Is the antioxidant capacity of flavonoids responsible for their anticancer effects? The case of quercetin in leukemia

Gian Luigi Russo

Institute of Food Sciences – CNR - Avellino, Italy

1 – 15 December 2020

Conflict of Interest Disclosure Statement

I declare the absence of any potential or direct conflict of interest, including honoraria or other funding, related to my participation in the CAHD 2020 Virtual Conference

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Nicholas Carpino	Stony Brook University L.I., New York, USA

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Carmela Spagnuolo
Idolo Tedesco
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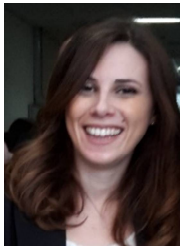

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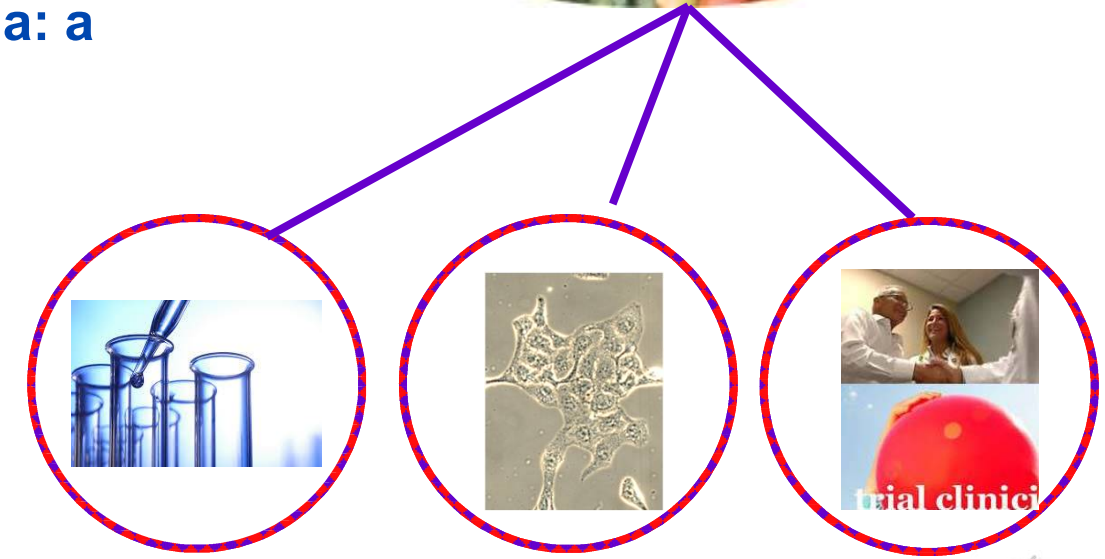
Overview

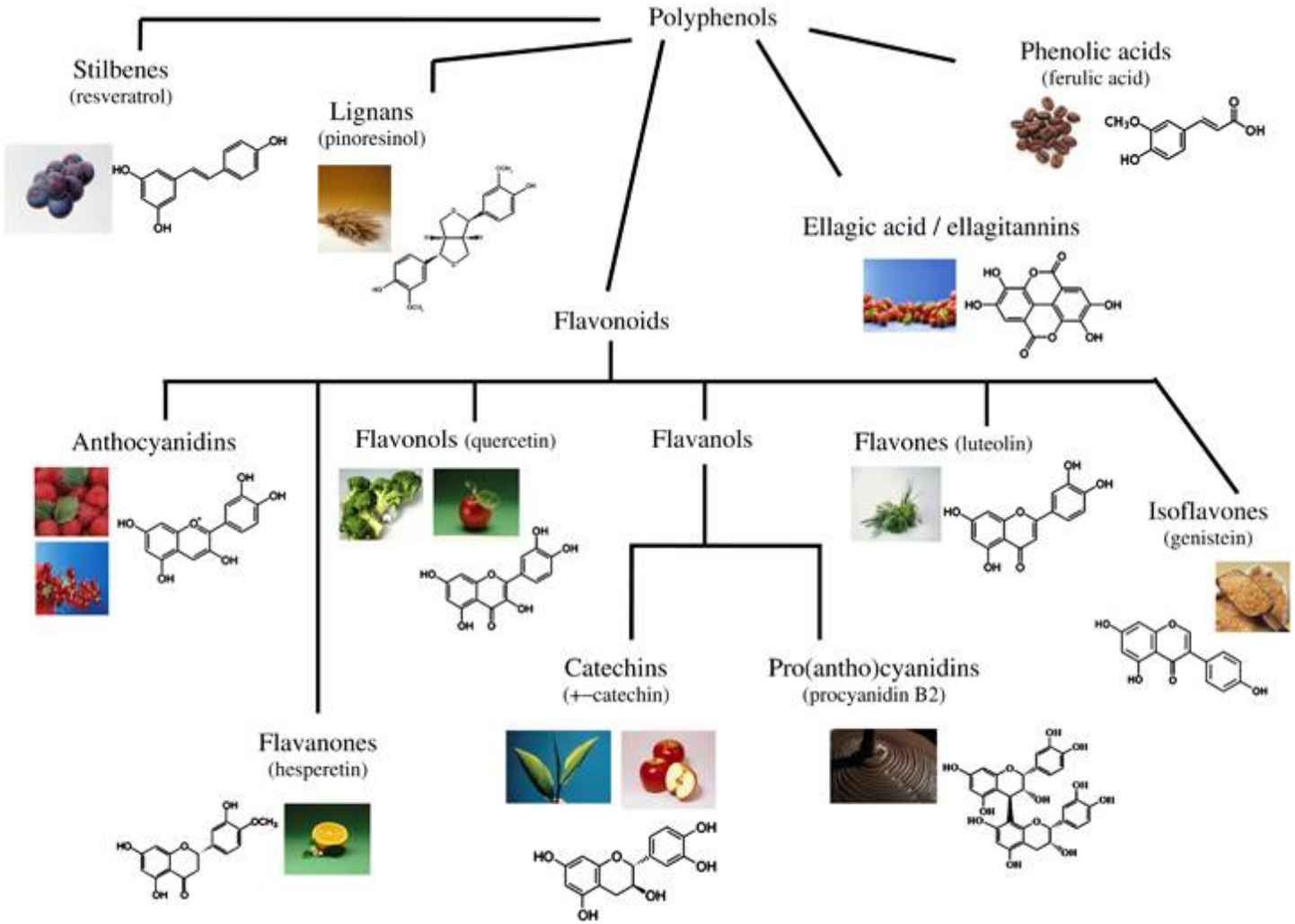
- **Polyhenols in cancer: a field in *identity crisis***

- **Molecular mechanism(s) of quercetin in Chronic Lymphocytic Leukemia: a question of *place* and *time***

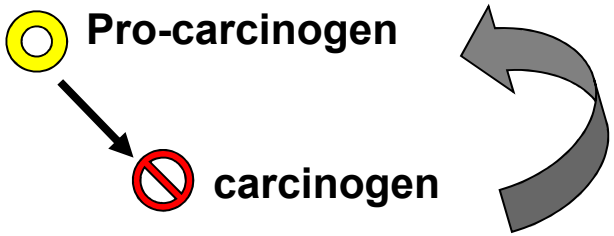


Polyphenols (flavonoids) are pleiotropic compounds

Polyphenols are organic compounds present in plants where they play different biological functions. These include the regulation of enzymes involved in the metabolism and the mechanisms of defense against chemical and physical agents (UV rays, oxidative stress) or microbial (viruses, bacteria, fungi)



Chemopreventive mechenisms of polyphenols



Blocking Agents
Sulphoraphane
Flavonoid

Suppressing Agents
Curcumin
ECGG
Resveratrol
Capsaicin
α-Bisabolol

Antioxidant activity
scavenging of free radicals and reducing oxidative stress

Phase II enzyme induction
enhancing detoxification

Phase I enzyme inhibition
blocking activation of carcinogens

Inhibition of cell proliferation

Induction of cell differentiation

Inhibition of oncogene expression

Induction of TSG

Induction of cell cycle arrest

Induction of apoptosis

Antiangiogenetic activity

Inhibition of cell adhesion and invasion

Others……

Adapted from
Liu J. Nutr. 2004, 134:3479S

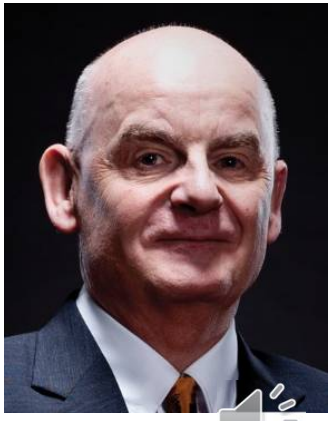
The term "nutraceutical" was coined from "nutrition“ and "pharmaceutical" in 1989 by Stephen DeFelice, MD, founder and chairman of the Foundation for Innovation in Medicine (FIM). According to DeFelice, nutraceutical can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease…”

Recent Advances in Chemoprevention of Cancer

Waun Ki Hong*, Michael B. Sporn
+ See all authors and affiliations

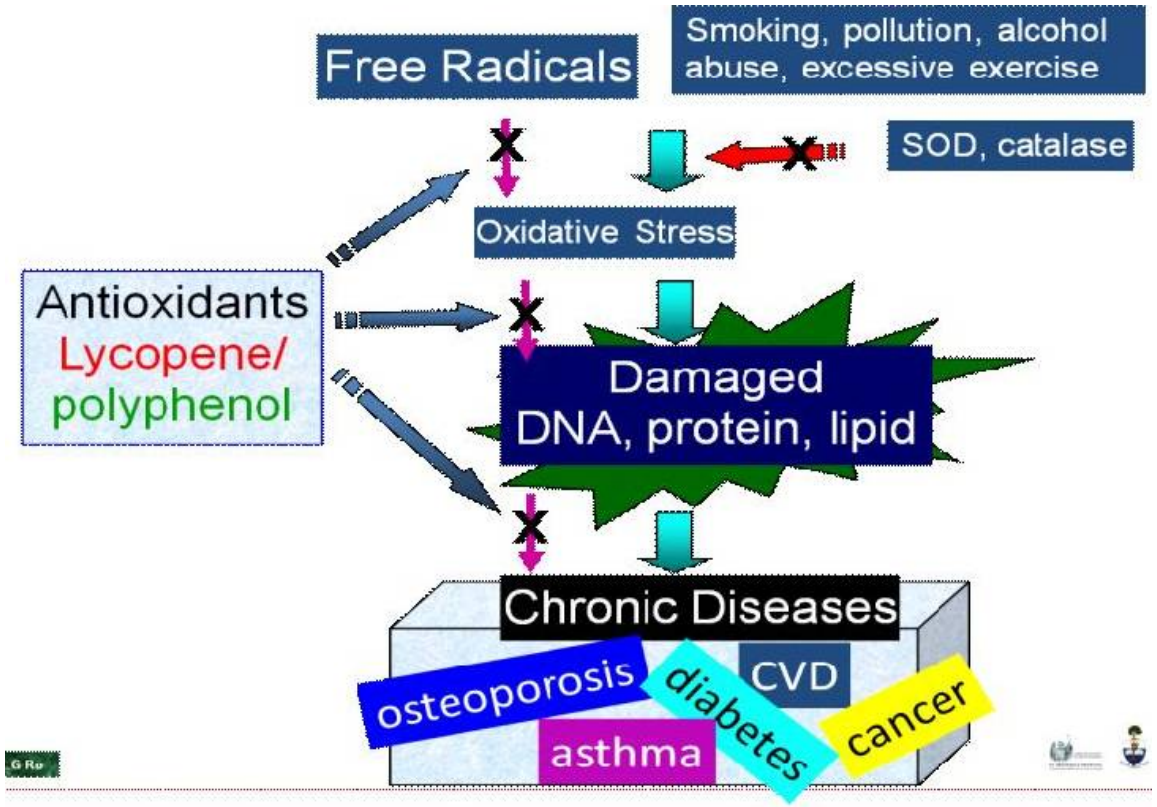
Chemoprevention is the use of pharmacologic or natural agents that inhibit the development of invasive cancer either by blocking the DNA damage that initiates carcinogenesis or by arresting or reversing the progression of premalignant cells (1997)

“Our endogenous antioxidant defenses are inadequate to prevent oxidative damage completely. Hence, sources of dietary antioxidants may be especially important to us” (Barry Halliwell, 1994)



The Antioxidant Hypothesis ("free radical theory")

"**Epidemiological studies have shown that high consumtion of fruit and vegetables reduces risk of developing cancer and cardiovascular disease…**
A popular explanation refers to the presence of antioxidant nutrients, including vitamin C, vitamin E, carotenoids, selenium and flavonoids, which prevent carcinogenesis and atherogenesis by interfering passively with oxidative damage to DNA, lipids and proteins…. [*Public Health Nutrition, 2004*]



Disease prevention by fruits and vegetables

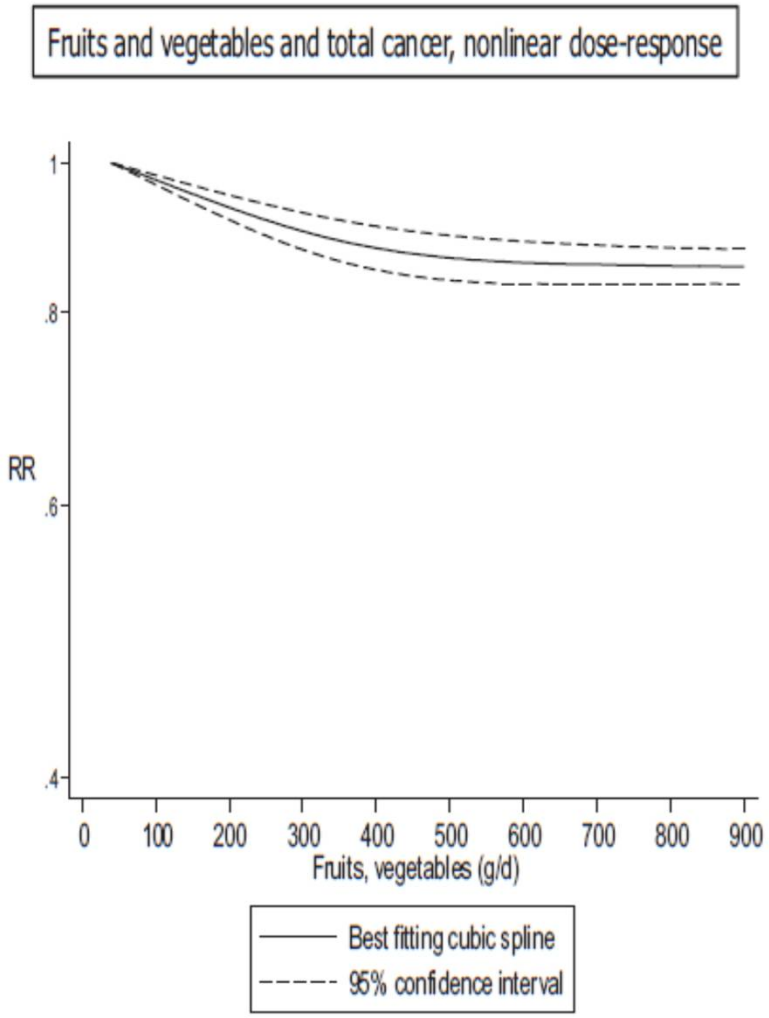
International Journal of Epidemiology, 2017, 1–28
doi: 10.1093/ije/dyw319
Original article

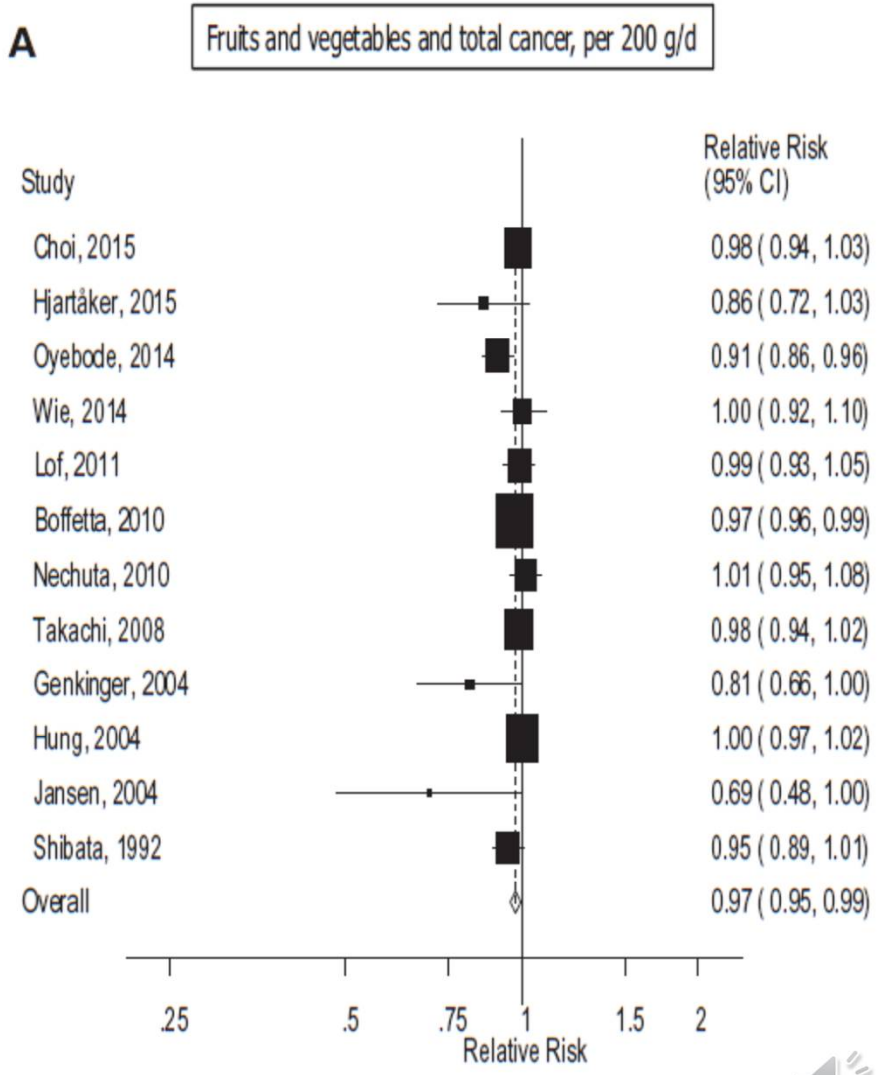
Original article

Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality–a systematic review and dose-response meta-analysis of prospective studies

Dagfinn Aune[1,2,3]*, Edward Giovannucci[4,5,6], Paolo Boffetta[7], Lars T. Fadnes[8], NaNa Keum[5,6], Teresa Norat[2], Darren C. Greenwood[9], Elio Riboli[2], Lars J. Vatten[1] and Serena Tonstad[10]

Cancer prevention by fruits and vegetables





Seminars in Cancer Biology 46 (2017) 1–13

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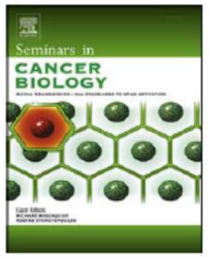
journal homepage: www.elsevier.com/locate/semcancer



Review

Antioxidant polyphenols in cancer treatment: Friend, foe or foil?

Gian Luigi Russo*, Idolo Tedesco[1], Carmela Spagnuolo[1], Maria Russo[1]

Institute of Food Sciences, National Research Council, 83100, Avellino, Italy

"..The controversial and contradictory issues related to the pros and cons on the use of polyphenols against cancer reflect the confounding assumption that cancer treatment and cancer prevention may overlap…"

Preclinical vs Clinical Studies

Table 1
Observational and interventional studies published in the last 10 years where polyphenols, as single compounds or in mixtures, have been tested against different types of cancers.

Uncertain – null - negative

Table 2
Ongoing clinical studies retrieved (*with results*) from the ClinicalTrials.gov database on polyphenols and cancer.

No significant differences

Russo GL et al. Seminars Cancer Biol, 2017

Preclinical vs Clinical Studies

Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC)[1–4]

Kathryn E Bradbury, Paul N Appleby, and Timothy J Key

. There was a borderline inverse association of fiber intake with breast cancer risk. For the other 9 cancer sites studied (stomach, biliary tract, pancreas, cervix, endometrium, prostate, kidney, bladder, and lymphoma) there were no reported significant associations of risk with intakes of total fruit, vegetables, or fiber. *Am J Clin Nutr* 2014;100(suppl):394S–8S.

A comprehensive meta-analysis on dietary flavonoid and lignan intake and cancer risk: level of evidence and limitations

Molecular Nutrition & Food Research, 2017

Giuseppe Grosso, Justyna Godos, Rosa Lamuela-Raventos, Sumantra Ray, Agnieszka Micek, Andrzej Pajak, Salvatore Sciacca, Nicolantonio D'Orazio, Daniele Del Rio, Fabio Galvano

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Russo GL et al. Seminars Cancer Biol, 2017

Preclinical vs Clinical Studies

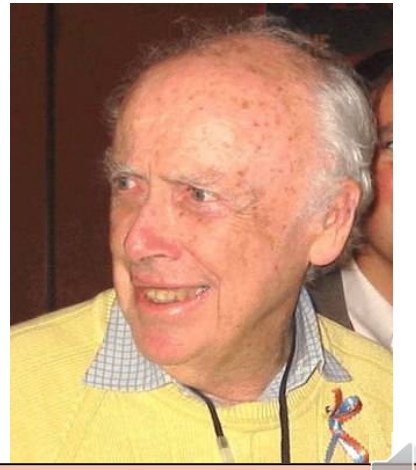
open Biology

Oxidants, antioxidants and the current incurability of metastatic cancers

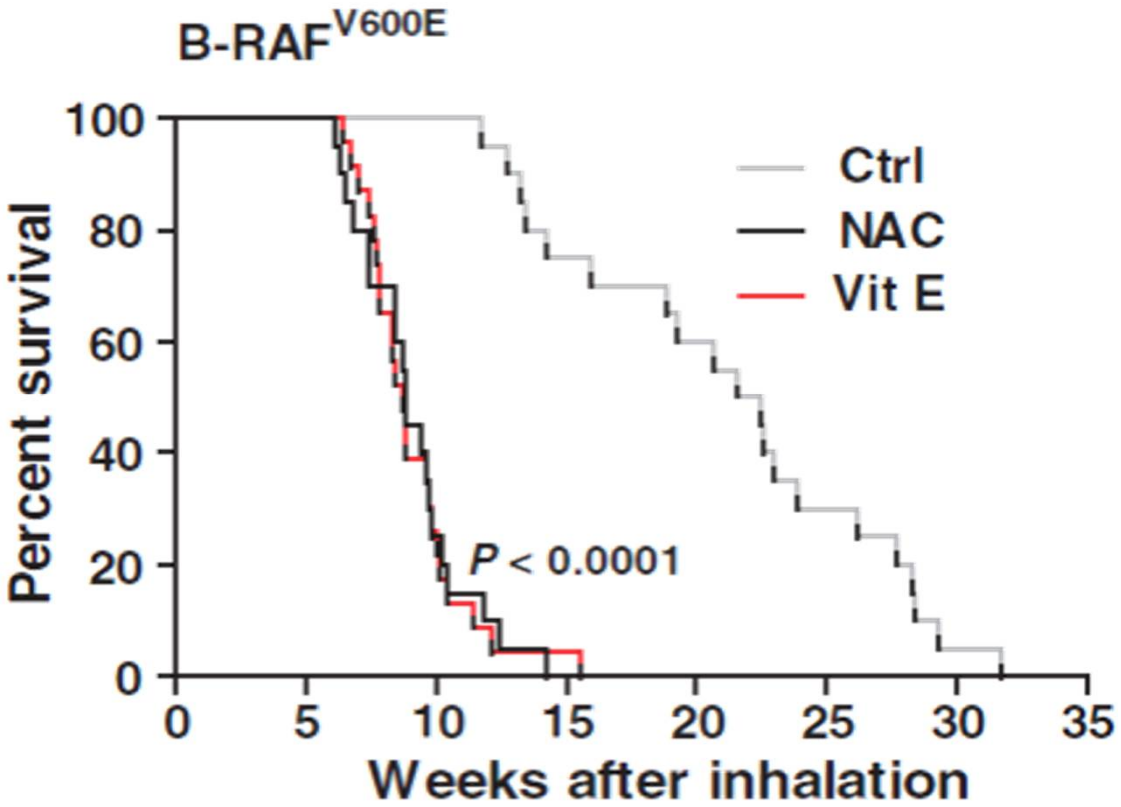
Jim Watson

Open Biol. 2013 **3**, 120144, published online 8 January 2013

"free radical-destroying antioxidative nutritional supplements may have caused more cancers than they have prevented…."



Antioxidants vs cancer cells

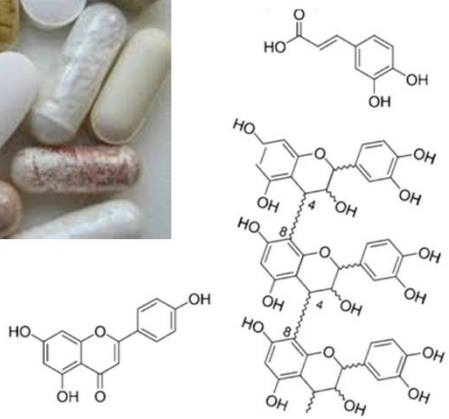


Sayin et al. ScienceTranslational Medicine, 2014

Polyphenols (flavonoids): cure or prevention…?

Functional foods

Drugs/Nutraceuticals



Food/Diet

PHARMACOLOGICAL DOSES

GUIDELINES:

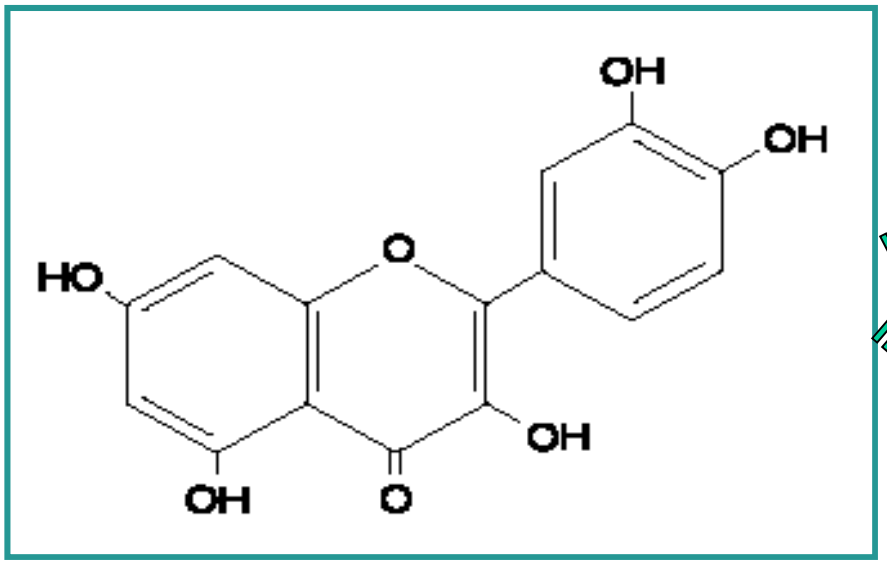
(i) a careful selection of the cellular models based on a robust hypothesis that the given phenolic compound can interfere with cell growth and division;

(ii) quantification of the real uptake of the tested compound (or its metabolites);

(iii) identification of the very early target(s) of the selected compounds, possibly within minutes from the uptake, and before measuring changes in regulatory pathways (e.g., apoptosis).

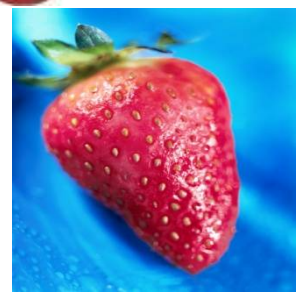
Russo GL et al. Seminars Cancer Biol, 2017

Quercetin



Food	Quercetin	
Lettuce	0.7-0.3	mg/100g
Onion	28.4-48.6	mg/100g
Apple	2.1-7.2	mg/100g
Strawberry	0.8-1.0	mg/100g
Black tea	1.7-2.5	mg/100ml
Red wine	0.4-1.6	mg/100ml
Apple juice	0.3	mg/100ml

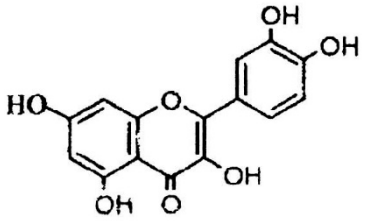
Polyphenols	1-2 g/day	
Flavonoids	1 g/day	
Flavonol glycoside	100 mg/day	
Quercetin	5-40 mg/day	200-500 mg/day (7 mg / Kg)



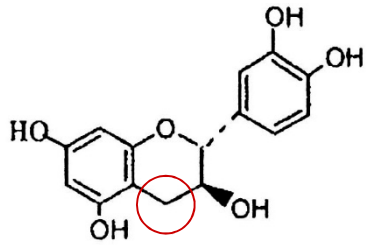
Quercetin: not apoptotic but pro-apoptotic

Traditional cancer therapy can activate apoptosis, but can encounter ***tumour resistance***. Promising results to bypass resistance are expected from protocols of "*combination therapy*" approach, where canonical drugs and/or naturally occurring chemotherapeutic agents can be associated with natural occurring agents.

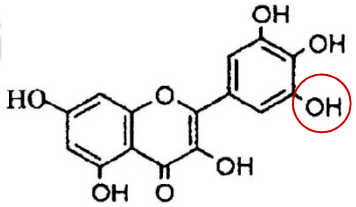
Molecule	↓ROS(%)	↑Apoptosis (%)
Quercetin 50 μM	60	>50
(+) Catechin 100 μM	40	0
Myricetin 50 μM	50	10
Quercetrin 50 μM	40	0
Resveratrol 30 μM	0	10
Gallic Acid 12 μM	0	10



Quercetin

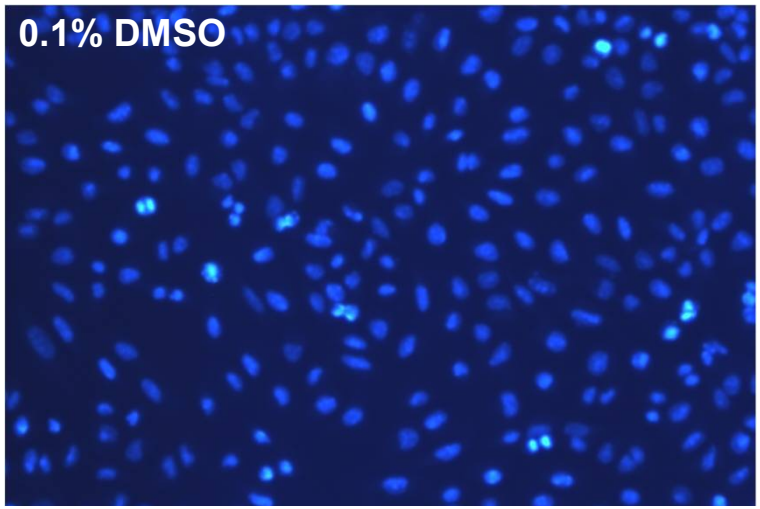


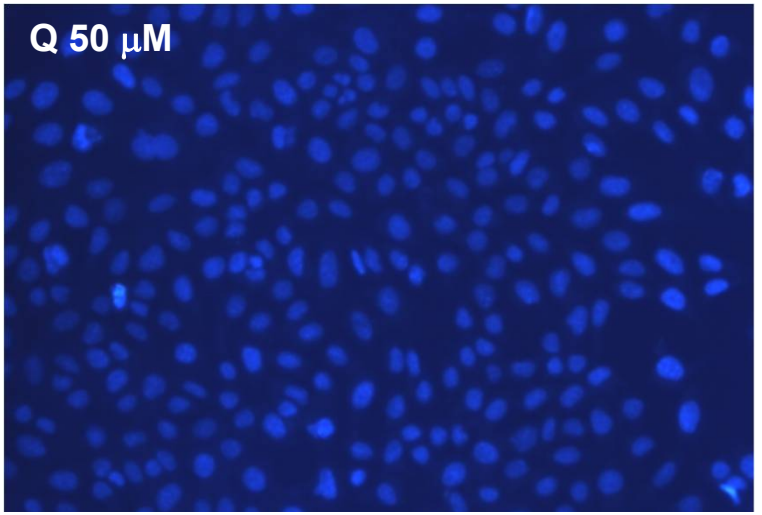
Catechin

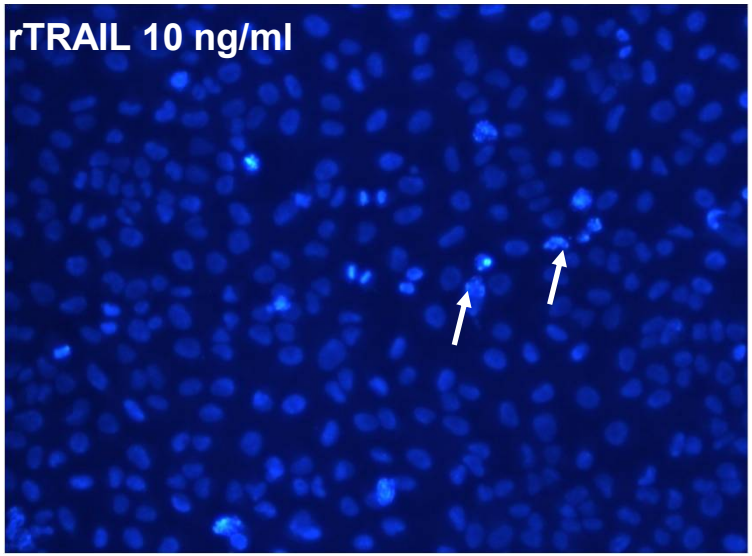


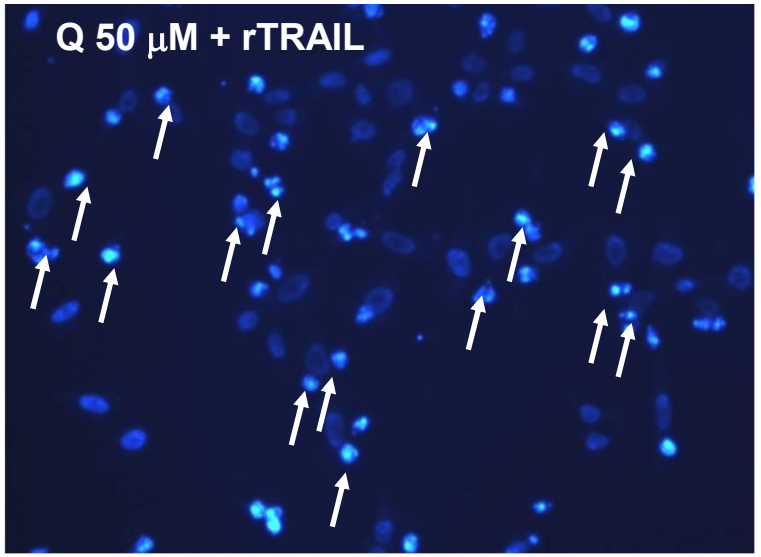
Myricetin

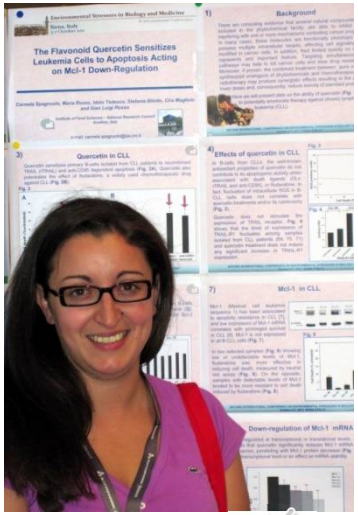
Quercetin enhances apoptosis in cell lines











Chronic Lymphocytic Leukemia (CLL)

❖ CLL is one of the most common leukemia in adult population (22-30% of all leukemia cases). In 2016, 18,960 new cases in USA with 4660 deaths.

❖ CLL is defined a malignant lymphoproliferative disorder of mature clonal B lymphocytes that accumulate in the blood and other lymphoid tissues.

❖The diagnosis of CLL occurs when B-cells count is >5,000/μL

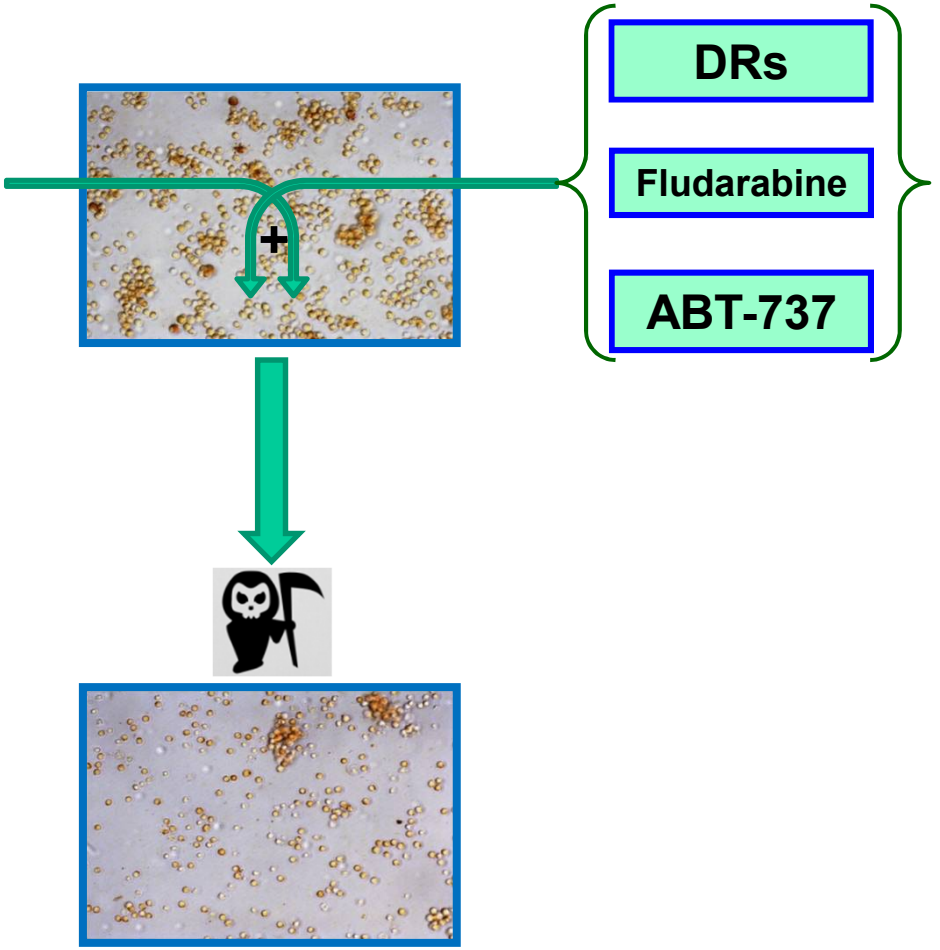
❖Poor diagnosis and shorter time to treatment and survival is more pronounced for CLL expressing Zap-70 and those with high level of CD38 (both markers are highly expressed in IGHV-UM CLL)

❖CLL is an highly heterogeneous and still an incurable disease. Most treated patients became resistant to common chemotherapeutic drugs (combination immunochemotherapy: fludarabine, cyclophosphamide and rituximab, anti-CD20 MAb).

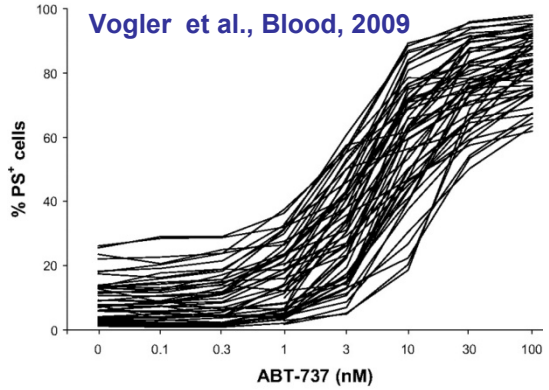
❖B-cells are resistant to DR-induced apoptosis

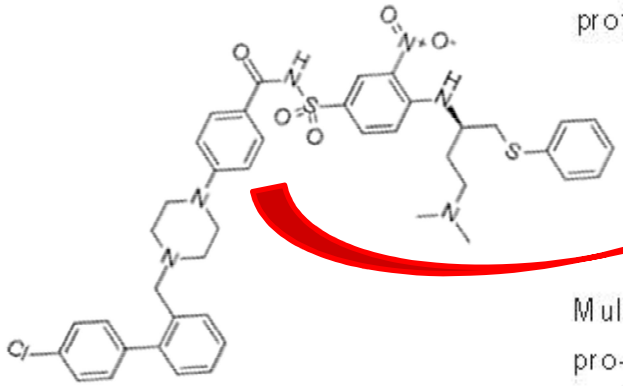
Can Quercetin induce Apoptosis in B-CLL?

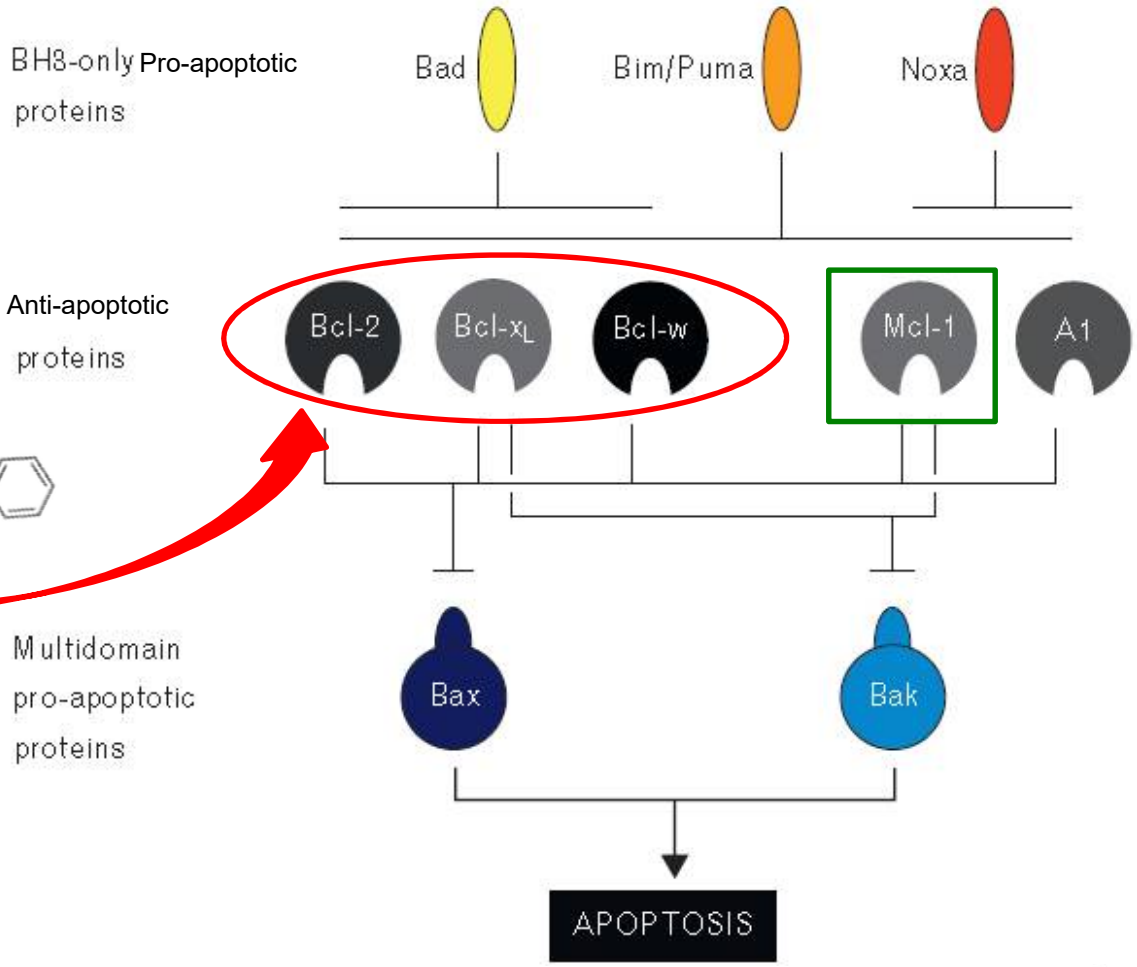
Silvestro Volpe
(Moscati Hospital, Avellino, Italy)



BH-3 “mimetic” ABT-737

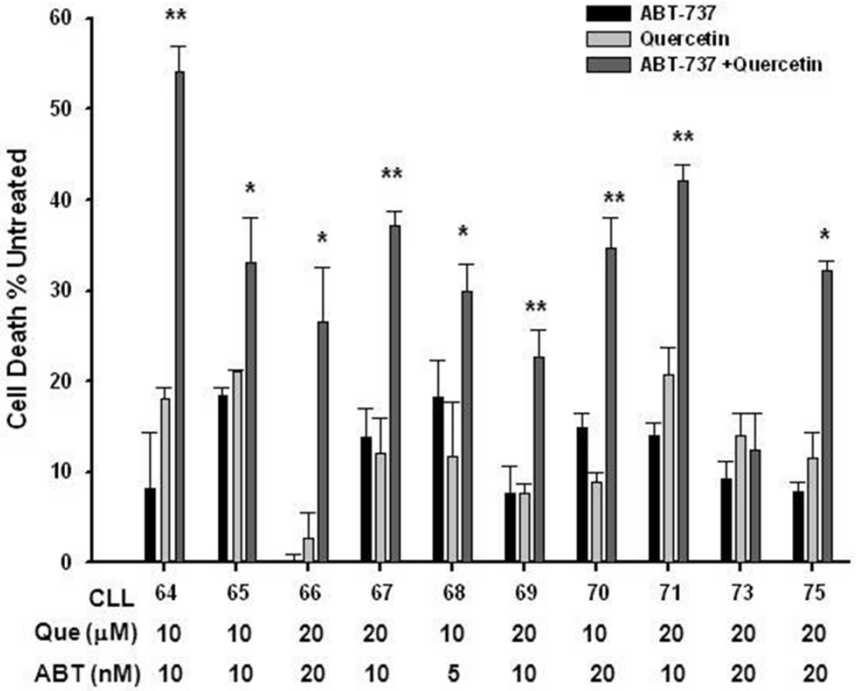


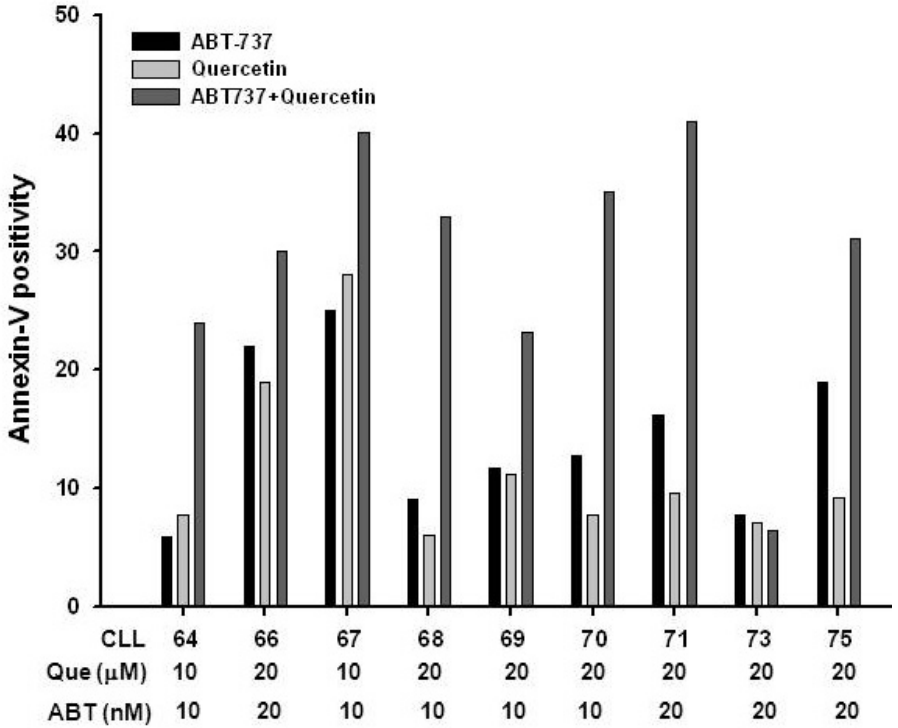




Adapted from S.-L. Khan t al., Pathology, 2011

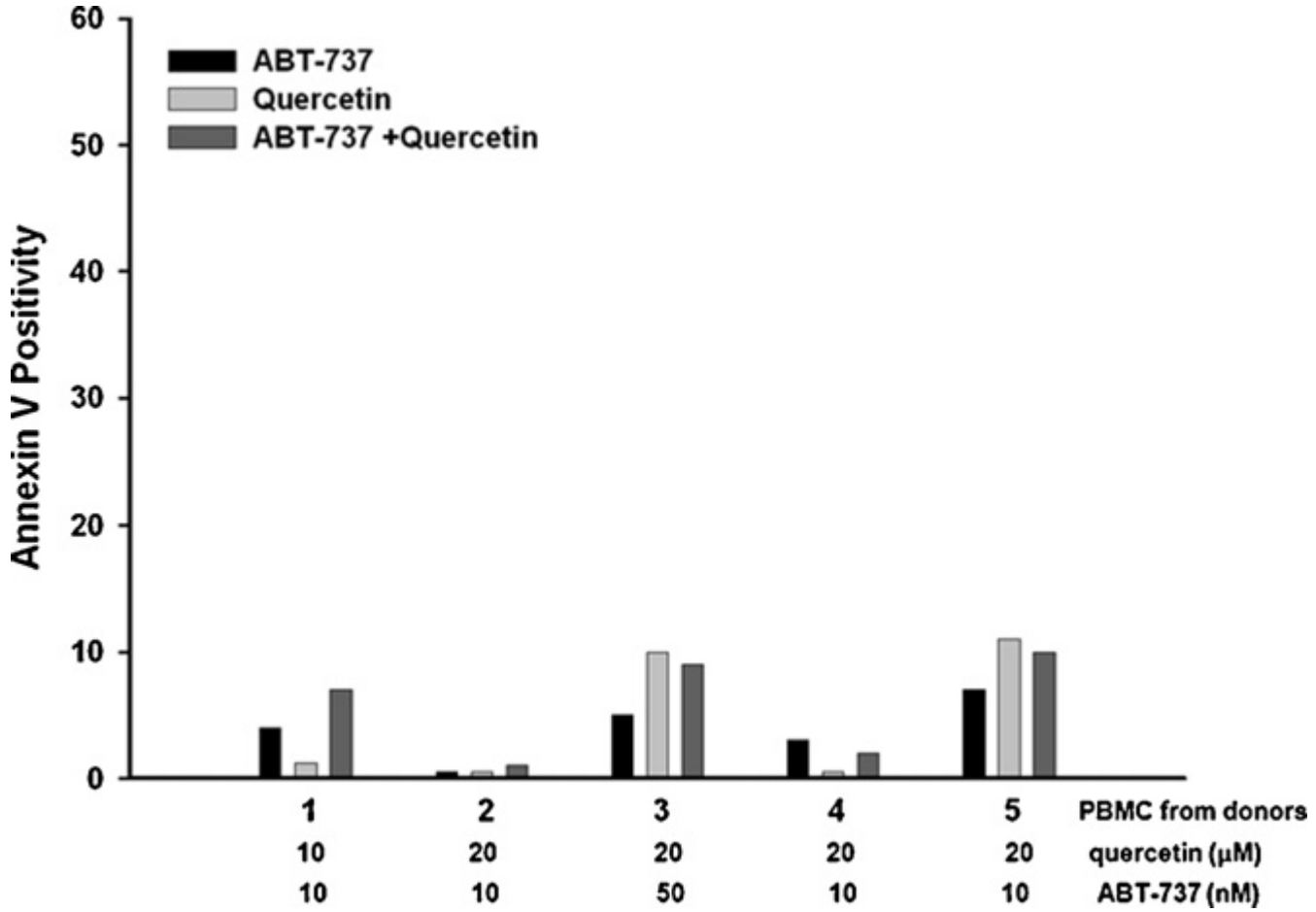
Quercetin and ABT-737 in B-CLL





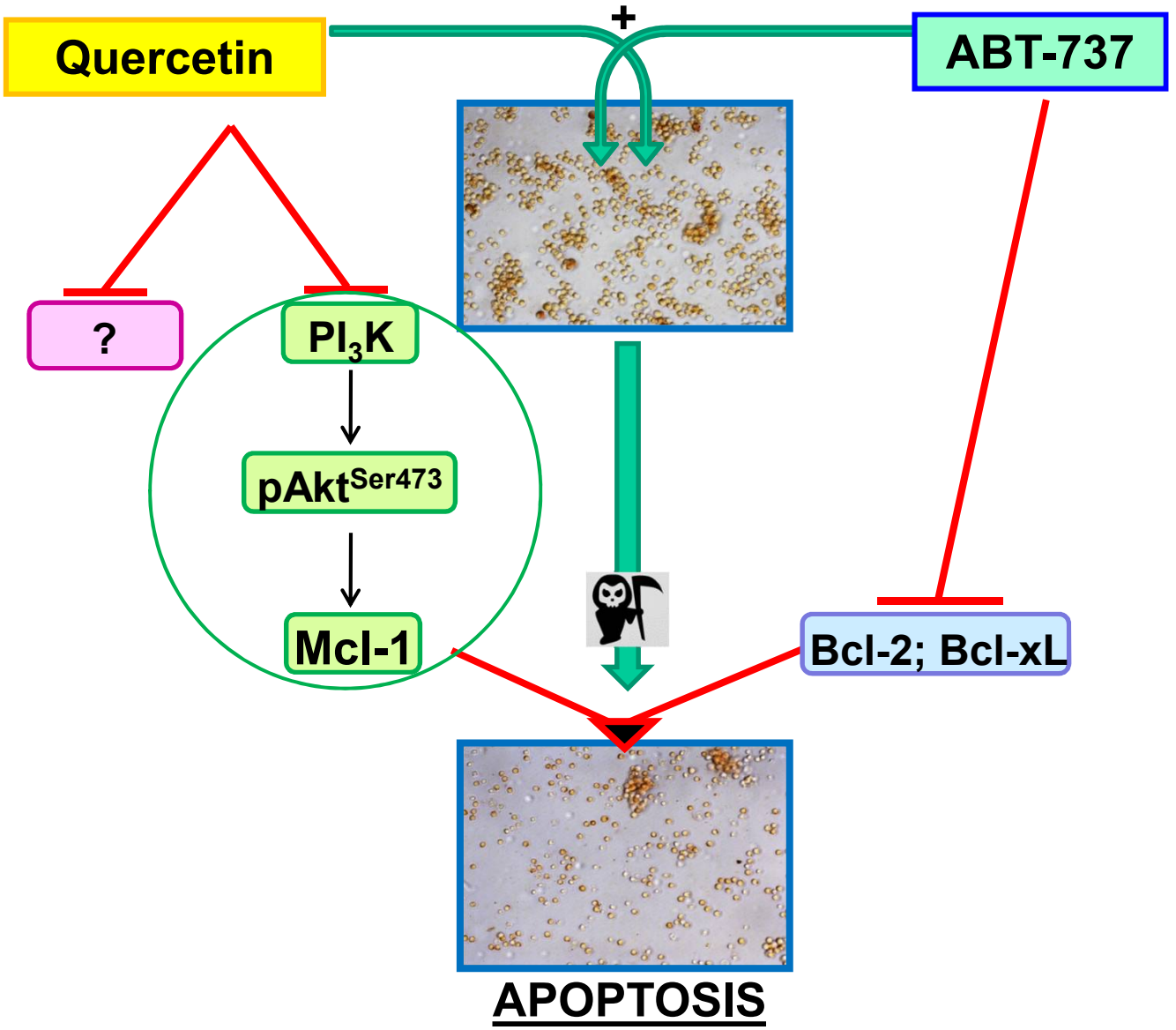
Russo M, Spagnuolo Biochem. Pharmacol., 2013

Quercetin and ABT-737 in PBMC



Russo M, Spagnuolo Biochem. Pharmacol., 2013

Quercetin and ABT-737 can bypass resistance in B-cells



HG3-CLL Cell Line (the "ideal" cellular model)

Anders Rosén
(Linköping University, Sweden)

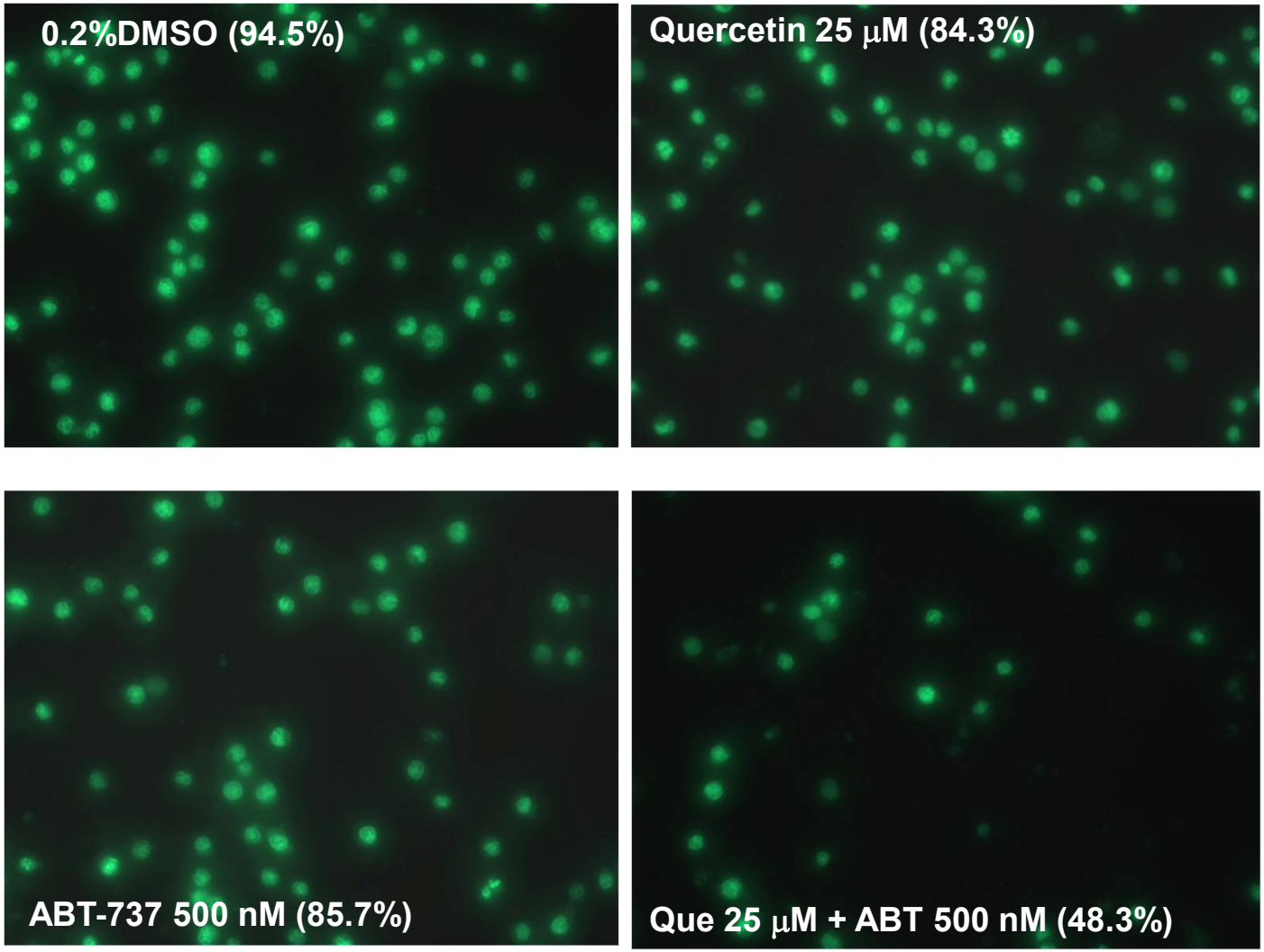
Oncolmmunology 1:1, 18–27; January/February 2012; © 2012 Landes Bioscience

Lymphoblastoid cell line with B1 cell characteristics established from a chronic lymphocytic leukemia clone by in vitro EBV infection

Anders Rosén,[1,*] Ann-Charlotte Bergh,[1] Peter Gogolák,[2,†] Chamilly Evaldsson,[1] Anna Lanemo Myhrinder,[1] Eva Hellqvist,[1] Abu Rasul,[2] Magnus Björkholm,[3] Mattias Jansson,[4] Larry Mansouri,[4] Anquan Liu,[2,‡] Bin Tean Teh,[5] Richard Rosenquist[4] and Eva Klein[2,*]

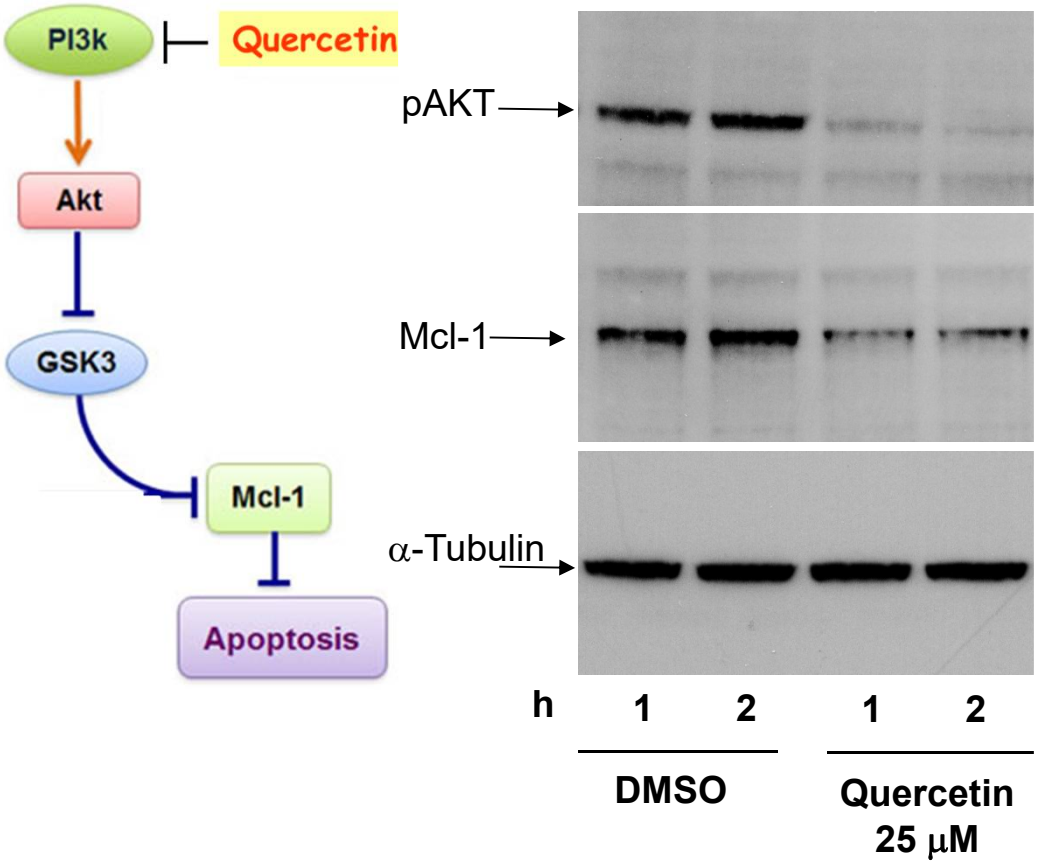
- HG3 established by *in vitro* EBV-infection;
- Derives from an IGHV1–2 unmutated CLL patient clone;
- Biallelic 13q14 deletions with genomic loss of DLEU7, miR15a/miR16–1, the two micro-RNAs that are deleted in 50% of CLL cases;
- Expression of CD5/CD20/CD27/CD43
- G-banding are showing identical biallelic deletions of chromosome 13 in HG3 cell line and in HG patient

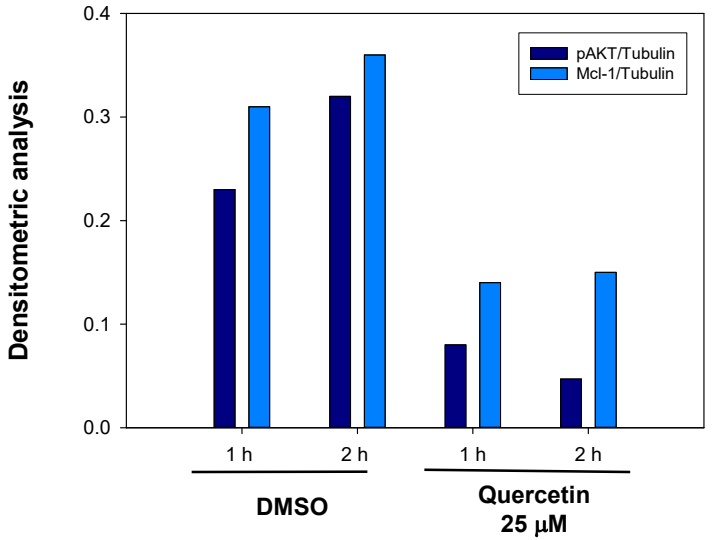
Quercetin Enhances Apoptosis in HG3-CLL



Russo M. et al. **Unpublished**

Quercetin Down-regulates PI_3K-Akt Pathway in HG3-CLL





Russo M. et al. **Unpublished**

Quercetin as a direct inhibitor of PI_3K

Table 2 Cellular kinases directly targeted by quercetin

Targets	Binding site	Concentration	Cellular effects	Reference
MEK-1	Activation loop	1–2 μM	Apoptosis	[49, 56, 58]
			Cell cycle	
			Growth arrest	
$PI_3K\gamma$	ATP-binding site	3.8 μM	Apoptosis	[105]
			Cell cycle	
			Growth arrest	
IKK α/β	ATP- and IκBα-binding sites	IC_{50} 11 μM (α)	Apoptosis	[77]

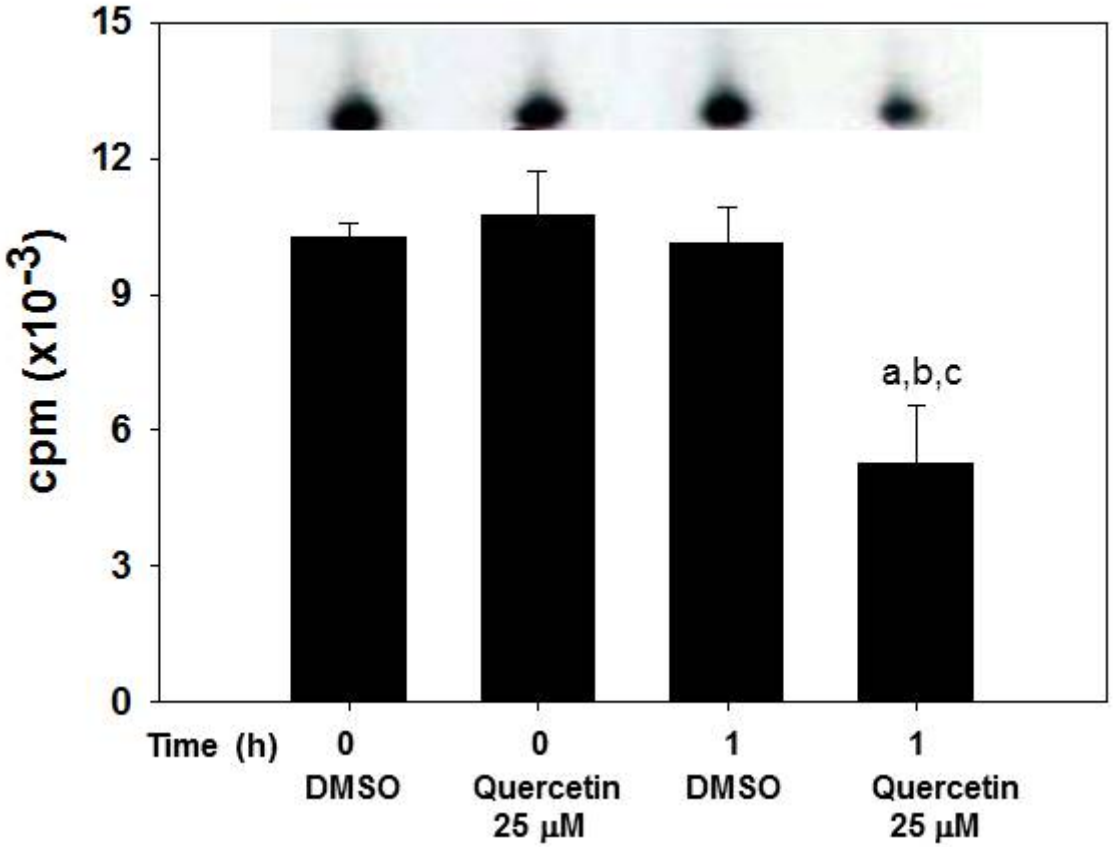
Molecular Cell, Vol. 6, 909–919, October, 2000, Copyright ©2000 by Cell Press

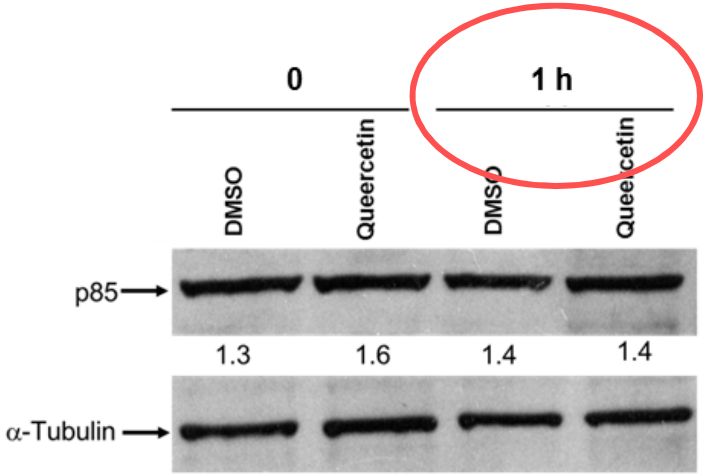
Structural Determinants of Phosphoinositide 3-Kinase Inhibition by Wortmannin, LY294002, Quercetin, Myricetin, and Staurosporine

Edward H. Walker,* Michael E. Pacold,* Olga Perisic,*

GL Russo et al., Cancer Treat. Res., 2014

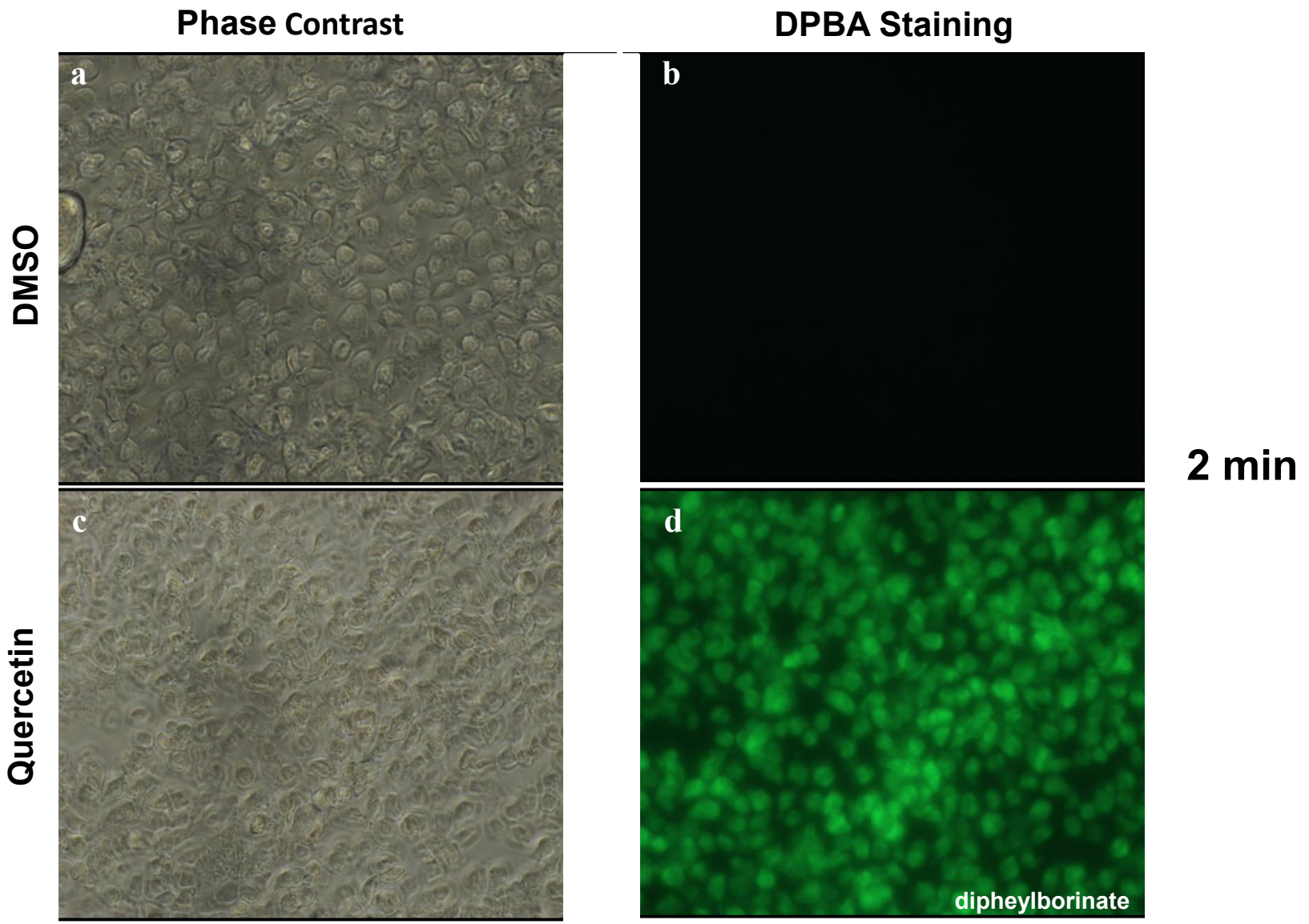
Quercetin inhibits PI_3K activity in HG3-CLL





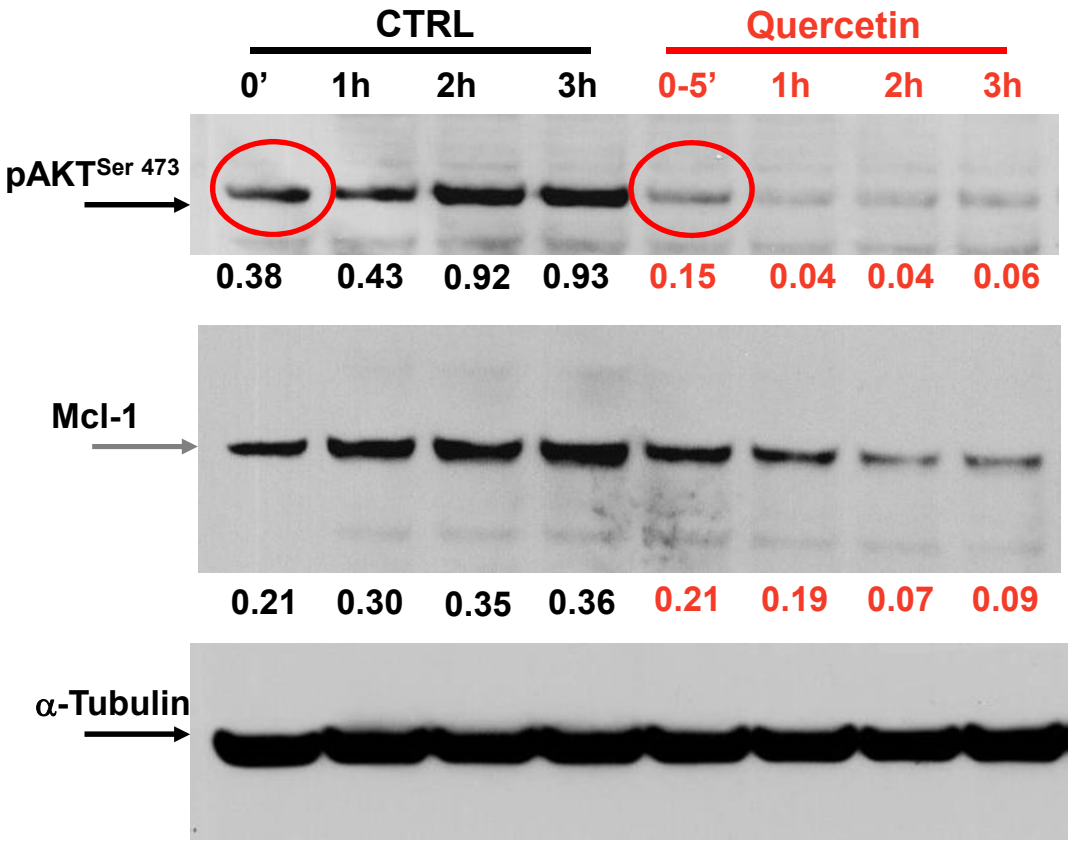
Russo M. et al., Oncotarget, 2017

DPBA Staining of Quercetin in HG3-CLL

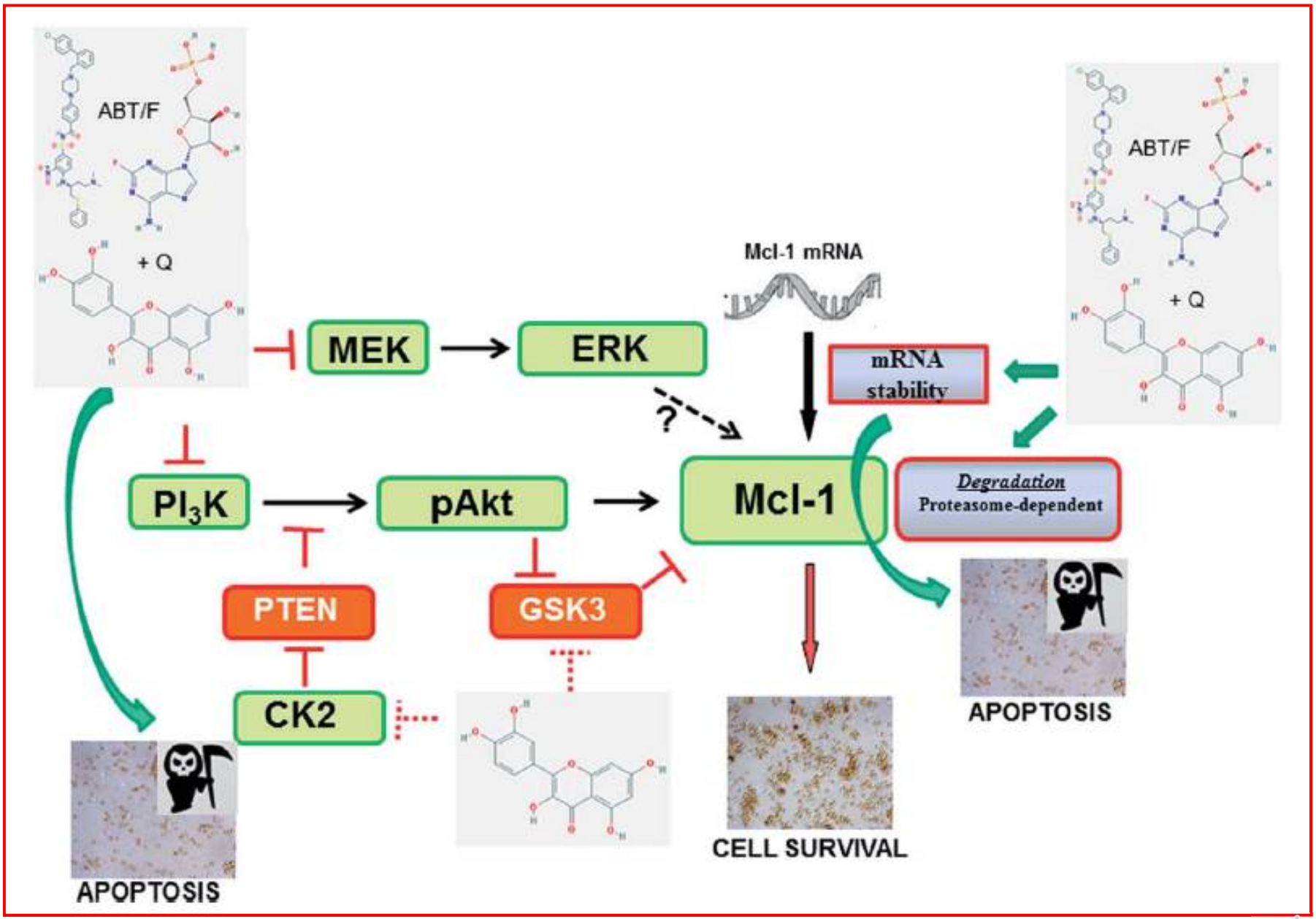


Russo M. et al., Oncotarget, 2017

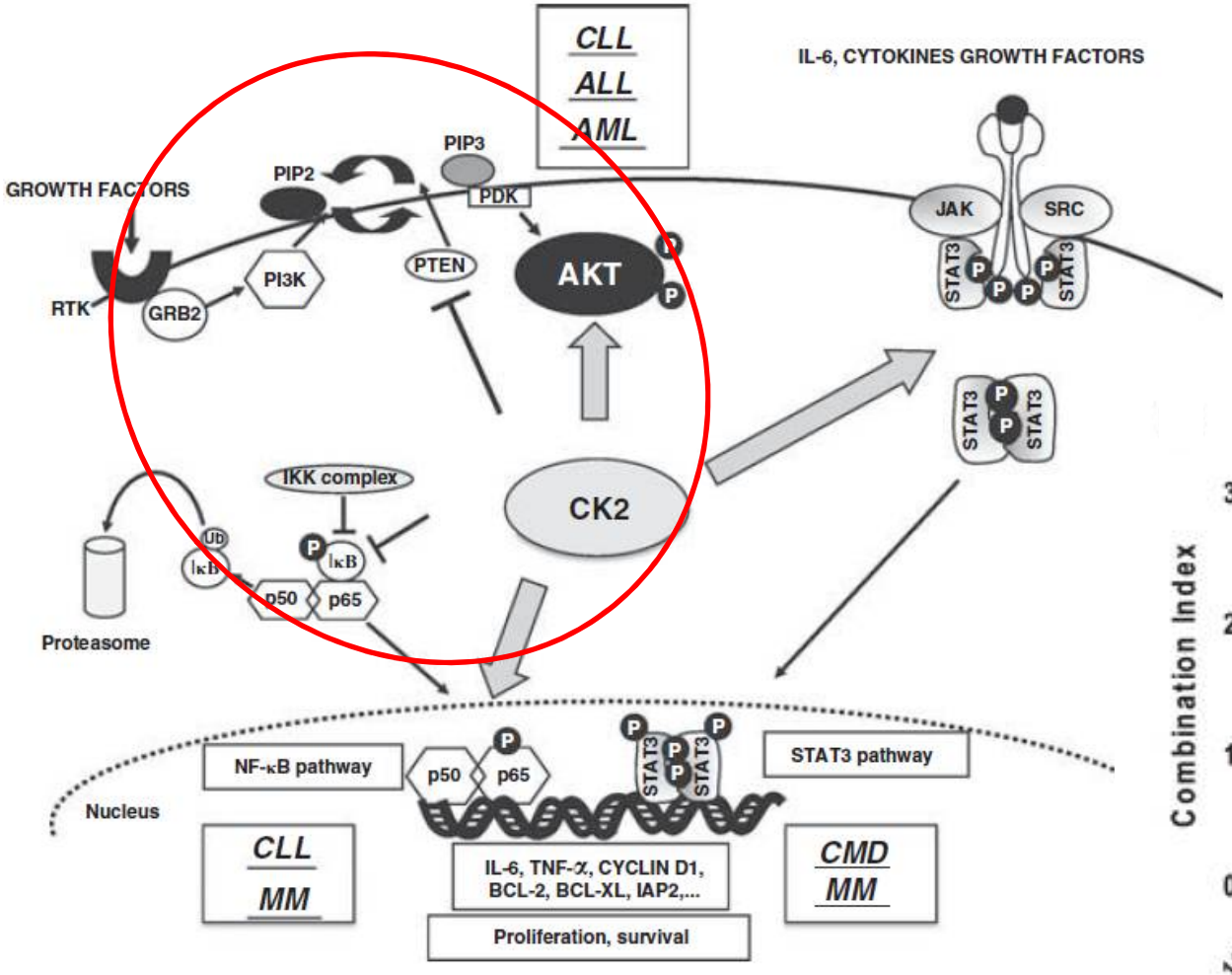
Time-dependent down-regulation of Akt/Mcl-1 (the time..)



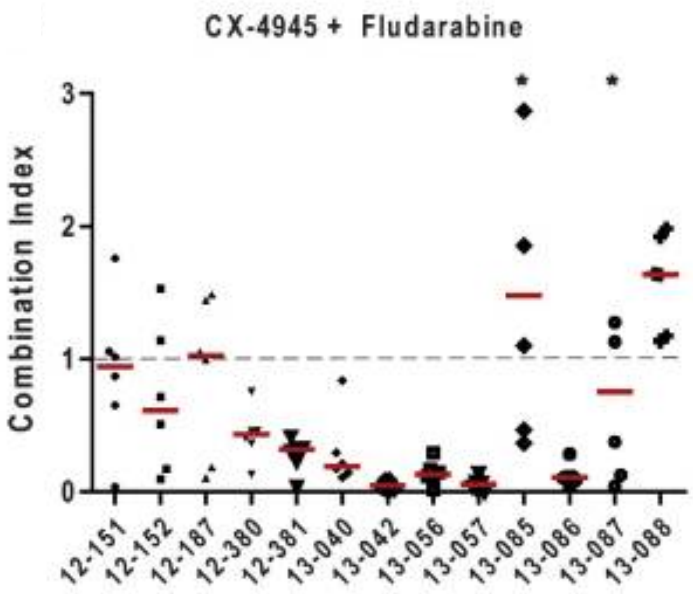
Russo M. et al., Oncotarget, 2017


ABT/F
+ Q
Mcl-1 mRNA
MEK
ERK
?
mRNA stability
ABT/F
+ Q
PI3K
pAkt
Mcl-1
Degradation
Proteasome-dependent
PTEN
GSK3
CK2
APOPTOSIS
CELL SURVIVAL
APOPTOSIS

CK2 (Casein Kinase 2) in CLL

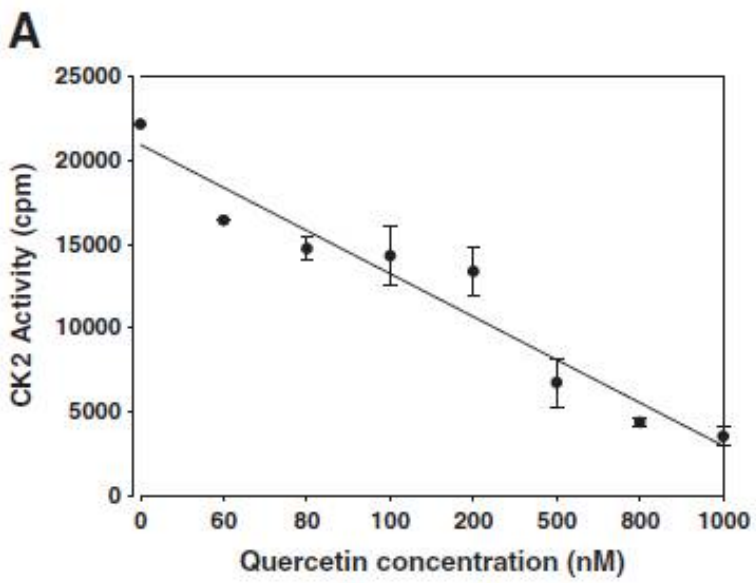


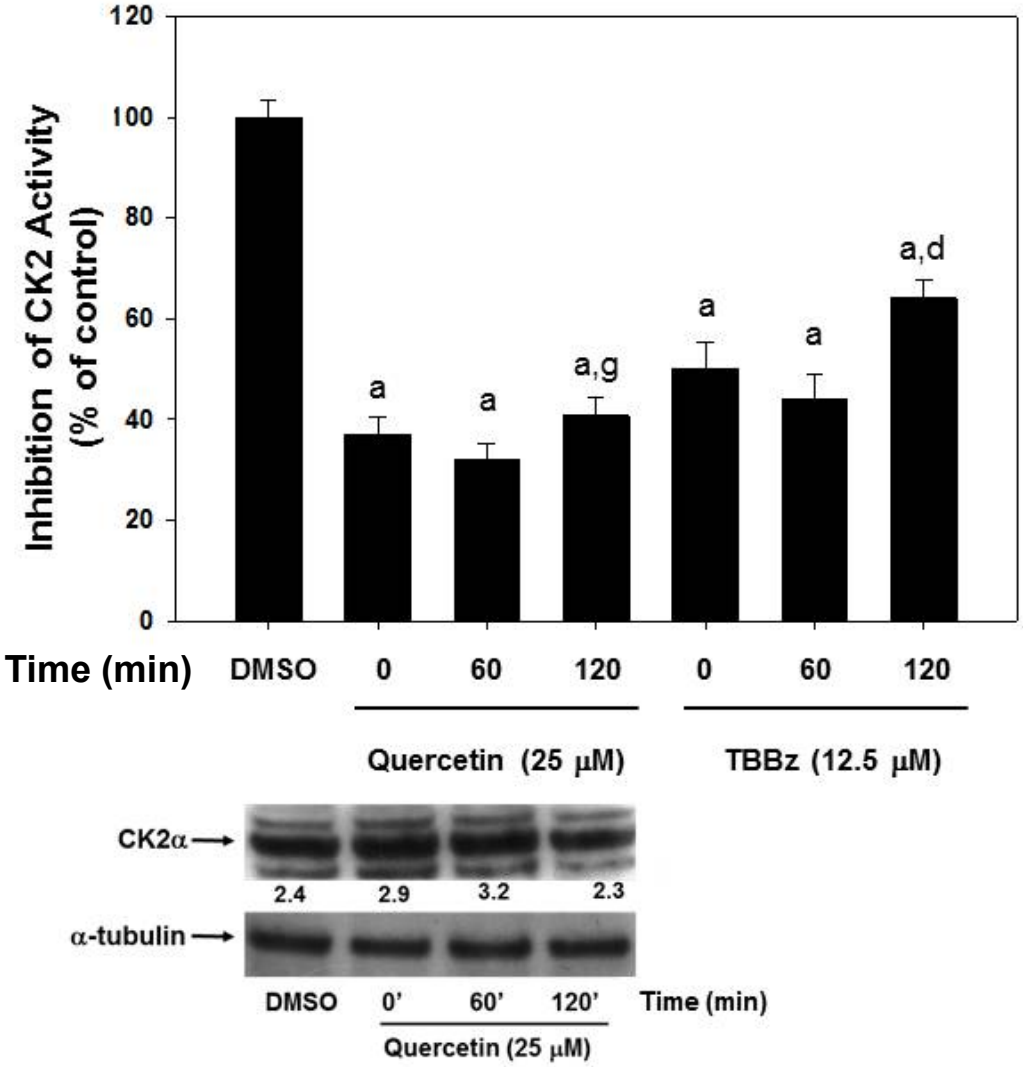
Adapted from Piazza et al., Leukemia, 2012



Adapted from Prins et al., Leukemia, 2013

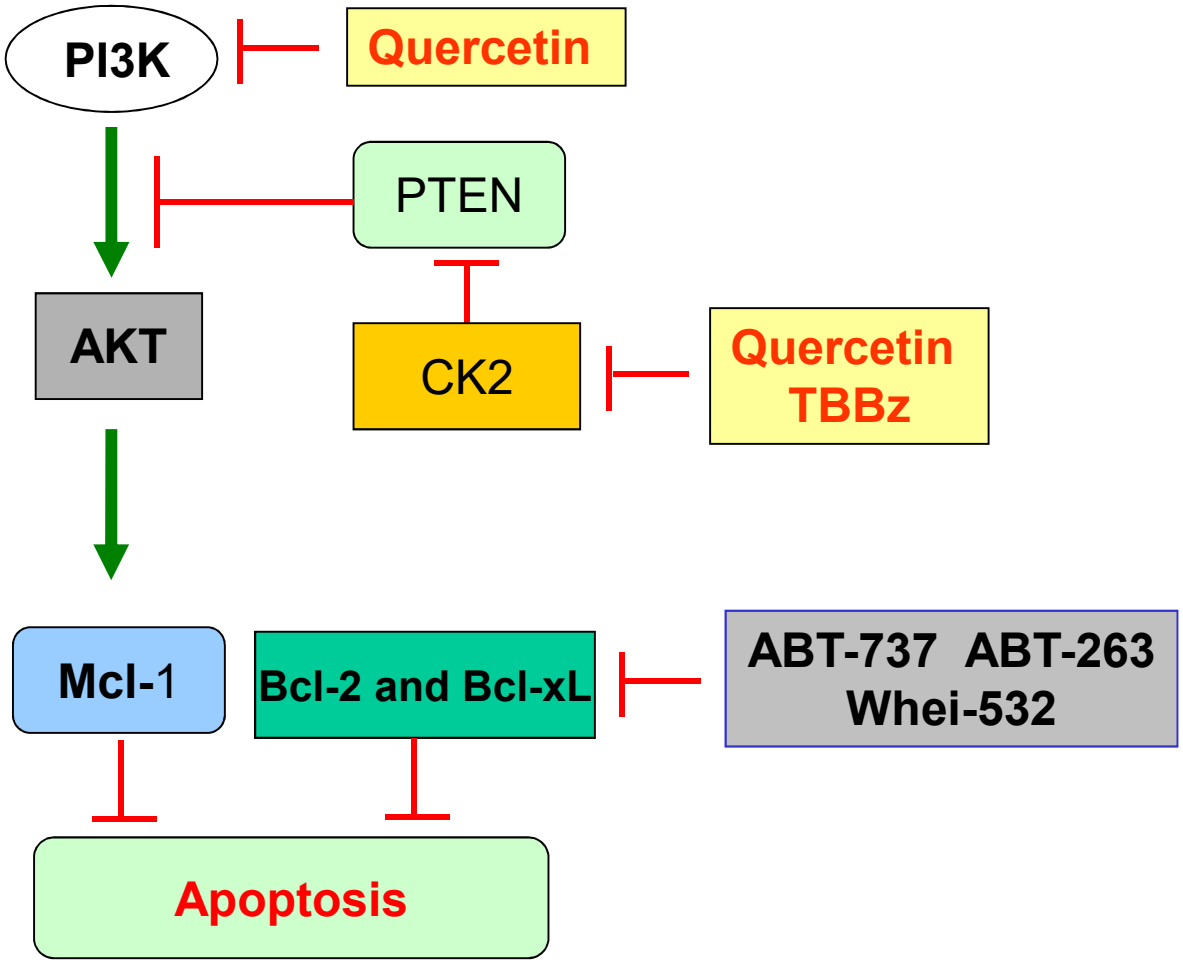
Quercetin Inhibits CK2 activity in HG3-CLL





Russo M. et al., Oncotarget, 2017

Quercetin mechanism of action



Russo M. et al., Oncotarget, 2017

Conclusions

- Quercetin sensitizes synergistically human malignant cell lines **AND** B-cells isolated from CLL patients to conventional (fludarabine) and novel (BH3-mimetics, ABT-737) treatments;
- In CLL-derived cells, quercetin targets primarily protein kinases (CK2 and PI_3K) which positively regulate the **PI_3K-AKT-Mcl1** pathway;
- Quercetin cytotoxicity on normal peripheral blood leukocytes is acceptable;

CONSIDERING THAT

- Phase I clinical trial in humans established a recommended dose of 1400 mg/m^2 (2.5 g/70 Kg) intravenous weekly interval.
- **Overall, these data indicate the potential therapeutic use of quercetin against CLL**

Acknowledgments

*Regional Products with Healthy Properties to Develop New Functional Foods (**RiSaNA**)*

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