, 08907 Barcelona, Spain
7 Departament de Patologia i Terapèutica Experimental, Universitat de Barcelona, L'Hospitalet de Llobregat, 08908 Barcelona, Spain
8 Departament de Ciències Fisiològiques, Universitat de Barcelona, L'Hospitalet de Llobregat, 08908 Barcelona, Spain
* Correspondence: fvinyals@iconcologia.net

Received: 7 June 2017; Accepted: 24 July 2017; Published: 30 July 2017

Abstract: There have been no major improvements in the overall survival of ovarian cancer patients in recent decades. Even though more accurate surgery and more effective treatments are available, the mortality rate remains high. Given the differences in origin and the heterogeneity of these tumors, research to elucidate the signaling pathways involved is required. The Transforming Growth Factor (TGFβ) family controls different cellular responses in development and cell homeostasis. Disruption of TGFβ signaling has been implicated in many cancers, including ovarian cancer. This article considers the involvement of TGFβ in ovarian cancer progression, and reviews the various mechanisms that enable the TGFβ signaling pathway to control ovarian cancer cell proliferation. These mechanistic explanations support the therapeutic use of TGFβ inhibitors in ovarian cancer, which are currently in the early phases of development.

Keywords: TGFβ; ovarian cancer; IGF1R; proliferation

1. Ovarian Cancer

Ovarian cancer has the second highest incidence of gynecological cancers (≈6 per 100,000 individuals) and is the fifth most common cause of cancer deaths in women in western countries [1,2]. Despite the significant advances in detection, surgical techniques and treatments, diagnosis is generally made at an advanced stage and its mortality rate has remained fairly static in recent years. Although early-stage disease has a good prognosis, most patients relapse after first-line treatment, for which carboplatin-paclitaxel is the standard of care. The global five-year survival rate is 42% for early-stage disease, dropping to 29% in advanced-stage disease [3].

One of the reasons for this low survival rate is the advanced stage at time of diagnosis with disseminated peritoneal disease. Another is the diversity of tumor types classified as ovarian cancer on the basis of their common anatomical location. In fact, ovarian tumor subtypes are essentially different diseases and their histological and molecular characteristics are remarkably heterogeneous. Thus, ovarian cancer can be divided into germ cell (3%), sex-cord stroma (2%) and epithelial (95%) tumors. Moreover, epithelial tumors can be subdivided into five main histotypes entirely on the basis of their tumor cell morphology, according to the predominant pattern of differentiation: low-grade serous carcinoma (LGSC, prevalence less than 5%), high-grade serous carcinoma (HGSC, 68%), endometrioid carcinoma (EMC, 20%), clear-cell carcinoma (CCC, 4%) and mucinous carcinoma (MC, 3%) [4]. Despite these differences, the same treatment is employed for all histological subtypes of ovarian cancer [5]. In order to increase the ovarian cancer survival rate, it is necessary to study the carcinogenesis process in all ovarian cancer types. One of the approaches is to identify the involvement of different signaling pathways in the transformation process of the various ovarian tumor types. Of these signaling pathways, TGFβ signaling could play an important role in ovarian tumor progression.

2. The Highly Discrepant Literature about the Origin of Ovarian Cancer

An ovarian carcinoma can originate from various cell types, giving rise to different tumor subtypes. Germ cells, which develop into germ cell tumors (dysgerminomas, yolk sac tumors and immune teratomas), represent only 3% of all ovarian cancers, while sex cord-stromal tumors (1–2% of ovarian cancers) are generated from granulosa-theca cells, which produce estrogen and progesterone. Nevertheless, more than 90% of ovarian tumors are localized on the epithelial surface, have an epithelial histology and are therefore called epithelial ovarian cancers (EOCs).

The cell of origin in the case of epithelial ovarian tumors is a controversial topic, and several theories have been proposed. It was originally widely believed that these tumors originated from the ovarian surface epithelium (OSE) and differentiated into the various tumor histological subtypes. Another theory was that the ovarian tumors were derived from a Müllerian-type tissue (columnar epithelium, often ciliated) in the paraovarian and paratubal locations [6]. Although an ovarian origin cannot be discounted, new evidence demonstrates that some supposedly primary ovarian cancers actually originate in other pelvic organs, such as the digestive tract, and only secondarily in the ovary. This is the case for the majority of mucinous carcinomas, which are metastases from extraovarian sites [7–9].

In recent years, it has been proposed that some EOCs originate from precursor epithelial lesions in the distal, fimbriated end of the Fallopian tube [10–13]. Other ovarian tumor types originate from ovarian endometriosis, through the process of retrograde menstruation and endometriosis generated after neoplastic transformation of tubal origin [9,13–15]. Recently, Eckert and colleagues analyzed the genetics of different HGSC samples and discovered that cancer cells located in the Fallopian tube, which were considered to have initiated the ovarian cancers, were actually metastases of the ovarian tumor [16]. These discoveries call into question the theory of the precursor lesions in the fimbriated Fallopian tube, at least in this cancer type. Therefore, more research has to be done in order to gain a deeper understanding of the mechanism causing these morphological transformations during cancer progression, and thereby to develop a tool that improves the chances of better detection and treatment.

At the genetic level, the p53 gene has been implicated in the formation of EOC. This was one of the first mutations to be observed, and is the most prevalent in this tumor type, and in retinoblastoma (Rb) [17,18]. It has also been demonstrated that, in the case of clear cell carcinoma, concomitant mutations of ARID1A and PIK3CA are necessary to initiate tumor formation [19]. Other signaling pathways implicated include that for the receptor tyrosine kinases c-Met and Ron, which are thought to play a role in ovarian cancer initiation and progression [20]. Thus, it is important to identify the main signaling pathways in normal ovarian cells, and to determine how they are altered to give rise to ovarian cancer.

3. TGFβ Transforming Growth Factor) Member Signaling Occurs in Normal Ovary

Members of the TGFβ superfamily are key factors in follicle development, regulating bi-directional communication between ovarian cell types (oocyte, granulosa or theca cells) [21]. TGFβ superfamily ligands bind to TGFβ receptors type I (TGFβRI) and type II (TGFβRII), transmembrane serine-threonine kinases specific for each ligand. After ligand binding, both receptors form a heterodimeric complex in which type II receptor phosphorylates and activates the type I component. In turn, active type I receptor phosphorylates SMA and mothers against decapentaplegic homologs (SMADs), transcription factors that translocate to the nucleus where they regulate the expression of target genes in collaboration with other transcriptional partners. TGFβ superfamily members, such as activins and bone morphogenetic proteins (BMP) members, have been implicated in mammal ovary functionality during oocyte maturation and in regulating follicle development [22,23]. In contrast, less is known about other TGFβ family members, such as the TGFβ sub-family. All three TGFβ ligand isoforms (TGFβ 1, 2 and 3) have been detected in normal ovarian epithelium [24], although little is known about their functionality. We have observed by immunodetection of active SMAD2 (phosphorylated and accumulated in the nuclei) in paraffin-embedded samples that the TGFβ signaling pathway is active in normal Fallopian tube epithelium (Figure 1A) [25]. Li and colleagues observed that SMAD2 and SMAD3 are essential for normal follicle development and oocyte maturation in order to produce developmental competence [26]. In consequence, the TGFβ superfamily is a fundamental component of a key signaling pathway in normal ovarian cells that could also be important in ovarian cancer when it is dysregulated.

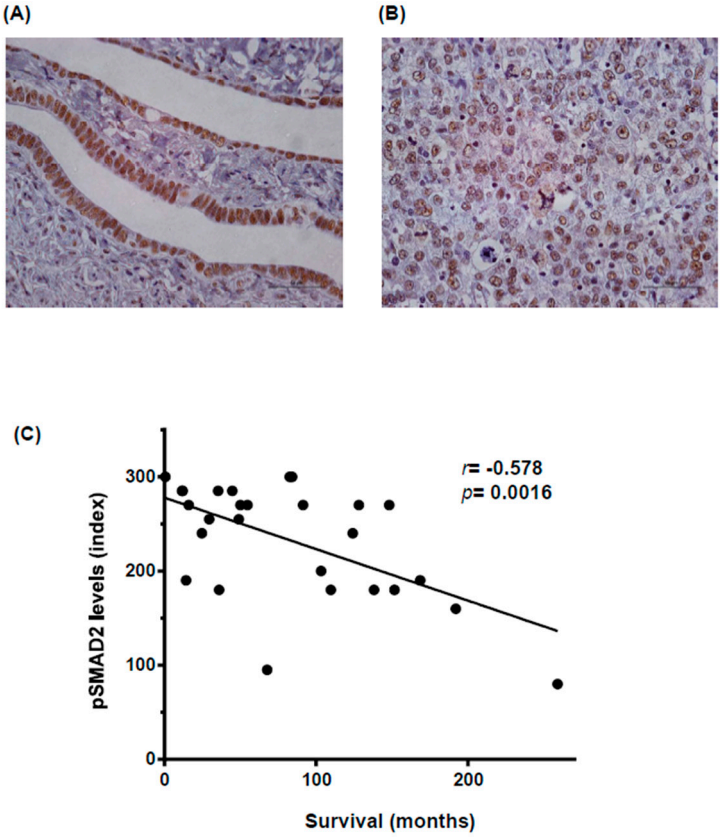


Figure 1. The level of phosphorylated and active SMAD2 (pSMAD2) staining is correlated with poor patient outcome. (**A**) pSMAD2 staining of normal human Fallopian tube epithelium. 400×, bar 100 µm. Staining was performed as previously described [25]; (**B**) pSMAD2 staining of a high-grade serous human tumor. 400×, bar 100 µm. Staining was performed as previously described [25]; (**C**) Correlation

between pSMAD2 levels in tissue microarray from high-grade serous ovarian patients and overall patient survival. The tissue microarray (TMA) comprised triplet cores from ovarian tumors resected between 1992 and 2007 at the Bellvitge Hospital (Barcelona, Spain). The study protocol was cleared by the hospital's Ethics Committee and signed informed consent was obtained from each patient. A total of 27 high-degree serous paraffin-embedded epithelial ovarian tumor specimens were represented and available for analysis on the TMA. All patients were treated using primary surgery and samples were collected before any radiotherapy or chemotherapy. This study included patients aged 30–88 years, with 79% aged 50–70 years. We observed no segregation due to age in our parameters. The Pearson correlation coefficient was used for statistical analysis.

4. TGF-β and Ovarian Cancer

A key objective of ovarian cancer research is to determine which signaling pathways are involved in its progression, with the aim of finding new therapies to reduce its high relapse rate. Focusing on the TGFβ family members, in granulosa cells depletion of FOXO1/3 and PTEN increase levels of activin (INHβB) and elevated phosphorylation/activation of SMAD2/3, effects that prevent differentiation and promote granulosa cell proliferation and tumor formation [27]. Another example of the involvement of the TGFβ family in ovarian cancer development is BMP/SMAD1/5/8 signaling, whereby double *SMAD1* and *SMAD5* or triple *SMAD1, 5* and *8* conditional knockout in mice generates metastatic granulosa cell tumors [28]. Recent work by our group highlights the TGFβ signaling pathway as a key contributor to this progression [25]. Thus treatment with a TGFβRI&II dual inhibitor, LY2109761, inhibits ovarian cancer cell proliferation and causes a reduction in tumor size. Our results indicate the presence of high levels of nuclei stained with active phosphoSMAD2 in tumoral cells ([25] and Figure 1B).

TGFβ signaling is important in a wide range of cellular processes from the physiological and pathological points of view. It is widely believed that TGFβ switches its role from tumor suppressor in normal cells to tumor promoter in advanced cancers, favoring invasiveness and metastasis depending on the tumor stage [29]. While TGFβ blocks cell growth in normal ovarian epithelial cells, in 40% of ovarian carcinomas TGFβ loses its cytostatic effect but maintains epithelial mesenchymal transition (EMT) induction and the production of extracellular matrix [30]. This loss of the TGFβ cytostatic effect could be due to mutations in important genes in its pathway. Unlike other tumor types, inactivating mutations in the TGFβ signaling pathway in ovarian cancer are rare and most of those that have been found are associated with chromosomal instability [31]. In the case of SMAD4, its mutations are not observed in ovarian tumors, but in ovarian cancer cell lines with metastatic potential. Its expression was reduced simultaneously with the dysregulation of p21 and c-Myc expression in ovarian tumor samples [31]. Furthermore, an allele of *TGFBR1* has been linked with a high-frequency and low-penetrance tumor susceptibility allele that predisposes to ovarian, breast and colorectal cancer, as well as to hematological malignancies [32].

Even though not many mutations are known in ovarian cancer cells, it is clear that the TGFβ signaling pathway is broadly active in ovarian cancer, as observed by high levels of pSMAD2 staining in different ovarian tumor types, and that its stimulation is important for ovarian cancer progression [25]. To confirm these results, we studied pSMAD2 expression in 27 human high-grade serous ovarian cancer patient samples and correlated its levels with overall survival. As shown in Figure 1C, a high level of pSMAD2 staining was significantly correlated with shorter survival in these patients. Our results concord with those of other studies obtained from independent advanced high-grade serous ovarian cancer patient series, in which it has also been described that a high level of pSMAD2 staining is correlated with poor patient outcome [33,34].

Mechanisms for activating the TGFβ pathway in tumors include overexpression of microRNA-181a, repression of the negative regulator SMAD7 [33] and the autocrine/paracrine secretion of TGFβ family members by tumoral or stromal cells [35,36]. There are three isoforms of the TGFβ sub-family ligands, TGFβ1, TGFβ2 and TGFβ3, which share the same receptor complex

and signal in similar ways, but vary in expression levels depending on the tissue. All three isoforms have been observed in ovarian cancer patient samples [37,38] and linked to increased ovarian cancer progression and metastasis [38,39]. In fact, a low level of TGFβ1 mRNA expression in advanced ovarian tumors was associated with better prognosis [40]. A skin carcinogenesis study suggested differential functions for each TGFβ isoform in epidermal carcinogenesis: TGFβ1 was associated with a more differentiated state, TGFβ2 was associated with highly malignant and invading cells, and TGFβ3 was linked to tumor stroma [41]. In addition, TGFβ isoforms are differentially expressed by OSE cells, and TGFβ seems to play an important role in regulating epithelial cell homeostasis and possibly stromal–OSE interactions [24]. Therefore, more work needs to be done to establish which TGFβ ligand is playing a role in tumor progression, whether there are differences between tumor and stromal cell types, and the implications of each TGFβ ligand for ovarian cancer progression. In any case, the TGFβ signaling pathway is highly activated in ovarian tumors reinforcing the idea of its potential importance in ovarian cancer.

5. TGFβ Controls Proliferation of Ovarian Cancer Cells

TGFβ blocks cell growth in normal ovarian epithelial cells but its effect on ovarian cancer cells remains controversial. For instance, it was demonstrated that proliferation was not inhibited after the addition of TGFβ in primary ovarian carcinoma cells, in contrast with its inhibitory effect on normal human OSE cells [42]. In two ovarian carcinoma cell types (OVCCRI and IGROVI) a different effect of TGFβ has been observed, in which TGFβ1 could induce cell cycle arrest at the G1/S transition in OVCCRI but not in IGROVI cells. The conclusion from this is that TGFβ growth inhibition is not a general feature of all ovarian cancer cells [43].

The mechanism responsible for the block of the anti-proliferative action of TGF-β remains unclear. The involvement of different components of the TGFβ signaling pathway has been examined in tissues of epithelial ovarian cancer patients and ovarian tumoral cell lines [44]. No modifications of TGFβ1 levels, its receptors or SMAD2/3 proteins were observed in either case. Therefore, the authors proposed that a failure had arisen in the control of the cell cycle by downstream molecules of the TGFβ signaling cascade. Likewise, Baldwin and colleagues concluded that TGFβ signaling remained functional, with the correct induction of some gene responses in primary ovarian carcinoma cells [42]. In contrast, other TGFβ-induced responses, such as the induction of c-Myc, were lost when they compared ovarian cancer cells and the human ovarian surface epithelium cells, in parallel with the failure to block the cell cycle [42].

Recent work by our group has demonstrated that TGFβ positively controls ovarian cancer proliferation through the control of insulin like growth factor 1 receptor (IGF1R) expression levels in some orthotopic mouse models (PDX) and ovarian cancer cell lines [25]. We also found a correlation between the levels of pSMAD2 and of IGF1R expression in the same patient tumor. IGF1R is a tyrosine kinase receptor already implicated in the control of ovarian cancer cell proliferation [45]. Similar indirect mechanisms of control of cell growth and proliferation by TGFβ through other growth factors have been described, for example, in glioma models, where TGFβ stimulates production of platelet derived growth factor-B (PDGF-B) and activation of platelet derived growth factor receptor β (PDGFRβ) [46]. Another mechanism involved is the expression of epidermal growth factor (EGF), which inhibits the TGFβ anti-proliferative effect in primary ovarian cancer cells [47]. Recently, androgens have been linked to the control of proliferation by TGFβ. Directly, or as a consequence of an androgen-induced reduction in TGFβ receptors, these cause the inhibition of a TGFβ anti-proliferative response [44]. Furthermore, ubiquitin specific protease 22 (USP22), high levels of which are associated with EOC and poor prognosis, have been shown to regulate the cell cycle pathway downstream of TGFβ1, consequently stimulating ovarian cancer cell proliferation [48]. It is not the first time that deubiquitinating enzymes (DUBs) have been found to regulate TGFβ signaling in order to control proliferation and other cellular processes. These include ubiquitin-specific peptidase 15 (USP15) in

glioblastoma [49], USP11 in the TGFβ-induced EMT process [50], and ubiquitin-specific protease 4 (USP4) that participates in the crosstalk between the TGFβ and AKT signaling pathways [51].

Together, these results lead to the proposition that TGFβ signaling controls cell proliferation through distinct, direct or indirect mechanisms in ovarian cancer.

6. Therapeutic Approaches

Although progress has been made in the treatment of ovarian cancer by way of improved surgical debulking techniques and the introduction of platinum-taxane regimens, the overall five-year survival rate is only 29% in advanced-stage disease [3]. Furthermore, 80% of advanced stages will relapse, mainly in the first 18–24 months, after primary treatment. The efficacy of chemotherapy in EOCs is limited, and although most patients show an initial response to treatment, upon relapse, this platinum response rate progressively declines, and ultimately disappears [52,53]. These reasons illustrate the great need for novel therapeutic strategies to overcome platinum resistance. Subsequently, therapeutic targeting of the TGFβ pathway in ovarian tumors should be one of the options to be tested. Some publications have already demonstrated the effectiveness of this treatment in ovarian cancer. For example, a pre-clinical study by Liao and colleagues showed blocked tumor growth in an SK-OV3 cell line transfected with nanoparticle-mediated soluble extracellular domain of the transforming growth factor-β type II receptor (sTGFβRII) [54]. Our recent work concluded that TGFβ inhibition blocked tumor growth in pre-clinical orthotopic models of ovarian cancer (PDX) [25]. It has recently been reported that a combination of a TGFβ inhibitor and cisplatinum in ovarian cancer cell lines had a stronger anti-proliferative effect than the additive effects of each treatment alone, and promoted tumor regression in established parental and resistant ovarian cancer xenograft models [55]. Thus, inhibition of the TGFβ pathway may enhance the treatment benefit of cisplatinum, which is the current standard treatment for ovarian cancer patients.

Some clinical trials blocking the TGFβ signaling pathway in ovarian cancer are being evaluated [56]. For example, a phase II study of high-risk stage III/IV ovarian cancer is underway that features an adjuvant FANG™ vaccine, which downregulates TGFβ1 and 2. Two clinical studies are being conducted in advanced solid tumors: a phase I study of anti-TGFβRII monoclonal antibody IMC-TR1 (LY3022859) in patients with advanced solid tumors, and a phase I trial with the TGFβ pathway inhibitor TEW 7197 in subjects with refractory solid tumors. Some TGFβ inhibitors are already at the late stages of disease-specific clinical trials: phase I/II in combination with radiotherapy and fresolimumab (TGFβ inhibitor) in non-small cell lung cancer and metastatic breast cancer. There is also a phase III trial in glioblastoma with trabedersen (AP-12009), another with galunisertib (LY2157299) [57], which is under clinical development in phase II studies of hepatocellular carcinoma, and phase I trials in glioblastoma, hepatocellular carcinoma, pancreatic cancer and non-small cell lung cancer.

7. Concluding Remarks

TGFβ signaling seems to play a role in ovarian physiology as well as acting as a tumor promoter that controls proliferation in ovarian cancer. Although mutations in this pathway are rare in this tumor, there are other mechanisms by which TGFβ, directly or indirectly, is associated with the promotion of ovarian cancer cell proliferation. Further investigation and progress in delineating the mechanisms involved in every specific ovarian tumor subtype is essential, given the heterogeneity of ovarian cancer at the molecular level.

A therapeutic approach blocking TGFβ signaling in ovarian cancer would provide an opportunity for these patients that takes into account the role that TGFβ plays in ovarian cancer proliferation. A better knowledge of the molecular mechanisms is essential if we are to be able to provide optimal patient stratification for these clinical assays. It is known that not all patients will respond in the same way to some target therapies and, in the case of ovarian cancer, it is even more difficult, since there are different histological subtypes with variable molecular characteristics.

Acknowledgments: This study was supported by research grants to Francesc Viñals from the Spanish Ministerio de Economía y Competitividad (SAF2013-46063R), the Spanish Institute of Health Carlos III (ISCIII) and the European Regional Development Fund (ERDF) under the Integrated Project of Excellence no. PIE13/00022 (ONCOPROFILE), and the Generalitat de Catalunya (2014SGR364). Work was supported by the Xarxa de Bancs de Tumors de Catalunya, sponsored by Pla Director d'Oncología de Catalunya (XBTC), IDIBELL and PLATAFORMA BIOBANCOS PT13/0010/0013. EAS is a recipient of a predoctoral fellowship from the Ministerio de Economía y Competitividad.

Author Contributions: Elisenda Alsina-Sanchís, Agnès Figueras and Alvaro Lahiguera performed the experiments; Marta Gil-Martín, Beatriz Pardo, Josep M. Piulats, Lola Martí, Jordi Ponce, Xavier Matias-Guiu and Alberto Villanueva acquired data and managed patients; Elisenda Alsina-Sanchís, Marta Gil-Martín, Josep M. Piulats, August Vidal and Francesc Viñals analyzed the data; Elisenda Alsina-Sanchís, Marta Gil-Martín and Francesc Viñals wrote the paper.

Conflicts of Interest: The Catalan Institute of Oncology received an unrestricted grant from Roche to finance the Pro Cure Programme (2015).

References

1. Berrino, F.; De Angelis, R.; Sant, M.; Rosso, S.; Bielska-Lasota, M.; Coebergh, J.W.; Santaquilani, M. Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995–99: Results of the eurocare-4 study. *Lancet Oncol.* **2007**, *8*, 773–783. [CrossRef]
2. Ferlay, J.; Parkin, D.M.; Steliarova-Foucher, E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur. J. Cancer* **2010**, *46*, 765–781. [CrossRef] [PubMed]
3. Oberaigner, W.; Minicozzi, P.; Bielska-Lasota, M.; Allemani, C.; de Angelis, R.; Mangone, L.; Sant, M. Survival for ovarian cancer in Europe: The across-country variation did not shrink in the past decade. *Acta. Oncol.* **2012**, *51*, 441–453. [CrossRef] [PubMed]
4. Kuhn, E.; Meeker, A.K.; Visvanathan, K.; Gross, A.L.; Wang, T.L.; Kurman, R.J.; Shih Ie, M. Telomere length in different histologic types of ovarian carcinoma with emphasis on clear cell carcinoma. *Mod. Pathol.* **2011**, *24*, 1139–1145. [CrossRef] [PubMed]
5. Stewart, B.W.; Kleihues, P. *World Cancer Report*; IARC Press: Lyon, France, 2003.
6. Dubeau, L. The cell of origin of ovarian epithelial tumours. *Lancet. Oncol.* **2008**, *9*, 1191–1197. [CrossRef]
7. Prat, J. Ovarian carcinomas: Five distinct diseases with different origins, genetic alterations, and clinicopathological features. *Virchows. Arch.* **2012**, *460*, 237–249. [CrossRef] [PubMed]
8. Kurman, R.J.; Shih Ie, M. The origin and pathogenesis of epithelial ovarian cancer: A proposed unifying theory. *Am. J. Surg. Pathol.* **2010**, *34*, 433–443. [CrossRef] [PubMed]
9. Karnezis, A.N.; Cho, K.R.; Gilks, C.B.; Pearce, C.L.; Huntsman, D.G. The disparate origins of ovarian cancers: Pathogenesis and prevention strategies. *Nat. Rev. Cancer* **2017**, *17*, 65–74. [CrossRef] [PubMed]
10. Medeiros, F.; Muto, M.G.; Lee, Y.; Elvin, J.A.; Callahan, M.J.; Feltmate, C.; Garber, J.E.; Cramer, D.W.; Crum, C.P. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am. J. Surg. Pathol.* **2006**, *30*, 230–236. [CrossRef] [PubMed]
11. Lee, Y.; Miron, A.; Drapkin, R.; Nucci, M.R.; Medeiros, F.; Saleemuddin, A.; Garber, J.; Birch, C.; Mou, H.; Gordon, R.W.; et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J. Pathol.* **2007**, *211*, 26–35. [CrossRef] [PubMed]
12. Karst, A.M.; Levanon, K.; Drapkin, R. Modeling high-grade serous ovarian carcinogenesis from the fallopian tube. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 7547–7552. [CrossRef] [PubMed]
13. Kurman, R.J.; Shih, Ie.M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer–shifting the paradigm. *Hum. Pathol.* **2011**, *42*, 918–931. [CrossRef] [PubMed]
14. Terada, T. Endometrioid adenocarcinoma of the ovary arising in atypical endometriosis. *Int. J. Clin. Exp. Pathol.* **2012**, *5*, 924–927. [PubMed]
15. Yuan Z, W.Y.; Cragun, J.M.; Chambers, S.K.; Zheng, W. Cell origin of endometriosis: Contribution by the fallopian tube epithelium. *Am. J. Clin. Exp. Obstet. Gynecol.* **2013**, *1*, 37–42.
16. Eckert, M.A.; Pan, S.; Hernandez, K.M.; Loth, R.M.; Andrade, J.; Volchenboum, S.L.; Faber, P.; Montag, A.; Lastra, R.; Peter, M.E.; et al. Genomics of ovarian cancer progression reveals diverse metastatic trajectories including intraepithelial metastasis to the fallopian tube. *Cancer Discov.* **2016**, *6*, 1342–1351. [CrossRef] [PubMed]

17. Bast, R.C., Jr.; Hennessy, B.; Mills, G.B. The biology of ovarian cancer: New opportunities for translation. *Nat. Rev. Cancer* **2009**, *9*, 415–428. [CrossRef] [PubMed]
18. Vaughan, S.; Coward, J.I.; Bast, R.C., Jr.; Berchuck, A.; Berek, J.S.; Brenton, J.D.; Coukos, G.; Crum, C.C.; Drapkin, R.; Etemadmoghadam, D.; et al. Rethinking ovarian cancer: Recommendations for improving outcomes. *Nat. Rev. Cancer* **2011**, *11*, 719–725. [CrossRef] [PubMed]
19. Chandler, R.L.; Damrauer, J.S.; Raab, J.R.; Schisler, J.C.; Wilkerson, M.D.; Didion, J.P.; Starmer, J.; Serber, D.; Yee, D.; Xiong, J.; et al. Coexistent ARID1A-PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling. *Nat. Commun.* **2015**, *6*, 6118. [CrossRef] [PubMed]
20. Moxley, K.M.; Wang, L.; Welm, A.L.; Bieniasz, M. Short-form Ron is a novel determinant of ovarian cancer initiation and progression. *Genes Cancer* **2016**, *7*, 169–181. [PubMed]
21. Knight, P.G.; Glister, C. TGF-β superfamily members and ovarian follicle development. *Reproduction* **2006**, *132*, 191–206. [CrossRef] [PubMed]
22. Reader, K.L.; Gold, E. Activins and activin antagonists in the human ovary and ovarian cancer. *Mol. Cell Endocrinol.* **2015**, *415*, 126–132. [CrossRef] [PubMed]
23. Chang, H.M.; Qiao, J.; Leung, P.C.K. Oocyte–somatic cell interactions in the human ovary—Novel role of bone morphogenetic proteins and growth differentiation factors. *Hum. Reprod. Update* **2017**, *23*, 1–18. [CrossRef] [PubMed]
24. Nilsson, E.; Doraiswamy, V.; Parrott, J.A.; Skinner, M.K. Expression and action of transforming growth factor β (TGFβ1, TGFβ2, TGFβ3) in normal bovine ovarian surface epithelium and implications for human ovarian cancer. *Mol. Cell Endocrinol.* **2001**, *182*, 145–155. [CrossRef]
25. Alsina-Sanchis, E.; Figueras, A.; Lahiguera, A.; Vidal, A.; Casanovas, O.; Graupera, M.; Villanueva, A.; Viñals, F. The TGFβ pathway stimulates ovarian cancer cell proliferation by increasing igf1r levels. *Int. J. Cancer* **2016**, *139*, 1894–1903. [CrossRef] [PubMed]
26. Li, Q.; Pangas, S.A.; Jorgez, C.J.; Graff, J.M.; Weinstein, M.; Matzuk, M.M. Redundant roles of SMAD2 and SMAD3 in ovarian granulosa cells in vivo. *Mol. Cell Biol.* **2008**, *28*, 7001–7011. [CrossRef] [PubMed]
27. Liu, Z.; Ren, Y.A.; Pangas, S.A.; Adams, J.; Zhou, W.; Castrillon, D.H.; Wilhelm, D.; Richards, J.S. Foxo1/3 and PTEN depletion in granulosa cells promotes ovarian granulosa cell tumor development. *Mol. Endocrinol.* **2015**, *29*, 1006–1024. [CrossRef] [PubMed]
28. Pangas, S.A.; Li, X.; Umans, L.; Zwijsen, A.; Huylebroeck, D.; Gutierrez, C.; Wang, D.; Martin, J.F.; Jamin, S.P.; Behringer, R.R.; et al. Conditional deletion of SMAD1 and SMAD5 in somatic cells of male and female gonads leads to metastatic tumor development in mice. *Mol. Cell Biol.* **2008**, *28*, 248–257. [CrossRef] [PubMed]
29. Tian, M.; Neil, J.R.; Schiemann, W.P. Transforming growth factor-β and the hallmarks of cancer. *Cell. Signal.* **2011**, *23*, 951–962. [CrossRef] [PubMed]
30. Helleman, J.; Jansen, M.P.; Burger, C.; van der Burg, M.E.; Berns, E.M. Integrated genomics of chemotherapy resistant ovarian cancer: A role for extracellular matrix, TGFβ and regulating micrornas. *Int. J. Biochem. Cell Biol.* **2010**, *42*, 25–30. [CrossRef] [PubMed]
31. Antony, M.L.; Nair, R.; Sebastian, P.; Karunagaran, D. Changes in expression, and/or mutations in TGF-β receptors (TGF-β RI and TGF-β RII) and SMAD 4 in human ovarian tumors. *J. Cancer Res. Clin. Oncol.* **2010**, *136*, 351–361. [CrossRef] [PubMed]
32. Kaklamani, V.G.; Hou, N.; Bian, Y.; Reich, J.; Offit, K.; Michel, L.S.; Rubinstein, W.S.; Rademaker, A.; Pasche, B. TGFBR1*6A and cancer risk: A meta-analysis of seven case-control studies. *J. Clin. Oncol.* **2003**, *21*, 3236–3243. [CrossRef] [PubMed]
33. Parikh, A.; Lee, C.; Joseph, P.; Marchini, S.; Baccarini, A.; Kolev, V.; Romualdi, C.; Fruscio, R.; Shah, H.; Wang, F.; et al. Microrna-181A has a critical role in ovarian cancer progression through the regulation of the epithelial-mesenchymal transition. *Nat. Commun.* **2014**, *5*, 2977. [CrossRef] [PubMed]
34. Petrillo, M.; Zannoni, G.F.; Beltrame, L.; Martinelli, E.; DiFeo, A.; Paracchini, L.; Craparotta, I.; Mannarino, L.; Vizzielli, G.; Scambia, G.; et al. Identification of high-grade serous ovarian cancer mirna species associated with survival and drug response in patients receiving neoadjuvant chemotherapy: A retrospective longitudinal analysis using matched tumor biopsies. *Ann. Oncol.* **2016**, *27*, 625–634. [CrossRef] [PubMed]
35. Henriksen, R.; Gobl, A.; Wilander, E.; Oberg, K.; Miyazono, K.; Funa, K. Expression and prognostic significance of TGF-β isotypes, latent TGF-β 1 binding protein, TGF-β type I and type II receptors, and endoglin in normal ovary and ovarian neoplasms. *Lab. Investig.* **1995**, *73*, 213–220. [PubMed]

36. Rodon, L.; Gonzalez-Junca, A.; Inda Mdel, M.; Sala-Hojman, A.; Martinez-Saez, E.; Seoane, J. Active creb1 promotes a malignant TGFβ2 autocrine loop in glioblastoma. *Cancer Discov.* **2014**, *4*, 1230–1241. [CrossRef] [PubMed]
37. Gordinier, M.E.; Zhang, H.Z.; Patenia, R.; Levy, L.B.; Atkinson, E.N.; Nash, M.A.; Katz, R.L.; Platsoucas, C.D.; Freedman, R.S. Quantitative analysis of transforming growth factor β 1 and 2 in ovarian carcinoma. *Clin. Cancer Res.* **1999**, *5*, 2498–2505. [PubMed]
38. Do, T.V.; Kubba, L.A.; Du, H.; Sturgis, C.D.; Woodruff, T.K. Transforming growth factor-β1, transforming growth factor-β2, and transforming growth factor-β3 enhance ovarian cancer metastatic potential by inducing a SMAD3-dependent epithelial-to-mesenchymal transition. *Mol. Cancer Res.* **2008**, *6*, 695–705. [CrossRef] [PubMed]
39. Gao, J.; Zhu, Y.; Nilsson, M.; Sundfeldt, K. TGF-β isoforms induce EMT independent migration of ovarian cancer cells. *Cancer Cell Int.* **2014**, *14*, 72. [CrossRef] [PubMed]
40. Komiyama, S.; Kurahashi, T.; Ishikawa, M.; Tanaka, K.; Komiyama, M.; Mikami, M.; Udagawa, Y. Expression of TGFSS1 and its receptors is associated with biological features of ovarian cancer and sensitivity to paclitaxel/carboplatin. *Oncol. Rep.* **2011**, *25*, 1131–1138. [CrossRef] [PubMed]
41. Gold, L.I.; Jussila, T.; Fusenig, N.E.; Stenback, F. TGF-β isoforms are differentially expressed in increasing malignant grades of HaCaT keratinocytes, suggesting separate roles in skin carcinogenesis. *J. Pathol.* **2000**, *190*, 579–588. [CrossRef]
42. Baldwin, R.L.; Tran, H.; Karlan, B.Y. Loss of c-Myc repression coincides with ovarian cancer resistance to transforming growth factor β growth arrest independent of transforming growth factor β/SMAD signaling. *Cancer Res.* **2003**, *63*, 1413–1419. [PubMed]
43. Jozan, S.; Guerrin, M.; Mazars, P.; Dutaur, M.; Monsarrat, B.; Cheutin, F.; Bugat, R.; Martel, P.; Valette, A. Transforming growth factor β 1 (TGF-β 1) inhibits growth of a human ovarian carcinoma cell line (ovccr1) and is expressed in human ovarian tumors. *Int. J. Cancer* **1992**, *52*, 766–770. [CrossRef] [PubMed]
44. Kohan-Ivani, K.; Gabler, F.; Selman, A.; Vega, M.; Romero, C. Role of dihydrotestosterone (dht) on TGF-β1 signaling pathway in epithelial ovarian cancer cells. *J. Cancer Res. Clin. Oncol.* **2016**, *142*, 47–58. [CrossRef] [PubMed]
45. Bruchim, I.; Werner, H. Targeting IGF-1 signaling pathways in gynecologic malignancies. *Expert. Opin. Ther. Targets* **2013**, *17*, 307–320. [CrossRef] [PubMed]
46. Bruna, A.; Darken, R.S.; Rojo, F.; Ocana, A.; Penuelas, S.; Arias, A.; Paris, R.; Tortosa, A.; Mora, J.; Baselga, J.; et al. High TGF β-SMAD activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the pdgf-b gene. *Cancer Cell.* **2007**, *11*, 147–160. [CrossRef] [PubMed]
47. Dunfield, L.D.; Nachtigal, M.W. Inhibition of the antiproliferative effect of TGFβ by EGF in primary human ovarian cancer cells. *Oncogene* **2003**, *22*, 4745–4751. [CrossRef] [PubMed]
48. Ji, M.; Shi, H.; Xie, Y.; Zhao, Z.; Li, S.; Chang, C.; Cheng, X.; Li, Y. Ubiquitin specific protease 22 promotes cell proliferation and tumor growth of epithelial ovarian cancer through synergy with transforming growth factor β1. *Oncol. Rep.* **2015**, *33*, 133–140. [CrossRef] [PubMed]
49. Eichhorn, P.J.; Rodon, L.; Gonzalez-Junca, A.; Dirac, A.; Gili, M.; Martinez-Saez, E.; Aura, C.; Barba, I.; Peg, V.; Prat, A.; et al. Usp15 stabilizes TGF-β receptor I and promotes oncogenesis through the activation of TGF-β signaling in glioblastoma. *Nat. Med.* **2012**, *18*, 429–435. [CrossRef] [PubMed]
50. Al-Salihi, M.A.; Herhaus, L.; Macartney, T.; Sapkota, G.P. Usp11 augments TGF β signalling by deubiquitylating Alk5. *Open Biol.* **2012**, *2*, 120063. [CrossRef] [PubMed]
51. Zhang, L.; Zhou, F.; Drabsch, Y.; Gao, R.; Snaar-Jagalska, B.E.; Mickanin, C.; Huang, H.; Sheppard, K.A.; Porter, J.A.; Lu, C.X.; et al. Usp4 is regulated by Akt phosphorylation and directly deubiquitylates TGF-β type I receptor. *Nat. Cell. Biol.* **2012**, *14*, 717–726. [CrossRef] [PubMed]
52. Galluzzi, L.; Senovilla, L.; Vitale, I.; Michels, J.; Martins, I.; Kepp, O.; Castedo, M.; Kroemer, G. Molecular mechanisms of cisplatin resistance. *Oncogene* **2012**, *31*, 1869–1883. [CrossRef] [PubMed]
53. Foley, O.W.; Rauh-Hain, J.A.; del Carmen, M.G. Recurrent epithelial ovarian cancer: An update on treatment. *Oncology (Williston Park)* **2013**, *27*, 288–294. [PubMed]
54. Liao, S.; Liu, J.; Lin, P.; Shi, T.; Jain, R.K.; Xu, L. TGF-β blockade controls ascites by preventing abnormalization of lymphatic vessels in orthotopic human ovarian carcinoma models. *Clin. Cancer Res.* **2011**, *17*, 1415–1424. [CrossRef] [PubMed]

55. Gao, Y.; Shan, N.; Zhao, C.; Wang, Y.; Xu, F.; Li, J.; Yu, X.; Gao, L.; Yi, Z. Ly2109761 enhances cisplatin antitumor activity in ovarian cancer cells. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 4923–4932. [PubMed]
56. Neuzillet, C.; Tijeras-Raballand, A.; Cohen, R.; Cros, J.; Faivre, S.; Raymond, E.; de Gramont, A. Targeting the TGFβ pathway for cancer therapy. *Pharmacol. Ther.* **2015**, *147*, 22–31. [CrossRef] [PubMed]
57. Dituri, F.; Mazzocca, A.; Fernando, J.; Papappicco, P.; Fabregat, I.; De Santis, F.; Paradiso, A.; Sabbà, C.; Giannelli, G. Differential inhibition of the TGF-β signaling pathway in hcc cells using the small molecule inhibitor LY2157299 and the D10 monoclonal antibody against TGF-β receptor type II. *PLoS ONE* **2013**, *8*, e67109. [CrossRef]