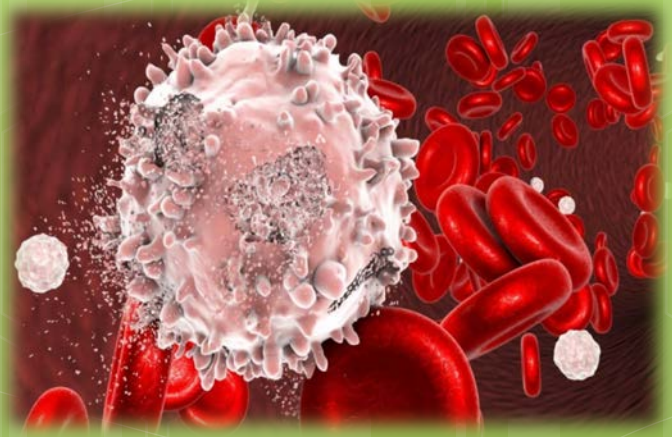

8th WHBA Summer School in Medical & Biosciences Research & Management
May 17-24, 2019
Neo Itilo, Mani Laconia & Athens, Greece

Olive oil polyphenols in cancer

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THE GERONTOLOGICAL SOCIETY OF AMERICA®

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doi:10.1093/gerona/glx227
Advance Access publication December 13, 2017

OXFORD

Translational Section
The Mediterranean Diet
Review in Depth

Health Benefits of the Mediterranean Diet: Metabolic and Molecular Mechanisms

Valeria Tosti, MD,[1] Beatrice Bertozzi, PhD,[1] and Luigi Fontana, MD, PhD[1,2]

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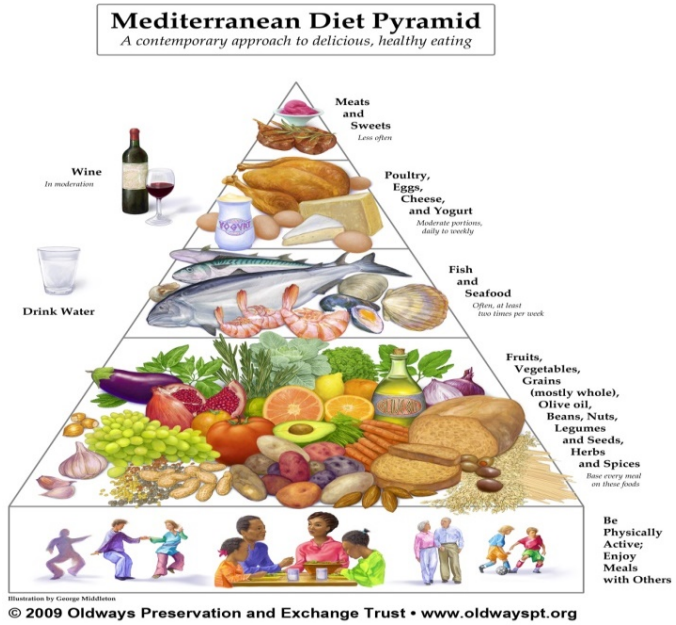

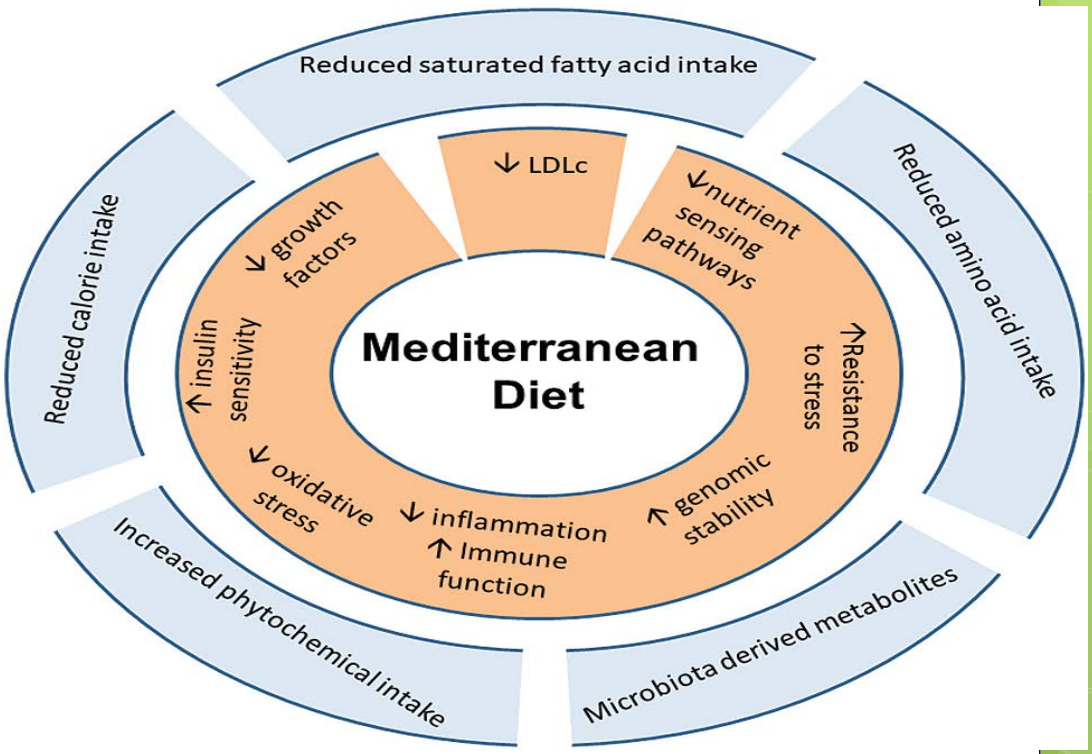

The effectors of the Mediterranean Diet, including reduced saturated fatty acid intake, reduced amino acid and calorie intake, increased phytochemical intake, and microbiota derived metabolites

CANCER GENOMICS & PROTEOMICS *14*: 403-408 (2017)
doi:10.21873/cgp.20050

Impact of Mediterranean Diet on Cancer: Focused Literature Review

YORAM BARAK[1] and DANA FRIDMAN[2]

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[2]*School of Design, Victoria University of Wellington, Wellington, New Zealand*

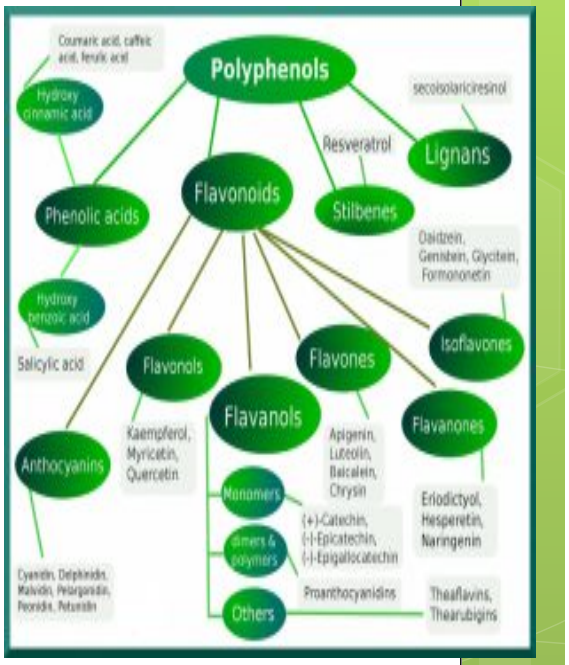
Table I. *Summary of studies reviewed.*

References	Study design	Sample size: cases and controls	Cancer type	Outcome	Comments
Stojanovic *et al.* (24)	Case-Control	446	Gastric	OR: 0.70	
van den Brandt & Schulpen (14)	Case-Cohort	3,986	Breast	OR: 0.94 NS	Significant effect for ER negative tumors
Molina-Montes *et al.* (13)	Prospective cohort study	865	Pancreatic	HR: 0.99 NS	EPIC study
Turati *et al.* (25)	Case-Control	792	Nasopharyngeal	OR: 0.66	
Rosato *et al.* (26)	Case-Control	10,549	Colorectal	OR: 0.52	
Giraldi *et al.* (27)	Case-Control	933	Head and Neck	OR: 0.61	
Hodge *et al.* (28)	Case- Series	403	Lung	HR: 0.64	Especially for current smokers
Toledo *et al.* (29)	Case-Control	4,152	Breast	HR: 0.32	The PREDIMED study
Filomeno *et al.* (30)	Case-Control	5,079	Endometrial	OR: 0.43	
Castello *et al.* (31)	Case-Control	2034	Breast	OR: 0.56	Significant effect for ER negative tumors
Filomeno *et al.* (6)	Case-Control	2,846	Oral cavity and pharyngeal	OR: 0.20	
Mourouti *et al.* (32)	Case-Control	500	Breast	OR: 0.91	
Grosso *et al.* (33)	Case-Control	1014	Colorectal	OR: 0.46	
Praud *et al.* (34)	Case-Control	3627	Gastric	OR: 0.78	
Buckland *et al.* (35)	Prospective cohort study	477,312	Bladder	HR: 0.84	EPIC study
Kenfield *et al.* (36)	Prospective cohort study	4538	Prostate	HR: 0.78	
Idilbi *et al.* (37)	Case-Control	200	Overall cancers	OR: 0.4	
Bosetti *et al.* (38)	Case-Control	2,892	Pancreatic	OR: 0.57	
Bamia *et al.* (39)	Prospective cohort study	4355	Colorectal	HR: 0.89	EPIC study
Möller *et al.* (12)	Case-Control	2,590	Prostate	OR: 1.07 NS	
Buckland *et al.* (40)	Prospective cohort study	335,062	Breast	HR=0.94	EPIC study. Significant effect for ER negative tumors. Premenopausal women NS. Postmenopausal women (HR=0.80).
Kontou *et al.* (35)	Case-Control	500	Colorectal	OR: 0.87	
Kontou *et al.* (41)	Case-Control	500	Colorectal	OR: 0.89	
Cade *et al.* (42)	Cohort Study	33,731	Breast	HR: 0.65	
Trichopoulou *et al.* (7)	Prospective cohort study	14,807	Breast	HR=0.88	EPIC study. Premenopausal women (HR=1.01). Postmenopausal women (HR=0.78).
Samoli *et al.* (43)	Case-Control	433	Upper Aerodigestive Tract	OR: 0.70	
Buckland *et al.* (9)	Prospective cohort study	485,044	Gastric	HR: 0.67	EPIC study
Benetou *et al.* (3)	Prospective cohort study	25,623	Overall cancers	HR: 0.88	EPIC study
Dalvi, Alison & Pamela (11)	Case-Control	949	Endometrial	OR=1.4	

EPIC, European Prospective Investigation into Cancer and Nutrition. NS, Not statistically significant.

Olive Oil compounds

- **Extra virgin olive oil (EVOO):** virgin olive oil which has a **free acidity**, expressed as oleic acid, of not more than 0.8 grams per 100 grams, and the other characteristics of which correspond to those fixed for this category in the IOC standard.
- **Triacylglycerols** (98–99%). The predominant fatty acid present in olive oil TGAs is monounsaturated **oleic acid** (up to 83% w/w).
- EVOO also contains **palmitic acid, linoleic acid, stearic acid, and palmitoleic acid** making up the remainder of olive oil TGAs.
- There is a plethora of lipophilic or amphiphilic **microconstituents** present in virgin olive oil, among them, **phytosterols, squalene, tocopherols, phenolic compounds, terpenic acid derivatives, etc.**



Kiritsakis FPMA. Olive fruit and olive oil composition and their functional compounds. In: Shahidi F, Kiritsakis A, editors. Olives and Olive Oil as Functional Foods. Hoboken, New Jersey: John Wiley & sons. 2017

Olive Oil compounds

- Among the compounds of EVOO are the **lignans** like **taxifolin, luteolin, apigenin**, and other molecules
- In olive oil, the content of polyphenols ranges from 50 to 1000 mg/kg.
- EVOO contains simple phenols that include **tyrosol, hydroxytyrosol, and phenolic acids**

Olive Oil Polyphenols

- Another subgroup is the **secoiridoids** that are derivatives from tyrosol, hydroxytyrosol, and elenolic acid, like as 3,4-DHPEA-EDA or **oleacein** p-HPEA-EDA or **oleocanthal**.
- The secoiridoids subgroup includes also the **oleuropein and ligstroside aglycons** (3,4-DHPEA-EA, p-HPEA-EA,respectively) and their isoforms **oleomissional and oleokoronal**
- Phenolic compounds are mainly responsible for the **characteristic gustatory property of virgin olive oil, namely the bitter taste**.

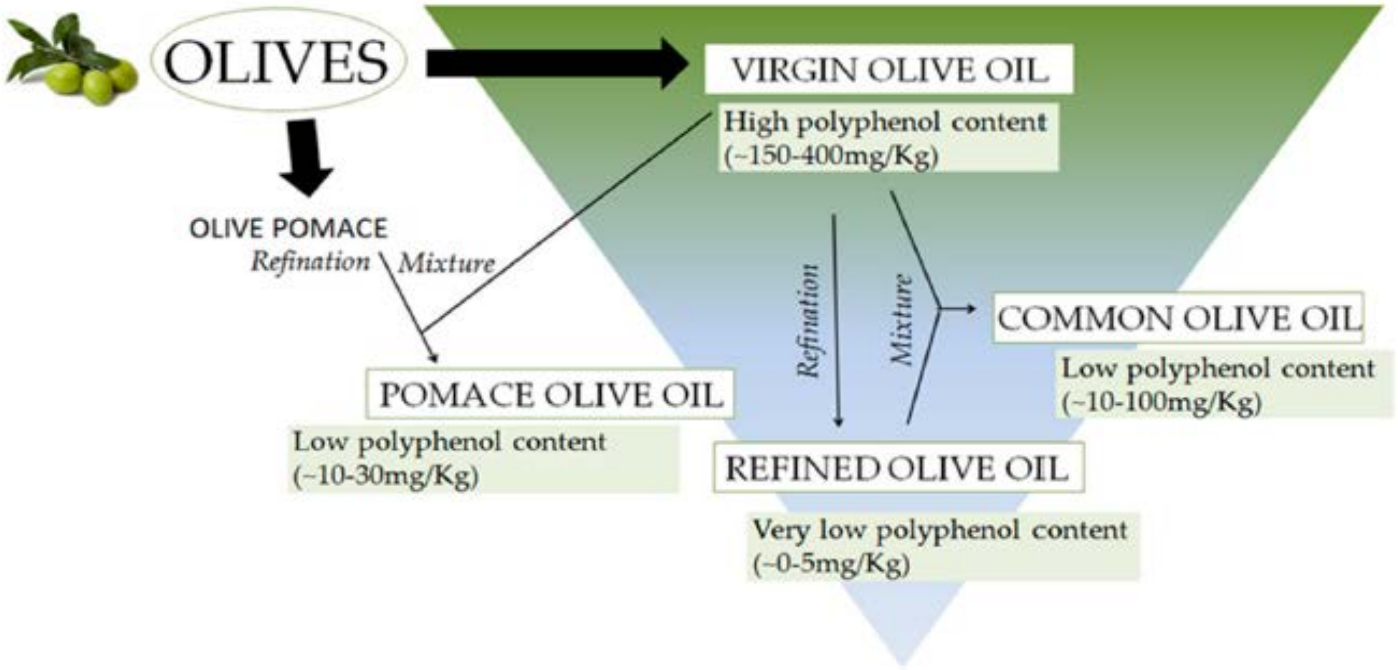
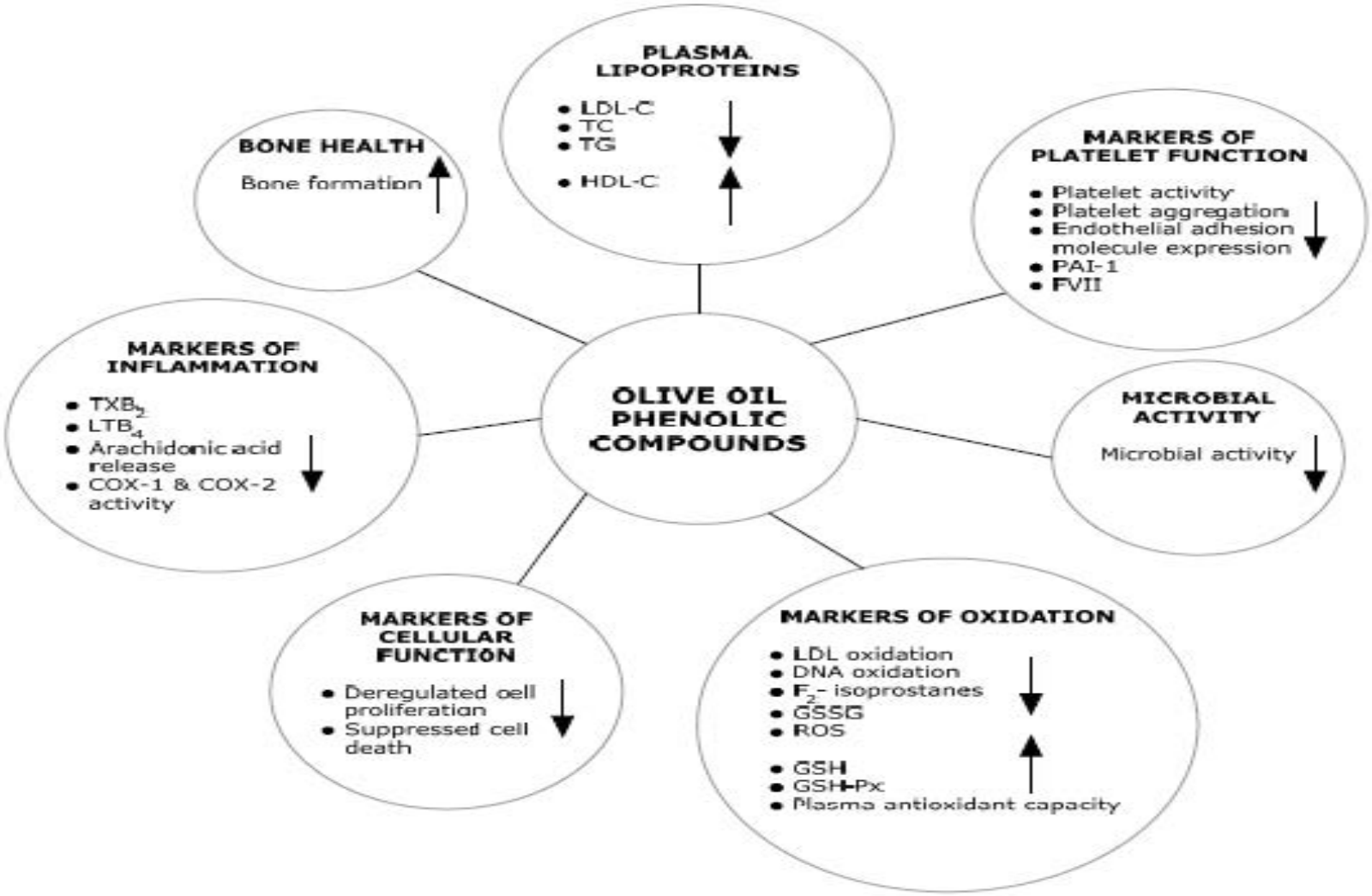


Figure 1. The concentration of polyphenols in differnet kinds of oilve oil depending on technological process of the oil extraction [25].

- The quantity and quality of polyphenols in olive oil is closely related to the process of olive milling and further processing.
- Therefore, virgin olive oils have substantially higher amounts of polyphenols than refined olive oils.
- As a matter of fact, it depends on the agronomic factors, the ripeness of olives, as well as extraction technology, along with storage or packaging processes.

Types of Olive Oil. Available online: http://www.nuproas.se/nuproas/olive/types-of-olive-oil/ (accesed on 26 February 2018.

Figure 1. Biological activities of olive oil phenolic compounds (adapted from Cicerale *et al.* [62]).



Int. J. Mol. Sci. **2010**, *11*, 458-479; doi:10.3390/ijms11020458

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Review

Biological Activities of Phenolic Compounds Present in Virgin Olive Oil

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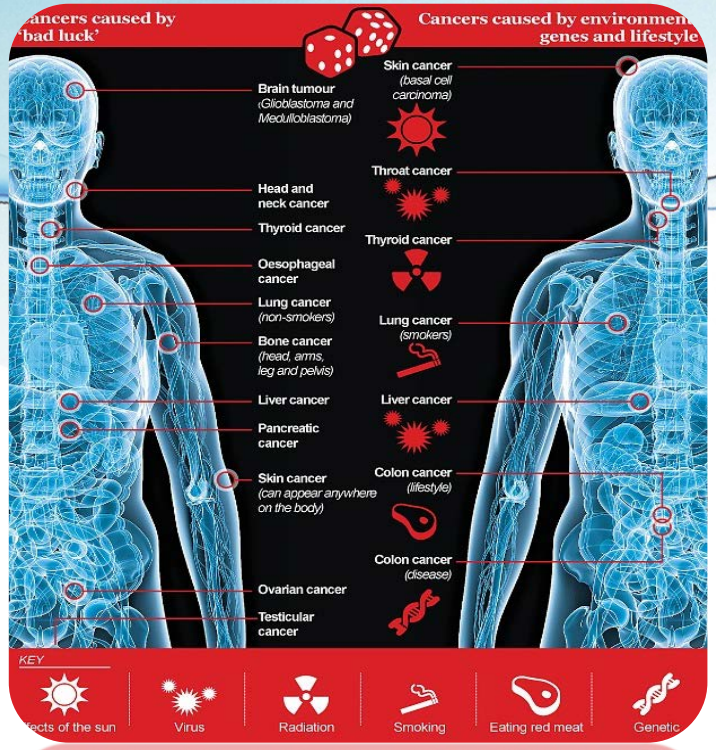
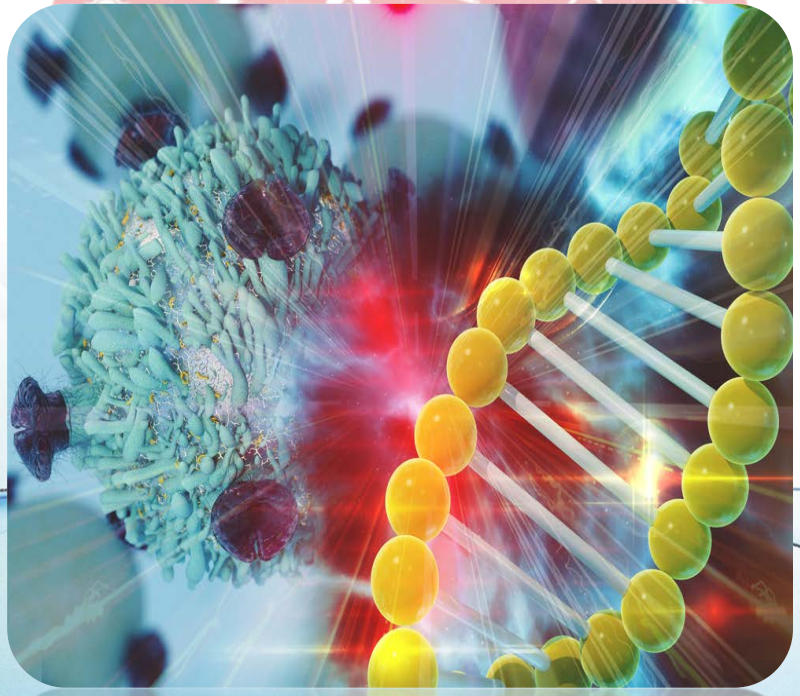
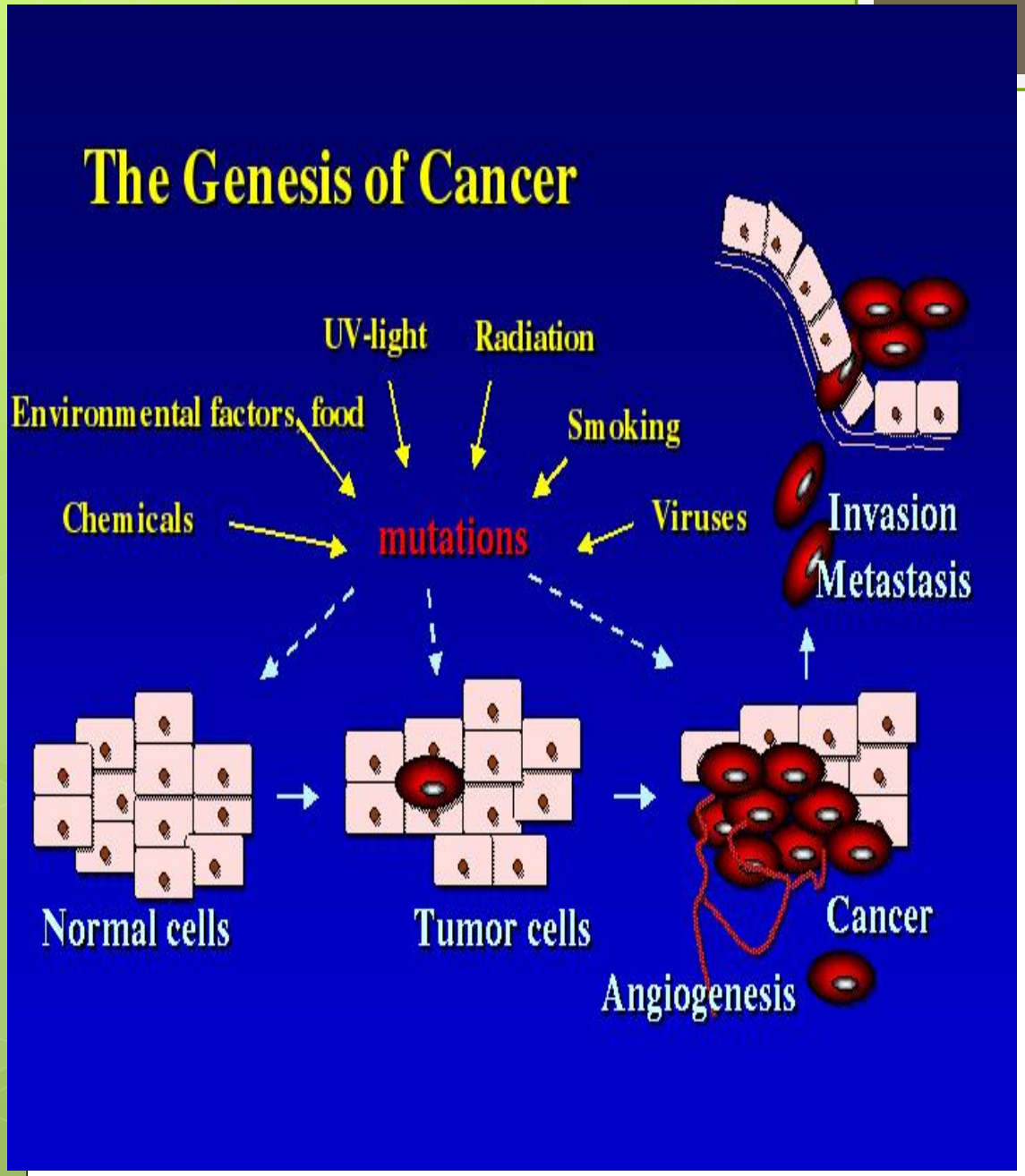
E-Mails: cicerale@deakin.edu.au (S.C.); ljlu@deakin.edu.au (L.L.)

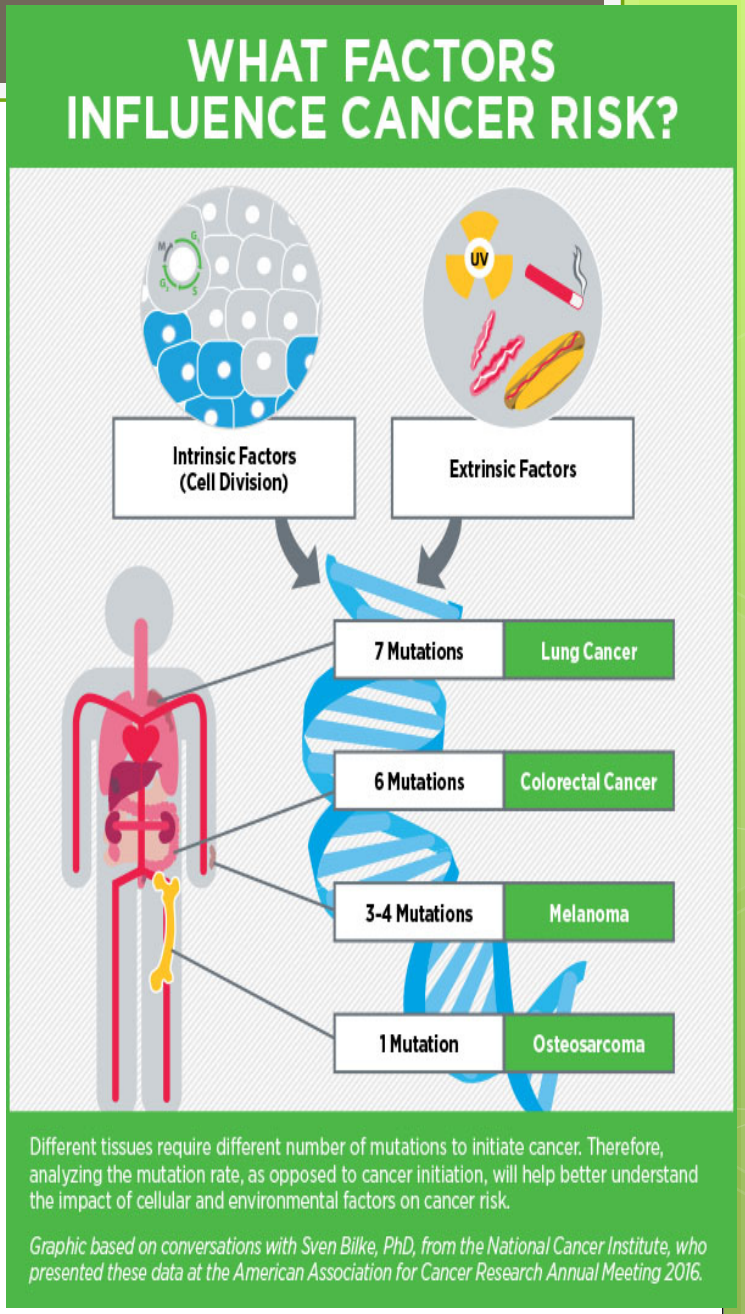

ancers caused by
'bad luck'
Cancers caused by environmen
genes and lifestyle
Brain tumour
(Glioblastoma and
Medulloblastoma)
Head and
neck cancer
Thyroid cancer
Oesophageal
cancer
Lung cancer
(non-smokers)
Bone cancer
(head, arms,
leg and pelvis)
Liver cancer
Pancreatic
cancer
Skin cancer
(can appear anywhere
on the body)
Ovarian cancer
Testicular
cancer
Skin cancer
(basal cell
carcinoma)
Throat cancer
Thyroid cancer
Lung cancer
(smokers)
Liver cancer
Colon cancer
(lifestyle)
Colon cancer
(disease)
KEY
ects of the sun
Virus
Radiation
Smoking
Eating red meat
Genetic

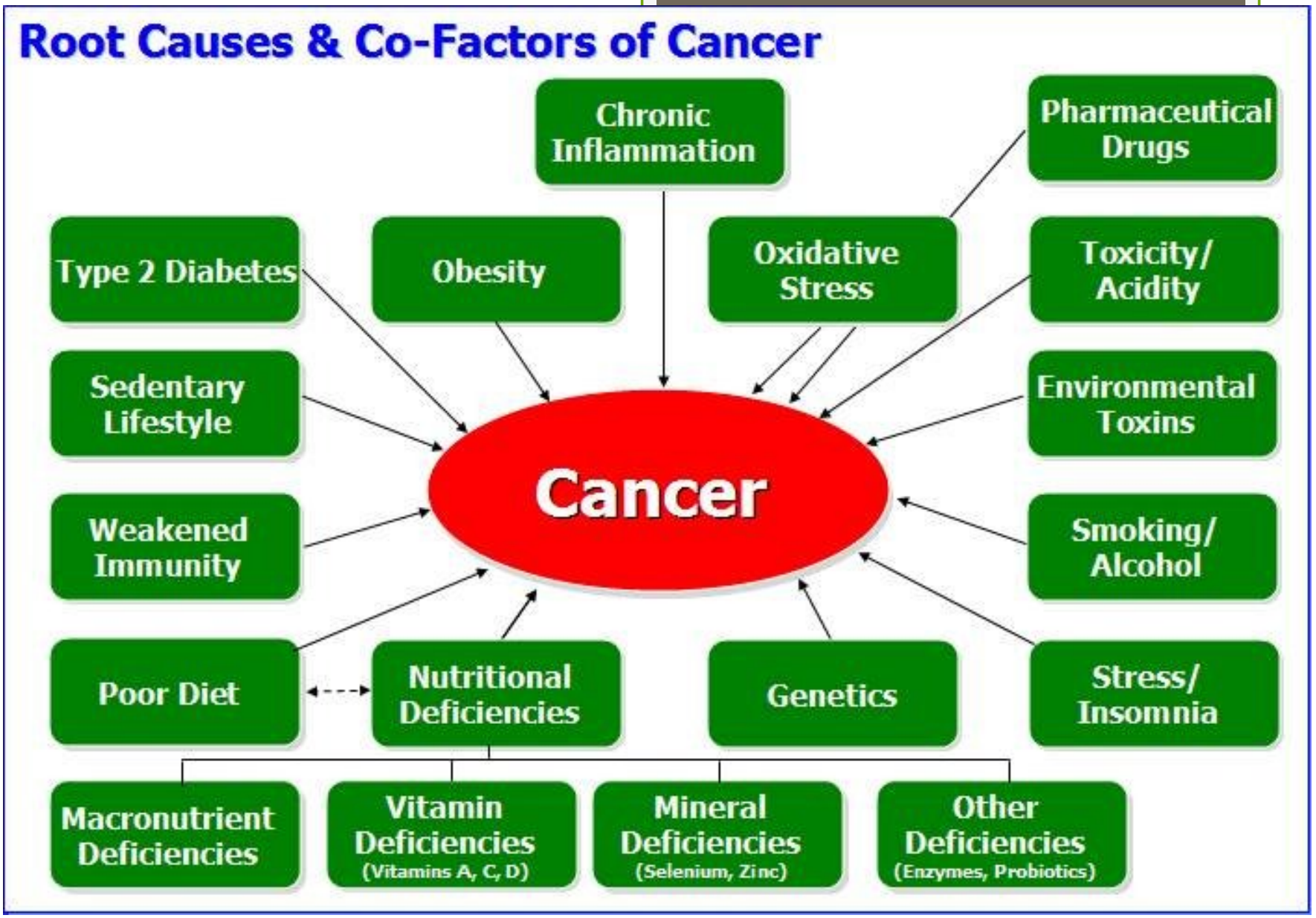

CANCER



- Cancer is currently the second leading cause of death worldwide, and new pharmacological remedies are urgently required
- Although targeted therapeutic agents have reduced the overall level of mortality in the last decade, the efficacy of the new treatments is still limited, mainly due to the adverse side-effects and the frequent development of resistance
- There is thus a demand for novel approaches with non-toxic, affordable, readily accessible, and more effective compounds.

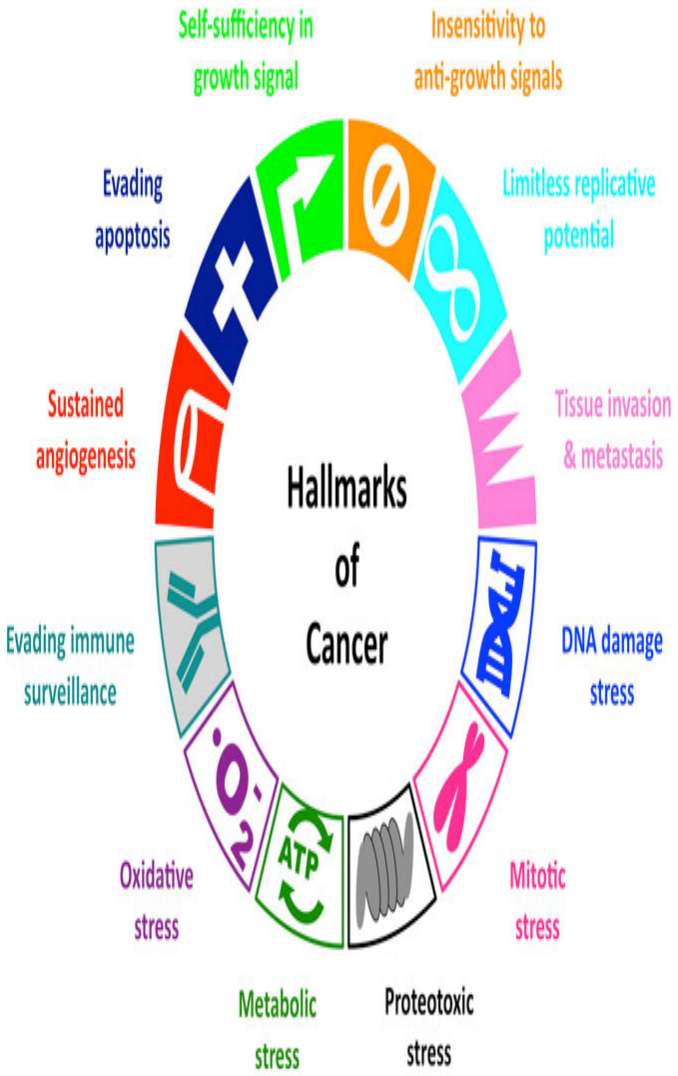

The Genesis of Cancer
UV-light
Radiation
Environmental factors, food
Smoking
Chemicals
mutations
Viruses
Invasion
Metastasis
Normal cells
Tumor cells
Cancer
Angiogenesis


WHAT FACTORS INFLUENCE CANCER RISK?
UV
Intrinsic Factors (Cell Division)
Extrinsic Factors
7 Mutations
Lung Cancer
6 Mutations
Colorectal Cancer
3-4 Mutations
Melanoma
1 Mutation
Osteosarcoma
Different tissues require different number of mutations to initiate cancer. Therefore, analyzing the mutation rate, as opposed to cancer initiation, will help better understand the impact of cellular and environmental factors on cancer risk.
Graphic based on conversations with Sven Bilke, PhD, from the National Cancer Institute, who presented these data at the American Association for Cancer Research Annual Meeting 2016.


Root Causes & Co-Factors of Cancer
Chronic Inflammation
Pharmaceutical Drugs
Type 2 Diabetes
Obesity
Oxidative Stress
Toxicity/ Acidity
Sedentary Lifestyle
Environmental Toxins
Cancer
Weakened Immunity
Smoking/ Alcohol
Poor Diet
Nutritional Deficiencies
Genetics
Stress/ Insomnia
Macronutrient Deficiencies
Vitamin Deficiencies (Vitamins A, C, D)
Mineral Deficiencies (Selenium, Zinc)
Other Deficiencies (Enzymes, Probiotics)

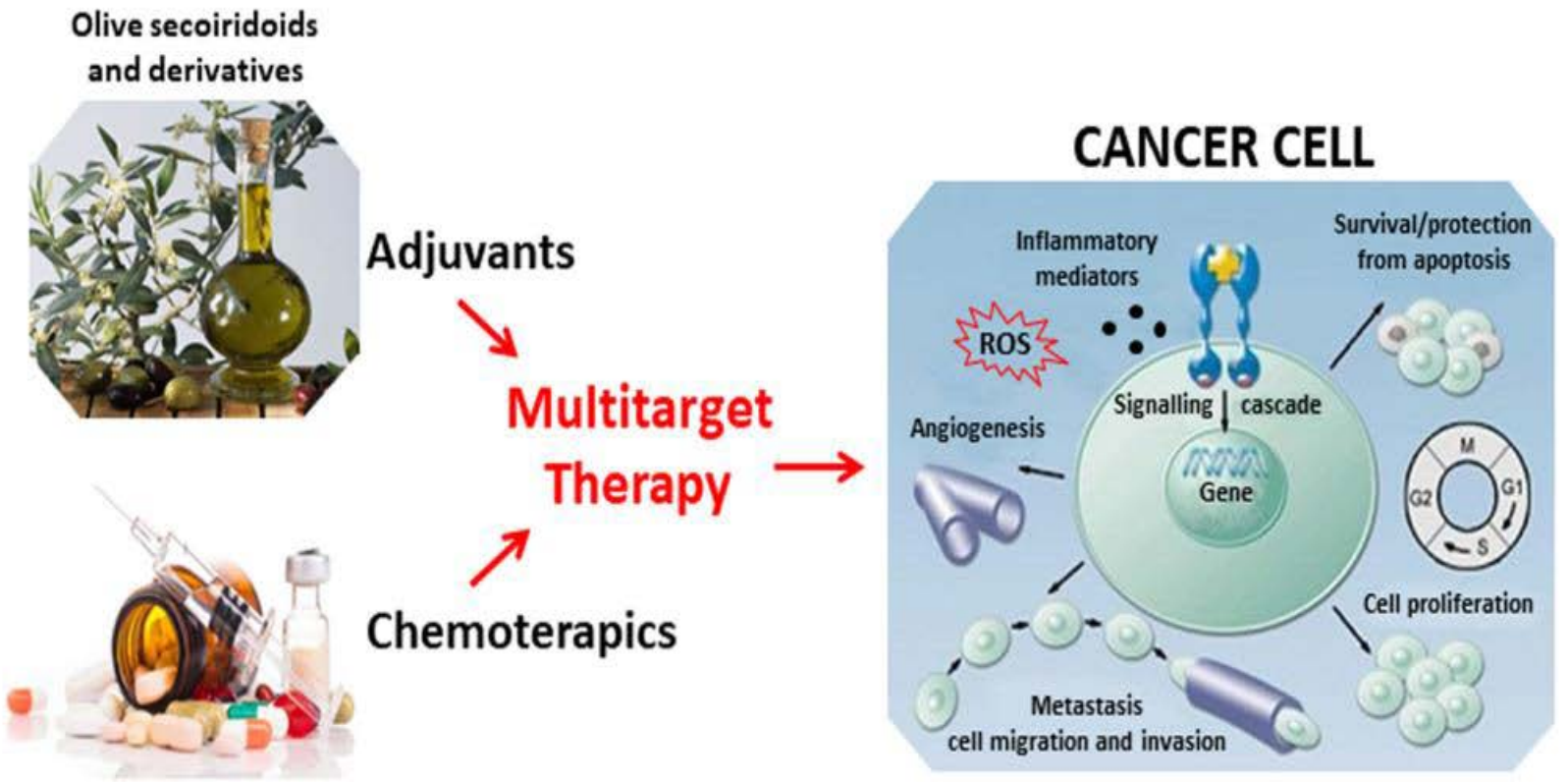
- The key processes occurring in cancer cells are caused by the oncogenic-driven deregulation of several signaling pathways and the subsequent altered expression and function of the molecules involved in the retention of chronic abnormal proliferation, escape from programmed cell death, and acquisition of invasive capacity.

- Moreover, the associated induction of angiogenesis, changes in the stromal microenvironment, stress oxidative damage and concomitant chronic inflammation, taken together, make it difficult to find an effective pharmacological counteraction of this disease.

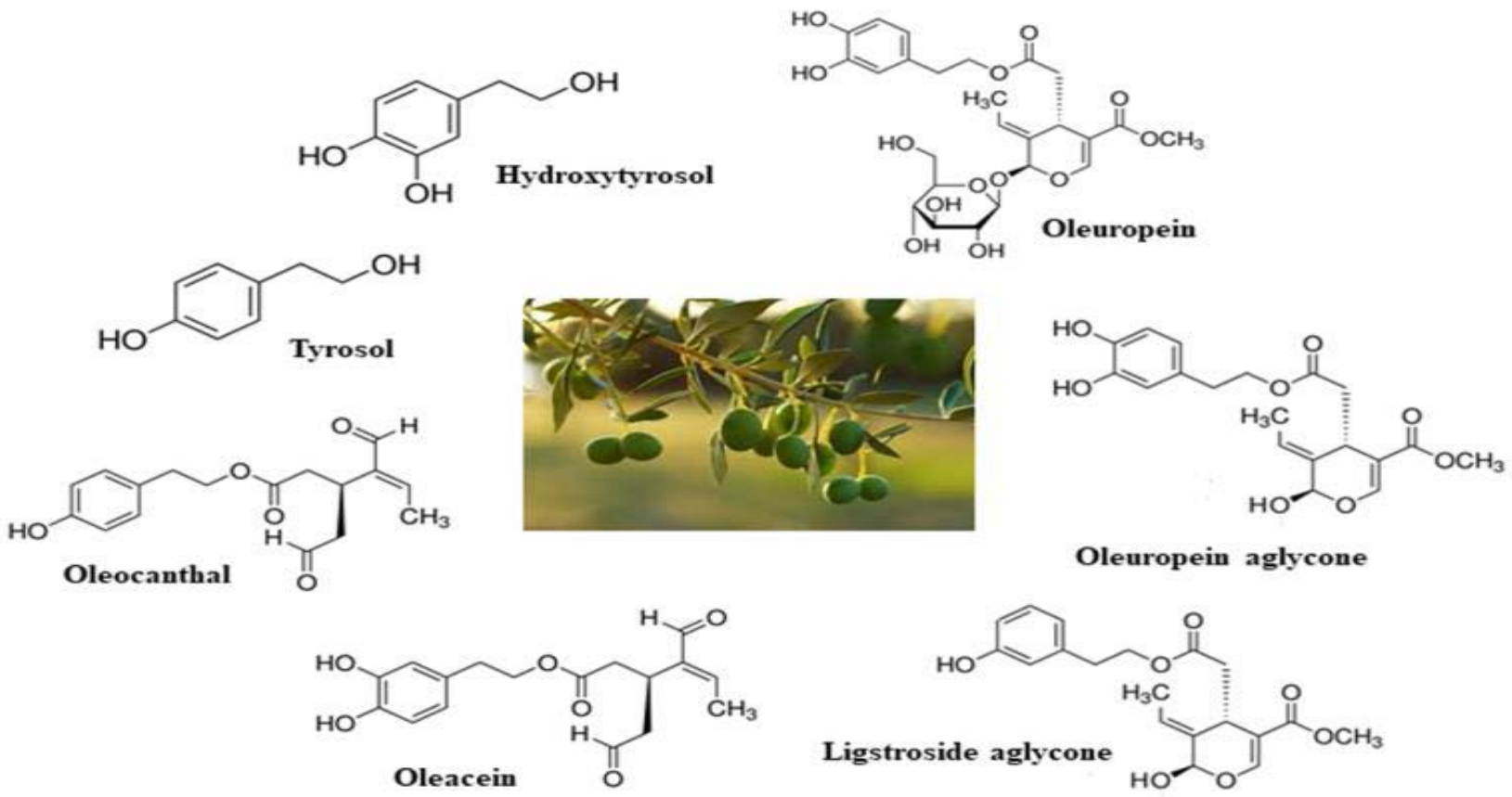


Pharmacological Research Volume 142, April 2019, Pages 77-86

Olive Oil Polyphenols and Cancer



Pharmacological Research Volume 142, April 2019, Pages 77-86

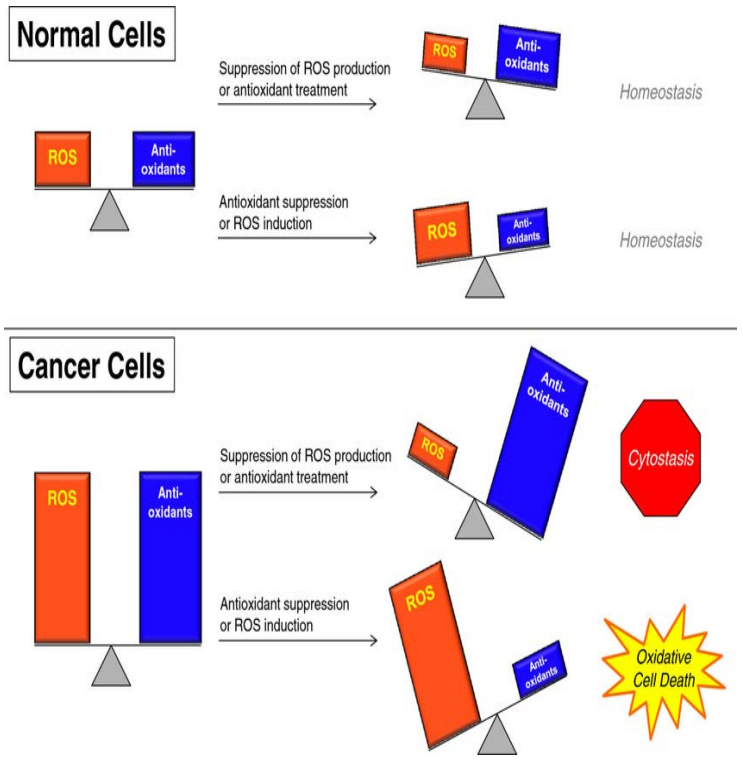


Chemical structures of the olive secoiridoids and derivatives used in preclinical studies as anticancer agents

Pharmacological Research Volume 142, April 2019, Pages 77-86

Antiredox activity

- There is a balance between ROS generation and the removal of overabundant endogenous free radicals and reactive metabolites
- thus preventing genotoxic effects as well as concomitant protein, lipid and membranes damage which may contribute to the onset, development and progress of several diseases including some aspects of cancer biology
- Because of metabolic, mitochondrial and signaling deregulation, cancer cells show an increased ROS levels

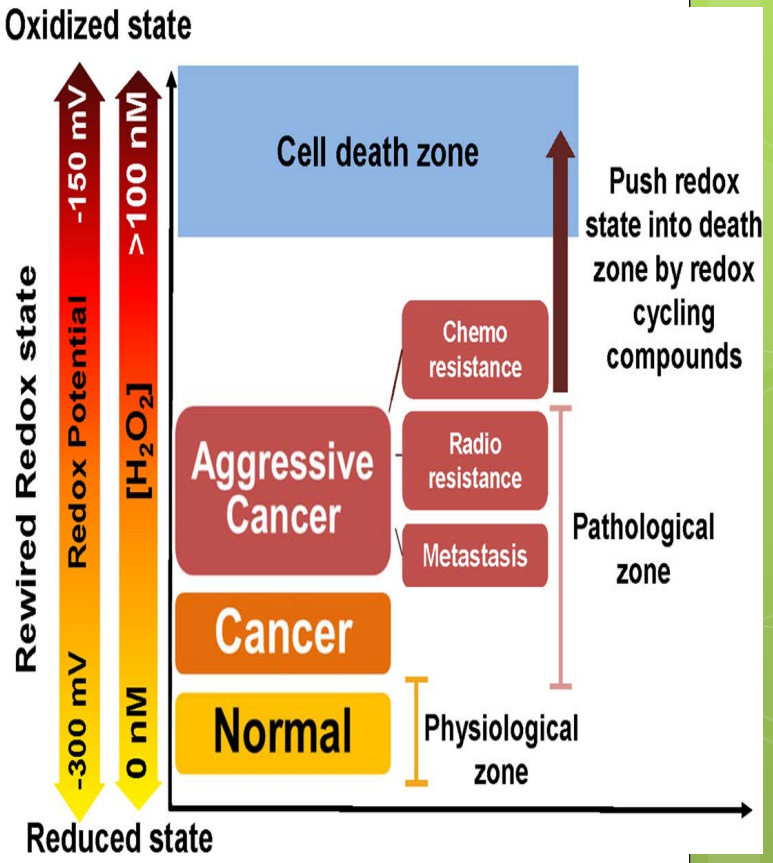


Sullivan and Chandel Cancer & Metabolism 2014, 2 :17

Pharmacological Research Volume 142, April 2019, Pages 77-86

Antiredox activity

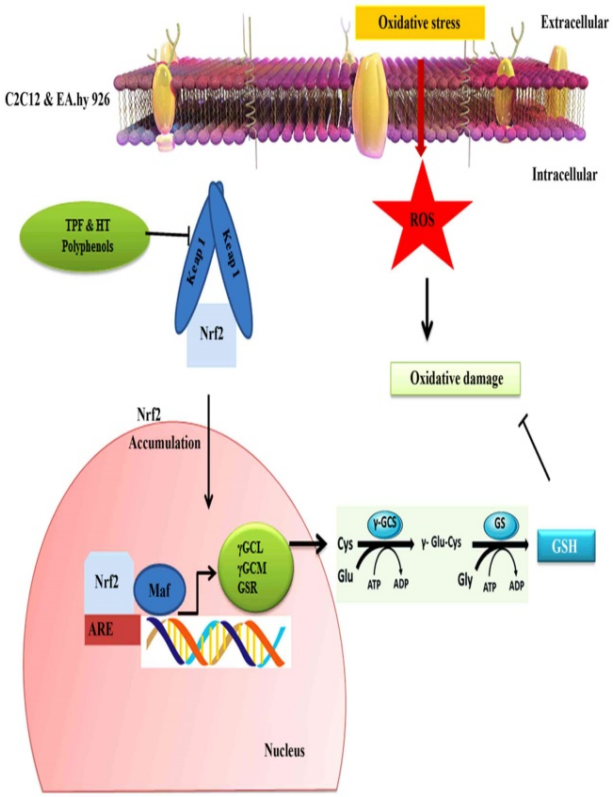
- Malignant cells depend on elevated intracellular levels of ROS to proliferate and self-renew
- Redox imbalance has proved to be a contributing factor to numerous cancer phenotypes; i.e., increased cell proliferation, invasion, and epigenetic changes that lead to pathologic and clinical progression of cancers.



Luksana Chaiswing et al Antioxidants and Redox Signaling 2017

Antiredox activity

- Olive phenols can directly protect cells from oxidative stress by acting as free radical scavengers, radical chain breakers, or metal chelators
- also act as oxidative stress defenders through the upregulation of a signaling pathway involving the nuclear factor Nrf2, thus resulting in an increased expression of protective phase II detoxifying enzymes
- protection against oxidative damage can also derive from the induction of mitochondrial biogenesis

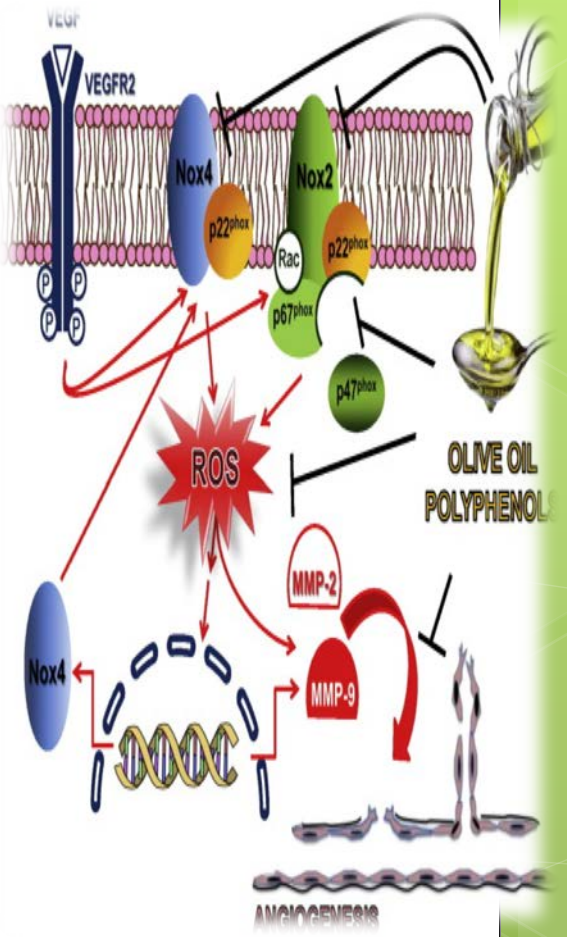


Possible mechanisms through which TPF and HT exert their effects on GSH levels. TPF, total polyphenolic fraction; HT, hydroxytyrosol; Nrf2, nuclear factor (erythroid-derived-2)-like 2; ROS, reactive oxygen species; GSH, glutathione; ARE, antioxidant response element.

Kouka, P et al (2017). Assessment of the antioxidant activity of an olive oil total polyphenolic fraction and hydroxytyrosol from a Greek Olea europea variety in endothelial cells and myoblasts. International Journal of Molecular Medicine, 40

Antiredox activity

- Olive phenols may work as "redox-active" compounds inducing:
- cancer cell growth arrest or cell death, by either stimulating ROS production
- or inhibiting antioxidant defense systems,
- or a combination of both
- Moreover, they have shown "redox-silent" activity inducing death pathways in cancer cells via mitochondrial and lysosomal dysfunction



Calabriso et al The Journal of Nutritional Biochemistry Volume 28, February 2016, Pages 19-29

Table 1: Activity on cellular redox status of olive secoiridoids and derivatives in preclinical cancer models

Compound	Model	Mechanism	Ref.
Hydroxytyrosol	HL-60*	n.a.	[29]
		↑ H_2O_2 discharge	[134]
	MCF-7*	↑ Nrf2, ERRα, SIRT3, GSTA2, HO-1	[39]
		↓ PGC1α,	
	(SW480, HCT116, PC3 LNCaP MCF7, MDA)*	↑ H_2O_2 discharge	[33]
	HepG2*	↓ BiP, ATF6α	[135]
	PC3*	↓ DNA damage, cGPx, PHGPx	[136]
	DLD1	↑ H_2O_2, ROS ↓ Δψ	[44]
	Glioma in Wistar rats	↓ TBARs, GSH, GSSG, SOD, CAT, GPx	[118]

AIF, apoptosis inducing factor; ASM, acid sphingomyelinase; ATF6α, activating transcription factor 6α; BiP, binding immunoglobulin protein; CAT, catalase; cGPx, classic glutathione peroxidase; Δψ, mitochondrial potential membrane; ERRα, estrogen-related receptor α; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; GSTA2, glutathione s-transferase alpha 2; HO-1, heme oxigenase-1; LDH, lactic dehydrogenase; LMP, lysosomal membrane permeabilization; n.a., not available; Nrf2, nuclear factor erythroid 2-related; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PHGPx, phospholipid hydroperoxide glutathione peroxidase; ROS, reactive oxygen species; SIRT3, sirtuin-3; SOD, superoxide dismutase; TBARs, thiobarbituric acid-reactive substances.
*Cancer cell lines: BCPAP, thyroid; DLD1, HCT116, HT29, SW480, WiDr, colon; Hep3B, HepG2, liver; HL-60, blood; DU145, LNCaP, PC3, prostate; MCF-7, MDA, T47D, breast; SW982, synovia.

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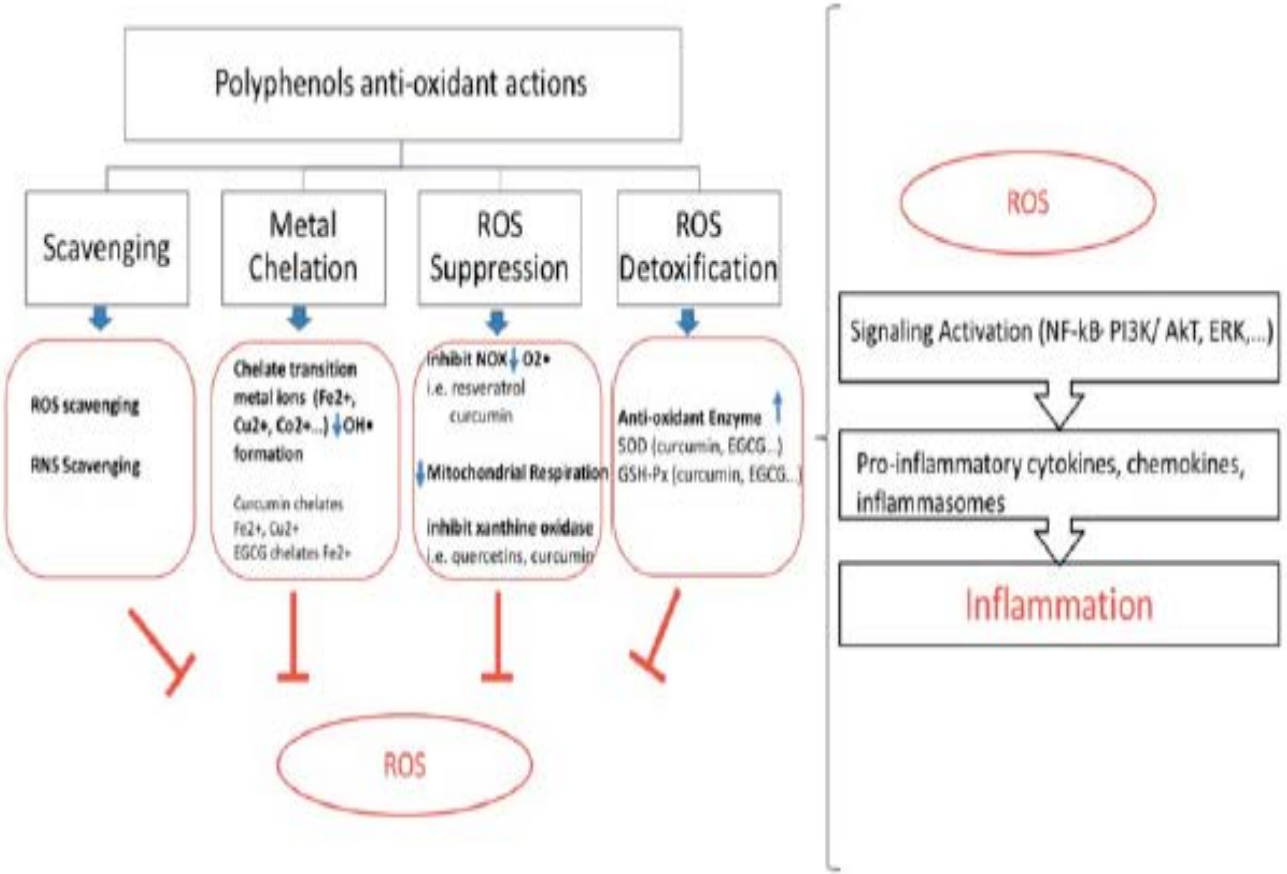
Hydroxytyrosol + Cetuximab	HT29, WiDr*	↓ AIF	[46]
Oleacein	HL-60*	n.a.	[29]
Oleocanthal	HepG2, Hep3B, SW480*	↓ Δψ ↑ ROS	[87]
	MDA-MB231 (ER-, PR-, HER2-)	↓ ASM ↑ LMP	[45]
Oleuropein	(MCF-7, T47D, BCPAP)*	↓ ROS	[30,31]
	SW982*	↑ Nrf2, HO-1	[117]
	LNCaP, DU145*	↑ LDH, ROS, HO-1	[40]
	HepG2*	↑ MDA	[41]
	Colorectal cancer in A/J mice	n.a.	[103]
	UVB irradiation in C57BL/6J mice	n.a.	[86]

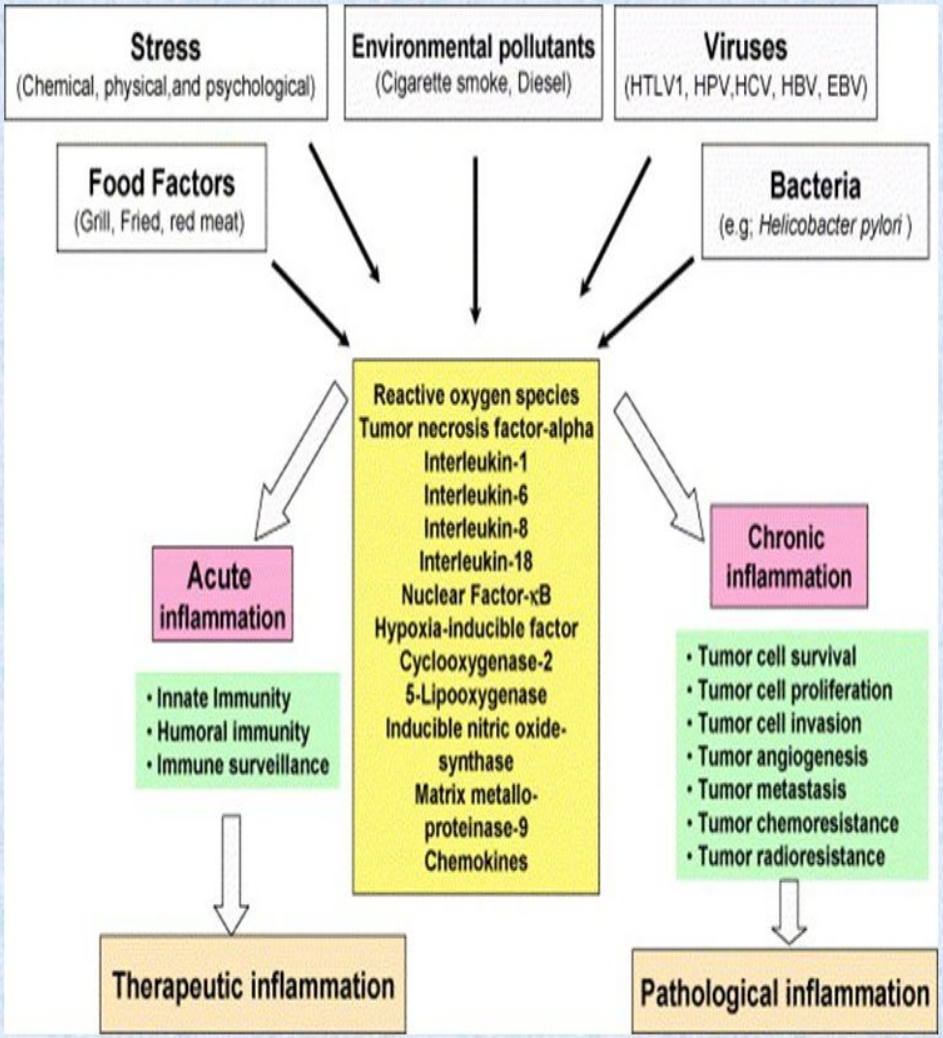
AIF, apoptosis inducing factor; ASM, acid sphingomyelinase; ATF6α, activating transcription factor 6α; BiP, binding immunoglobulin protein; CAT, catalase; cGPx, classic glutathione peroxidase; Δψ, mitochondrial potential membrane; ERRα, estrogen-related receptor α; GPx, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; GSTA2, glutathione s-transferase alpha 2; HO-1, heme oxigenase-1; LDH, lactic dehydrogenase; LMP, lysosomal membrane permeabilization; n.a., not available; Nrf2, nuclear factor erythroid 2-related; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator-1α; PHGPx, phospholipid hydroperoxide glutathione peroxidase; ROS, reactive oxygen species; SIRT3, sirtuin-3; SOD, superoxide dismutase; TBARs, thiobarbituric acid-reactive substances.

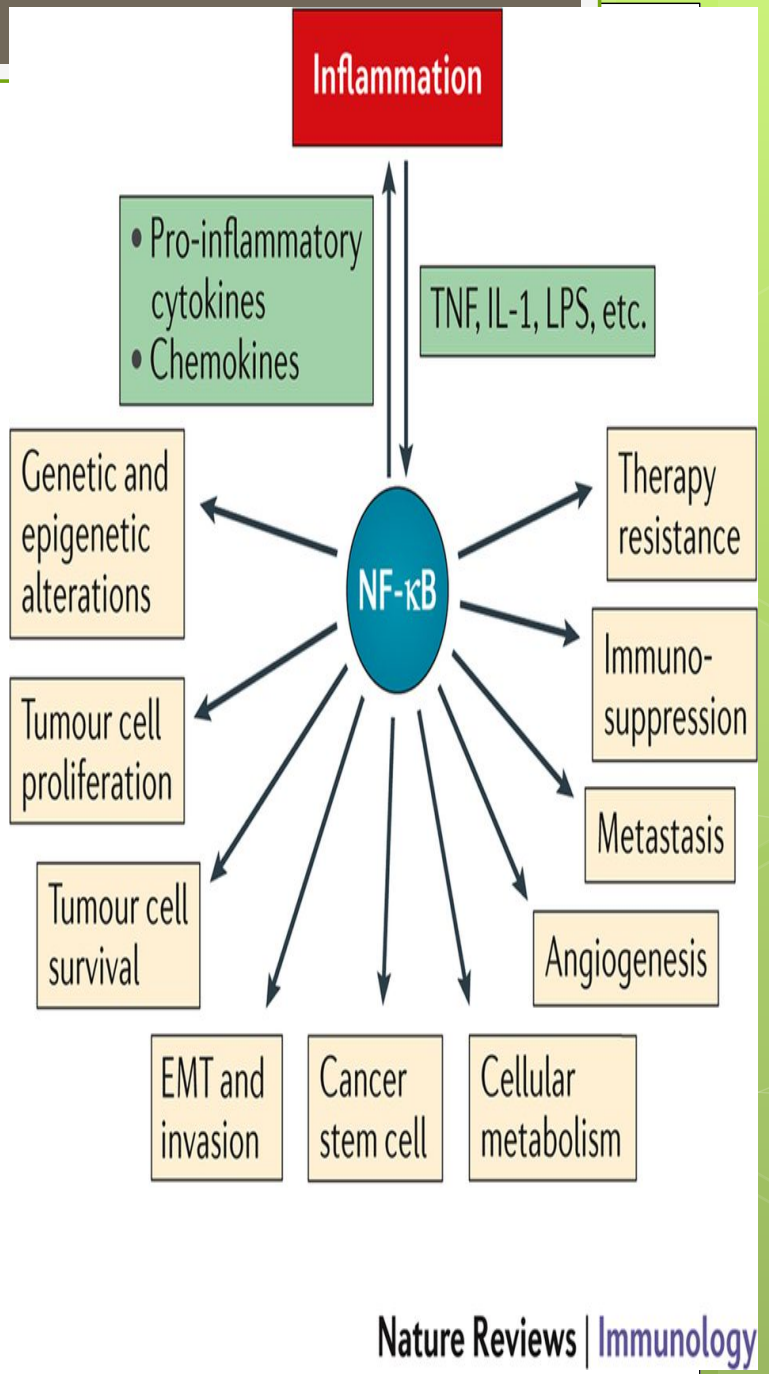
*Cancer cell lines: BCPAP, thyroid; DLD1, HCT116, HT29, SW480, WiDr, colon; Hep3B, HepG2, liver; HL-60, blood; DU145, LNCaP, PC3, prostate; MCF-7, MDA, T47D, breast; SW982, synovia.



Nutrients **2018**, *10*, 1618; doi:10.3390/nu10111618

Role of chronic inflammation in promoting cancer development



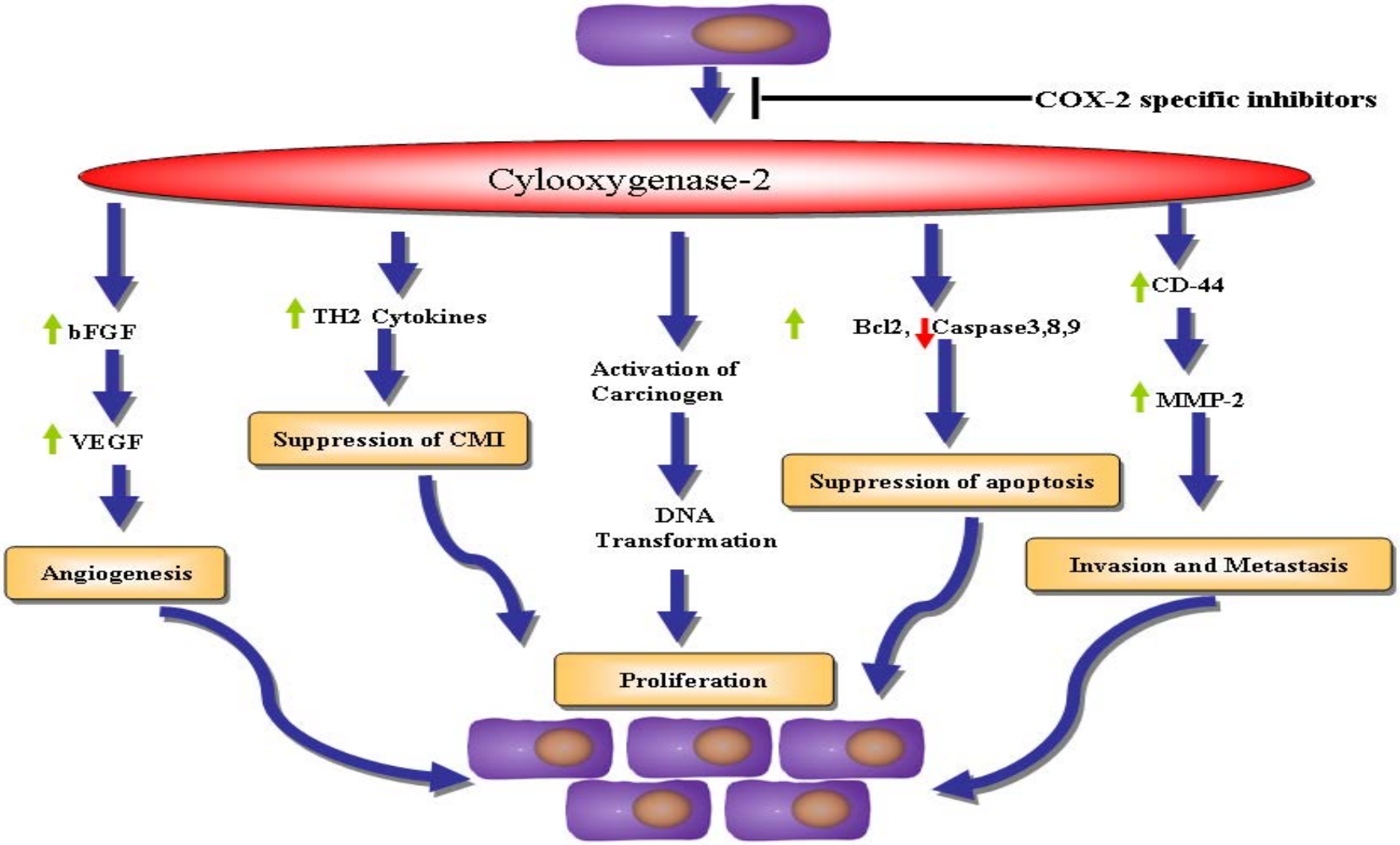


Anti-inflammatory activity

- Inflamatory mediators act on damaged tissue cells, endothelial cells and the same immune cells, and trigger oncogenic pathways which may contribute to an abnormal proliferation regulation.

- Beauchamp and colleagues demonstrated that OC inhibited cyclooxygenase (COX)1 and COX2 activity in a very similar way to the anti-inflammatory drug ibuprofen.
- The attenuating action of Ole and HT on NF-KB activation has been also described

- L. Parkinson, R. Keast, Oleocanthal, a phenolic derived from virgin olive oil: A review of the beneficial effects on inflammatory disease, Int. J. Mol. Sci. 15 (7) (2014) 12323–12334
- Celano M, Maggisano V, Lepore SM, Russo D, Bulotta S, Secoiridoids of olive and derivatives as potential coadjuvant drugs in cancer: a critical analysis of experimental studies, Pharmacological Research (2019)
- E. Giner, I. Andújar, M.C. Recio, J.L. Ríos, J.M. Cerdá-Nicolás, R.M. Giner, Oleuropein ameliorates acute colitis in mice, J. Agric. Food Chem. 59 (24) (2011) 12882-12892



Role of COX-2 in the initiation of cancers, the downregulation of apoptosis and the promotion of angiogenesis, invasion and metastasis.(Smita et al 2009, Experimental Dermatology).

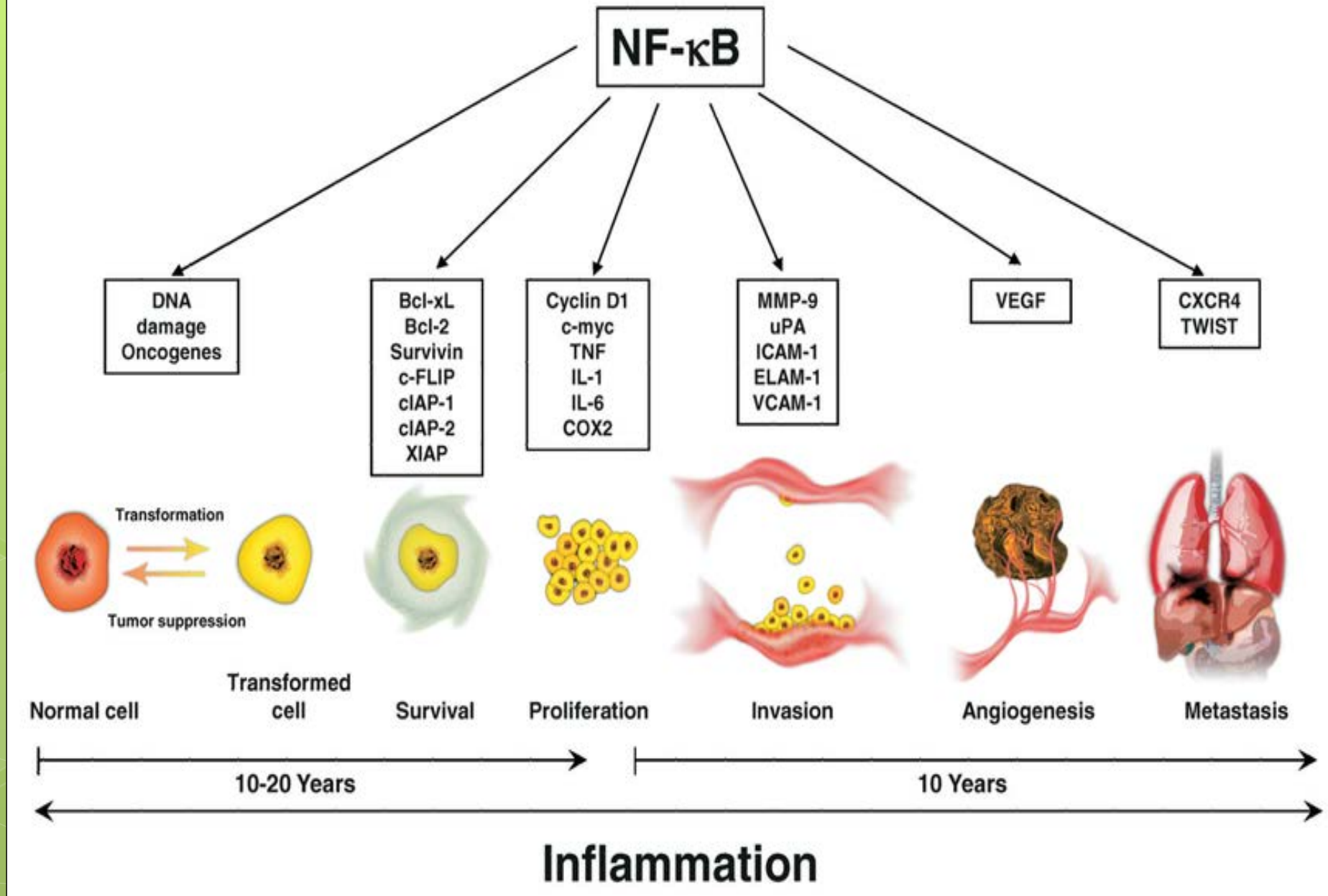

NF-κB
DNA damage Oncogenes
Bcl-xL Bcl-2 Survivin c-FLIP cIAP-1 cIAP-2 XIAP
Cyclin D1 c-myc TNF IL-1 IL-6 COX2
MMP-9 uPA ICAM-1 ELAM-1 VCAM-1
VEGF
CXCR4 TWIST
Transformation
Tumor suppression
Normal cell
Transformed cell
Survival
Proliferation
Invasion
Angiogenesis
Metastasis
10-20 Years
10 Years
Inflammation

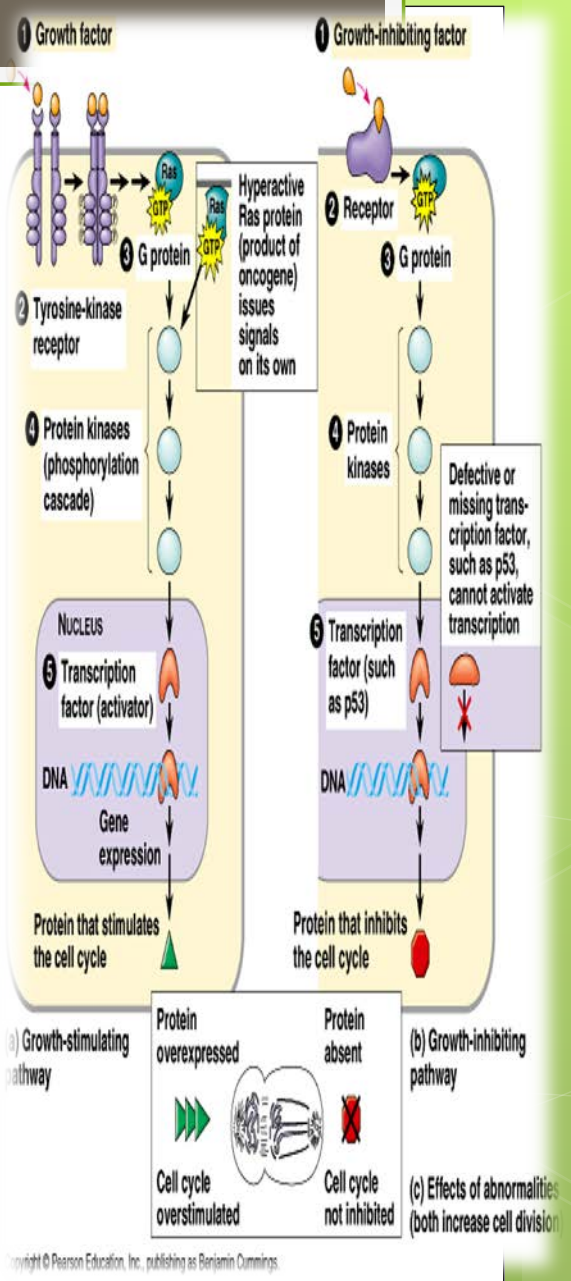
Table 2. Anti-inflammatory effects of olive secoiridoids and derivatives in preclinical cancer models

Compound	Model	Molecular target	Ref.
Hydroxytyrosol	Colorectal adenocarcinoma in athymic nude mice	↓ mPGES-1, HIF-1α	[104]
	(Hep3B, HepG2)*	↓ IL-6	[137]
	Glioma in Wistar rats	↓ IL-6, TNFα, ASAP, IRAP	[119]
Oleuropein	Skin cancer in HOS:HR-1 mice	↓ MMP2,9,13, COX2	[107]
	AOM/DSS-colorectal cancer in C57BL/6 mice	↓ IL-6-17, IFNγ, TNFα, COX2, NF-κB	[88]
	SW982*	↓ IL-6, TNFα, COX2, mPGES-1, NF-κB	[117]
Tyrosol	U-87MG*	↓ TNFα ↑ COX2, NF-κB	[138]

AOM, azoxymethane; ASAP, aspartyl aminopeptidase; COX2, cyclooxygenase 2; DSS, dextran sulfate sodium; HIF-1α, hypoxia inducible factor-1α; IFNγ, interferon γ; IL-6, interleukin-6; IRAP, insulin-regulated aminopeptidase; MMP, matrix metalloproteinase; mPGES-1, microsomal prostaglandin E synthase-1; NF-κB, nuclear factor-kappa B; TNFα, tumor necrosis factor α.
*Cancer cell lines: Hep 3B, HepG2, liver; SW982, synovia; U-87MG, brain.

Antiproliferative activity

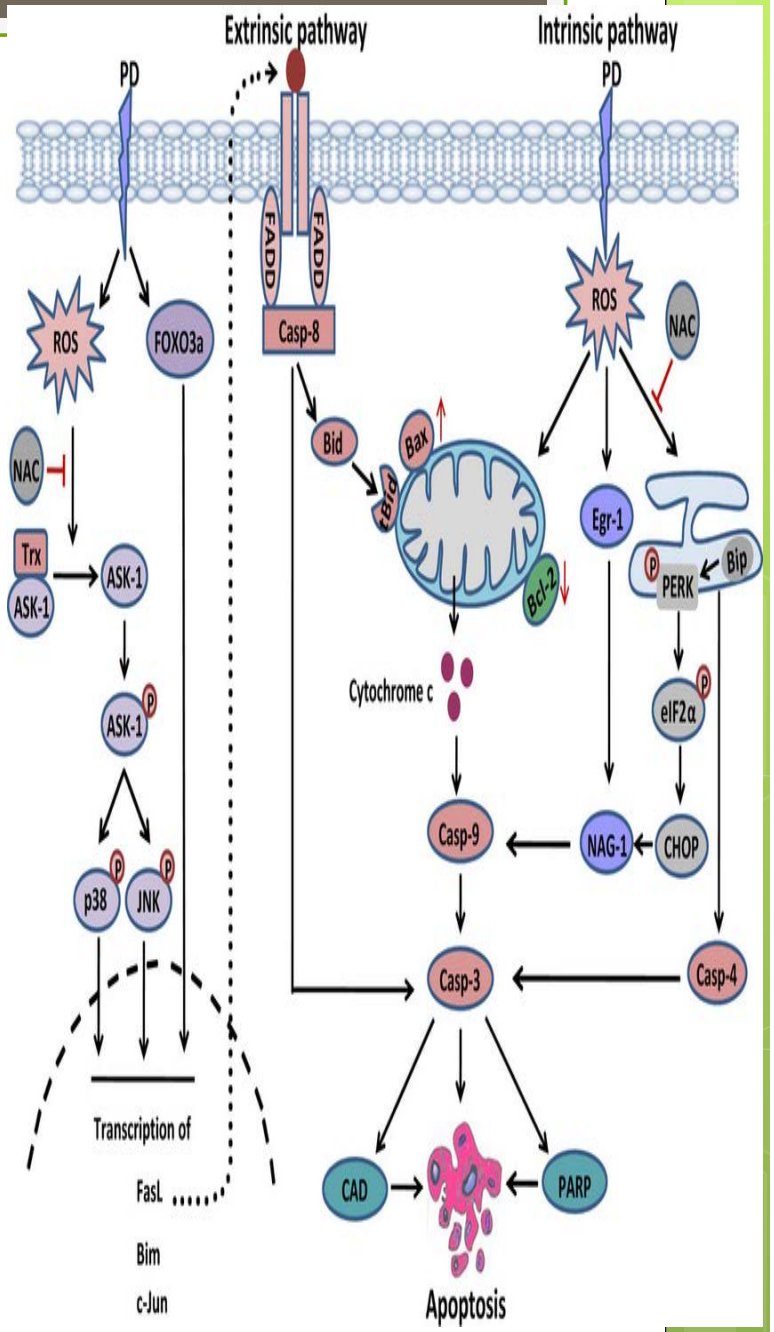
- The treatment of cancer cell lines with olive phenols has highlighted the capacity to down regulate cancer cell proliferation
- by altering the expression and/or function of key molecules involved in the onset and development of cancer including
  - the human epidermal growth factor receptor (HER),
  - mitogen activated protein-kinases (MAPKs),
  - c-Met proto-oncogene and
  - the fatty acid synthase (FASN) enzyme



Pharmacological Research Volume 142, April 2019, Pages 77-86

Olive phenols

- reduces cell growth by stimulating both extrinsic and intrinsic apoptosis pathways and/or arresting the cell cycle through the upregulation of p53, p21 and cyclin dependent kinase (CDK) inhibitors or the downregulation of cyclin D1 and ki67
- Affect the Lysosomal membrane integrity by decreasing the activity of the lysosomal lipase acid sphingomyelinase (ASM), and its inhibition promotes organelle membrane permeabilization resulting in the release of lysosomal hydrolytic enzymes into the cytosol and cell death



Pharmacological Research Volume 142, April 2019, Pages 77-86

Table 3. Oncogenic molecular targets of olive secoiridoids and derivatives in *in vivo* models

Compound	Model	Molecular target	Ref.
Hydroxytyrosol	DMBA/Breast cancer in Sprague–Dawley rats	↓ Ki67, SFRP4	[95]
	Colorectal adenocarcinoma in athymic nude mice	↓ Ki67, VEGF, HIF-1α, mPGEs-1 ↓ Caspase,3 cleavage	[104]
	Cholangiocarcinoma in nude BALB/c mice	↓ p-ERK, Ki67 ↑ Bax, (Caspase 3,9, PARP) cleaved	[105]
	Colorectal adenocarcinoma in athymic nude mice	↓ Ki67, EGFR	[101]
Hydroxytyrosol + Paclitaxel	Breast cancer in Sprague–Dawley rats	↓ Ki67	[98]
Oleocanthal	Breast cancer in athymic nude mice Foxn1^{nu}/Foxn1^{+}	↓ Ki67, CD31	[76]
	Hepatocellular cancer in BALB/c athymic nude mice	↓ Ki67, pSTAT3	[106]
	Melanoma cancer in BALB/c athymic nude mice	↓ Ki67, CD31	[108]
Oleuropein	Skin cancer in HOS: HR-1 mice	↓ Ki67	[107]
	Skin UVB irradiation in C57BL/6J mice	↓ Ki67, MMP3	[86]
	AOM/DSS-colorectal cancer in C57BL/6 mice	↓ NF-κB, Wnt/βCatenin, PI3K/AKT ↓ STAT3, Bax, Ki67	[88]
Oleuropein + Doxorubicin	Breast cancer in BALB/c Foxn1 mice	↓ NF-κB, Cyclin D1, Bcl2	[99]

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Table 4. Effects and molecular targets of olive secoiridoids and derivatives in breast cancer cell lines

Compound	Cell line	Molecular Target	Ref.
Hydroxytyrosol	MCF-7 (ER$^+$)	↑ Caspase cleaved	[139]
		↓ ERK2*	[69]
	MCF7 (ER$^+$, HER2$^+$)	↓ HER2*	[140]
	SKBR3 (HER2$^+$)	↓ HER2*	[140]
Ligstroside aglycone	MCF7 (ER$^+$, HER2$^+$)	↓ (FASN, HER2)*	[76, 77]
	SKBR3 (HER2$^+$)	↓ (FASN, HER2)*	[76, 77]
Oleocanthal	MCF-7 (ER$^+$)	↑ (e-Cadherin, Zo-1)*	[76]
		↓ β-Catenin*	
	BT-474 (ER$^+$, PR$^+$, HER2$^+$)	↑ (e-Cadherin, Zo-1)*	[76]
		↓ (β-Catenin, ERα)*	[76, 96]
	MDA-MB231 (ER$^-$, PR$^-$, HER2$^-$)	↓ (Cyclin D1, CDK6, p-Met, Vimentin)*	
		pBRK*	[76]
		↑ (p-21, p-27)*	
		↑ (Caspase 3, PARP) cleaved	[45, 76]
		↓ p-Met*	[74, 75]
Oleacein	MCF7 (ER$^+$, HER2$^+$)	↓ FASN, HER2*	[76, 77]
	SKBR3 (HER2$^+$)	↓ FASN, HER2*	[76, 77]
Oleuropein	MCF-7 (ER$^+$)	↓ ERK2*	[69]
		↓ (MMP 2,9; TIMP3)#	[141]
		↓ Bcl2#	[83]
		↓ miR 21, 155	[94]
		↑ (Bax, p53)#	[83]
		↑ (APAF-1, PTEN, TP53INP1, FADD)#	[94]
	MDA-MB231 (ER$^-$, PR$^-$, HER2$^-$)	↑ Bax*	[80]
		↓ (Bcl-2, Cyclin D1, NF-κB)*	
	SKBR3 (HER2$^+$)	↑ (Bax, p21, p53, pERK)*	[82]
		(Caspase 3,9; PARP) cleaved	
		↓ (Bcl-2, Cyclin D1)*	
Oleuropein aglycone	MCF7 (ER$^+$, HER2$^+$)	↓ (FASN, HER2)*	[68, 76, 77]
	SKBR3 (HER2$^+$)	↓ (FASN, HER2)*	[68, 76, 77]

APAF-1, apoptotic peptidase activating factor-1; Bax, Bcl-2-associated X protein; Bcl2, B-cell lymphoma 2; BRK, breast tumor kinase; CDK6, cyclin dependent kinase 6; ER, estrogen receptor; ERK, extracellular regulated kinase; FADD, fas associated with death domain; FASN, fatty acid synthase; HER2, human epithelial receptor 2; miR, microRNA; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; PARP, poly(ADP-ribose) polymerase; PR, progesterone receptor; PTEN, phosphatase and tensin homologs; TIMP, tissue inhibitors of metalloproteinases; TP53INP1, tumor protein p53 inducible nuclear protein 1; Zo-1, zona occludens-1. *protein expression; #gene expression.

Celano M, et al Secoiridoids of olive and derivatives as potential coadjuvant drugs in cancer: a critical analysis of experimental studies, *Pharmacological Research* (2019),

nutrients

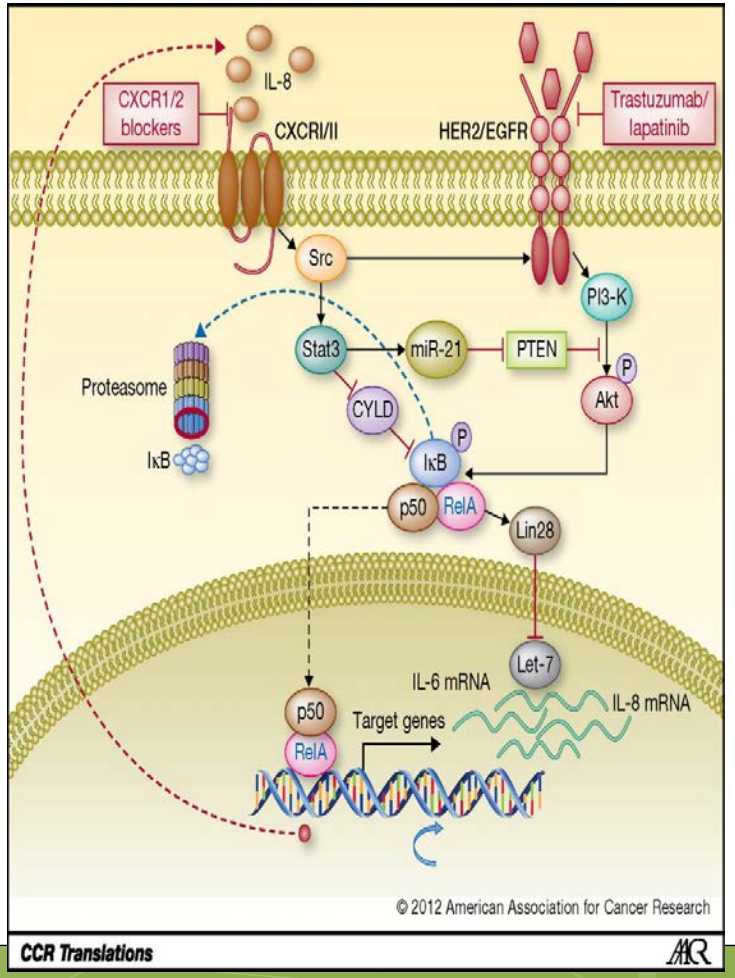
MDPI

Article

(—)-Oleocanthal Combined with Lapatinib Treatment Synergized against HER-2 Positive Breast Cancer In Vitro and In Vivo

Abu Bakar Siddique [1], Hassan Y. Ebrahim [1], Mohamed R. Akl [1], Nehad M. Ayoub [2], Amira A. Goda [1], Mohamed M. Mohyeldin [1], Suresh K. Nagumalli [1], Wael M. Hananeh [3], Yong-Yu Liu [1], Sharon A. Meyer [1] and Khalid A. El Sayed [1,*]

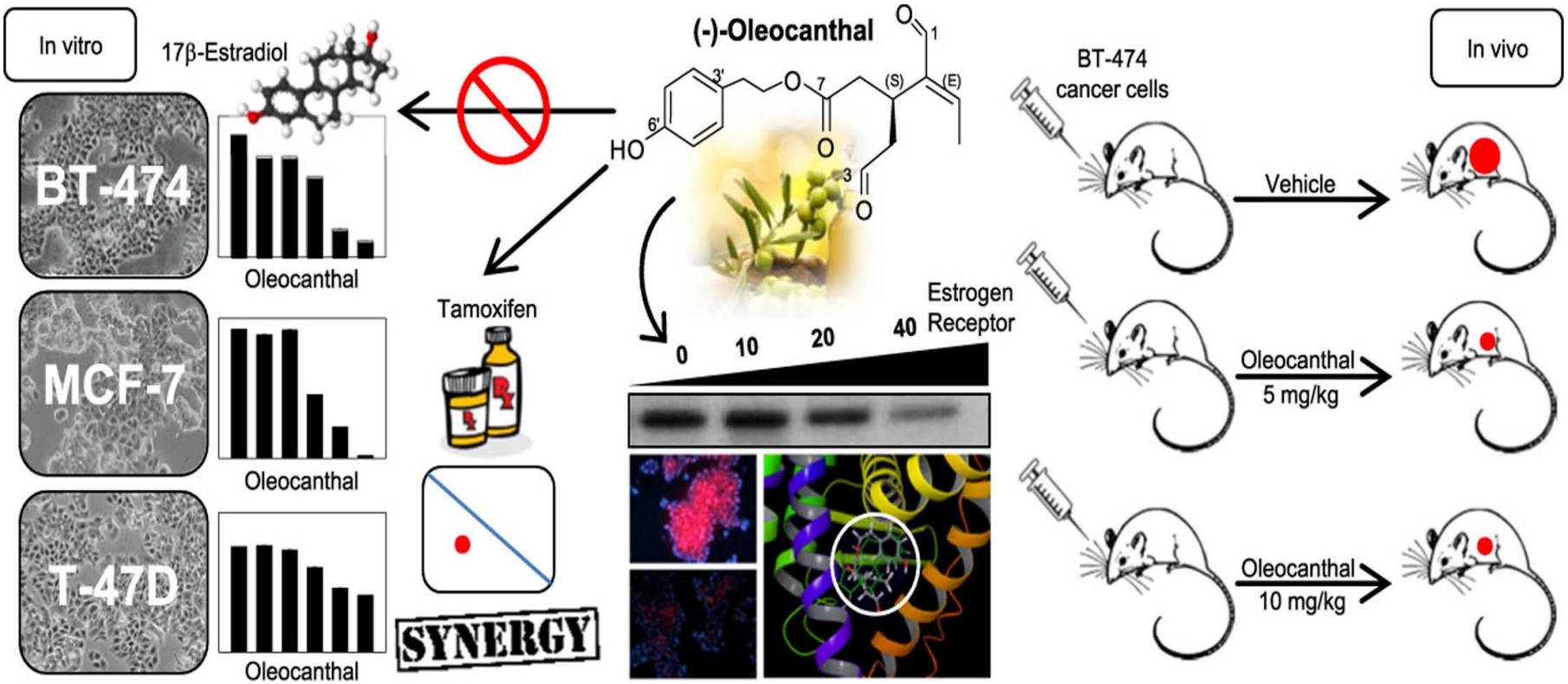
- Overexpression of HER2 protein is associated with aggressive tumor profile and poor clinical outcomes among BC patients diagnosed with HER2-positive phenotype .
- Lapatinib (LP) is a small-molecule dual inhibitor of both EGFR and HER2
- The significant tumor growth inhibition in combination-treated mice was mediated by reduced total and active levels of HER2, EGFR, and c-Met, compared to individual OC or LP treatments suggesting effective tumor cell sensitization



Author's Accepted Manuscript

The olive oil phenolic (-)-oleocanthal modulates estrogen receptor expression in luminal breast cancer *in vitro* and *in vivo* and synergizes with tamoxifen treatment

Nehad M. Ayoub, Abu Bakar Siddique, Hassan Y. Ebrahim, Mohamed M. Mohyeldin, Khalid A. El Sayed



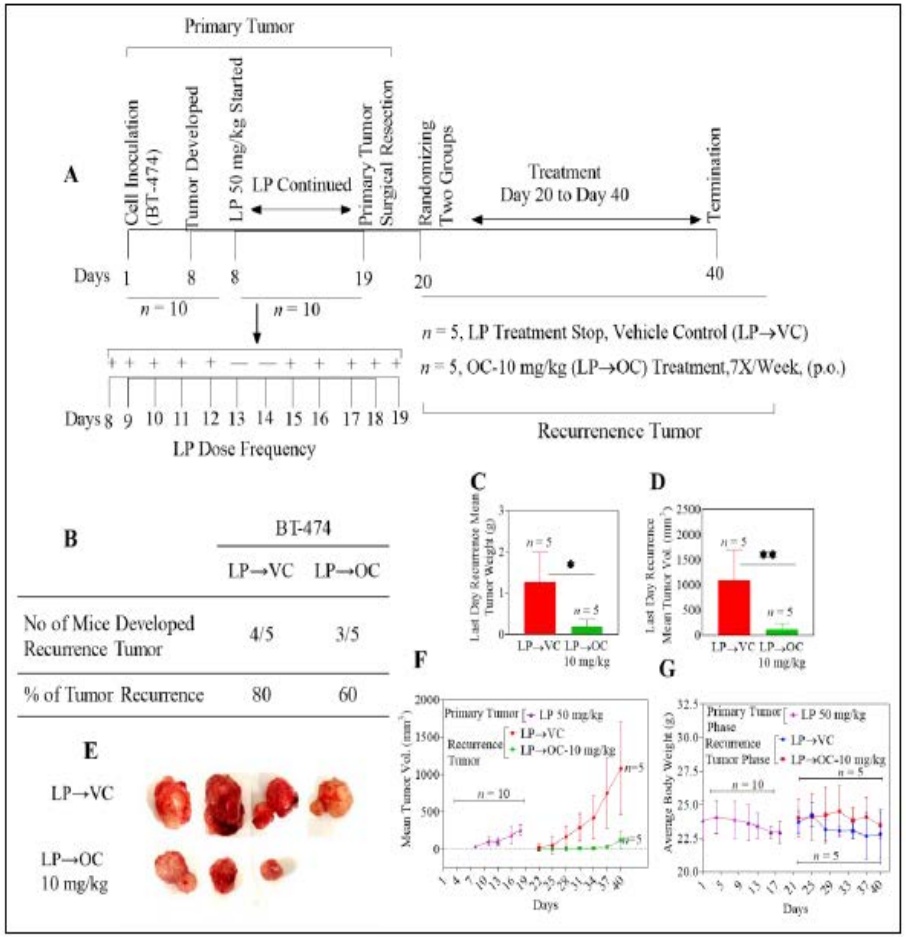
cancers

MDPI

Article

(−)-Oleocanthal Prevents Breast Cancer Locoregional Recurrence After Primary Tumor Surgical Excision and Neoadjuvant Targeted Therapy in Orthotopic Nude Mouse Models

Abu Bakar Siddique [1], Nehad M. Ayoub [2], Afsana Tajmim [1], Sharon A. Meyer [1], Ronald A. Hill [1] and Khalid A. El Sayed [1,*]



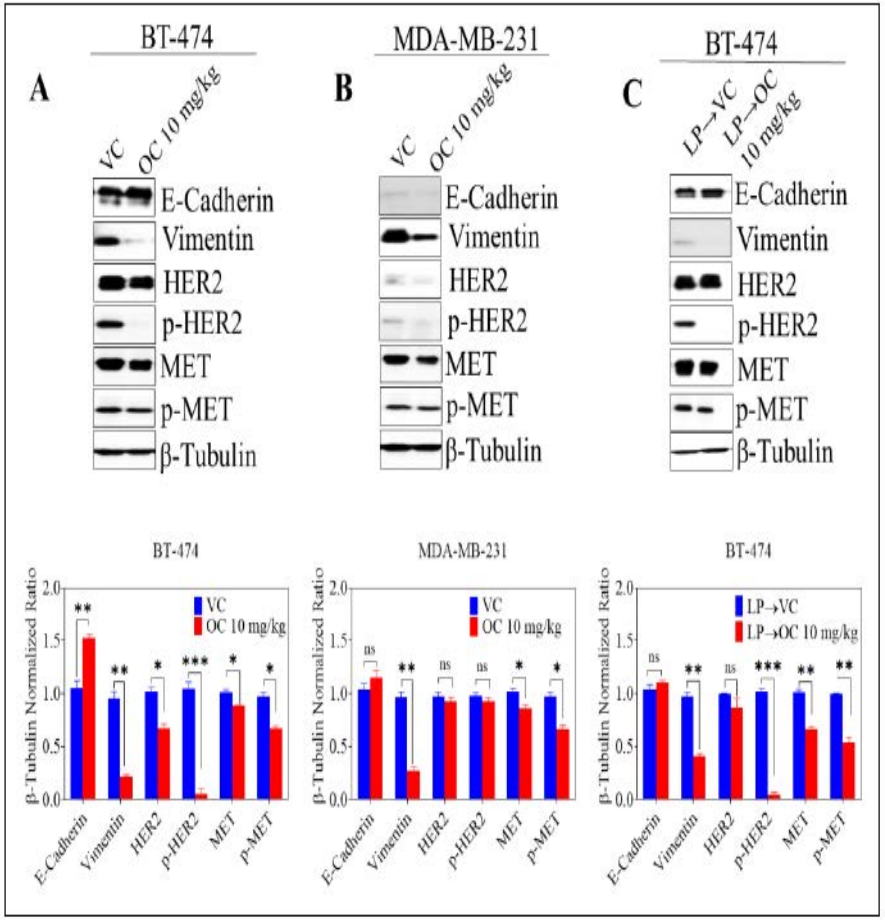


Table 5: Effects and molecular targets of olive secoiridoids and derivatives in colon, liver and pancreatic human cancer cell lines

Compound	Cancer	Cell line	Molecular Target	Ref.
Hydroxytyrosol	Colon	CaCo2	↓Cyclin D1*, p38*, p-ERK 1/2	[142]
		DLD1	↑(Caspase 3,7, PARP) cleaved ↑p-FOX3a, p-Akt	[44]
		HT29, CaCo2, Widr, CC18Co	↓EGFR*	[101]
	Liver	Hep3B, HepG2, SK-HEP-1	↓p-AKT, (Bcl2, Bcl-xL, c-myc, COX2, Cyclin D1, VEGF)*	[143]
	Pancreatic	MIA PaCa-2, BxPC-3, CFPAC-1	↑(Caspase-3/7, PARP) cleaved ↑Bax*, (c-Jun, c-Fos)#*	[144]
Oleocanthal	Colon	HT29, SW480, HCT116	↓p-(p53), (AMPK, Bcl-2)* (Caspase 3, PARP) cleaved	[145]
		SW480, HT29	↑(Caspase 3,7, PARP) cleaved ↑γ-H2AX*	[42]
	Liver	Huh-7, HepG2, HCCLM3	↑(Caspase-3, PARP) cleaved ↓p-STAT, (Cyclin D1, Bcl2, Survivin, MMP2)*	[106]
		HepG2, Hep3B, Huh7, PLC/PRF/5	↑(Caspase 3,7, PARP) cleaved ↑γ-H2AX*	[42]
	Pancreatic	BxPC3	↑(Caspase 3, PARP) cleaved ↑p-ERK	[45]
Oleuropein	Colon	HT29	↑(p53, PPARγ)* ↓HIF-1α*	[79]
	Liver	HepG2, Huh7	↑(Caspase 3,8,9, PARP) cleaved ↓p-AKT, Bcl2#* ↑Bax#*	[84]
	Pancreatic	MIA PaCa-2, BxPC-3, CFPAC-1	↑(Caspase 3,7, PARP) cleaved ↑Bax*, (c-Jun, c-Fos)*#	[144]

AMPK, adenosine monophosphate-activated protein kinase; Bax, Bcl-2-associated X protein; Bcl2, B-cell lymphoma 2; COX2, cyclooxygenase 2; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; FOX3a, forkhead box O3a; γ-H2AX, γ-H2A histone family member X; HIF-1α, hypoxia inducible factor-1α; MMP2, matrix metallo proteinase 2; PARP, poly(ADP-ribose) polymerase; PPARγ, peroxisome proliferator-activated receptor γ; STAT3, signal transducer and activators of transcription 3; VEGF, vascular endothelial growth factor. #gene expression; *protein expression.

Celano M, et al Secoiridoids of olive and derivatives as potential coadjuvant drugs in cancer: a critical analysis of experimental studies, *Pharmacological Research* (2019),

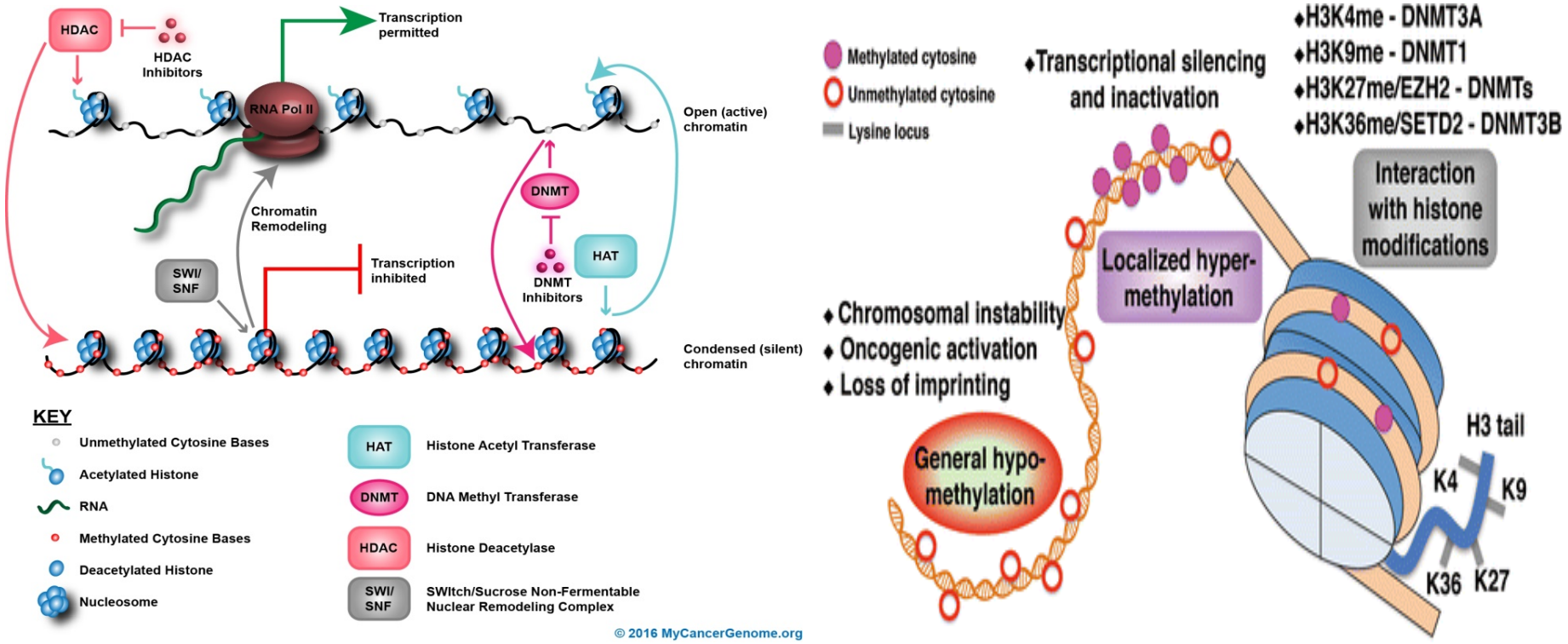
Table 6. Effects and molecular targets of olive secoiridoids and derivatives in several human cancer cell lines

Compound	Cancer	Cell lines	Molecular Targets	Ref.
Hydroxytyrosol	Thyroid	TPC-1, FB-2, WRO	↓ Cyclin D1#* ↑ p21#*, (p53, Bad)* (Caspase 3,8, PARP) cleaved	[113]
	Prostate	LNCaP, C4–2	(Caspase 3,7, PARP) cleaved ↑ (Bax, p21, p27)* ↓ (Bcl2, Bcl-x, cyclin D1,E, CDK 2,4)* NF-κB#*, p-AKT, p-STAT	[112]
	Blood	HL60	↑ (p21, p27) #*, Cyclin D3* ↓ CDK6*	[79]
Oleocanthal	Prostate	PC-3	↓ p-Met	[74]
	Blood	ARH-77	↓ MIP-1α*#, RANKL*, p-AKT, p-ERK1/2 (Caspase 3,9) cleaved ↑ p-P38	[110]
	Skin	HaCat, A431	↓ p-AKT, p-ERK	[146]
		A375, 501Mel	↓ Bcl2#, p-ERK, p-AKT (Caspase 3,9, PARP) cleaved	[85]
		A375, A2058, HaCaT	↓ pSTAT, pJAK2, pSrc	[108]
Oleuropein	Thyroid	TPC-1, BCPAP	↓ p-AKT and p-ERK	[31]
	Prostate	LNCaP, DU145	↓ p-AKT	[40]
	Ovary	HeLa	(Caspase 3,9, PARP) cleaved ↑ p-JNK, (Bax, p53, p-p53, p21)* ↓ Bcl2*	[70]
	Brain	U251, A172	↑ Bax* ↓ (Bcl2, pAKT, MMP2,9)* (Caspase 3,9) cleaved	[72]
	Lung	A549	PARP cleaved ↑ p-ATF2, p-P38, Bax* ↓ p-ERK1/2, Bcl2*	[114]
		H1299	PARP cleaved ↑ Bax, ATF2, p-P38* ↓ Bcl2*	[115]
Tyrosol	Ovary	HeLa	↓ p-S6K1, p-4E-BP1*	[111]

ATF2, activating transcription factor 2; Bad, Bcl-2-associated death promoter; Bax, Bcl2-associated X protein; Bcl2, B-cell lymphoma 2; CDK, cyclin dependent kinase ; 4E-BP1, eukaryiotic initiation factor 4E (eIF4E) binding protein 1; ERK, extracellular regulated kinase; MIP-1α, macrophage inflammatory protein 1α; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; PARP, poly(ADP-ribose) polymerase; RANKL, receptor activator of nuclear factor κB ligand; S6K1, ribosomal protein S6 kinase β1; STAT, signal transducer and activator of transcription. #gene expression; *protein expression.

Celano M, et al Secoiridoids of olive and derivatives as potential coadjuvant drugs in cancer: a critical analysis of experimental studies, *Pharmacological Research* (2019),

Phenols and epigenetics control



Global DNA hypomethylation, gene promoter hypermethylation and aberrant histone posttranslational modifications are hallmarks of neoplastic cells, which have been associated with genomic instability and altered gene expression

Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol 2010;28:1057–68.

OXFORD

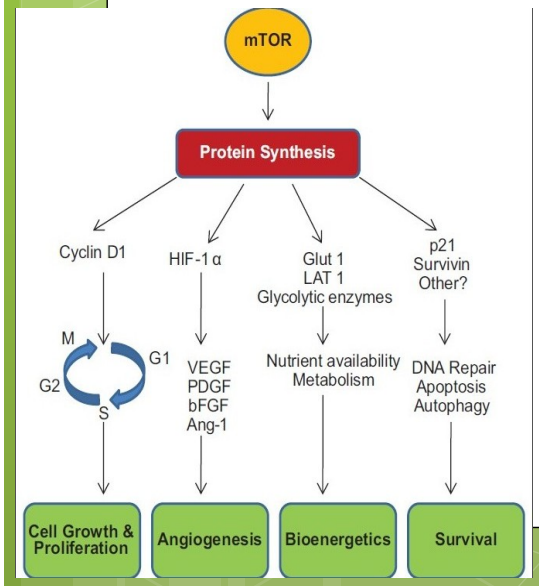
Carcinogenesis, 2018, Vol. 39, No. 4, 601–613
doi:10.1093/carcin/bgy023
Advance Access publication February 14, 2018
Original Article

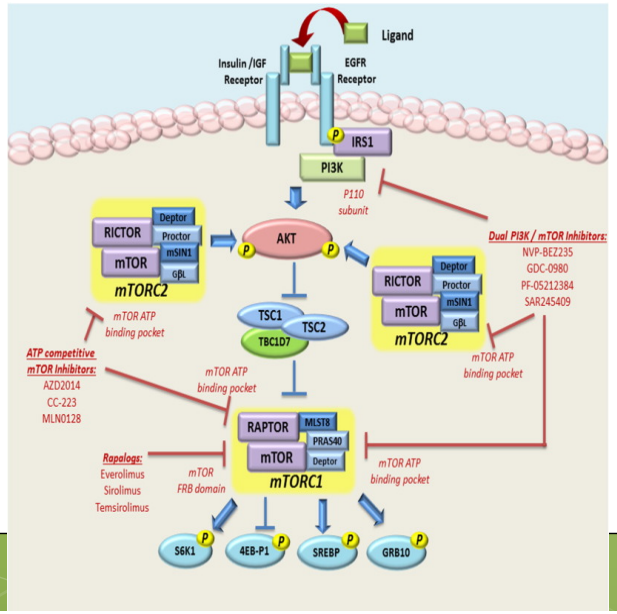
ORIGINAL ARTICLE

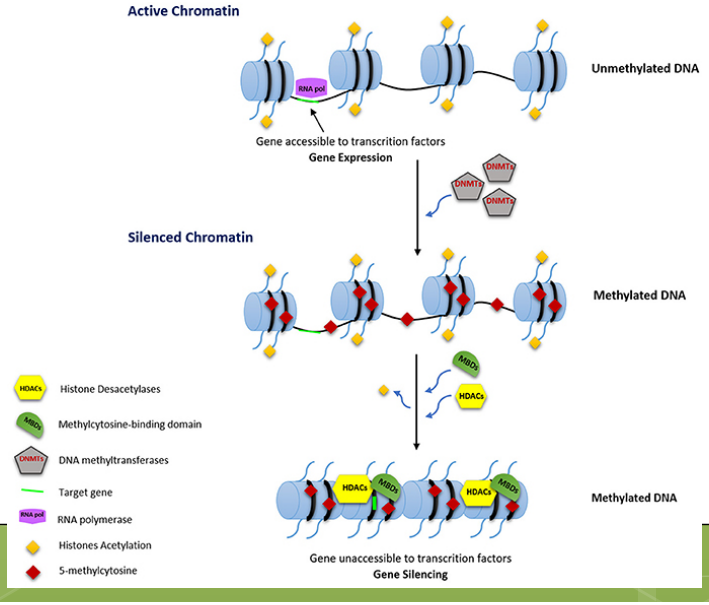
Extra-virgin olive oil contains a metabolo-epigenetic inhibitor of cancer stem cells

Bruna Corominas-Faja[1,2,†], Elisabet Cuyàs[1,2,†], Jesús Lozano-Sánchez[3,4,†], Sílvia Cufí[2,15], Sara Verdura[1,2], Salvador Fernández-Arroyo[5,6], Isabel Borrás-Linares[4], Begoña Martin-Castillo[7], Ángel G. Martin[8], Ruth Lupu[9,10], Alfons Nonell-Canals[11], Melchor Sanchez-Martinez[11], Vicente Micol[12,13], Jorge Joven[5,6], Antonio Segura-Carretero[3,4] and Javier A. Menendez[1,2,14,*]

- In silico studies and in vitro assays on BC cancer steam cells suggested that **oleacein** may
- Inhibit mTOR by binding and inhibiting both the ATP-binding kinase domain site in mTOR
- inhibit DNA methyltransferase (DNMT)







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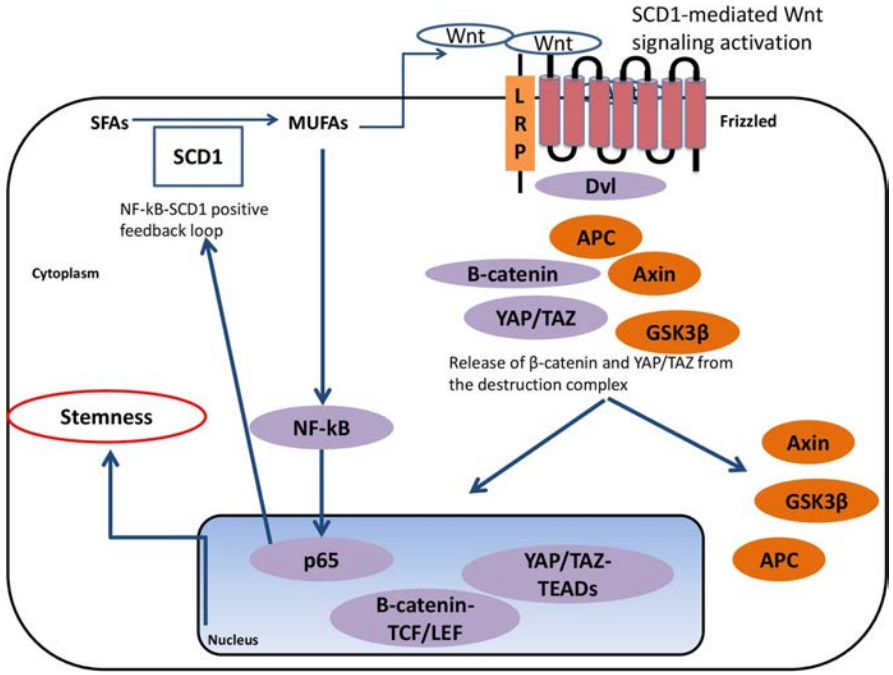
- Oleacin might functionally deplete tumor-initiating CSC-like (cancer stem cells) states that sustain tumorigenicity by impacting fundamental controllers of cell fate choice, a metabolo-epigenetic mechanism involving both the downregulation of stemness-driving transcription factors and the re-activation of epigenetically suppressed differentiation programs.



Metabolic features of cancer stem cells: the emerging role of lipid metabolism Rita Mancini1 et al . Oncogene 2018

Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry xx (2014) xxx–xxx
Journal of Nutritional Biochemistry

Extravirgin olive oil up-regulates CB_1 tumor suppressor gene in human colon cancer cells and in rat colon via epigenetic mechanisms

Andrea Di Francesco[a], Anastasia Falconi[a], Clara Di Germanio[b], Maria Vittoria Micioni Di Bonaventura[c], Antonio Costa[d], Stefano Caramuta[e], Michele Del Carlo[a], Dario Compagnone[a], Enrico Dainese[a,f], Carlo Cifani[c,1], Mauro Maccarrone[d,f,1,*], Claudio D'Addario[a,g,1,*]

- Phenolic extracts has been identified as a modulator in the expression of the gene encoding for the cannabinoid type 1 receptor (CNR1) a tumor suppressor gene exerting antiproliferative effects and whose site-specific promoter hypermethylation, cause loss in gene function
- In particular, it was observed hypomethylation of CNR1 promoter after HT treatment, and this effect was associated both with an increase of CNR1 expression and a reduced proliferation of colon cancer cell lines

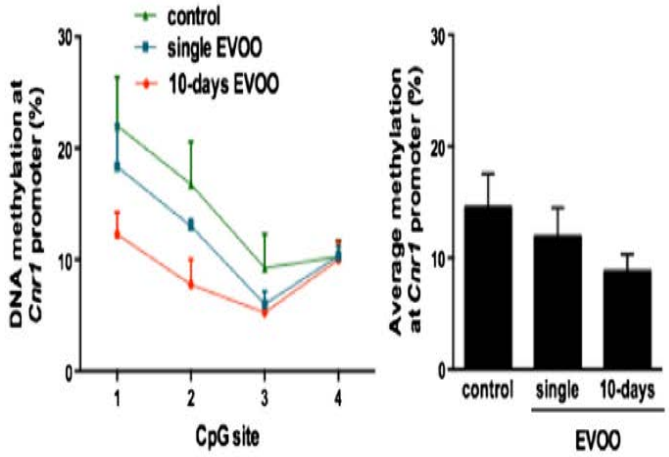


Fig. 6. DNA methylation changes of rat *Cnr1* gene promoter after EVOO diet. *Cnr1* DNA methylation was analyzed by pyrosequencing in colon samples of rats treated with vehicle (control) or EVOO for 2 h (single) and 10 days. * $P<.05$ vs. control, Turkey–Kramer test.

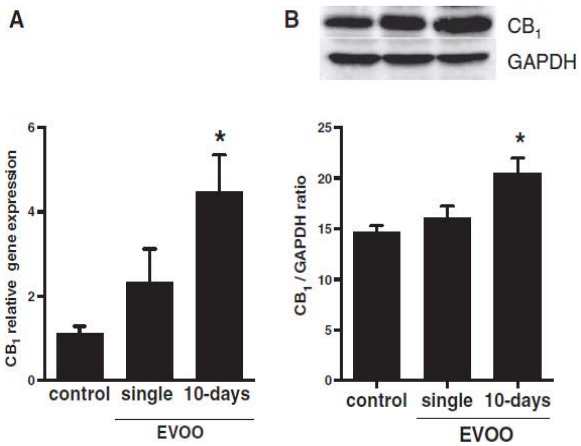


Fig. 5. (A) Effects of dietary EVOO supplementation on *Cnr1* gene expression analyzed in colon samples of rats treated with vehicle (control) or EVOO for 2 h (single) and 10 days (* $P<.05$ vs. control, Turkey–Kramer test). (B) Representative immunoblot showing effects of dietary EVOO supplementation on CB_1 protein levels in rat colon samples. Protein levels were assessed as ratio of CB_1 absorbance normalized to that of GAPDH. Values are reported as mean±S.E.M. ($n=6$). *$P<.05$ vs. control.

Microbiota and cancer

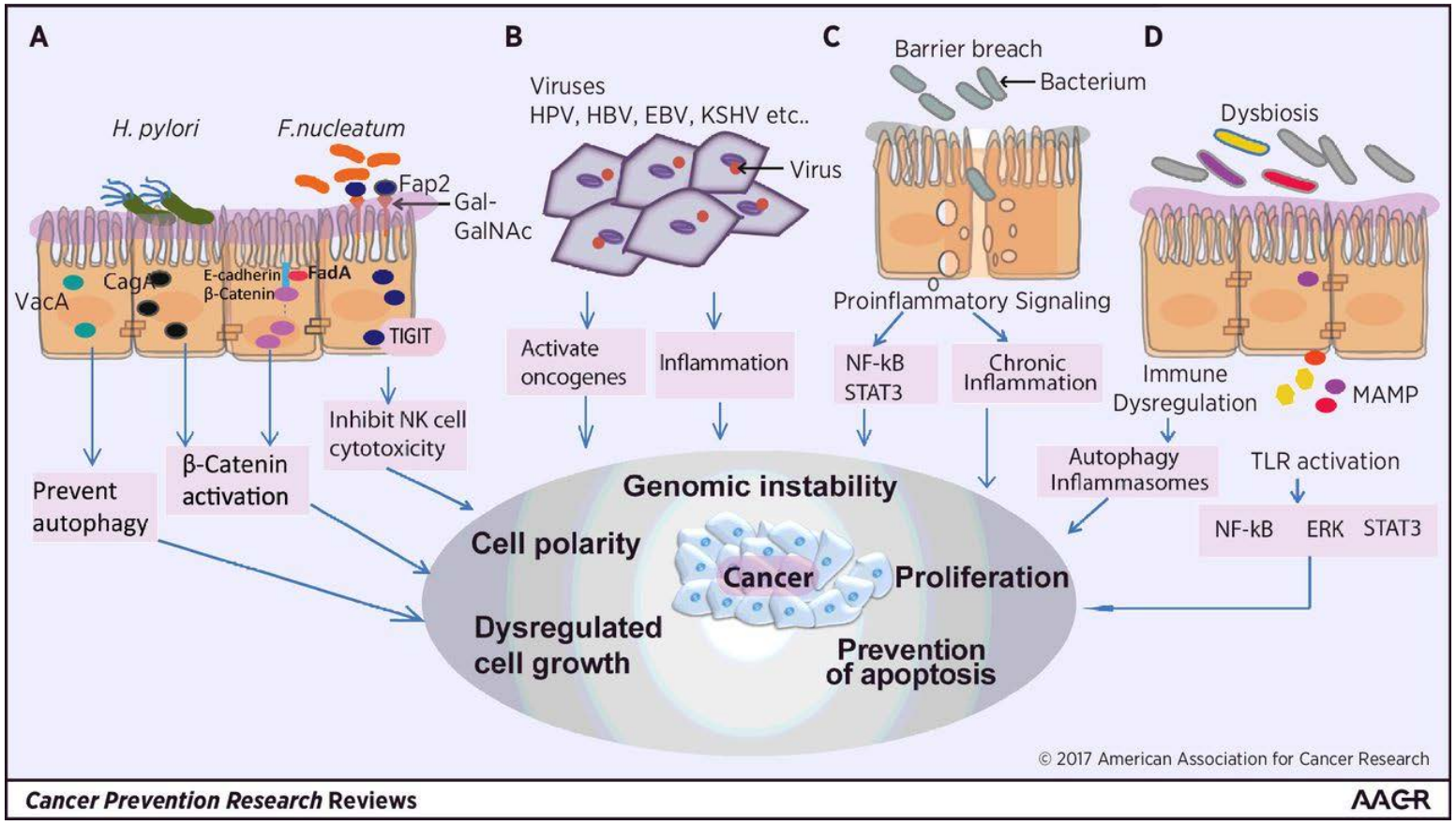
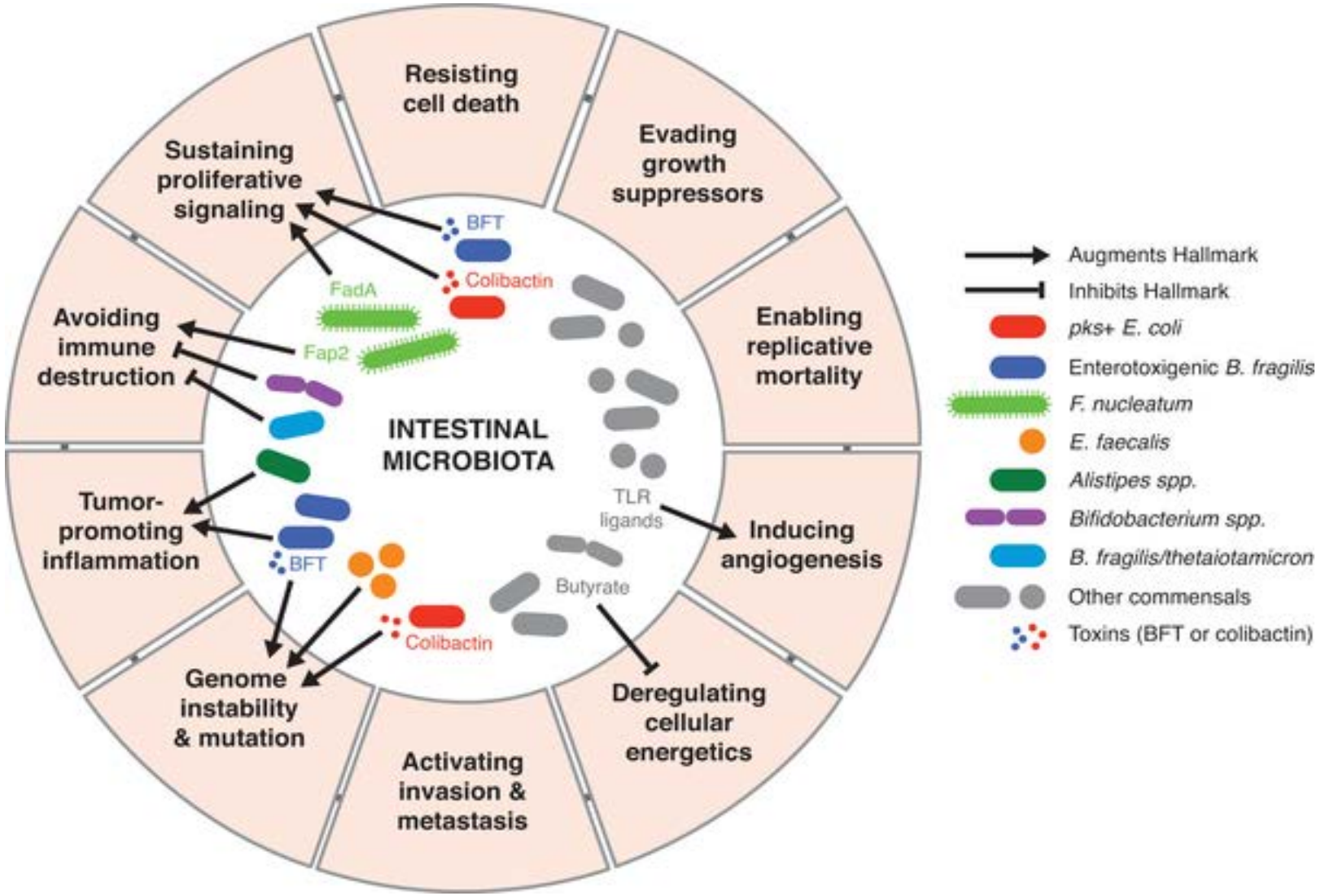


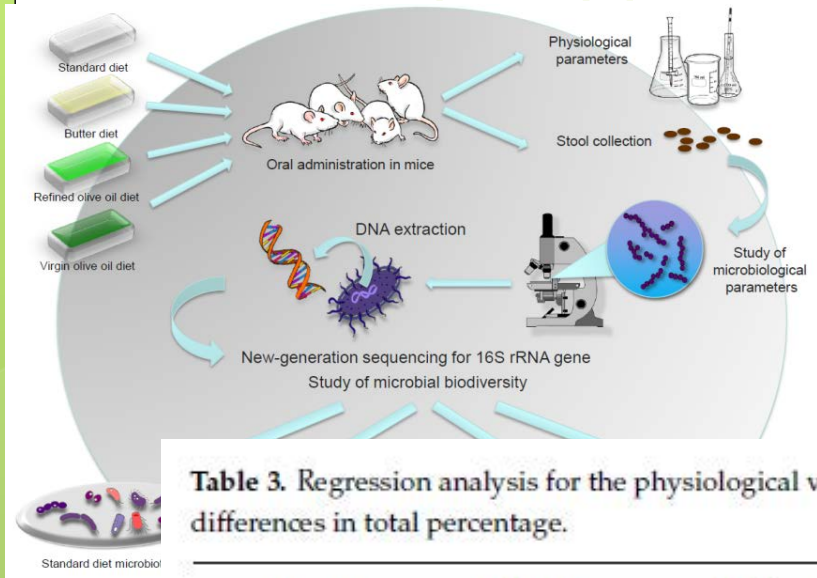
Fig 1. Microbial-derived signals modulate numerous hallmarks of cancer through diverse mechanisms.



Fulbright LE, Ellermann M, Arthur JC (2017) The microbiome and the hallmarks of cancer. PLOS Pathogens 13(9): e1006480. https://doi.org/10.1371/journal.ppat.1006480
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006480

PLOS | PATHOGENS

Olive oil polyphenols and microbiota



Diet.	SD		EVOO		ROO		BT	
Composition	g/100 g	% energy	g/100 g	% energy	g/100 g	% energy	g/100 g	% energy
Protein	16.5	20	16.5	14	16.5	14	16.5	14
Carbohydrates	60	72	55	48	55	48	55	48
Fat	3	8	20	35	20	35	20	35
Total Energy (kJ/g)	14.2		19.6		19.6		19.6	

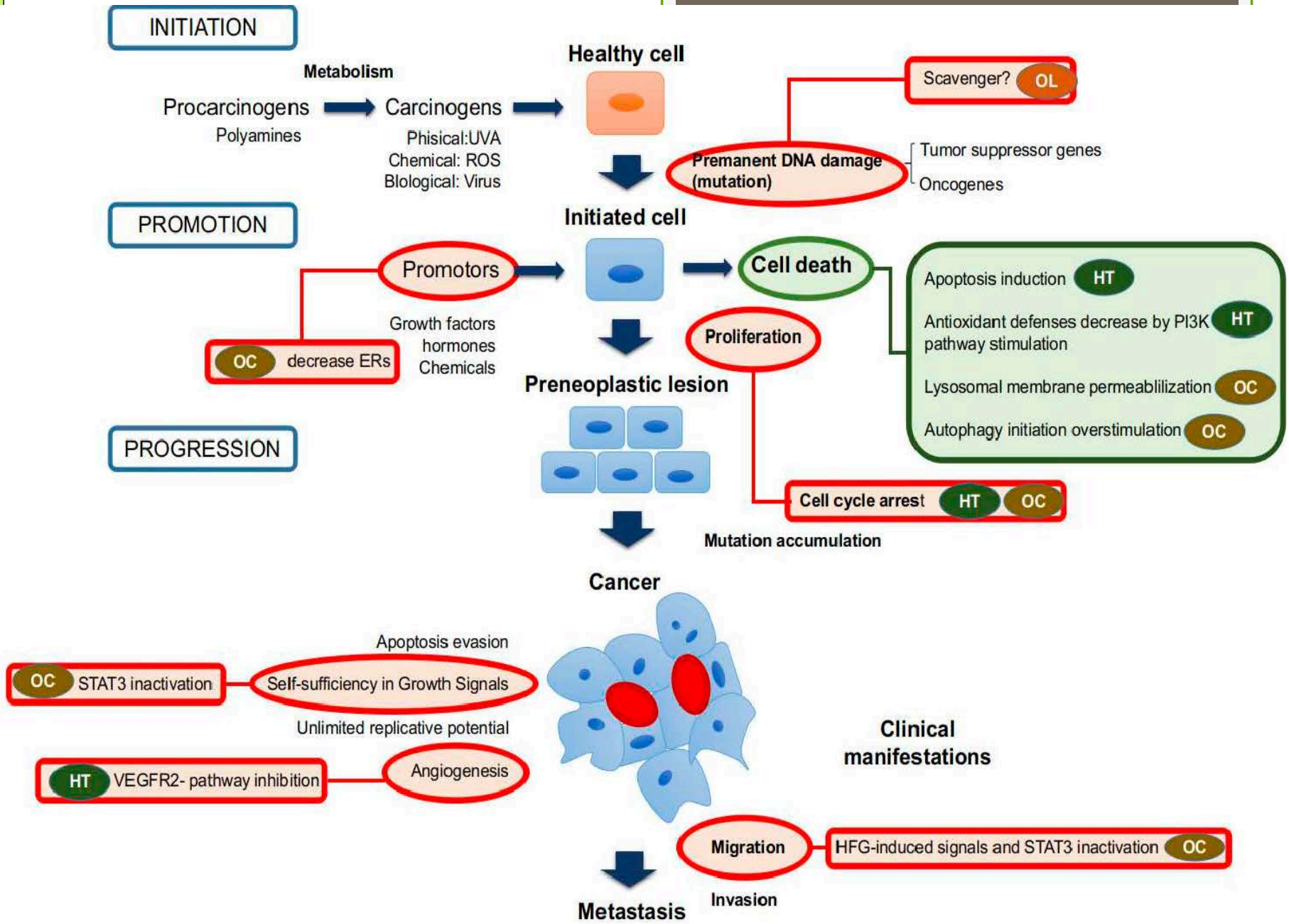
Table 2. Metabolic and physiological values in mice at the end of the experiment.

Diet	SD	EVOO	BT	ROO	p
Food Intake (g/day)	3.70 ± 0.65	3.74 ± 0.39	2.76 ± 0.38	3.83 ± 0.41	n.s.
Water intake (mL/day)	8.71 ± 1.61	11.28 ± 1.60	6.67 ± 1.95	7.14 ± 1.10	n.s.
Diuresis (mL/day)	2.44 ± 0.70	2.68 ± 0.65	1.65 ± 0.63	2.41 ± 0.50	n.s.
			42.15 ± 0.61	38.09 ± 0.86	A *
			190.50 ± 8.53	156.14 ± 19.54	A **
			1433.23 ± 226.95	897.33 ± 259.13	n.s.
			78.02 ± 42.82	89.33 ± 14.97	n.s.
			1518.44 ± 329.97	875.97 ± 132.60	B *
			192.0 ± 17.2	259.63 ± 20.50	C *
			48.94 ± 4.89	47.44 ± 8.89	n.s.
			98.67 ± 9.98	115.4 ± 11.39	D **
			0.32 ± 0.03	0.28 ± 0.02	E *

m [12]. n.s.: not significant; A: differences in
), and ROO diets; C: differences in ROO vs.
OO, and BT diets, and EVOO vs. ROO diet;

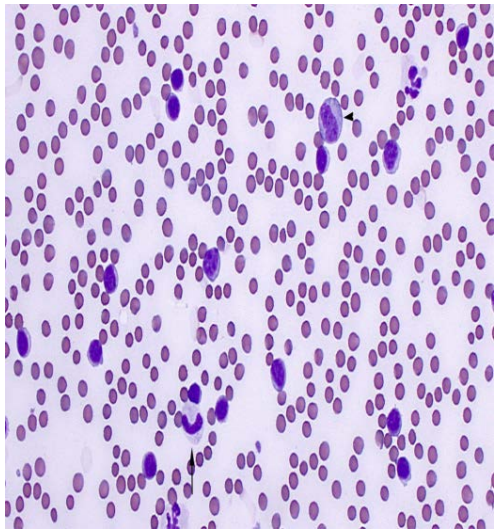
Table 3. Regression analysis for the physiological variables studied and those families with statistical differences in total percentage.

Variable	Diuresis (0.23/0.0086)	Leptin (0.79/0.0000)	Insulin * (0.12/0.0456)	Total Cholesterol (0.23/0.0185)	Triglycerides (0.62/0.0000)
Prevotellaceae	n.s.	n.s.	n.s.	−2.73 ± 1.18 (0.0276)	n.s.
Desulfovibrionaceae	n.s.	−309.07 ± 78.84 (0.0006)	n.s.	n.s.	n.s.
Marinilabiliaceae	n.s.	−2112.02 ± 1004.75 (0.0462)	n.s.	n.s.	n.s.
Erysipelotrichaceae	1.55 ± 0.54 (0.0086)	n.s.	−0.461 ± 0.221 (0.0456)	n.s.	n.s.
Sutterellaceae	n.s.	−1026.14 ± 189.08 (0.0000)	n.s.	n.s.	n.s.
Spiroplasmataceae	n.s.	−5033.70 ± 2006.53 (0.0000)	n.s.	60.63 ± 28.27 (0.0399)	n.s.



Rodriguez et al. Int. J. Mol. Sci. **2018**, *19*(8), 2305;

Effect of nutritional intervention with High-Oleocanthal and Oleacein olive oil in patients with chronic lymphocytic leukemia



Andreas Paola Rojas Gil[1], Ioannis Kontonis[2], Anastasios Ioannidis[1], Tzortzis Nomikos [3], Maria Efthymia Katsa [3], Georgios Kosmidis[1], Eleni Meliou[4], Prokopis Magiatis[4]

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2. General Hospital of Lakonia Hematology Department, Sparta, Greece
3. Department of Nutrition and Dietetics, School of Health Sciences and Education, Harokopio University, Athens, Greece
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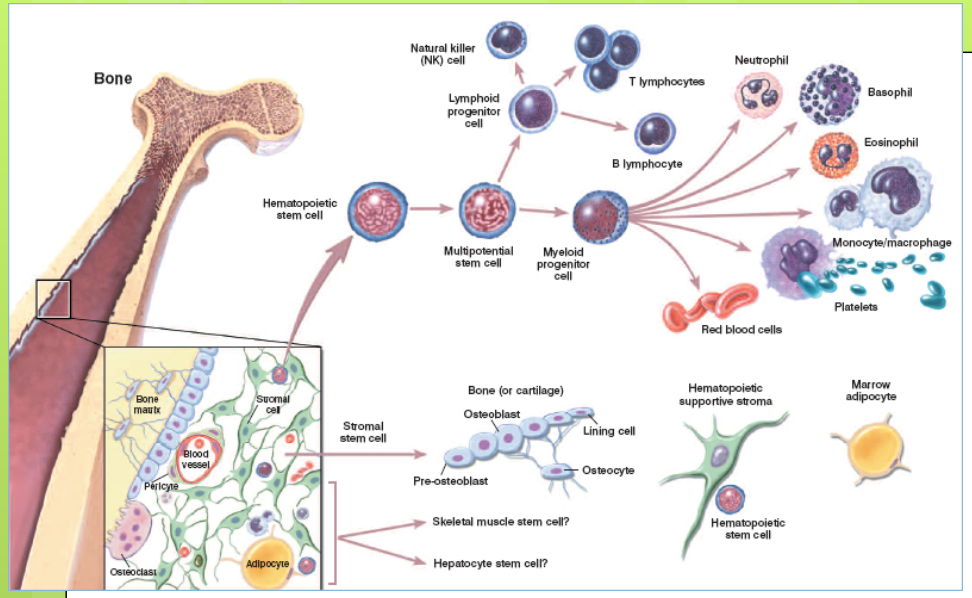
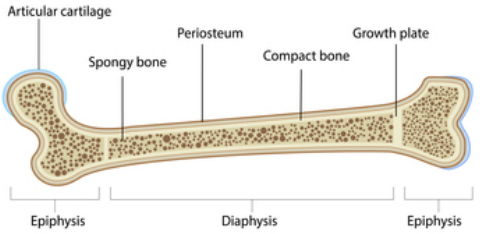
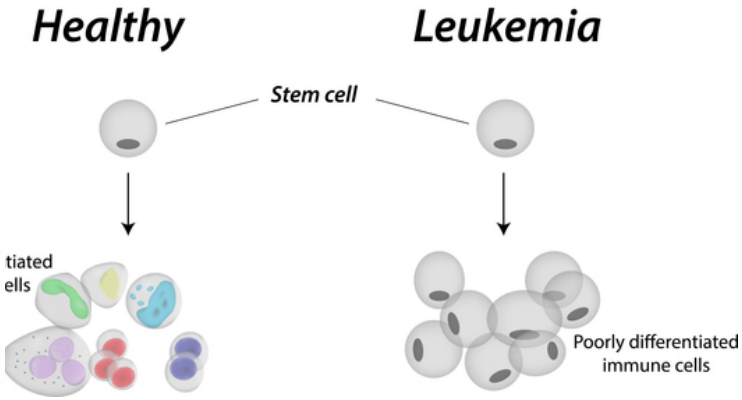


Leukemia





Leukemia, Lymphoma & Myeloma

Understanding and treating a family of blood cancers.

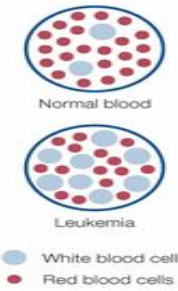
Blood cancers are the second deadliest form of cancer.

Estimated Deaths in the U.S., 2016

- Lung & Bronchus Cancer: 158,080
- Blood Cancers: 58,320
- Colorectal Cancer: 49,190
- Pancreatic Cancer: 41,780
- Breast Cancer: 40,890

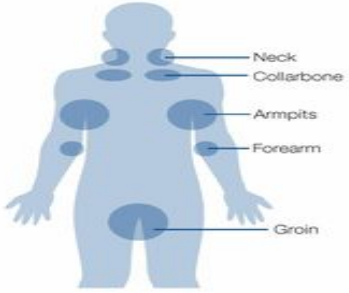
Blood cancers come in many forms and are grouped into three major types.

Leukemia



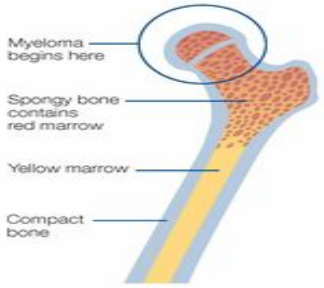
- Leukemia usually starts in bone marrow.
- It creates abnormal white blood cells that don't function correctly and crowd out other cells.
- There are 4 major types of leukemia:
  - Acute lymphocytic leukemia (ALL)
  - Acute myelogenous leukemia (AML)
  - Chronic lymphocytic leukemia (CLL)
  - Chronic myelogenous leukemia (CML)
- These can be acute (progresses

Lymphoma



- Lymphomas start in the lymphatic system, which is part of the circulatory and immune systems of the body.
- It creates abnormal white blood cells that grow and form masses and weaken the immune system.
- Hodgkin lymphoma is one of the most curable cancers.
- Non-Hodgkin lymphomas are a group of related cancers that can be fast or slow growing.

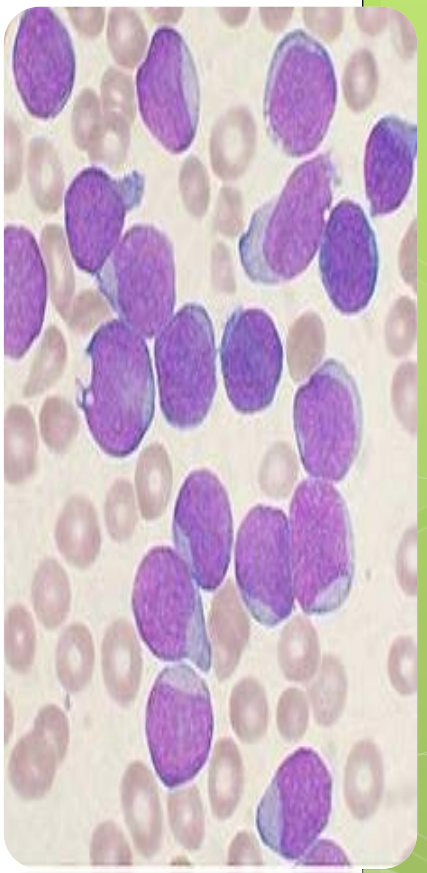
Myeloma



- Myeloma typically starts in the bone marrow.
- It consists of plasma cells, a type of white blood cell in bone marrow that is responsible for antibody production.
- There are many forms, the most common being multiple myeloma.
- Patients may have no symptoms in early stages.

Chronic Lymphocytic Leukemia (CLL)

- CLL is the most common adult leukemia in western countries. It is responsible for 25% of all leukemias.
- It is characterized by accumulation of monoclonal B-lymphocytes in bone marrow, peripheral blood, lymphatic tissues and spleen and is often asymptomatic and slow in its development.
- Patients with CLL do not always require immediate therapy. However, once symptomatic, the median survival of these patients ranges from 18 months to 6 years depending on the clinical stage



Hallek M (2015) Chronic lymphocytic leukemia: 2015 Update on diagnosis, risk stratification, and treatment. Am J Hematol 90(5): 446-460

Chronic Lymphocytic Leukemia (CLL)

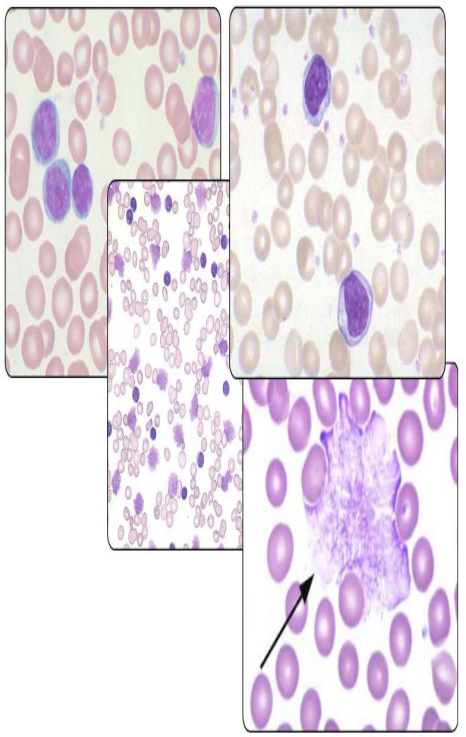
Table 1. Rai classification system*

Stage	Description	Median survival (months)	Risk status (Modified Rai)
0	Lymphocytosis, lymphocytes in blood >15,000/mcL and >40% lymphocytes in the bone marrow	140	Low
I	Stage 0 with enlarged node(s)	100	Intermediate
II	Stage 0–1 with splenomegaly, hepatomegaly, or both	70	Intermediate
III	Stage 0–II with hemoglobin <11.0 g/dL or hematocrit <33%	20	High
IV	Stage 0–III with platelets <100,000/mcL	20	High

** Adapted from the 2008 NCI guidelines; BC Cancer Agency 2008 guidelines.*[3,4]

Chronic Lymphocytic Leukemia (CLL)

- Criteria for the diagnosis of CLL are monoclonal B lymphocytes ≥5000 lymphocytes/ml in the peripheral blood for at least 3 months,
- prolymphocytes ≤55%,
- co-expression of CD5 and B-cell surface antigens CD19, CD20, and CD23, low levels of CD20, CD79b and
- low expression of surface immunoglobulins (sIg), and kappa or lambda light chain restriction



Gomes LC, P et al (2017) Prognosis biomarkers evaluation in chronic lymphocytic leukemia. Hematol Oncol Stem Cell Ther 10: 57-62

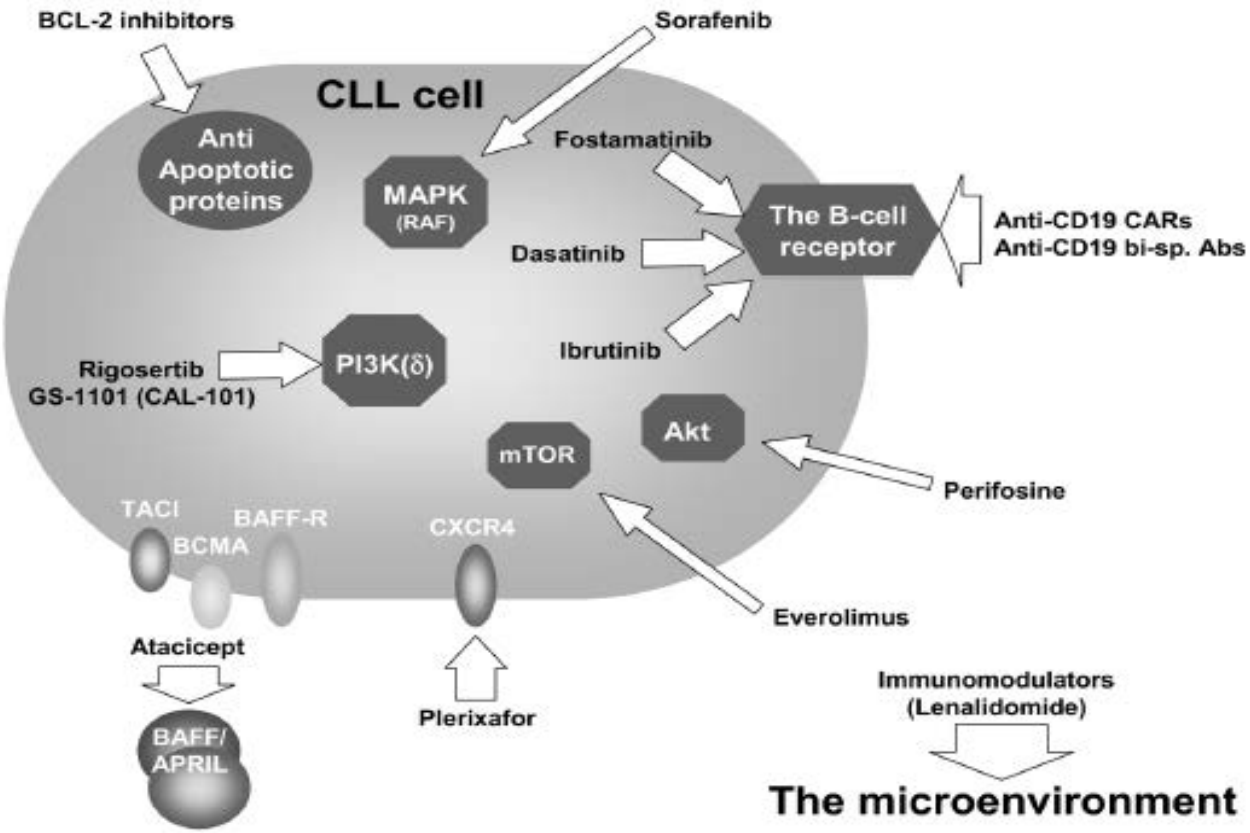
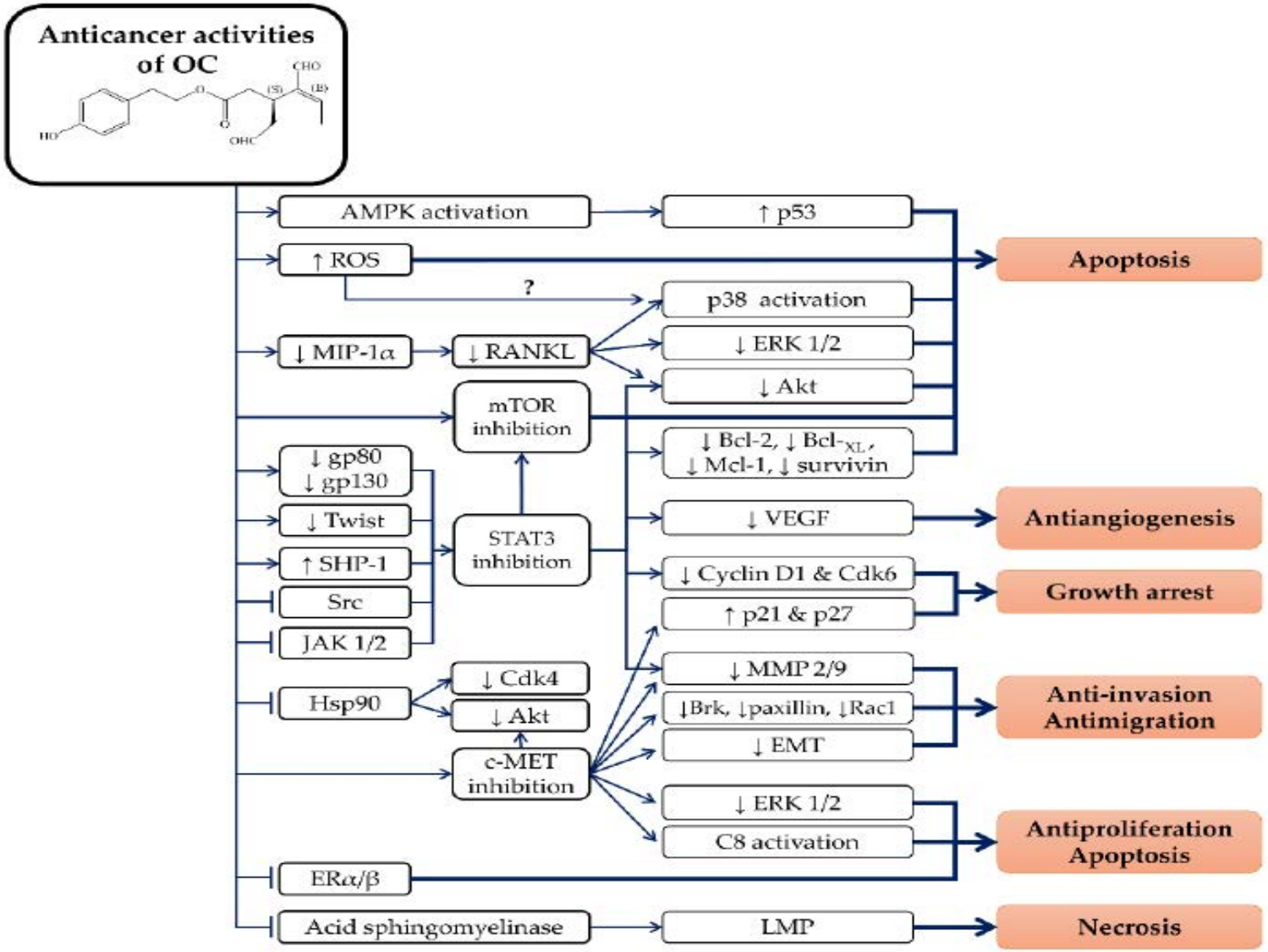


Figure 3.
Therapeutic targeting of microenvironmental-induced signaling in CLL. Current and experimental CLL therapeutics (arrows) target the various components of the microenvironment-CLL milieu and its associated signaling network. Thus the BCR and its associated components are targeted by antibodies (anti-CD19) or small molecules (e.g. SYK (e.g. fostamatinib) or BTK inhibitors (e.g. ibrutinib)). Small molecules are also utilized to inhibit mTOR, Akt, PI3K(δ) and the MAPK cascades. Extracellular inhibitors such as plerixafor or atacicept can block the association of SDF-1 or BAFF/APRIL, respectively, with their receptors on the CLL cell. Both the microenviroment (e.g. the immune system) and the outcome of its signaling responses in the CLL cells (e.g. upregulation of BCL-2) are avenues for therapeutic targeting.

Hematol Oncol Clin North Am. 2013 April ; 27(2): 173–206. d

Oleocanthal and cancer



Oleocanthal and leukemia

Human myeloma ARH-77 cells Murine myeloma MOPC-31C cells	• Cytotoxicity, antiproliferation, G1 arrest and apoptosis with caspase-9/3 activation • Downregulated MIP-1α and led to RANKL, Akt, and ERK1/2 downregulation, but p38 activation	[75]
Human histiocytic lymphoma U937 cells	• Downregulated Hsp90 client proteins (Akt and Cdk4)	[79]

Nutrients **2018**, *10*, 570; doi:10.3390/nu10050570

Aim

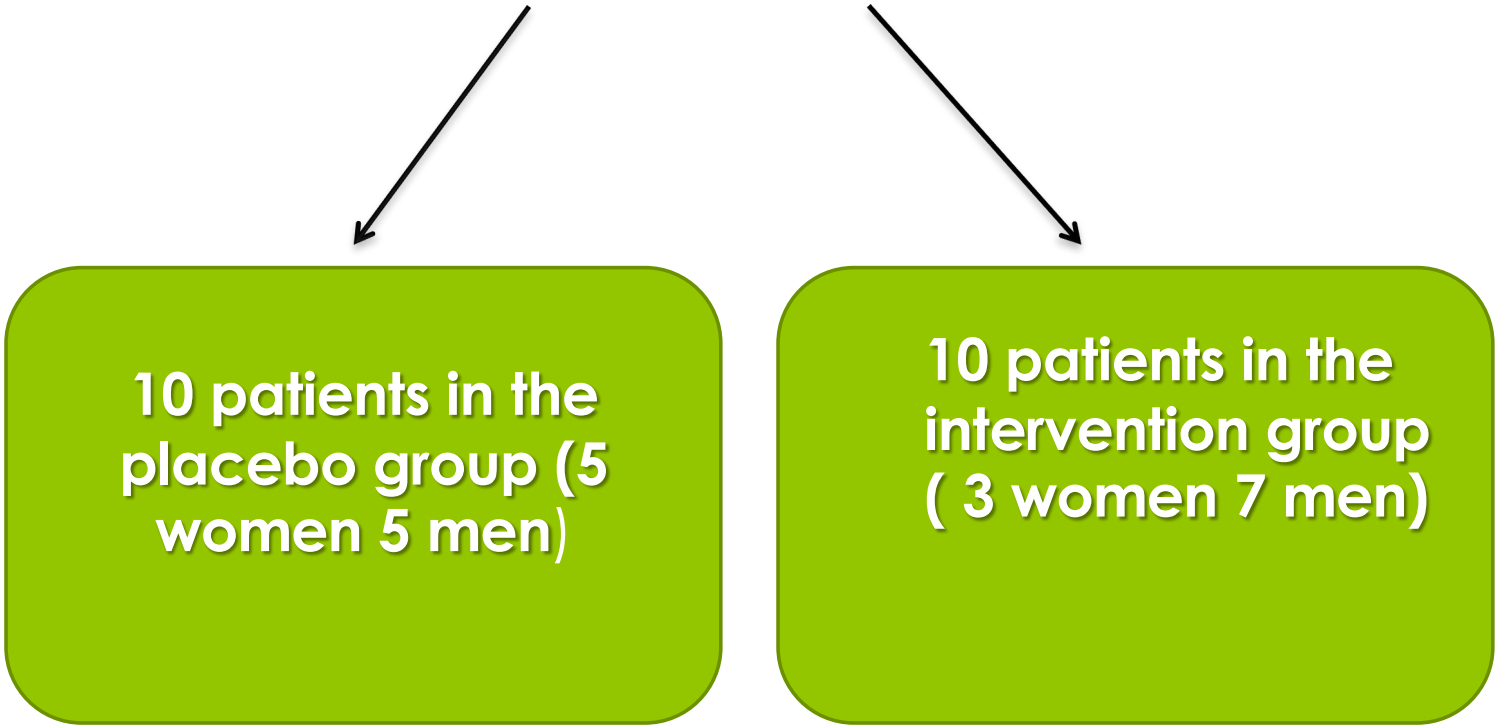
The aim of the present study was

- to investigate the nutritional effect of EVOO consumption, rich in oleocanthal / oleacein (OC / OL), on hematological and cellular markers and disease progression in patients with Chronic Lymphocytic Leukemia (CLL) who do not require treatment yet.
- Additionally, their potential cellular mechanism of action was also investigated.

Methodology

First intervention vs placebo

- 20 patients (aged 72,4 ± 7,4 years old) with CLL participated the research (no medication was taken)
- Patients were randomly divided into 2 groups



Olive oil rich in oleocanthal

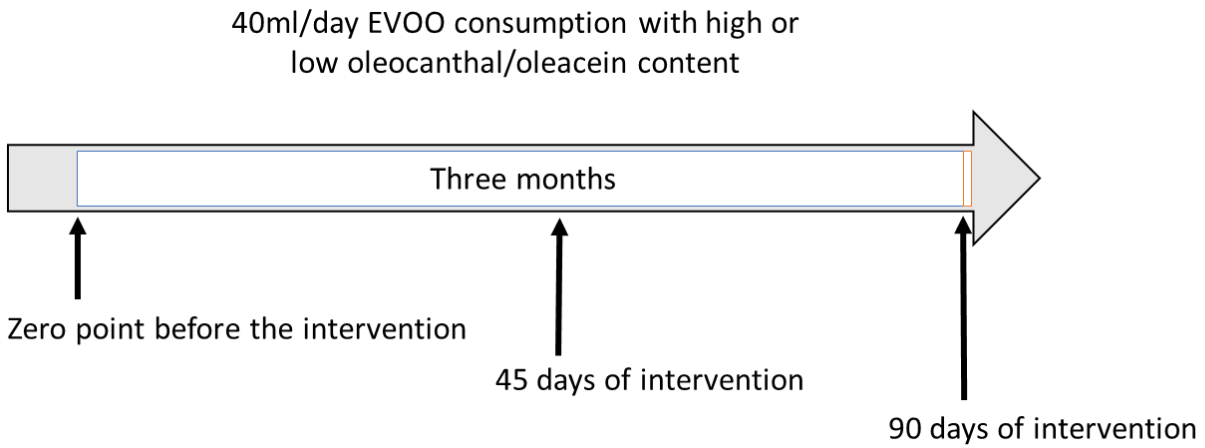
- Oleocanthal 416 mg/Kg
- Oleasin 284 mg/Kg
- D1 (Oleocanthal + Oleasin) =700 mg/Kg
- Analysis with NMR July2017
- Variety: Lianolia of Corfu
- Origin : St. Matthew, Corfu
- Harvest in October 2016

Sponsor of olive oil
Spyros & Giorgos Dafnis

Olive oil poor in oleocanthal

- Oleocanthal 82 mg/Kg
- Oleasin 33 mg/Kg
- D1 (Oleocanthal + Oleasin) =115 mg/Kg
- Free Tyrosol : 250 mg/Kg
- Total phenols : 505 mg/Kg
- Category: Extra Virgin
- Analysis with NMR July2017
- Harvest in October 2016

- Note: It does not matter just how many phenols are included in olive oil but which are these phenols.

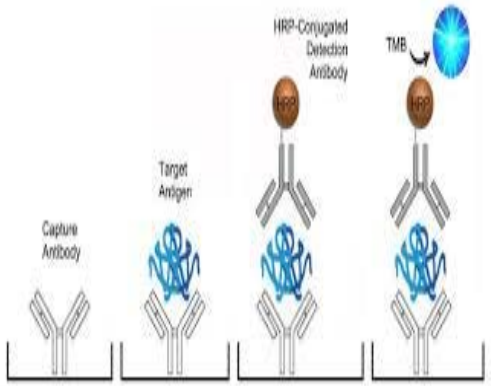


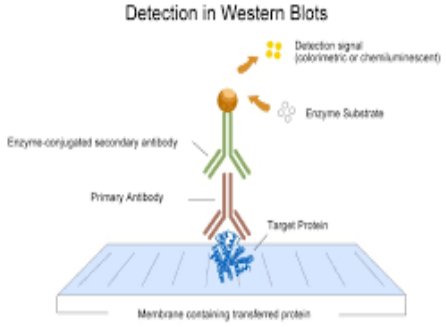
- Flow chart of the intervention protocol. Intervention A: High oleocanthal/oleacein EVOO. Intervention B (control group): Low oleocanthal/oleacein EVOO

Methods

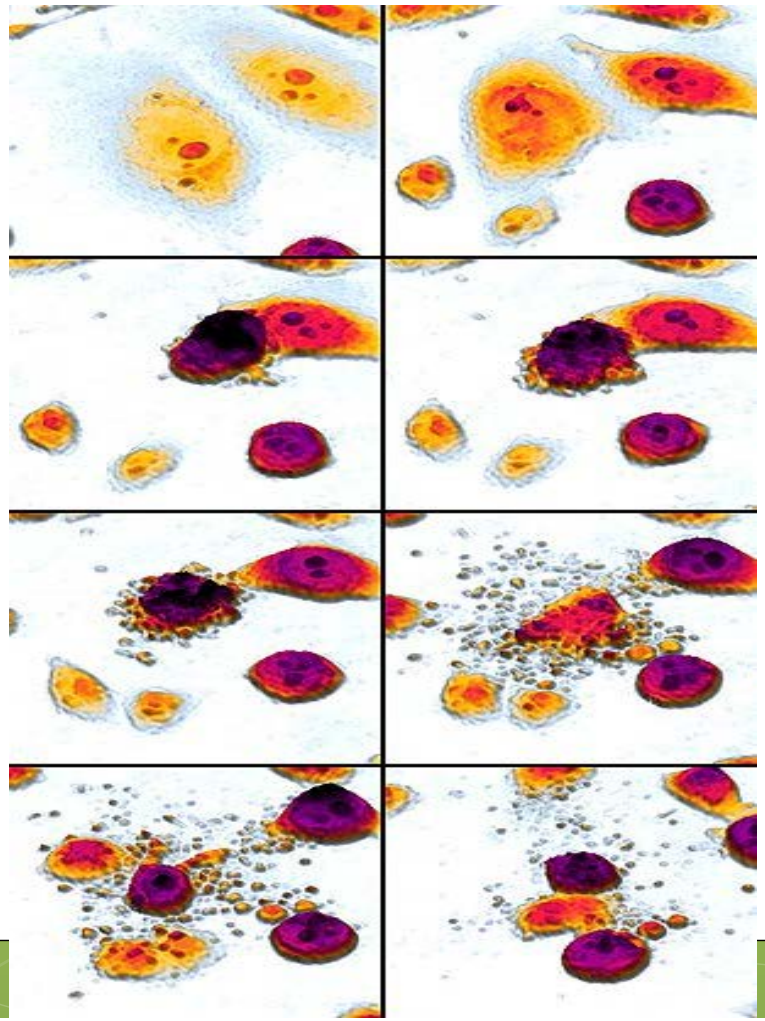
At all time point, we studied

- Blood cell count
- Lipid profile
- Fasting Blood Glucose
- Hepatic markers
- Apoptotic markers (survivin, ApoFas)
- Isolation of mononuclear white cells
- Western Immnoblotting in mononuclear white blood cells (cell cycle markers)



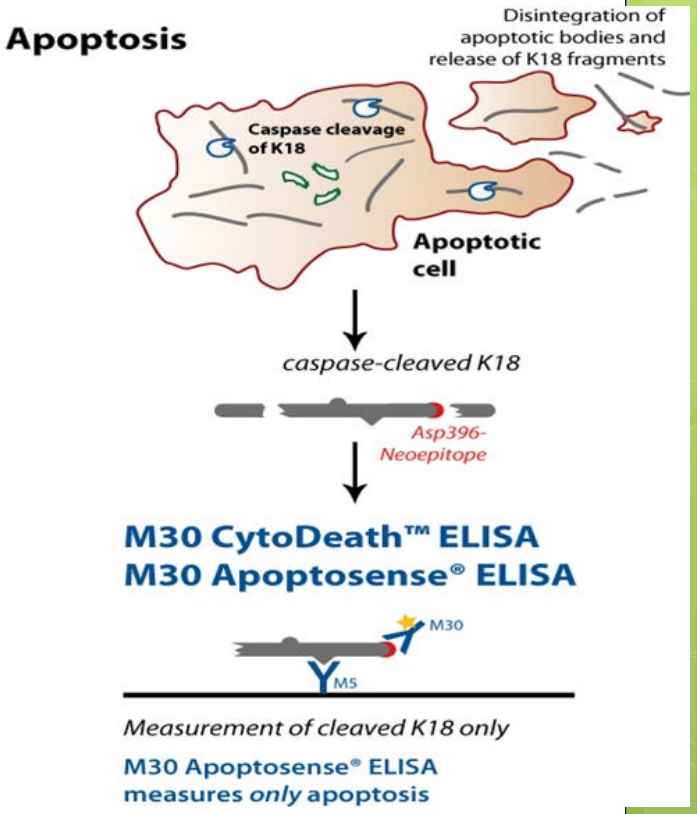


Apoptotic markers



Caspase activity - cck18

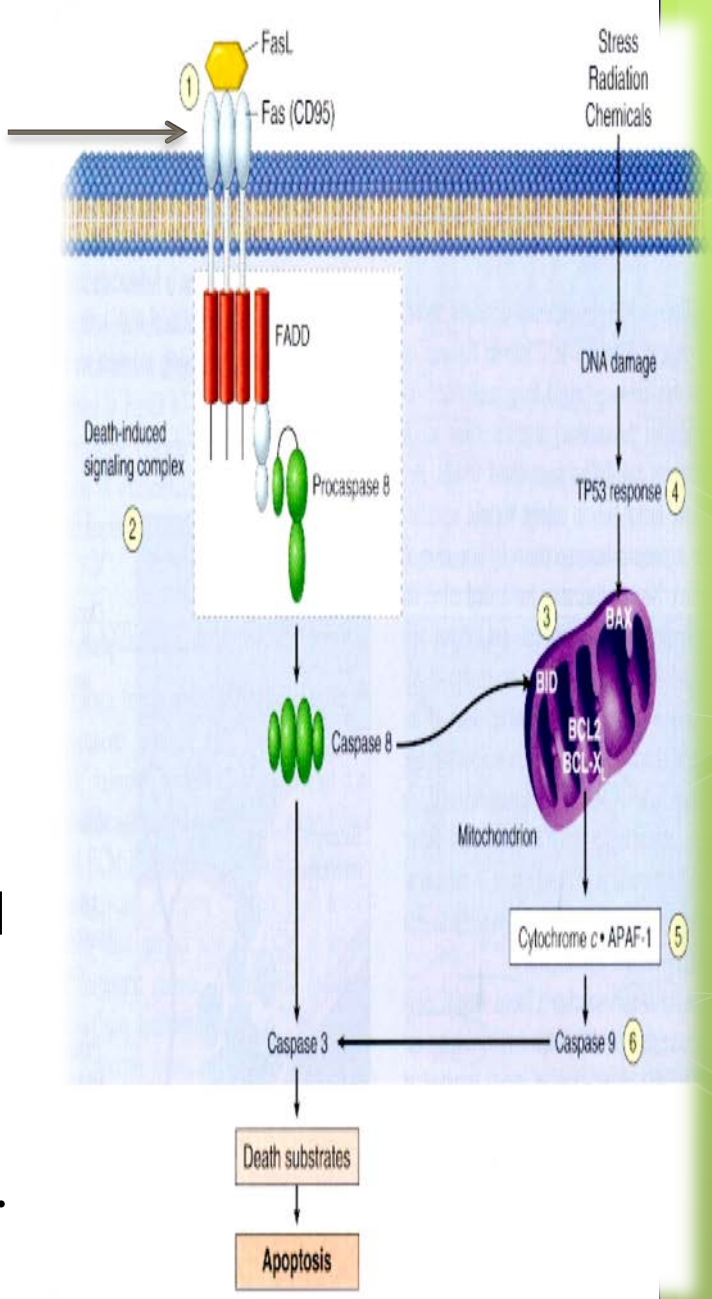
- Cytokeratin-18 is a cytoskeletal protein
- During apoptosis cytokeratin-18 is cleaved twice by caspases, generating an 18-kilodalton fragment termed caspase-cleaved cytokeratin-18 (cCK-18).
- This caspase-specific processing exposes a neo-epitope at the c-terminal end of cCK-18 that is recognized by a specific monoclonal antibody (M30)



APO1/Fas

APO1/Fas (CD95)→ APO1/Fas (CD95) is a glycosylated surface protein (48 kDa) which contains a single transmembrane region and is considered as a member of TNF/NGF receptor's superfamily.

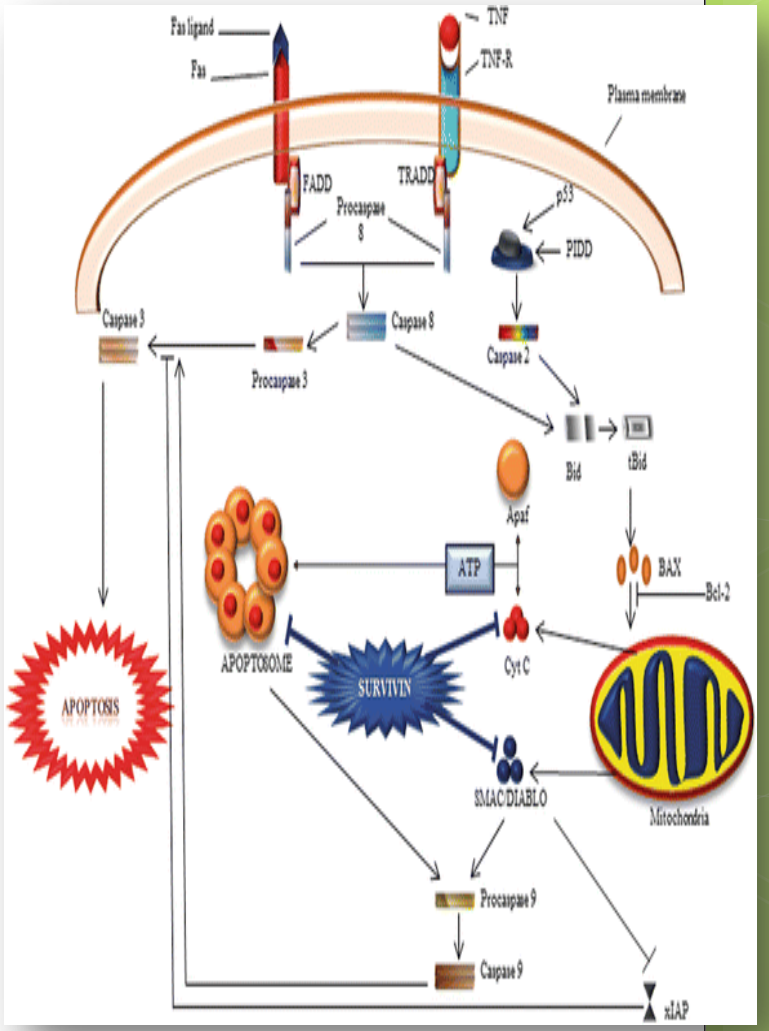
- It is expressed in a variety of human T and B cell lines, several different tumor cells and in various normal human tissues.
- APO1 activation (with its ligand or anti-APO1 monoclonal antibodies) may lead to rapid induction of apoptosis in cells with increased sensitivity.
- Fas receptor can activate inflammatory pathways in several cell lines and tissues.



J Clin Endocrinol Metab, 2014. 99(1): p. E36-44.

Survivin

- Survivin is a member of the apoptosis inhibitor family.
- It is overexpressed in most human cancer cells.
- It is involved in both apoptosis inhibition and promotion of cell division.



Altieri DC. Survivin, cancer networks and pathway-directed drug discovery. Nat Rev Cancer. 2008;8:61–70.

Table 2

Differences in hematological and molecular markers of CLL Patients during intervention with low oleocanthal content olive oil.

	Control Group			
	Baseline	45 days	90 days	p-value
Whole blood count				
WBC (x10^3/mm^3)	15.18 ± 8.12	16.16 ± 7.78	15.36 ± 6.36	0.840
LYMPH (x 10^3/mm^3)	10.90 ± 7.52	11.80 ±7.11	11.34 ± 6.23	0.956
PLT (x10^3/mm^3)	203.20 ± 52.58	211.40 ± 31.33	200.00 ± 34.52	0.316
Ht (%)	41.58 ± 3.20	42.56 ± 3.35	42.50 ± 2.55	0.185
HGB (g/dl)	13.86 ± 1.26	14.14 ± 1.31	14.02 ± 0.97	0.573
Biochemical Marker				
Glucose (mg/dl)	107.60 ± 8.85	110.20 ± 18.42	98.00 ± 9.30	0.083
Urea (mg/dl)	44.20 ± 8.37	45.60 ± 9.71	41.60 ± 9.42	0.759
Uric Acid (mg/dl)	4.72 ± 0.60	4.52 ± 0.83	4.88 ± 0.70	0.085
Creatinin (mg/dl)	3.32 ± 3.43	0.76 ± 0.11	0.80 ± 0.12	0.119
SGPT (U/L)	20.20 ± 5.63	20.40 ± 4.72	22.60 ± 8.56	0.084
SGOT (U/L)	29.40 ± 7.70	24.80 ± 12.48	23.80 ± 9.04	0.289
LDH (IU/L)	246.80 ± 32.42	229.60 ± 66.68	230.00 ± 30.89	0.273
γGT (U/L)	16.20 ± 4.66	16.60 ± 5.13	18.20 ±4.38*	**0.033**
ALP (U/L)	59.20 ± 14.85	66.80 ± 21.55**	68.00 ± 23.14*	**0.001**
Lipidemic Profile				
Total cholesterol (mg/dl)	247.80 ± 47.78	225.80 ± 49.93	248.20±45.38*	**0.041**
Triglycerides(mg/dl)	120.80 ± 15.96	112.40 ± 30.95	105.20 ± 40.76	0.398
HDL cholesterol (mg/dl)	68.40 ± 12.62	67.00 ± 8.74	67.40 ± 15.09	0.124
LDL cholesterol (mg/dl)	160.20 ± 37.28	136.20 ± 43.95	159.80 ± 45.15	0.129
Apoptotic Markers				
ccK18 (U/L)	116.45 ± 79.61	102.24 ± 31.91	78.93 ± 50.18	0.480
Apo1-Fas	98.32±12.17	83.64±14.21	89.234±10.88*	**0.040**
Survivin/API4 (pg/ml)	116.26 ± 17.91	104.66 ± 9.70**	125.20 ± 26.00	**0.021**

*Statistically significant difference in comparison with the baseline.

**Statistically significant difference between baseline and 45 days

Table 3

Differences in hematological and molecular markers of CLL Patients during intervention with high oleocanthal content olive oil.

	Intervention group			
	Baseline	45 days	90 days	p-value
Whole blood count				
WBC (x10^3/mm^3)	25.02 ± 6.43	26.52 ± 7.64	22.47 ± 5.27	0.840
LYMPH (x 10^3/mm^3)	19.06 ± 5.87	20.41 ±6.94	17.19 ± 4.98	0.956
PLT (x10^3/mm^3)	190.40 ± 55.23	185.40 ± 63.70	190.40 ± 54.59	0.316
Ht (%)	42.41 ± 3.76	43.45 ± 3.64	43.31 ± 3.86	0.185
HGB (g/dl)	14.14 ± 1.37	14.19 ± 1.41	14.30 ± 1.44	0.573
Biochemical Marker				
Glucose (mg/dl)	109.50 ± 16.07	110.40 ± 17.93	101.30 ± 9.39	0.083
Urea (mg/dl)	51.90 ± 13.80	46.60 ± 9.58	46.40 ± 13.90	0.759
Uric Acid (mg/dl)	5.61 ± 1.60	5.34 ± 1.46	5.57 ± 1.87	0.085
Creatinin (mg/dl)	2.51 ± 3.17	1.62 ± 2.25	0.96 ± 0.22	0.112
SGPT (U/L)	19.30 ± 7.05	22.00± 9.70	27.30 ± 17.15	0.084
SGOT (U/L)	26.80 ± 7.61	25.90 ± 7.75	27.10 ± 9.89	0.289
LDH (IU/L)	222.00 ± 60.84	222.50 ± 30.04	236.60 ± 29.89	0.273
γGT (U/L)	18.10 ± 8.02	21.10 ± 10.79**	19.30 ± 8.21	**0.007**
ALP (U/L)	69.50 ± 12.68	73.60 ± 14.85**	73.00 ± 15.01*	**0.001**
Lipidemic Profile				
Total cholesterol (mg/dl)	185.80 ± 35.72	184.60 ± 38.20	191.60 ± 43.29*	**0.041**
Triglycerides(mg/dl)	132.90 ± 73.06	152.90 ± 89.23	143.20 ± 67.98	0.398
HDL cholesterol (mg/dl)	55.80 ± 16.84	57.5 ± 17.65	54.10 ± 17.13	0.124
LDL cholesterol (mg/dl)	103.50 ± 24.64	95.20 ± 30.38	109.30 ± 33.14*	**0.032**
Apoptotic Markers				
ccK18 (U/L)	145.90 ± 92.24	144.59 ± 91.69**	193.68±169.09*	**0.035/0,01**
Apo1-Fas	78.194±17.4	80.88±17.86**	96.12±37.6*	**0.015/0,016**
Survivin/API4 (pg/ml)	182.61 ± 57.47	106.35 ± 8.89**	120.87 ± 26.46	**0.018**

*Statistically significant difference in comparison with the baseline.

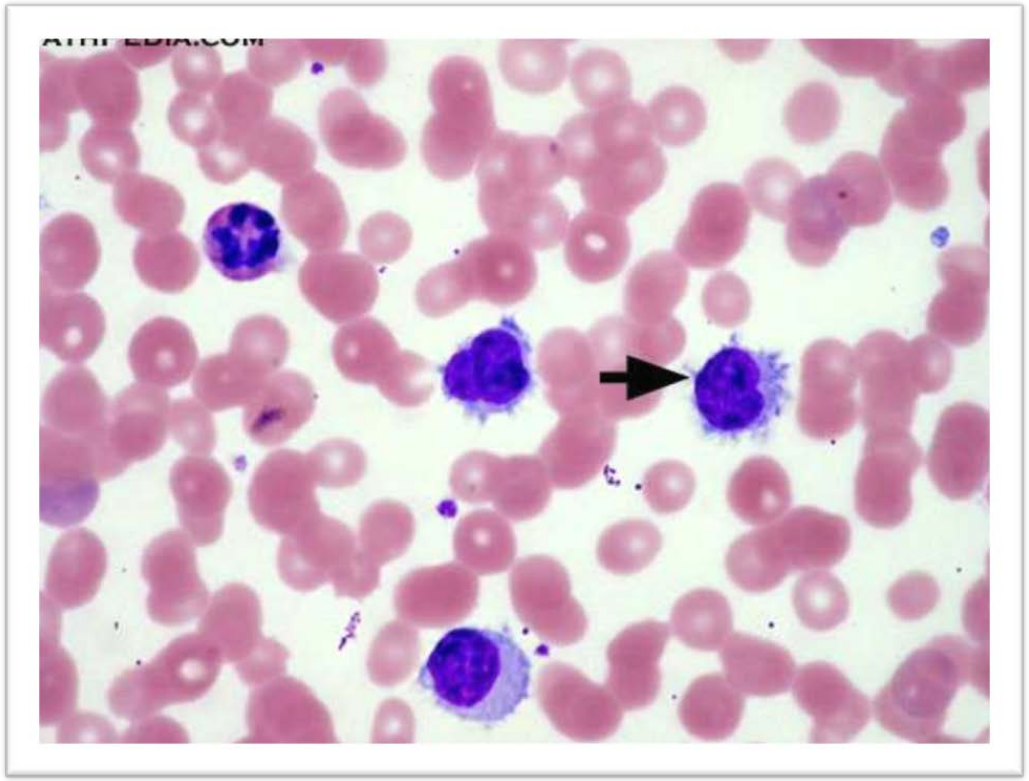
**Statistically significant difference between baseline and 45 days

Results

In the first survey

- the number of white blood cells in the CLL patients of the intervention group was reduced, and statistically significantly in 5/10 patients ($p<0.05$)
- increase in the apoptotic markers CCK18, Apo1-Fas
- decrease in antiapoptotic protein Survivin was observed in CLL patients of the intervention with EVOO rich in oleocanthal.

Responding group



Responding group n=5

Table 4

Differences in significant changing hematological and molecular markers of responding CLL Patients (5/10) during intervention with high oleocanthal content olive oil

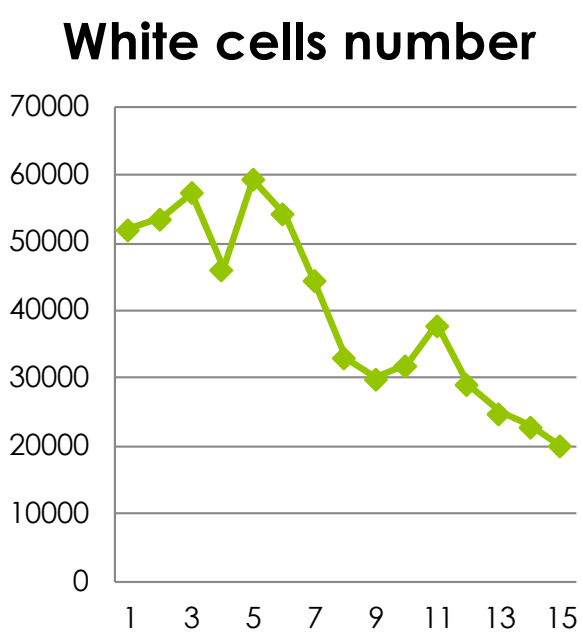
	Responding Group			
	Baseline	45 days	90 days	p-value
Whole blood count				
WBC ($x10^3/mm^3$)	38560± 28247.88	35540±26079.26	29540±20676.3*	**0.042**
PLT ($x10^3/mm^3$)	202800±55861.97	208200±56211.7	196800±59811.0	0.33
Ht (%)	42.24±4.37	43.06±3.26	35.56±4.26	0.48
Biochemical Marker				
Glucose (mg/dl)	112.8±13.81	117±17.91**	105.6±9.32*	**0.034/0.049**
LDH (IU/L)	231±76.45	211.6±27.98	239.6±24.66	0.39
γGT (U/L)	17.2±8.95	20.6±11.77**	18.2±7.90	**0.04**
ALP (U/L)	66.4±15.35	74.6±16.83**	67.4±17.04	**0.009**
Lipidemic Profile				
Total cholesterol (mg/dl)	172.4±13.76	165.4±12.54**	167.8±11.23	**0.034**
Triglycerides(mg/dl)	159±88.39	145.2±90.07	150.4±87.65	0.29
HDL cholesterol (mg/dl)	49.6±9.41	51.8±11.70**	51.2±9.57*	**0.038**
LDL cholesterol (mg/dl)	91±6.72	77.2±11.82	88.8±11.70	0.35
Apoptotic Markers				
ccK18 (U/L)	115.506±25.66	118.84±36.61	296.23±192.27*	**0.05**
Apo1-Fas	80.76±18.99	83.57±19.98	108.18±23.08*	**0.009**
Survivin/API4 (pg/ml)	181.156±20.62	108.91±3.51**	107.35±3.84*	**0.07/0.01**

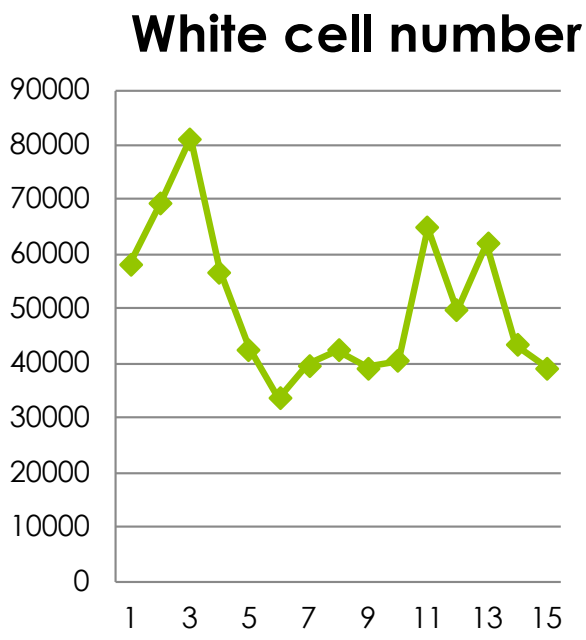
*Statistically significant difference in comparison with the baseline.

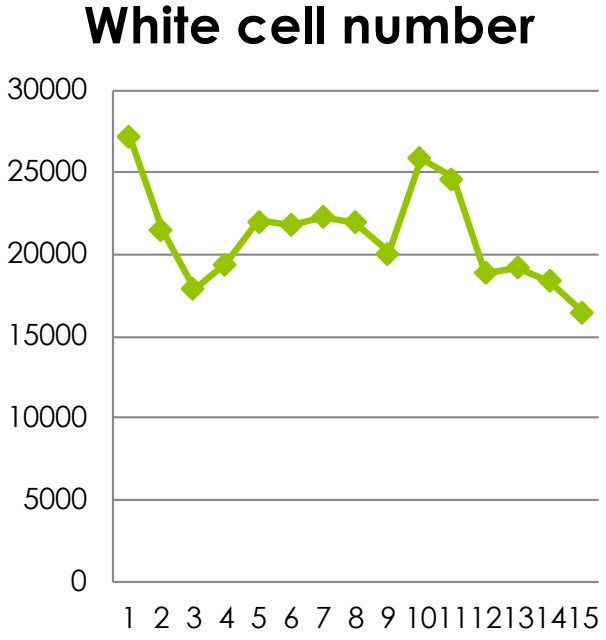
**Statistically significant difference between baseline and 45 days

Second Intervention

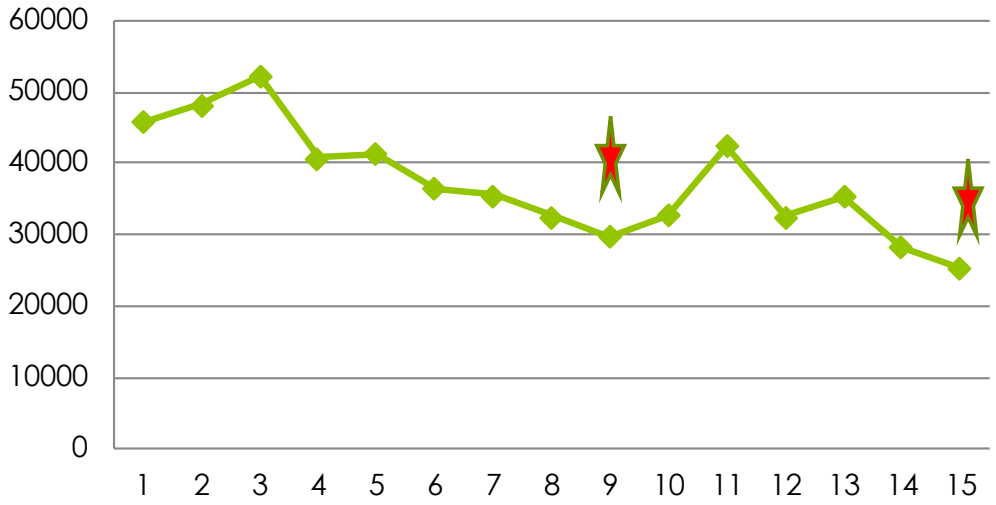
- The 5 responding patients continued, for 12 months (5 patients) and for 18 months (3 patients), the intervention with EVOO rich in OC/OL.
- All patients showed stabilization in the number of white blood cells.





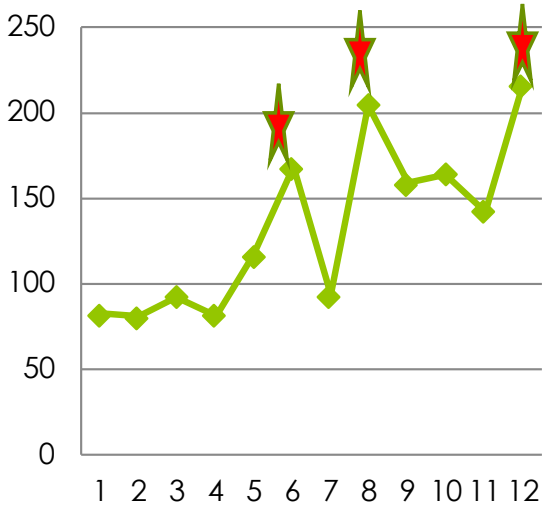


White cell number

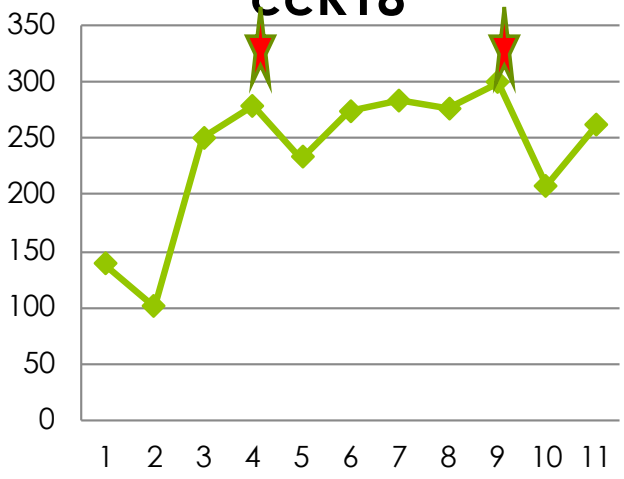


P=<0,005 in comparison to the first point

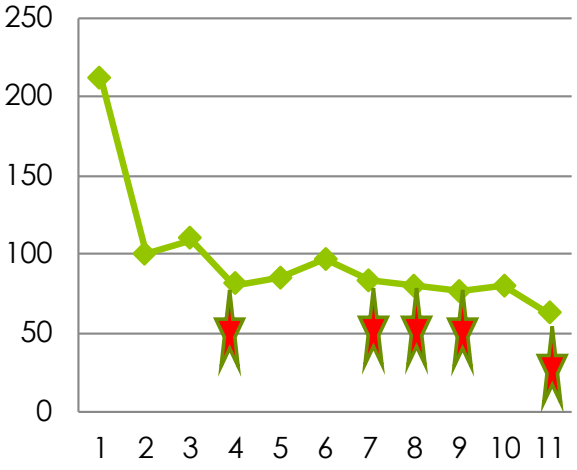
ApoFasl



ccK18

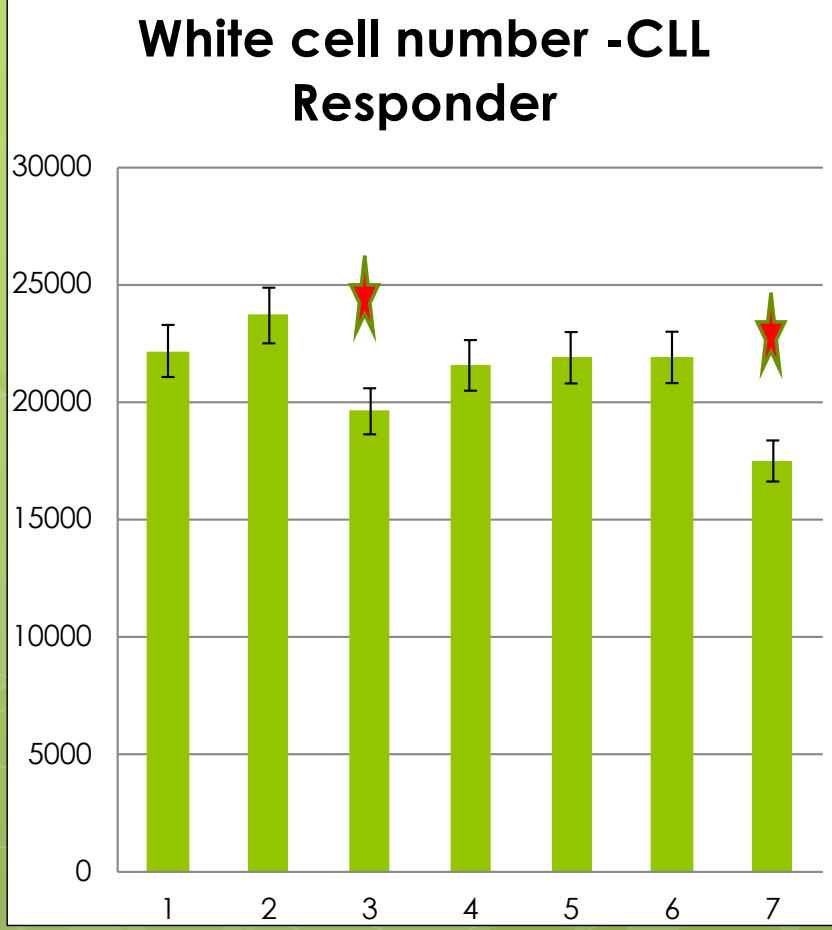


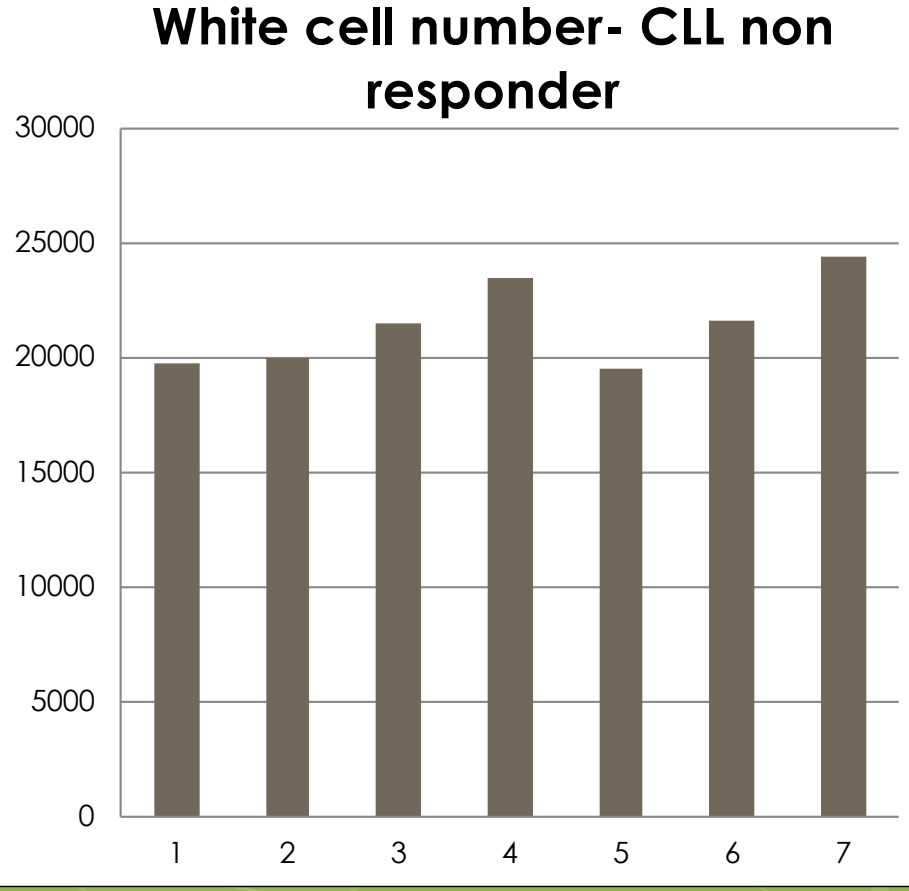
Survivin



- Third intervention with OC / EL-rich EVOO was performed in **20 CLL patients** for 7 months where a response was found in 15/20 patients with decreased white blood cells

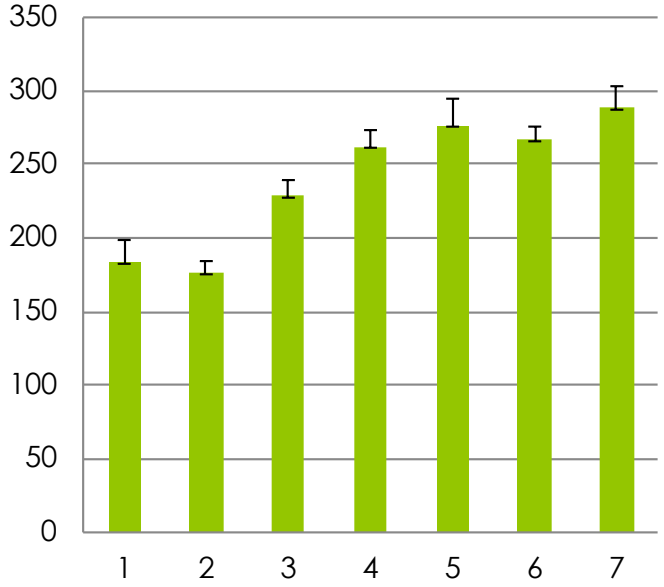
P=<0,005 in comparison to the first point



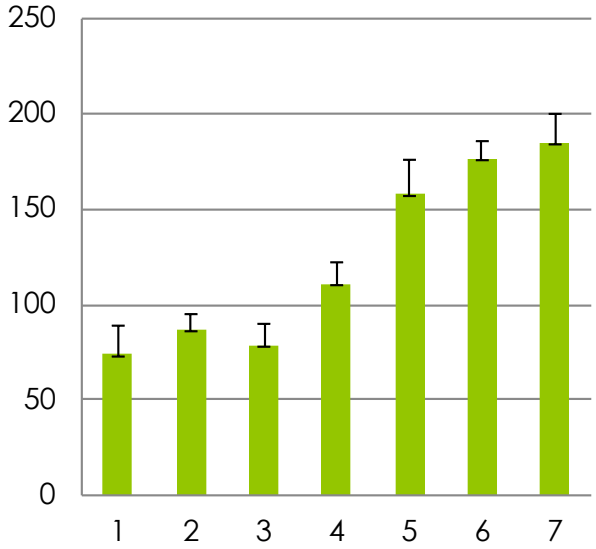


- Regarding the cell death markers,
  - the anti-apoptotic survivin index remained low
  - the cell death-induced ApoFasl and ccK18 proteins remained at consistently high levels in blood serum.
  - There was also an increase in apoptotic proteins at a cellular level and in the white blood cells of the patients

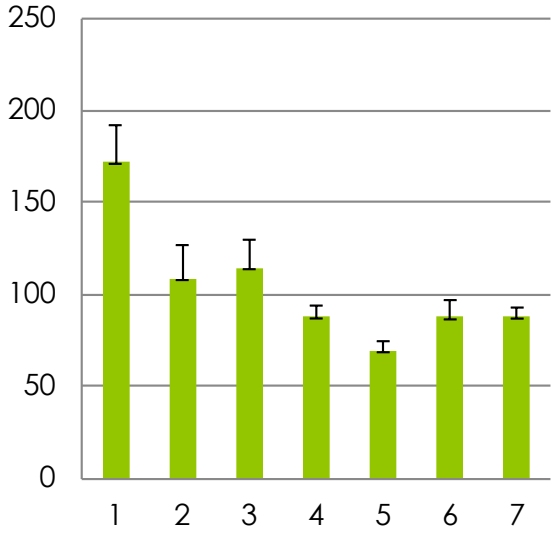
ccK18 Response group (N=15)



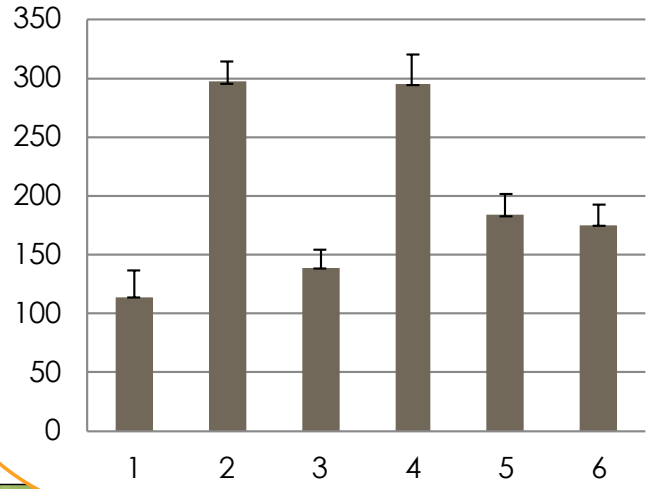
ApoFasl Response group (N=15)



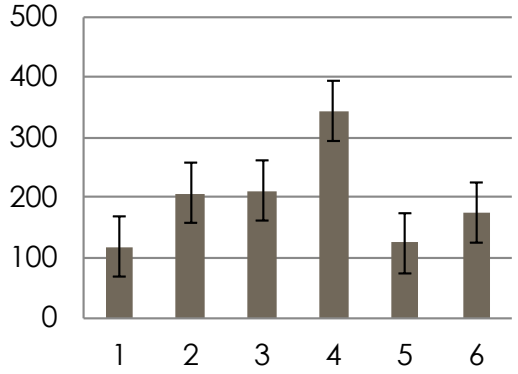
Survivin Response group N=15



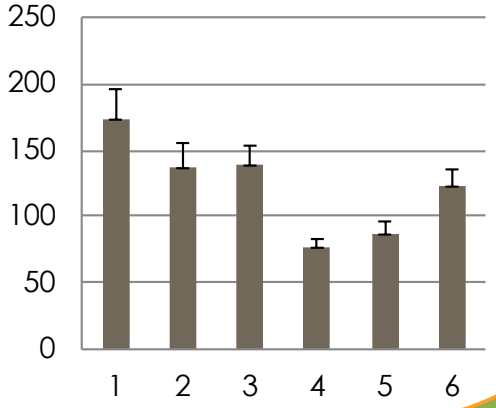
ccK18 non-Response group (N=5)



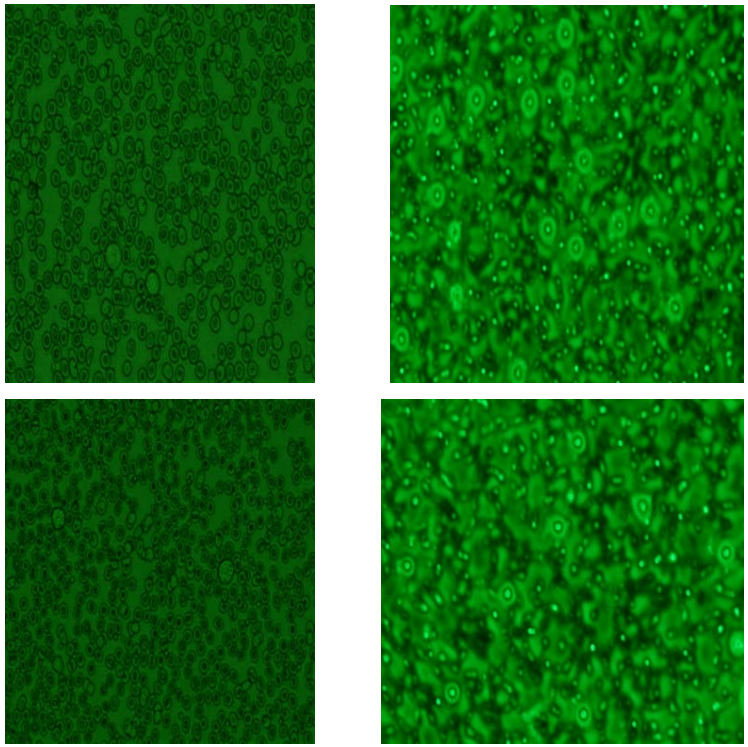
ApoFasl non-response group (N=5)

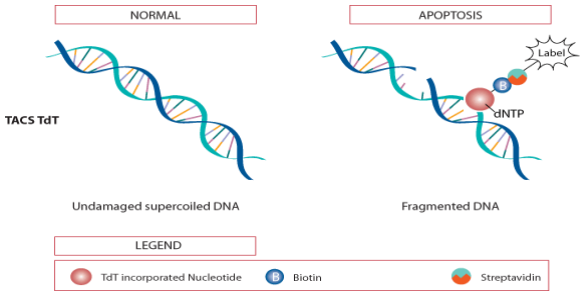


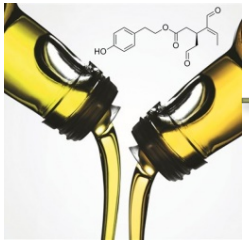
Survivin non-Response group N=5

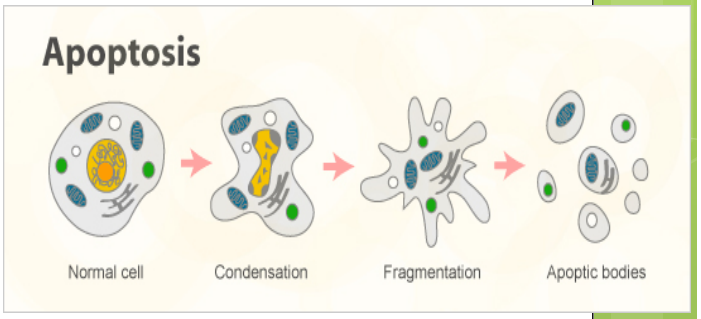


Tunnel Assay- Apoptotic assay



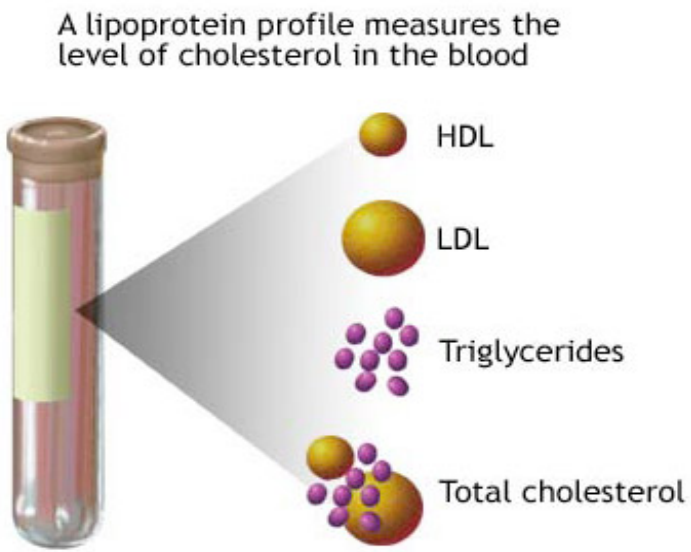


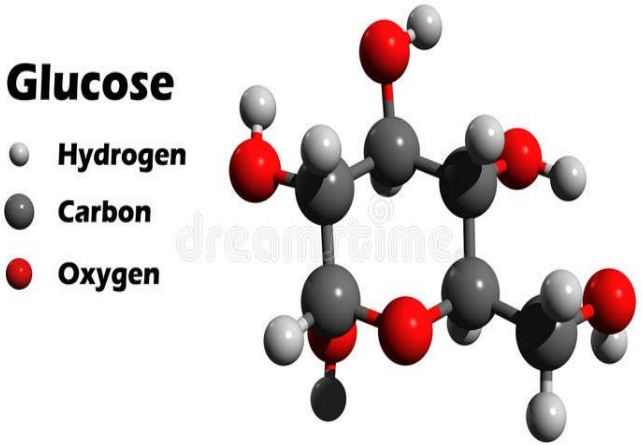




White blood cells after tunnel assay before treatment (0 time) and after treatment (3 month) with EVOO rich in OC/OI

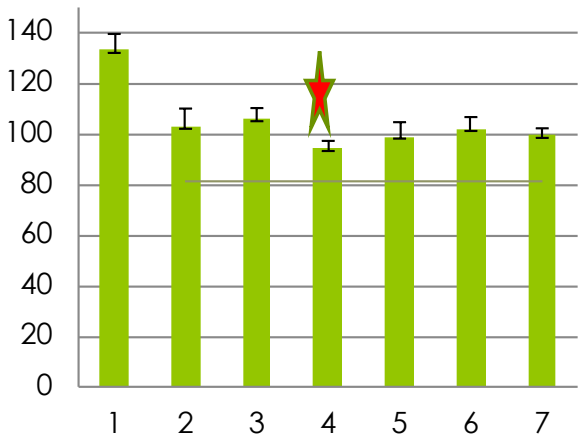
Biochemical markers



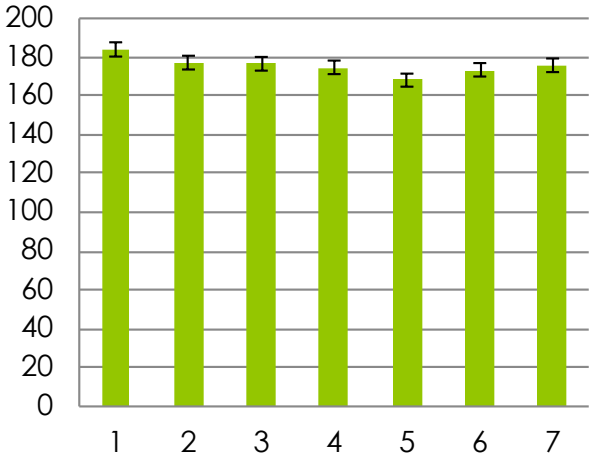


EVOO consumption rich in oleocanthal improve the glucose level and lipid profile mainly in the CLL response group

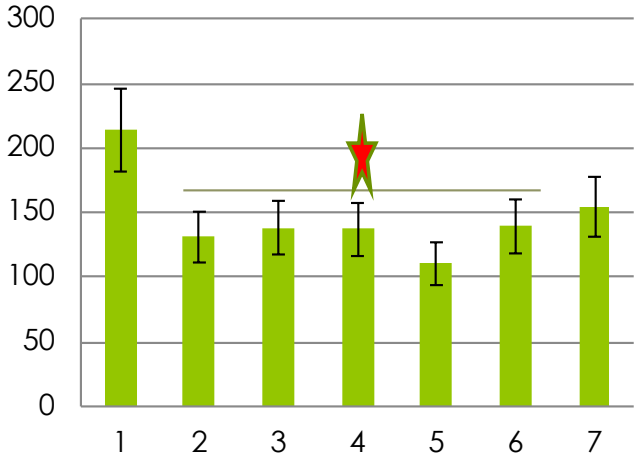
Glucose Response group



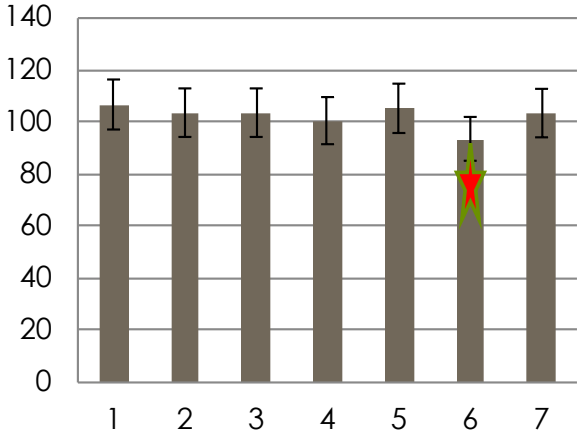
Cholesterol Response group



Triglycerides Response group

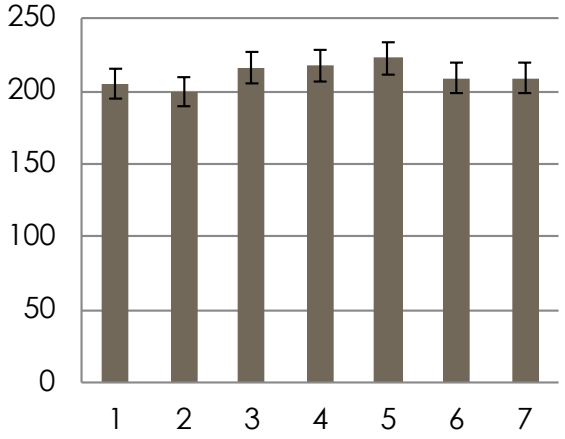


Glucose non-Response group



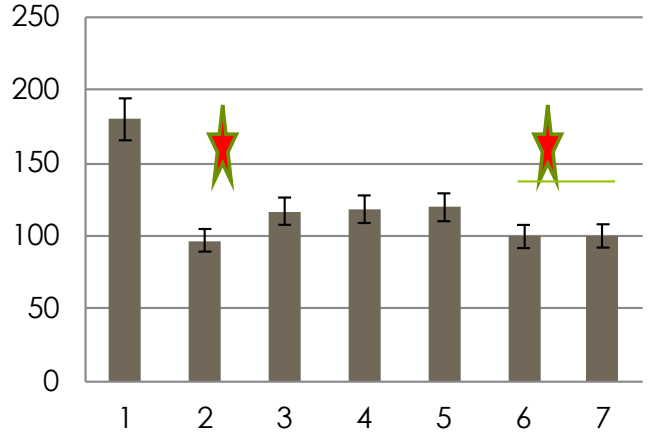
NV <100 mg/dl

Cholesterol non-Response group



NV <200 mg/dl

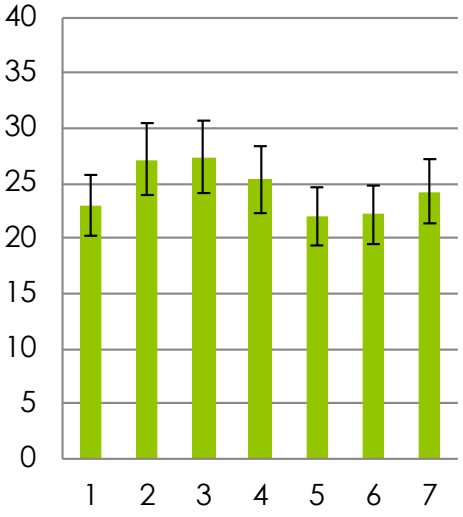
Triglycerides non-Response group



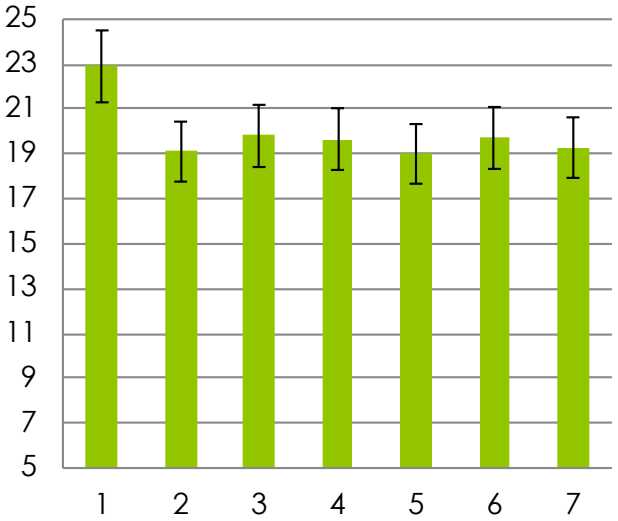
NV <150 mg/dl

P=<0,005 in comparison to the first point

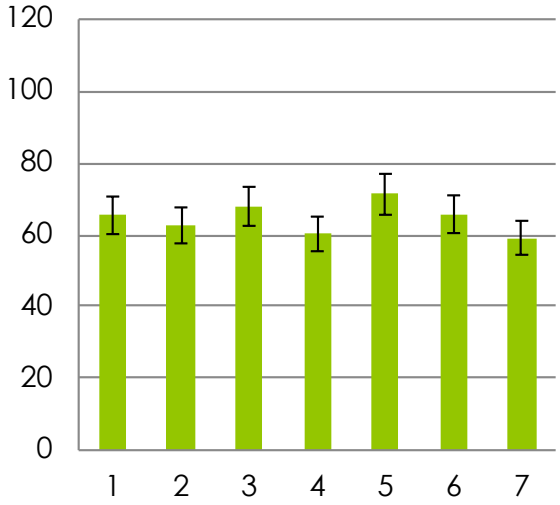
SGOT Response group



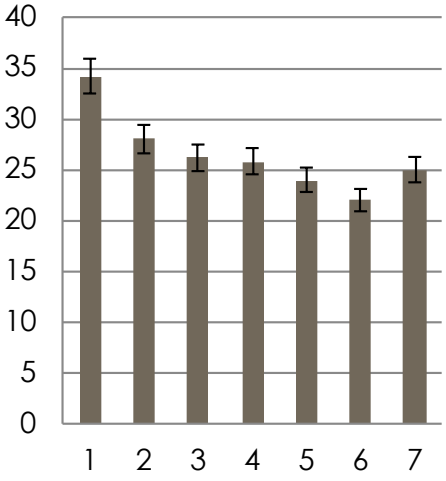
γ-GT Response group



ALP response group

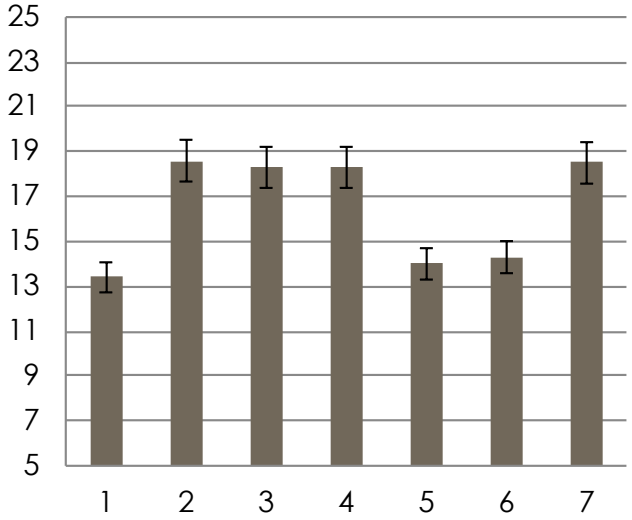


SGOT non response group



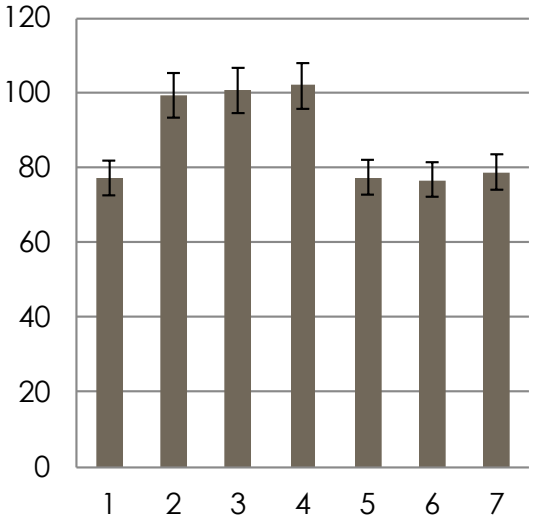
NV 10-40 IU/L

γ-GT non Response group



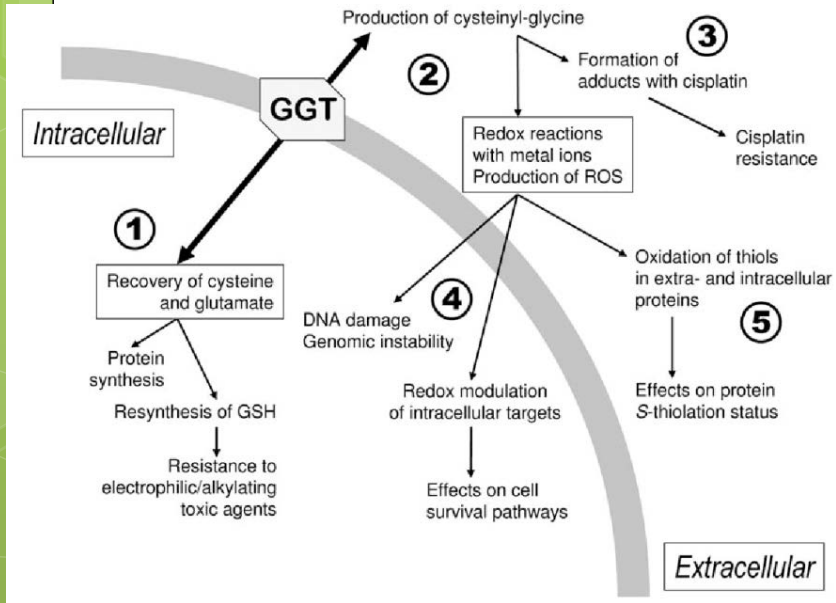
NV 5 - 40 IU/L.

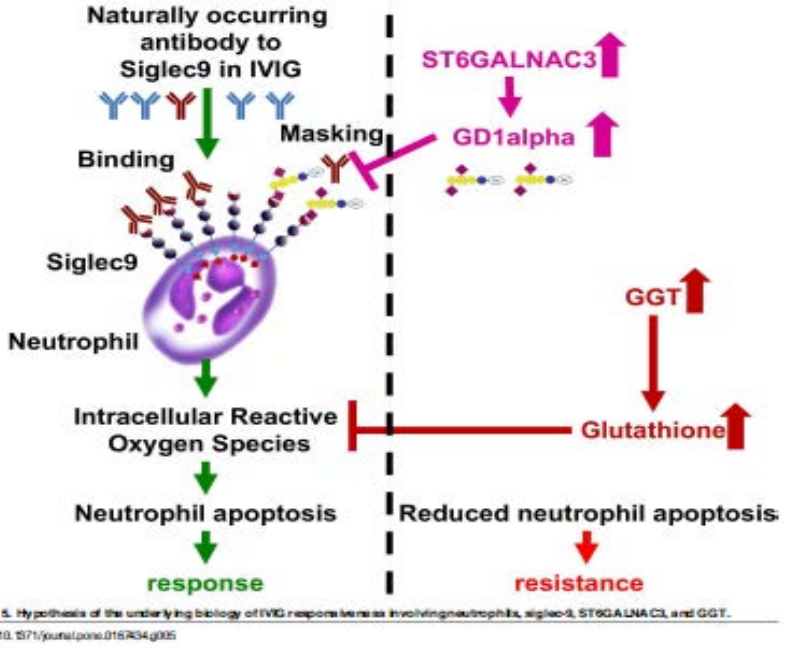
ALP non response group



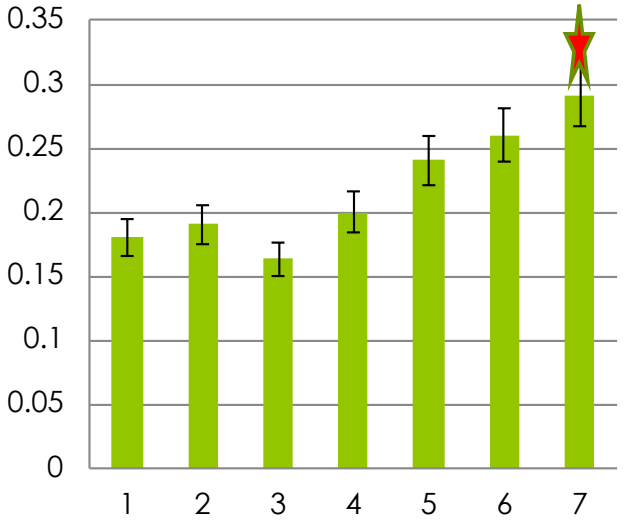
30-115 IU/L

- After 3, 6 and 12 months of intervention in responding patient **ccK18** was negatively correlated with
- γGT (r=0.810, p=0.001),
- triglycerides (r=0.600, p=0.002)
- Cholesterol (r= 0,5, p=0.001)

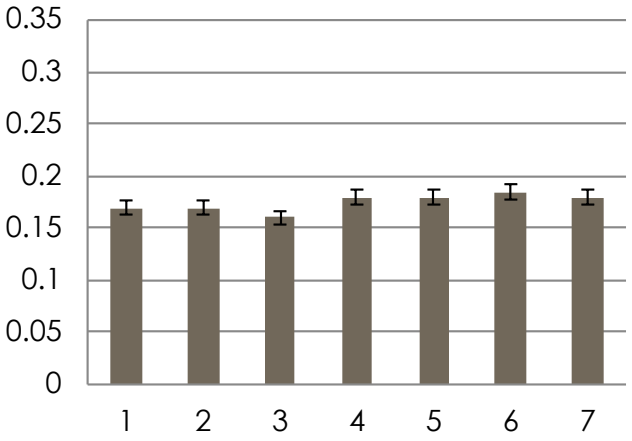


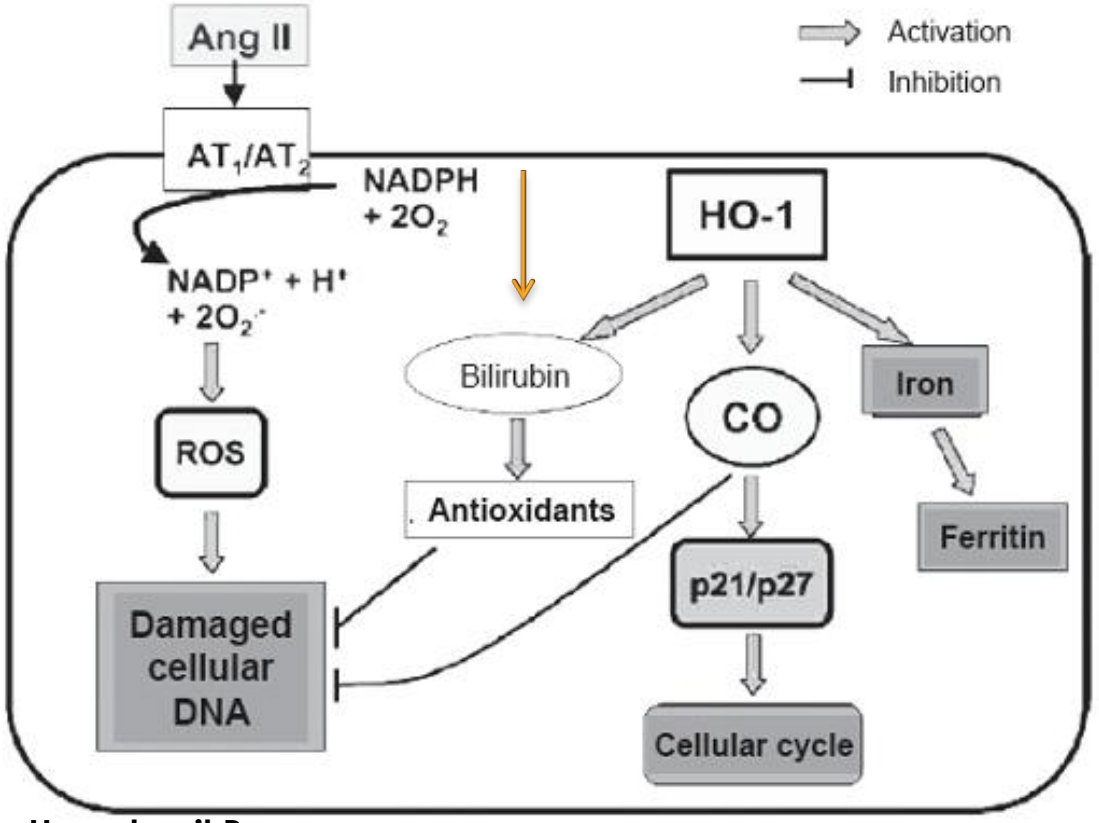


Bilirubin Response Group

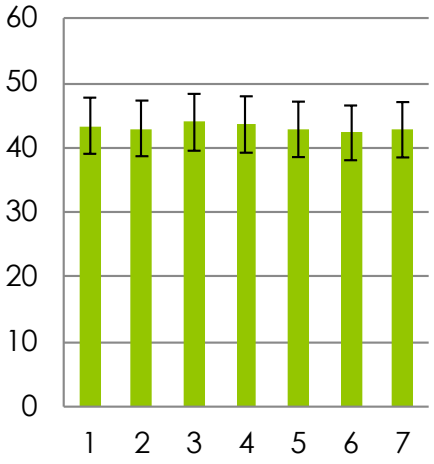


Bilirubin non-Response group

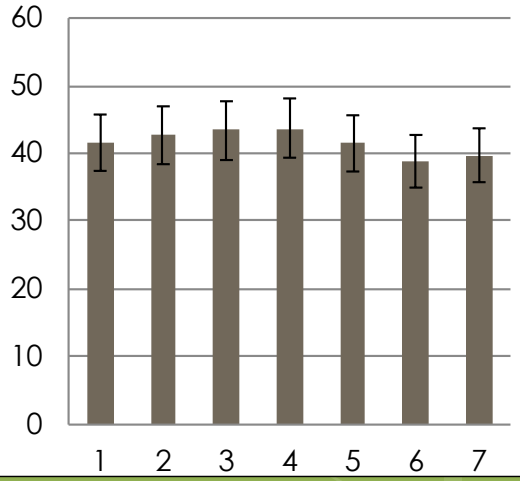




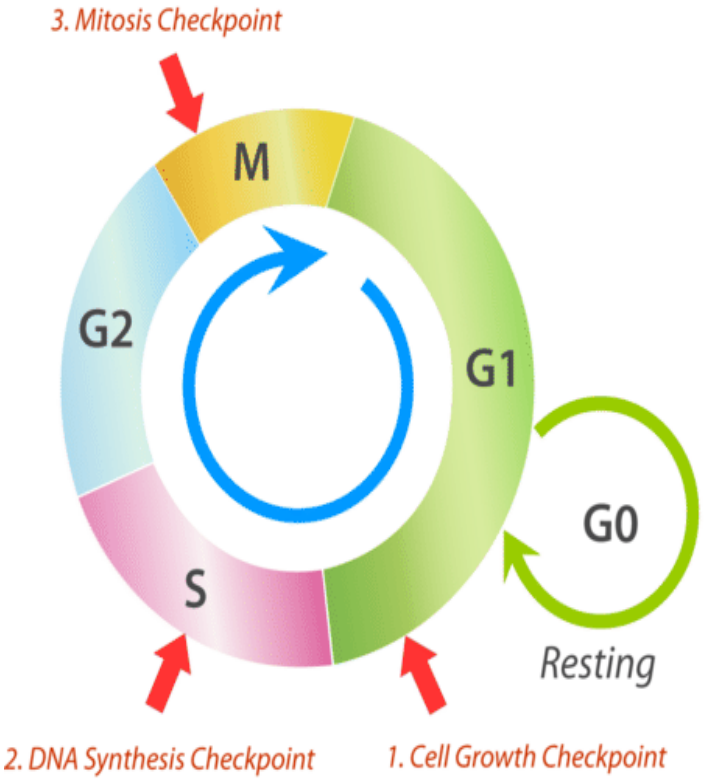
Hematocrit Response group



Hematocrit Non-response group



The Cell Cycle and the Checkpoints



1. Cell Growth Checkpoint

- Occurs toward the end of growth phase 1 (G1).
- Checks whether the cell is big enough and has made the proper proteins for the synthesis phase.
- If not, the cell goes through a resting period (G0) until it is ready to divide.

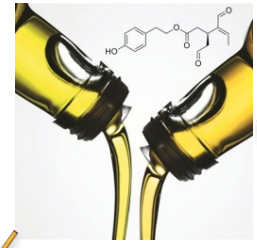
2. DNA Synthesis Checkpoint

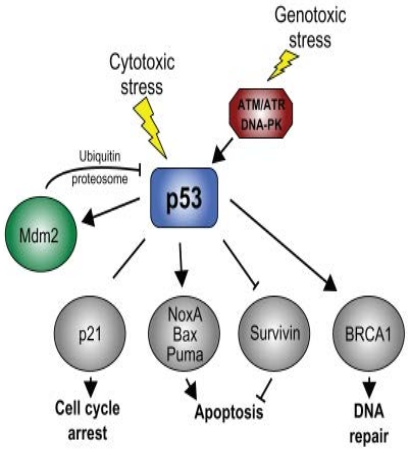
- Occurs during the synthesis phase (S).
- Checks whether DNA has been replicated correctly.
- If so, the cell continues on to mitosis (M).

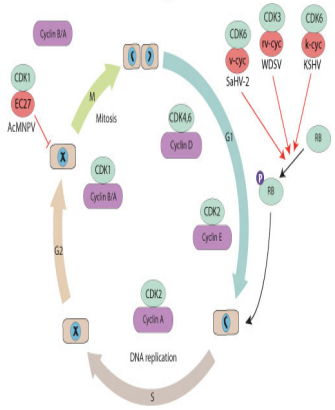
3. Mitosis Checkpoint

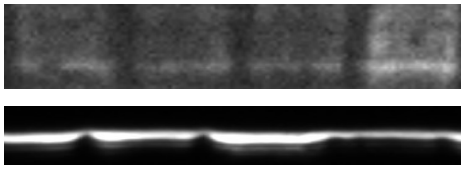
- Occurs during the mitosis phase (M).
- Checks whether mitosis is complete.
- If so, the cell divides, and the cycle repeats.

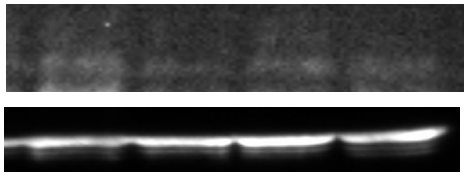
Cell cycle markers

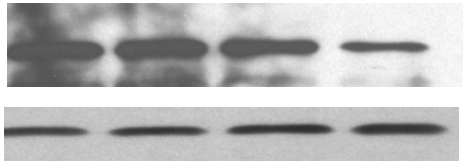






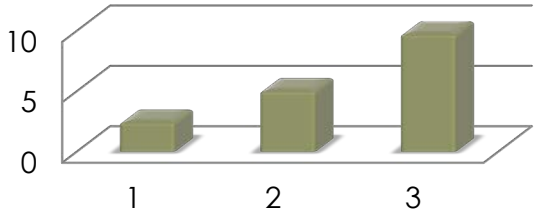




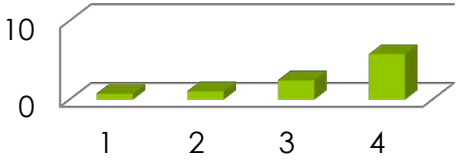


Western Blott

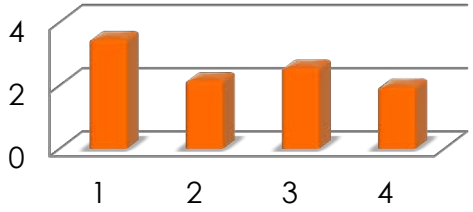
p53/actin CLL



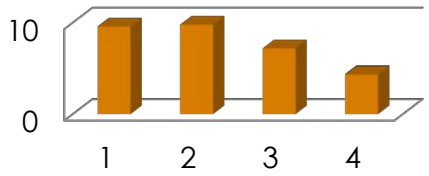
p21/actin CLL



Cyclin D/ actin CLL



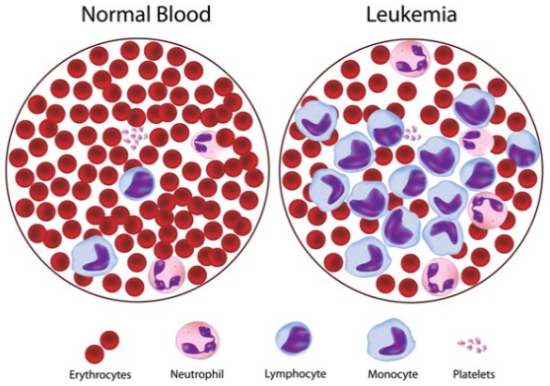
Cyclin A/actin CLL

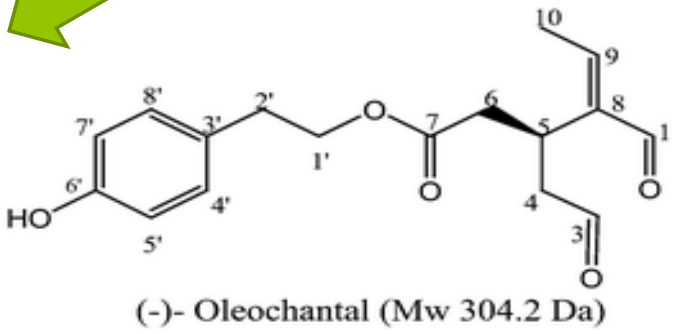


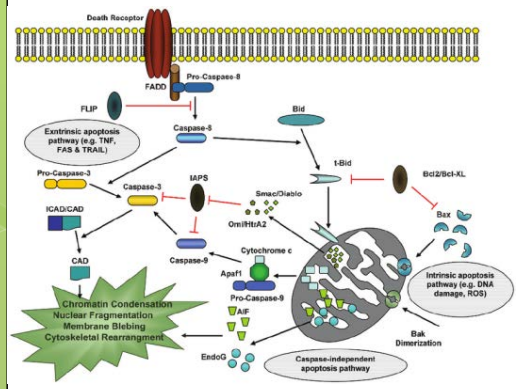
Conclusions

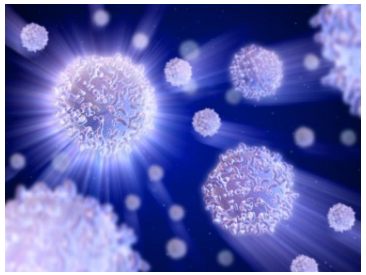
- None of the study patients needed to initiate chemotherapeutic intervention.
- Further studies are necessary to clarify the exact role and ability of such nutritional interventional actions to improve the health status during neoplastic blood diseases such as CLL.

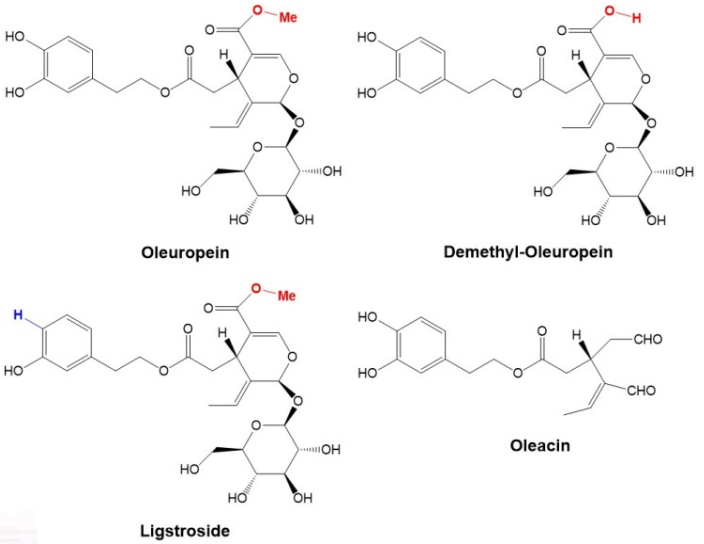
What next??

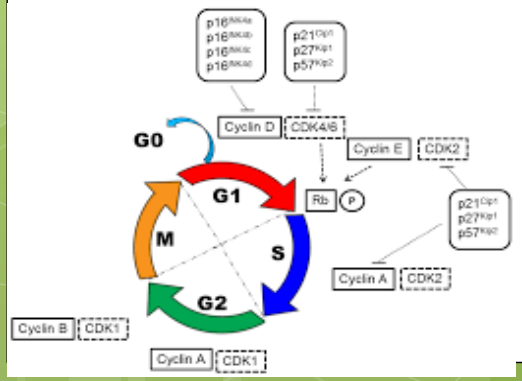

Normal Blood
Leukemia
Erythrocytes
Neutrophil
Lymphocyte
Monocyte
Platelets


HO
(-)- Oleochantal (Mw 304.2 Da)

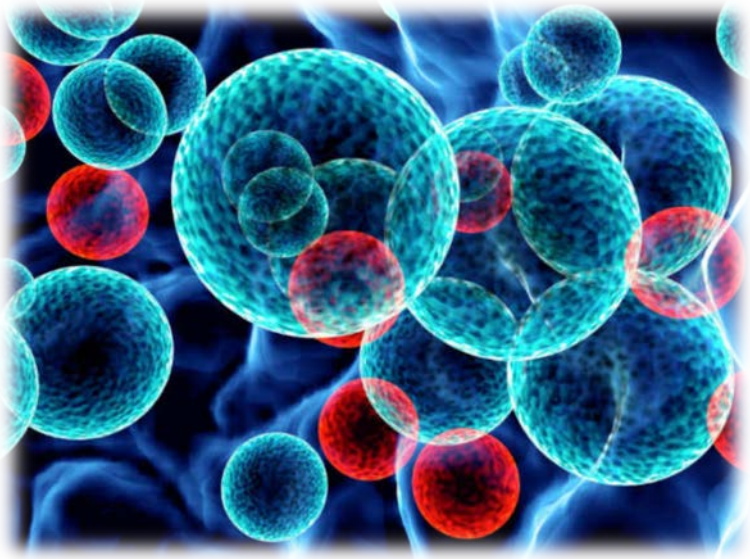

Death Receptor
FADD
Pro-Caspase-8
FLIP
Caspase-8
Bid
Extrinsic apoptosis pathway (e.g. TNF, FAS & TRAIL)
t-Bid
Pro-Caspase-3
Caspase-3
IAPS
Bcl2/Bcl-XL
Smac/Diablo
ICAD/CAD
Omi/HtrA2
Bax
Caspase-9
Cytochrome c
CAD
Apaf1
Intrinsic apoptosis pathway (e.g. DNA damage, ROS)
Pro-Caspase-9
AIF
Chromatin Condensation
Nuclear Fragmentation
Membrane Blebing
Cytoskeletal Rearrangment
Bak Dimerization
EndoG
Caspase-independent apoptosis pathway




Oleuropein
Demethyl-Oleuropein
Ligstroside
Oleacin


G0
G1
S
G2
M
Cyclin D
CDK4/6
Cyclin E
CDK2
Rb
P
Cyclin A
CDK2
Cyclin B
CDK1
Cyclin A
CDK1

Thank you for your attention



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1. The World Olive Center for Health for the economic support of the current research
2. The Governor (Dafni family) for the olive oil donation

Message for Home…..