Tolerance of oral lipoid acid and hydroxycitrate combination in cancer patients

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Tolerance of oral lipoid acid and hydroxycitrate combination in cancer patients : first approach

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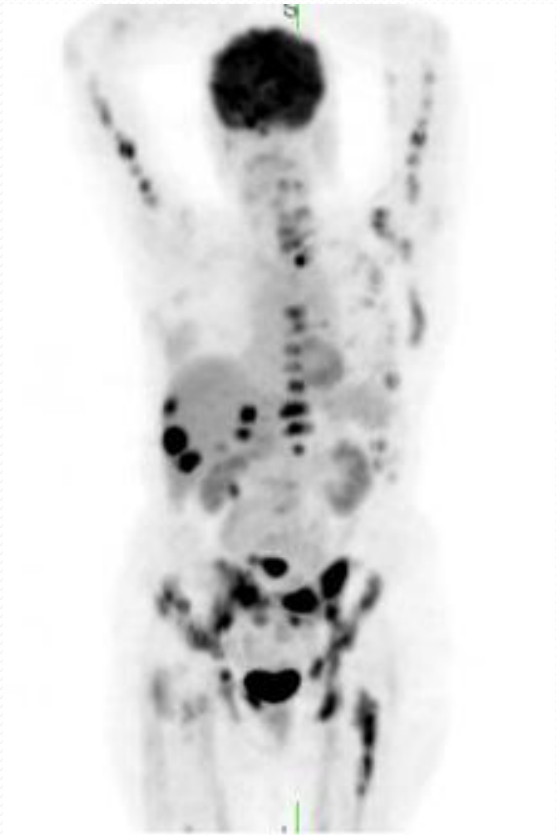
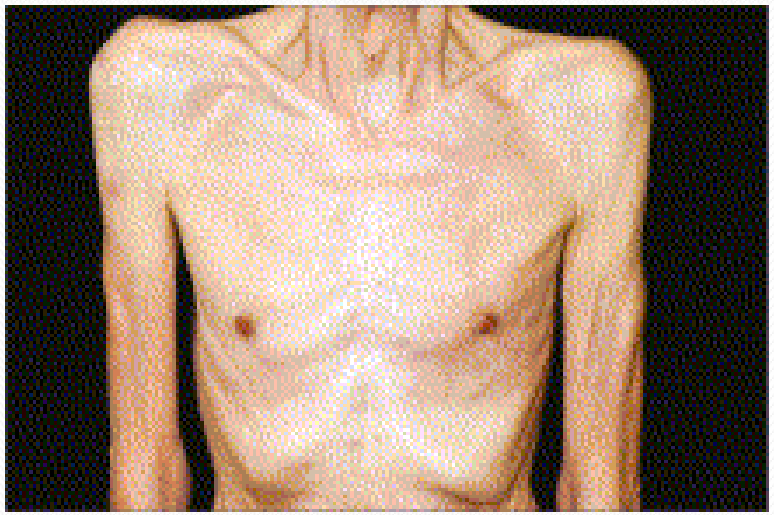
The metabolic alterations of cancer

- Aberrant glucose consumption (Pet-scan)



- Cancer associated cachexia



Cancer Metabolism: A Recurrent Approach

Preclinical evidences:

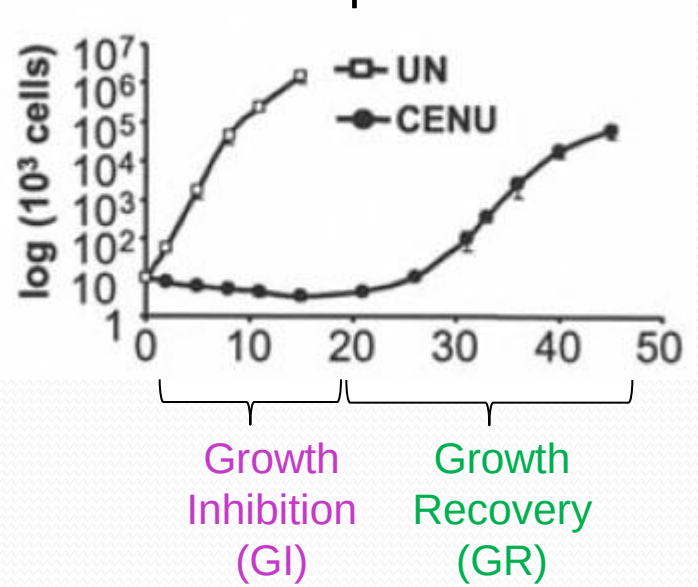
- 1925- In most cancer cells: high aerobic glycolysis and lactic acid secretion
  Warburg, Nobel Prize
- 1997- Oncogenes and tumor suppressor genes alter metabolic pathways
  Shim H. *PNAS*
- 2002- Mutated forms of metabolic enzymes could promote cancer
  Tomlinson I. *Nat. Genet*.
- 2009- Cell chronic exposure to insulin and glucose favors transformation
  Schwartz & Demidem, *AACR*

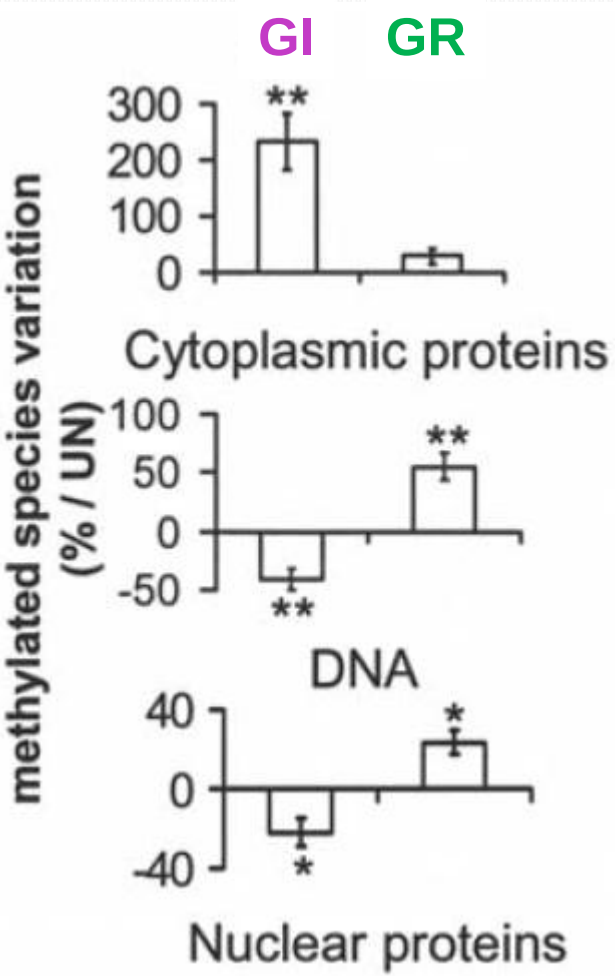
Clinical evidences:

- 2009- Metformin long term treatment of diabetes reduced cancer incidence
  Libby G. *Diabetes Care*
- 2010- Obesity is the second risk factor for cancer
  Khan N. *Cancer Lett.*
- 2010- Use of an indirect PDH activator: regression of glioblastoma in 3/5 patients
  Michelakis E. *Sci Transl Med.*

Chemotherapy alters cell methylation

B16F10 cell proliferation curve



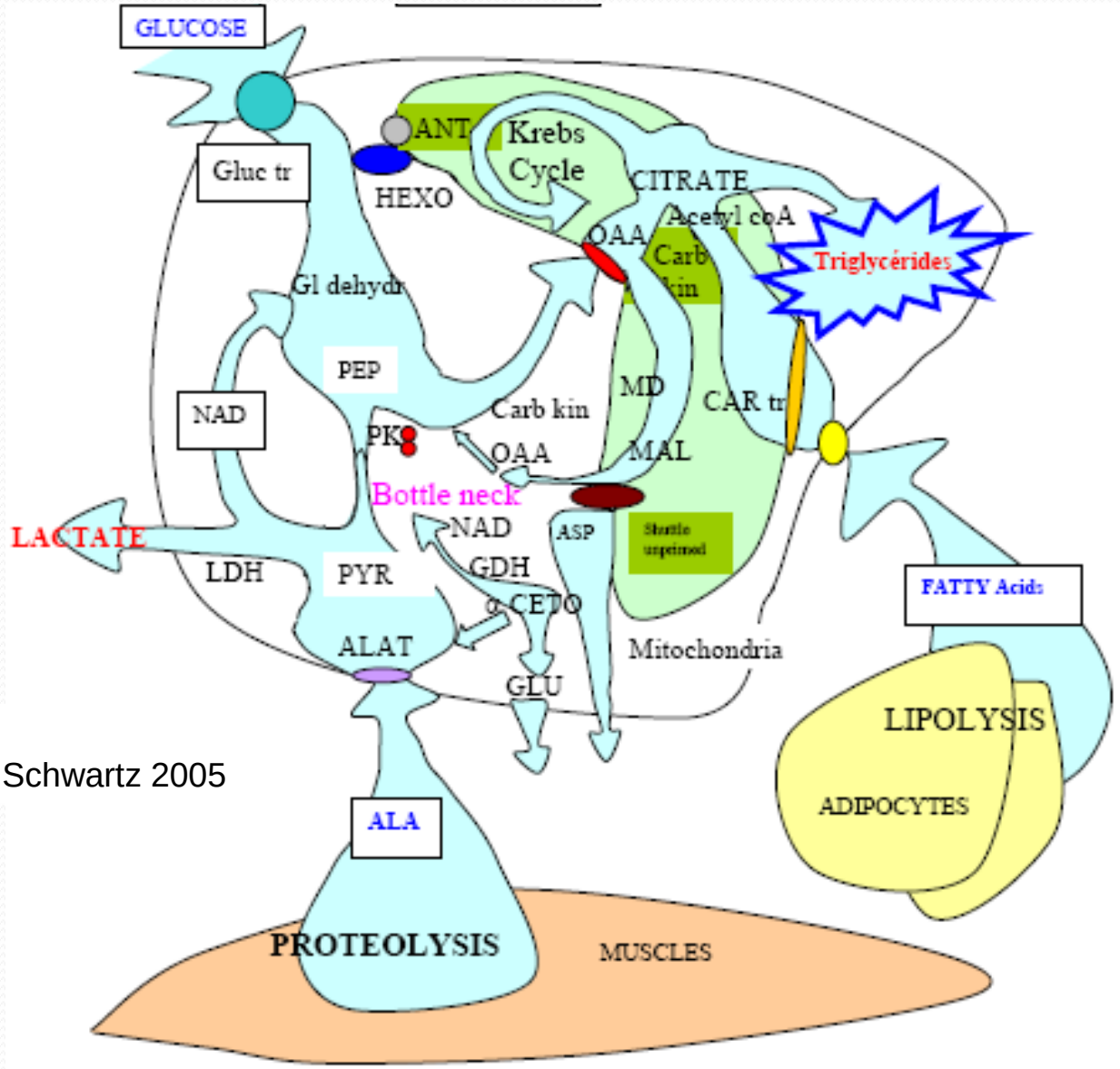


GI phase is correlated with hypermethylation of cytoplasmic proteins

GR phase is correlated with methylation of nuclear proteins and DNA

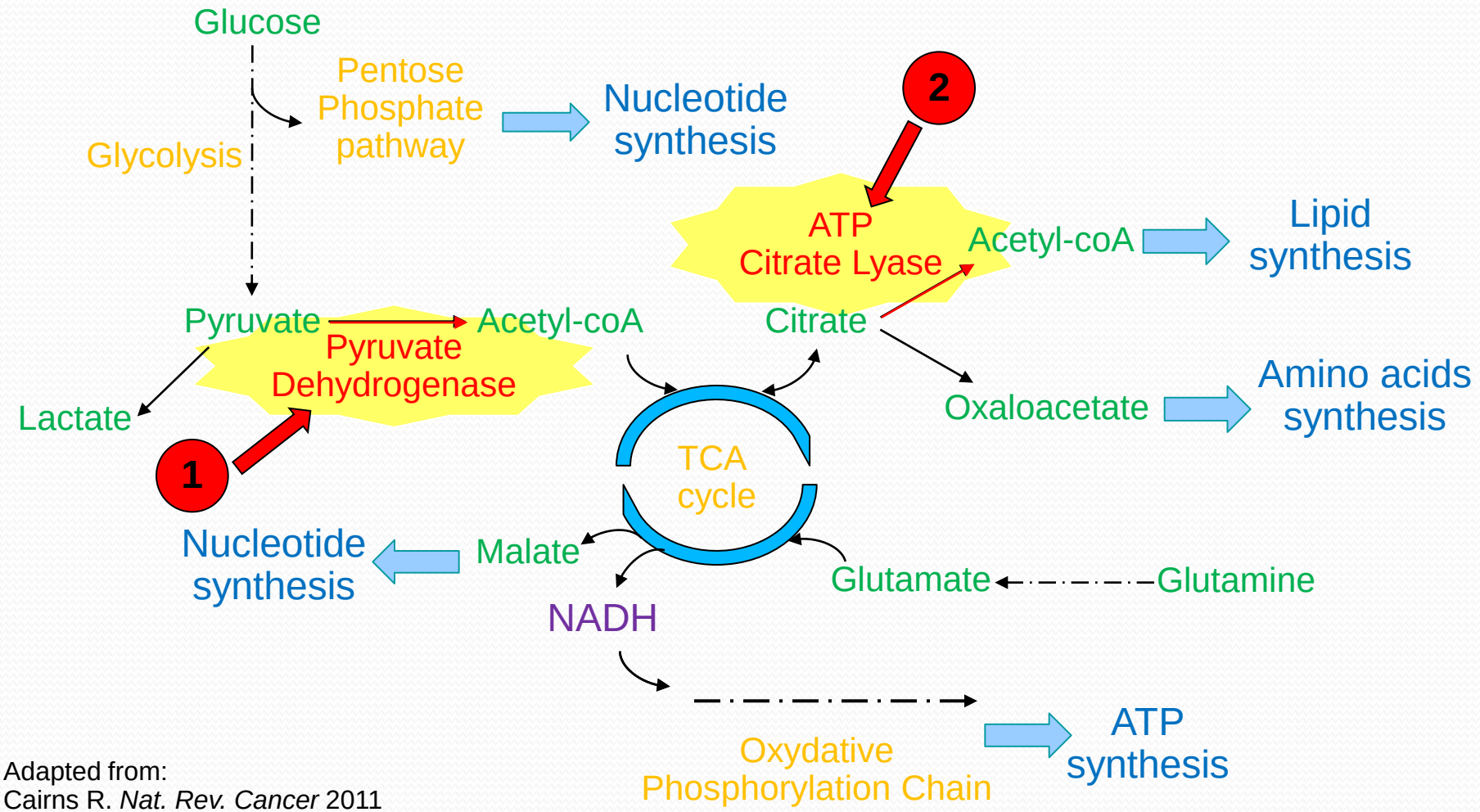
Guénin et al. Int J Oncol. 2008

Cancer is a metabolic disease



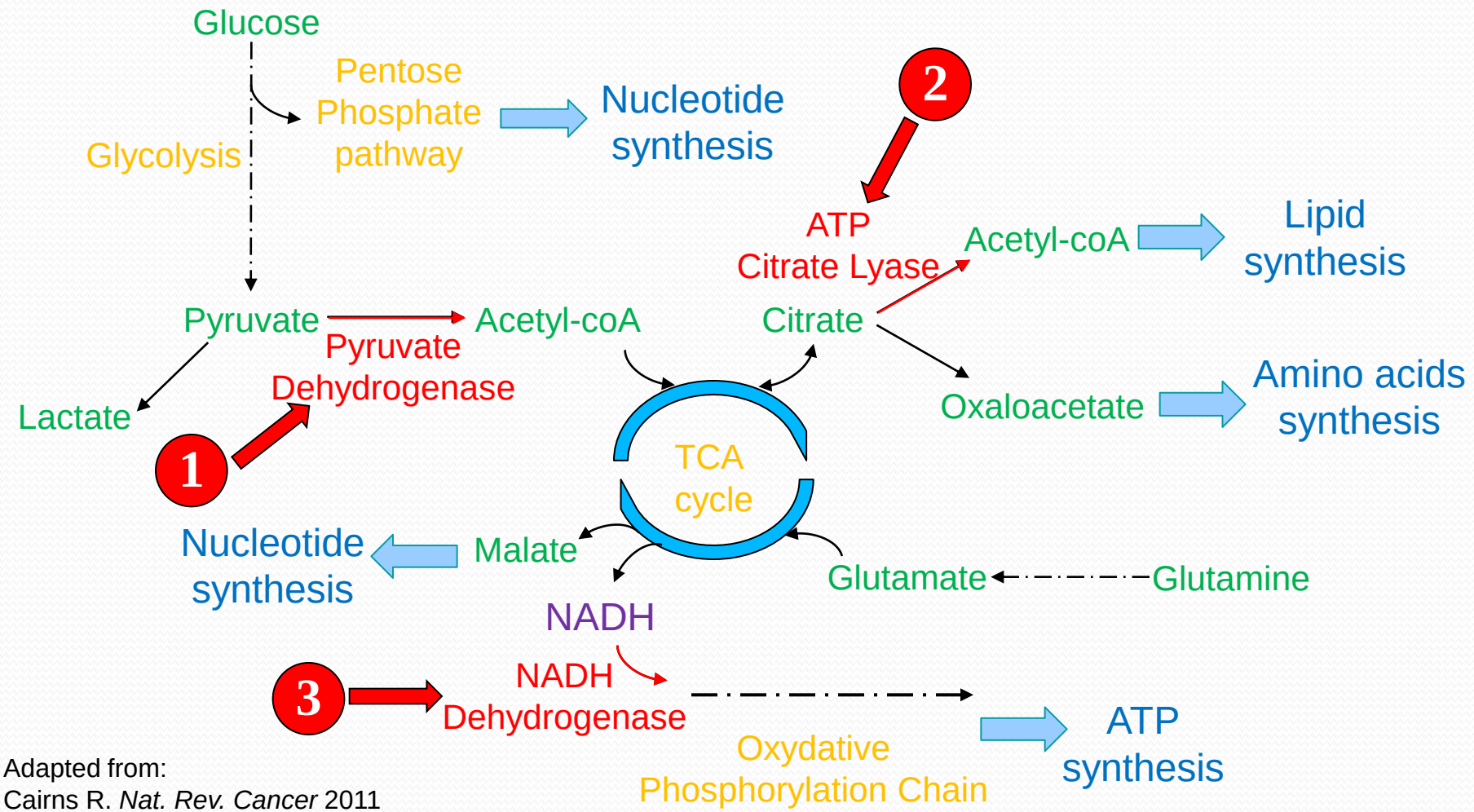
From:
Israël and Schwartz 2005

Metabolic Pathways Activated in Cancer



Adapted from:
Cairns R. *Nat. Rev. Cancer* 2011
Levine A. *Science* 2010
Van der Heiden M. *Science* 2009

Metabolic Pathways Activated in Cancer



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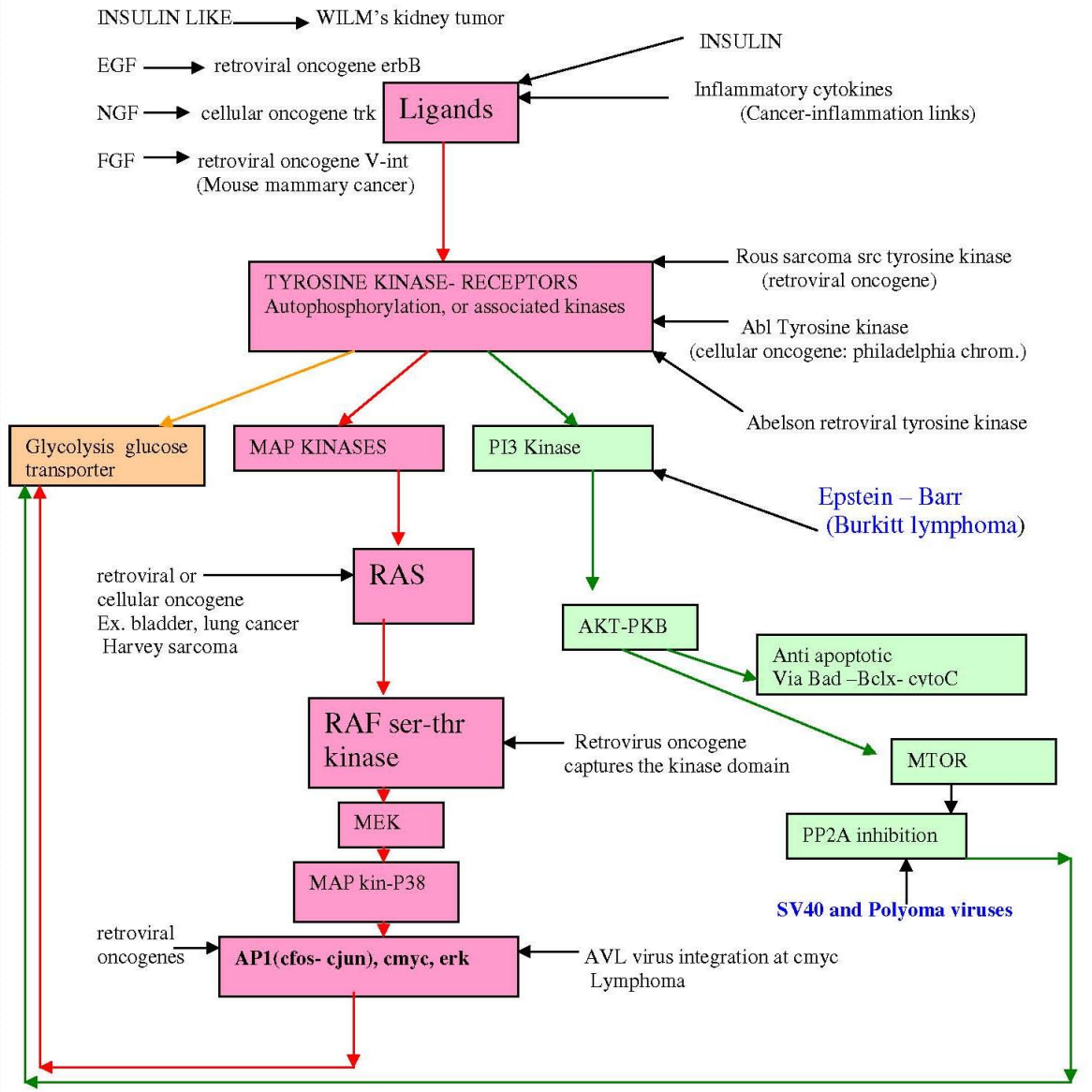
Links between oncogenes and metabolism



Around one hundred identified targets

Targets
Carbonic anhydrase
PEP carboxykinase
NADH dehydrogenase
PDE-3
lipotropic factor
NADH dehydrogenase
Pyruvate Kinase M2 isoform
Pyruvate dehydrogenase kinase 1
Pyruvate dehydrogenase
phospholipase D
Phospholipase Cgamma
LDH
PEP carboxykinase
ATP citrate lyase
Cyt. P450 demethylase
Hexokinase
AMPK
Choline kinase
Lipolysis
Phospholipase A2
IGFBP
Potassium channel blocker
PP2A

Targets
Glutaminase
Polyamine synthesis
Citrate synthase
Na+/H+ antiport
Hypothalamic D2 receptor
HDAC
Citrate synthase
AID
Alanine transaminase
PK-M1/M2 splicing
Serotonine reabsorption
Triglycerides
Cyt. P450 demethylase
Oxidative stress
Aromatase inhibition
Arginase inhibition
Tyr kinase receptor
Transketolase
Pyruvate kinase
GH receptor
Alanine transaminase
Cellular differentiation
GH secretion

Selected drugs for in vivo screening

Drugs	Mechanism of action
6-diazo-5-oxo-L-norleucine	Glutaminase inhibition
Agmatine	Polyamine synthesis inhibition
Alpha cetoglutarate	Citrate synthase inhibition
Amiloride	Na+/H+ antiport inhibition
Apigenine	IGFBP activation
Bicalutamide	IGFBP activation
Bromocriptine	Hypothalamic D2 receptor agonist
Butyrate sodium	HDAC inhibition
Chitosan	PK-M2 inhibition
Choline Chloride	lipotropic factor
Citrate	Citrate synthase inhibition
Cryogenine	PEP carboxykinase inhibition
Curcumin	AID inhibition
D-alanine	Alanine transaminase inhibition
Epigallocatechin gallate	PK-M1/M2 splicing regulation
Fluoxetine	Serotonine reabsorption inhibition
Ibuprofen	NSAIDS
Indole 3 carbinol	Triglycerides reduction
Ketoconazole	Cyt. P450 demethylase inhibition
Lactoferine	Oxydative stress reduction
Letrozole	Aromatase inhibition
L-norvaline	Arginase inhibition
Melatonine	anti-oxydant, anti-proliferative
Menadione	Tyr kinase receptor inhibition
Oméprazole	IGFBP activation
Oxythiamine	Transketolase inhibition
PEG8000	PK activation
Pegvisomant	GH receptor inhibition
Pralidoxime	Alanine transaminase inhibition
Retinoic acid	Cellular differentiation activation
Sulpiride	GH secretion inhibition
Suramine	Citrate synthase inhibition
Valproate sodium	HDAC inhibition
Vitamine B12	lipotropic factor

Selection Process

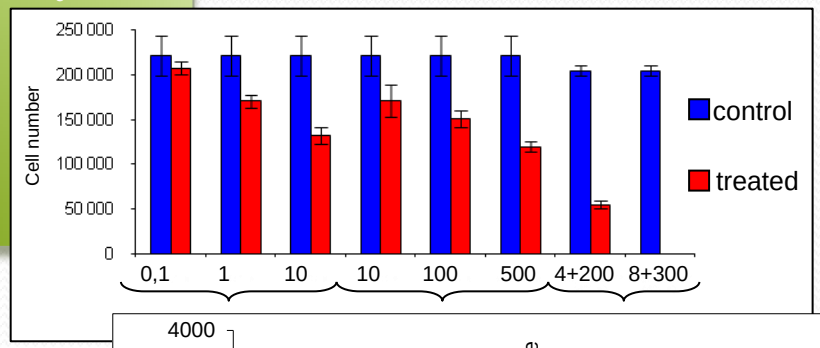
Literature Screening

- Metabolic Activity (80)
- Human Use and Tolerance (30)

Not effective	Weakly effective	Effective
Xylitol	Suramine	Acetazolamide
Niacin	Amobarbital	Albendazole
Lithium chloride	Quinine	Dichloroacetate
Hydrazine sulfate	Metformin	
Amrinone	Silibinine	
	Ketoconazole	
	Tolbutamide	
	Quinacrine	
	Simvastatine	
	D-Fructose-1,6-bisphosphate	
	Betaine	

In vitro screening

- Antiproliferative activity
- Individual drug (20)
- Pair of drugs (7)

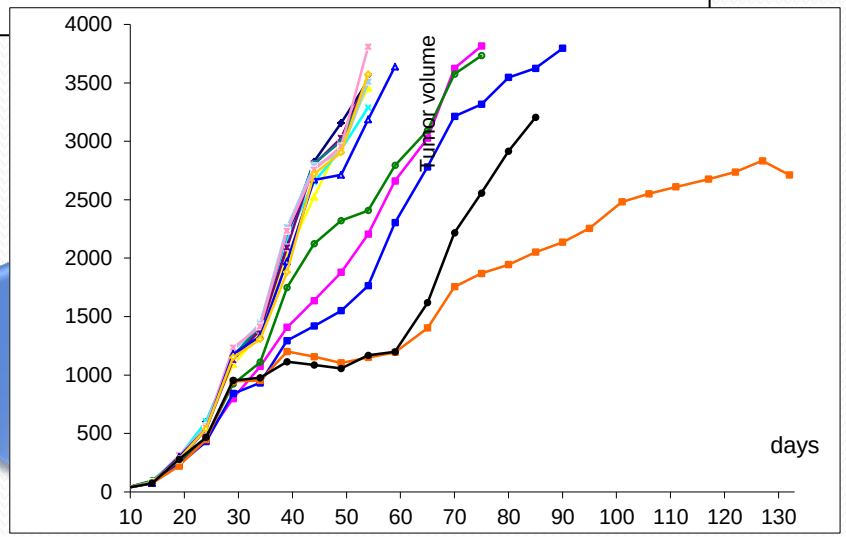


Double Combination Tests

- 3 in Vivo Cancer Models
- Antitumoral activity

Triple Combination Tests

- 40 more compounds
- 3 in Vivo Cancer Models
- Antitumoral activity



Drug Combination with Antitumoral Activity: ALA/HCA

In vivo drug combination tests

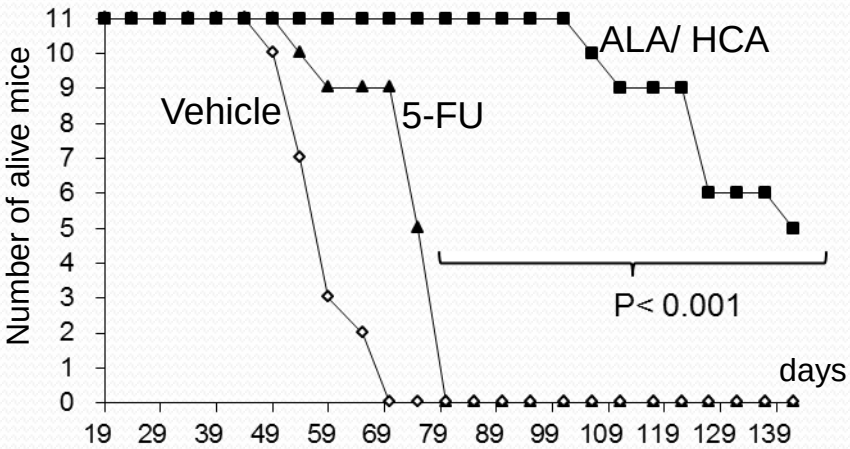
- Subcutaneous inoculation of tumor cells (~3000 mice)

  3 syngeneic models:

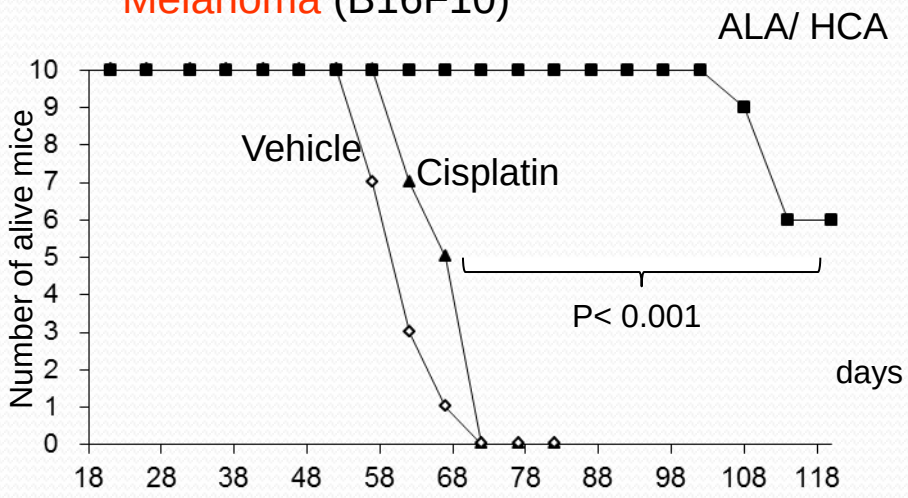
  - Lung carcinoma (LLC),
  - Bladder carcinoma (MBT-2),
  - Melanoma (B16-F10)
- Treatment via intraperitoneal administration of the drugs
- Tumor development follow-up:
  - Tumor volume (caliber, twice a week)
  - Animal weight
  - Survival

ALA/ HCA Efficacy

Bladder carcinoma (MBT2)



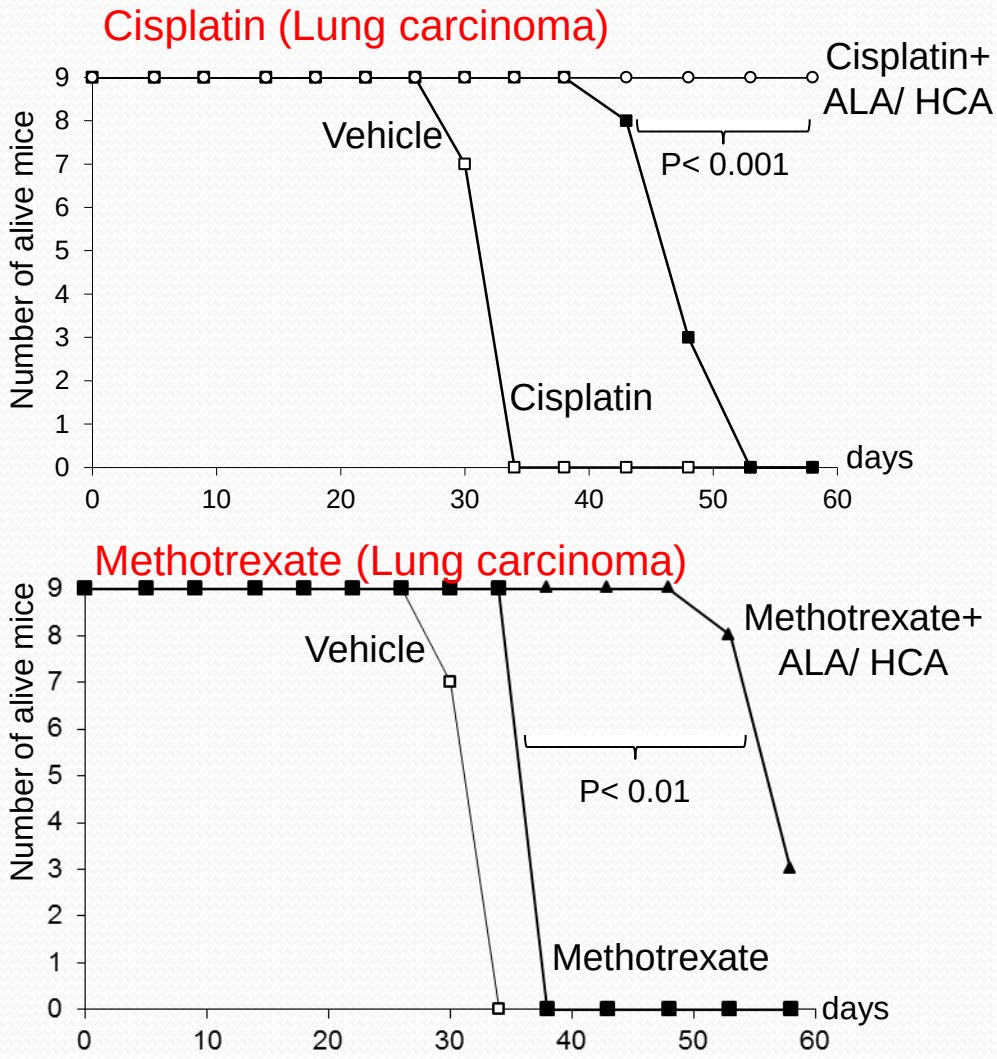
Melanoma (B16F10)



Schwartz et al. 2010
Oncology Reports

The ALA/ HCA combination **doubles survival** as compared to untreated mice

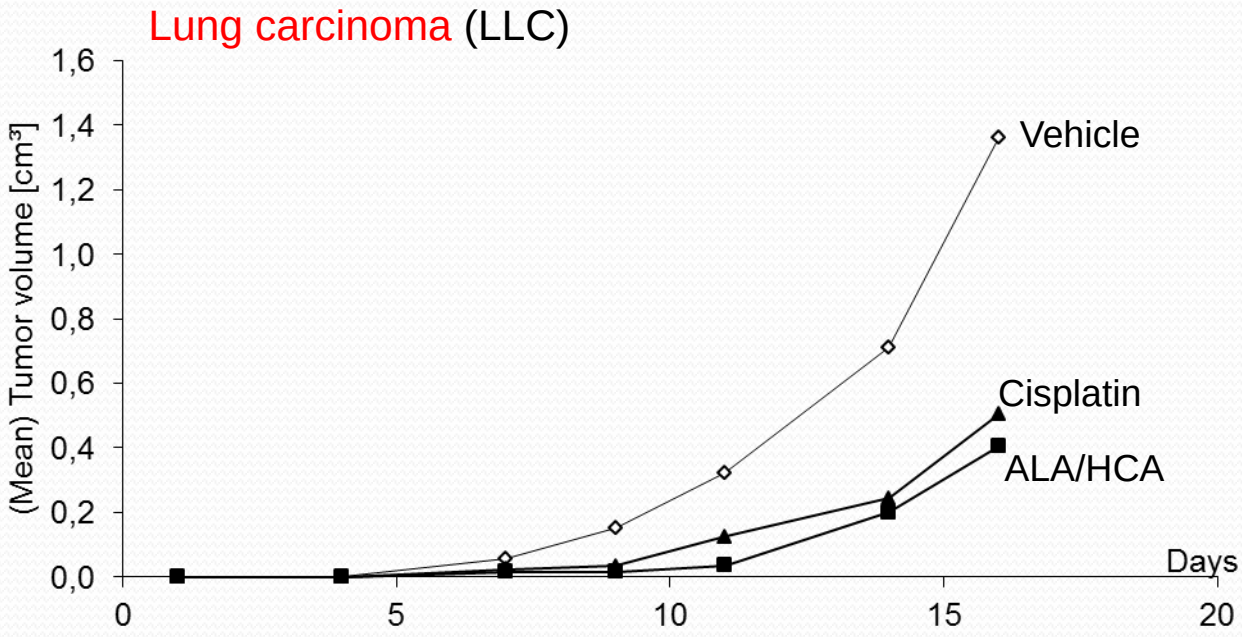
ALA/ HCA combined with chemotherapy



Addition of ALA/ HCA to chemotherapy **significantly enhances survival**

Guais et al. 2010
Investigational New Drugs

ALA/HCA Efficacy



Experiments performed by EPO GmbH, Berlin University

The combination **significantly reduces** tumor development (equivalent to cisplatin chemotherapy)

Animal models main results

Combination	Alpha lipoic acid + Hydroxycitrate
Results	Slows down tumor development extends survival
Efficiency	Similar to chemotherapy Potentiates CHT effectiveness
Models	Syngeneic mouse models: melanoma, bladder and lung carcinoma mice cells

Our previous publications

- tend to demonstrate that lipoïc acid (ALA) and hydroxycitrate (HCA) combination decreases
  - the tumor growth in mice
  - with either lung cancer, bladder cancer or melanoma

Lipoic acid

ALA , well known treatment of the diabetic neuropathy its interest in cancer is growing

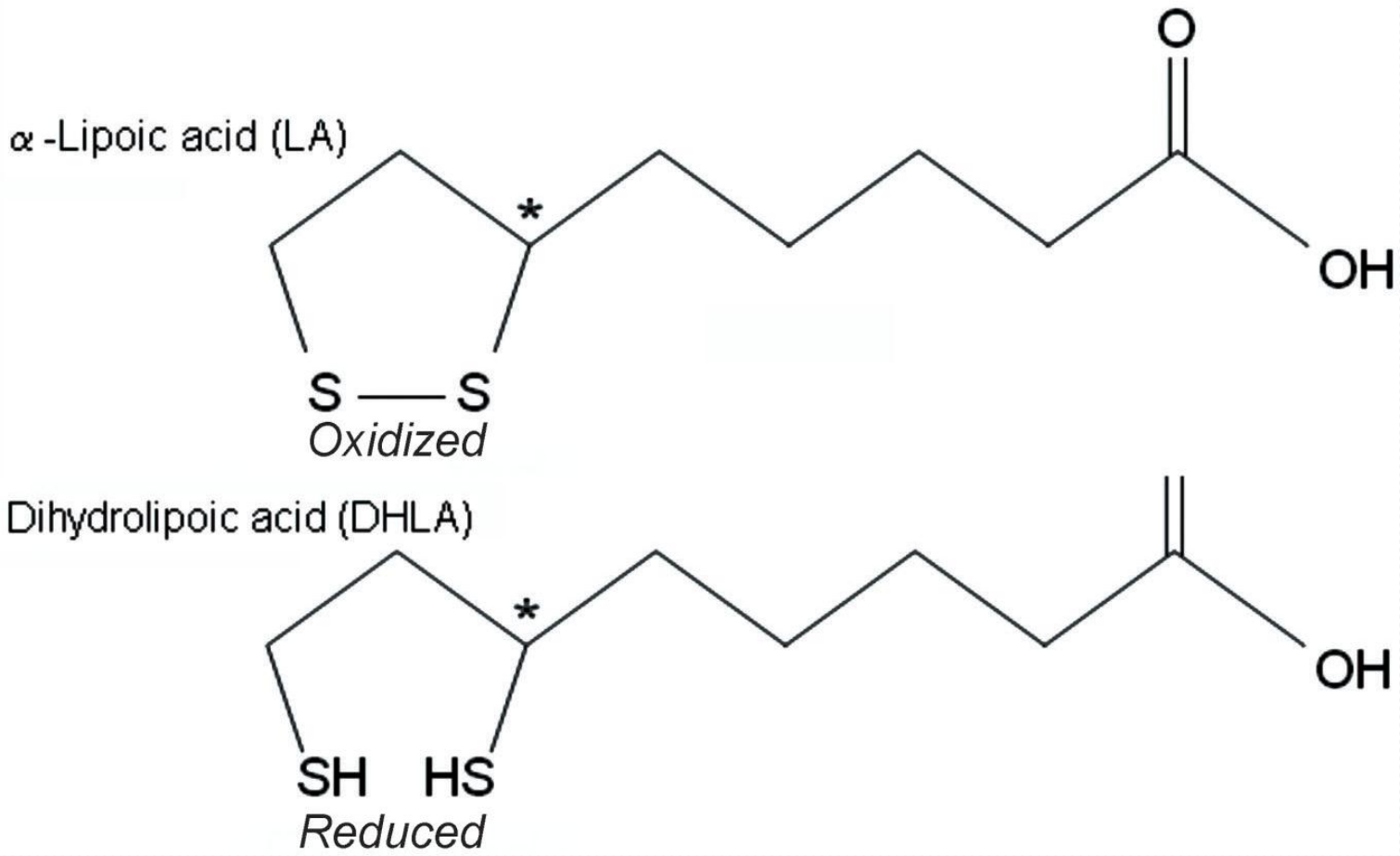
ALA is a cofactor in mitochondrial energy metabolism and a potent regulator of the cell's redox status with effects on P13K and AMPK signaling and related transcriptional pathways

- These mechanisms increase its interest in cancer and aging relative diseases

Alpha-Lipoic acid (LA; 5-(1,2-dithiolan-3-yl) pentanoic acid)

- was originally isolated from bovine liver by Reed in 1951.
- LA was once considered a vitamin. Subsequently, it was found that LA is not a vitamin and is synthesized by plants and animals.
- LA functions as a cofactor for mitochondrial enzymes by catalyzing the oxidative decarboxylation of pyruvate, alpha-ketoglutarate and branched-chain alpha-keto acids.
- Dihydrolipoic acid (DHLA) is the reduced form of LA.

Alpha-Lipoic acid (LA; 5-(1,2-dithiolan-3-yl) pentanoic acid and its reduced form – dihydrolipoic acid (DHLA)



Alpha lipoic acid (ALA)

- Mechanism of action
  - Anticancer activity via its activation of Pyruvate dehydrogenase (PDH)
  - Other know activities: antioxidant and cofactor of several enzymes (Biewenga 1997)
- Actual approved treatment for
  - Diabetic neuropathy and neurodegeneration (Thioctacid, MEDA Pharma in Germany),
  - Dietary supplement in many countries (EU, USA…)
- Anticancer activity:
  - In vitro in the µM range (apoptosis induction, about 100 publications from end of 1990s and 2000s and Biorébus data)
  - Low tumor inhibition in mice (20mg/kg/day) (Biorébus, no other publication)
  - In human, few articles:
    - Reduction of oxidative stress caused by anticancer agents (Mantovani G. *Onc. Rep.* 2002, *Nutrition* 2008)
    - ALA and Low-dose Naltrexone prolonged the survival of three cancer patients (pancreas); one case: > 78months (Berkson B. 2006, 2009)
- Very Good Tolerance (Cremer D. 2006) :
  - No Secondary Effects at 1200mg/day during 2 years
  - No Mutagenic or Genotoxic Activity
- Bioavailability: after 600mg oral dose of Na-RALA, Cmax = 70µM (Carlson D. 2007)

Therapeutic action of LA and DHLA

- Their therapeutic action is based on their antioxidant properties: they meet all the criteria for an ideal antioxidant because they can easily quench radicals, can chelate metals, have an amphiphilic character.

- They interact with other antioxidants and can regenerate them. For this reason, LA is called an antioxidant of antioxidants.

- Current studies support the use of LA in the ancillary treatment of many diseases, such as diabetes, cardiovascular, neurodegenerative, autoimmune diseases, cancer and AIDS. LA and DHLA do not exhibit any serious side effects.

Hydroxycitrate (HCA)

- Mechanism of action: ATP Citrate Lyase Inhibitor (only identified target) (Lowenstein 1969)
- Extracted from *Garcinia cambodgia* fruit rinds, an indian traditional condiment
- Indication: nutritional supplement for weight loss (for instance Slimax Solgar)
- Anticancer Activity:
  - In vitro about 100µM (2 publications and Biorébus data) (Board 1996, Beckner 2010)
  - Moderate tumor inhibition in mice (500mg/kg/day) (Biorébus, no other publication)
  - In human, no known anticancer application of HCA (few articles for whole garcinia extracts)
- Very Good Tolerance (Soni MG 2004):
  - No Secondary Effects at 5g/day during 8 weeks
  - No Mutagenic or Genotoxic Activity
- Bioavailability: after 2g oral dose of Ca/K HCA, Cmax = 16µM (Loe Y. 2001)

Potential role of hydroxycitrate ?

- This could also be a way to inhibit tumor cells growth as other evoked mechanisms :
  - increase of serotonin in the brain
  - inhibition of pancreatic alpha amylase and intestinal alpha glucosidase
  - leading to a reduction in carbohydrate metabolism.

Lipoic acid and hydroxycitrate

both molecules target the abnormal metabolic pathways such as seen in cancer

There combination appears in mice to be more effective than their use alone

We report here the early clinical tolerance evaluation of a daily metabolic combination in humans associated with chemotherapy

Italian experience:

median duration of 14,5 months (4 to 20 m)

4 p. : 3 F, 1 M , median age 57 y(37 - 81 y)

1 breast cancer

1 nasopharyngeal carcinoma

1 pancreatic adenocarcinoma

1 glioblastoma.

Doses : ALA 1,2 g/d HCA 1,2 g/d orally

All but one p. had concomitant chemotherapy

French experience : Jan 08 to Nov 11,

- 13 p. with local relapse and/or metastatic cancer with a combination of ALA -HCA
- 7 M, 6 F, median age 45 y (28 -74)
- 2 colon, 1 lung , 1 hepatocarcinoma, 5 sarcomas, 1 neuro-endocrine
- HCA administered orally , 3 g / d (1 g x3/d) from Oct 11 increased to 6 g /d for 3 last 6 p.
- ALA 1,8 g /d (600 m g x3/d)
- Median duration : 3 months (15 d - 5 m, 1 p. 20 m)

Results: This association was well tolerated with few clinical disturbances :

- vomiting , nausea
- 5 patients had a gastric protective treatment
- 2 because of corticotherapy
- The increased dose of ALA was well tolerated
- No hepatic toxicity found
- no weight loss ,no hypoglycemia

Inconveniences

- bad and discontinued observance for patients in relation with the cost of these medicines
- the difficulty to buy them (only by online pharmacy for ALA in France)
- The tolerance of HCA was mild because of gastric pain but patients continue the treatment

Availability of the drugs

- Alpha-lipoic acid : 13 companies having marketing authorization for iv formulation against diabetic neuropathy in Germany

  (Wörwag Pharma, Stadapharm, AAA-Pharma, MIP Pharma, CNP Pharma, Berlin-Chemie, Biomo Pharma, Esparma, Ratiopharm, Mit Gesundheit, Hexal AG, MEDA Pharma, Trommsdorff).

- Hydroxycitrate : Garcinia cambodgie extract freely available
  - In France, as a food supplement: Slimax® from Solgar (Pharmacy and health food stores)
  - In USA, as a food supplement and diet beverage ingredient: Super Citrimax® from Interhealth

Conclusion

Combination of safe drugs may be able to:

- Reduce tumor development rate
- Enhance survival
- Improve chemotherapy efficacy

➔ ALA / HCA : a **new adjuvant** to chemotherapy ?

Conclusion

- ALA - HCA combination well tolerated promising treatment in cancer patients
- The switch to IV ALA would permit to obtain higher blood peaks and better observance
- Nevertheless we don’t know which dosage would be the best for patients
- Randomized clinical trials are necessary to evaluate the efficacy on tumor progression

in correlation with pharmacologic studies