Will metastatic cancer be a curable disease?
Cancer Treatment is not a success (so far..)

Overall cancer mortality, Age standardized ratio by age class in 19 western countries

Summa et al. Submitted
Age standardized ratio by cancer site in 19 countries
What is Cancer?

**Cancer phenotype: Invariants**

1. Increased pressure
2. Fractal shape
3. Increased glucose uptake
4. Intracellular alkalosis
What is Cancer?

Cancer phenotype: 1 - Increased pressure

Measured Interstitial Pressures

<table>
<thead>
<tr>
<th>Site Measured</th>
<th>Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Parenchyma</td>
<td>4</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>13</td>
</tr>
<tr>
<td>Primary Hepatic Tumor</td>
<td>25-26</td>
</tr>
<tr>
<td>Metastatic Hepatic Tumor</td>
<td>15-22</td>
</tr>
</tbody>
</table>

Interstitial Pressures compared to vascular pressures

<table>
<thead>
<tr>
<th>Site</th>
<th>Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>15-26</td>
</tr>
<tr>
<td>Portal Vein</td>
<td>4</td>
</tr>
<tr>
<td>Mean Arterial</td>
<td>137</td>
</tr>
</tbody>
</table>
What is Cancer?

Cancer phenotype: 2 – Fractal shape

Fleury et al., « Fractal » 2003

Fractal image of a breast carcinoma
What is Cancer?

Cancer phenotype: 3 – Increased glucose uptake
Cancer phenotype: 4 – Intracellular alkalosis

Hematoxylin and eosin stains are a pH based phenomenon
Cancer: between glycolysis and physical constraints.

L. Schwartz Springer 2004
Cell Mitosis
The cell is an electrical machine
Bioenergetics of Cell Metabolism

Adenosine triphosphate (ATP) : the energetic currency exchange
Bioenergetics of Cell Metabolism

ATP hydrolysis is the molecular motor of Life.
Glycolysis vs. Oxidative phosphorylation

The **central carbon metabolism** (CCM) oscillates between **biomass** and **energy** synthesis and times cell division.
The **central carbon metabolism** (CCM) oscillates between **biomass** and **energy** synthesis and times cell division.
Differentiated cells have an oxidative metabolism based on mitochondrial respiration and energy synthesis (ATP).
Differentiated Cells

- ATP = 9 pmol
- Acidic pH = 6.8
- NAD+/NADH ratio = 8-10

Differentiated cells have an oxidative metabolism based on mitochondrial respiration and energy synthesis (ATP)
Rapidly proliferating cells have a glycolytic metabolism based on glucose fermentation and biomass synthesis.
Intertwined redox oscillators

In the case of normal cells

[ATP]

G0  G1  S  G2  M  G0

[H+]

mol/L

G0  G1  S  G2  M  G0

NADH/NAD+

ratio NADH/NAD+

G0  G1  S  G2  M  G0

G0  G1  S  G2  M  G0
3. pH Oscillation Regulates Cell Cycle

pHi oscillation during cell cycle

1. Lois et al. (1983)
2. Allfrey et al. (1962)
4. Grainger et al. (1979)
5. McBrian et al. (2013)
CANCER: A DECREASE IN ENERGY YIELD
Mitochondria are not functional in cancer

Arismendi-Morillo *Int J Biochem Cell Biol* 41, 2062-68, 2009
Warburg Effect: Reduced Energy Efficiency

Heiden et al. (2010)
Cancer Cells are ATP-deprived

[ATP] in normal and cancer cells
(n=8)
Intertwined redox oscillators

In the case of cancer cells

[ATP]

[H+]

NADH/NAD+

NADPH/NADP+
Cancer Drugs Change Cancer Metabolism
PET SCAN

Chemotherapy normalizes the radioactive glucose uptake

T = tumor in liver (pre- and post-therapy)
K = kidney (does not contain tumor but concentrates FDG)

Source: Science 324, 1020 (2009).
Can we do better than chemotherapy?
Our approach

Data-base

- Literature screening
  - Metabolic activity
  - Well-known molecules
  - Data on human toxicity

28 molecules

- Individual screening
  - 5 cancer cell types
  - Proliferation and MTT test
  - 1, 3, 5 days cultures

7 molecules

- Combination screening
  (same experimental conditions)

7 combinations

Results

- High clinical efficiency
- Universal and simple mechanisms
- Low regulatory risk and low cost
## Literature screening

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetazolamide</td>
<td>Anhydrase carbonic inhibition</td>
</tr>
<tr>
<td>Albendazole</td>
<td>PEP carboxykinase inhibition</td>
</tr>
<tr>
<td>Amobarbital</td>
<td>NADH dehydrogenase</td>
</tr>
<tr>
<td>Amrinone</td>
<td>PDE-3 inhibition</td>
</tr>
<tr>
<td>Betaine</td>
<td>lipotropic factor</td>
</tr>
<tr>
<td>Capsaicin</td>
<td>NADH dehydrogenase</td>
</tr>
<tr>
<td>Dfructose 1,6 biphosphate</td>
<td>PKM2 activation</td>
</tr>
<tr>
<td>Dichloroacetate</td>
<td>PDHK1 inhibition</td>
</tr>
<tr>
<td>Dimercaprol</td>
<td>PDH activation</td>
</tr>
<tr>
<td>Farnesol</td>
<td>phospholipase D inhibition</td>
</tr>
<tr>
<td>Genistein</td>
<td>Phospholipase Cgamma inhibition</td>
</tr>
<tr>
<td>Gossypol</td>
<td>LDH inhibition</td>
</tr>
<tr>
<td>Hydrazine sulfate</td>
<td>PEP carboxykinase inhibition</td>
</tr>
<tr>
<td>Hydroxycitrate</td>
<td>ATP citrate lyase</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Cyt. P450 demethylase inhibition</td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>PDHK1 inhibition</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>PEPCK inhibition</td>
</tr>
<tr>
<td>Lonidamine</td>
<td>Hexokinase inhibition</td>
</tr>
<tr>
<td>Metformine</td>
<td>AMPK activation</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>Choline kinase inhibition</td>
</tr>
<tr>
<td>Niacine</td>
<td>Lipolysis inhibition</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>Phospholipase A2 inhibition</td>
</tr>
<tr>
<td>Quinine</td>
<td>Phospholipase A2 inhibition</td>
</tr>
<tr>
<td>Silibinin</td>
<td>IGFBP activation</td>
</tr>
<tr>
<td>Simvastatine</td>
<td>Lipolysis inhibition</td>
</tr>
<tr>
<td>Suramine</td>
<td>Citrate synthase inhibition</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>Potassium channel blocker</td>
</tr>
<tr>
<td>Xylitol</td>
<td>PP2A</td>
</tr>
</tbody>
</table>


Aim:
Identify a combination of two drugs active against altered cancer cell metabolism
Experiments

• Three syngeneic cancer models
  – MBT-2 bladder carcinoma
  – B16-F10 melanoma
  – LL/2 lung carcinoma

• Analysis of tumor volume and animal survival
Combination of molecules in bladder cancer

Tumor volume evolution in bladder carcinoma model (MBT-2).
The ALA/HCA combination reduces tumor development rate and doubles survival time as compared to untreated mice.
ALA/HCA in melanoma (B16-F10)

The ALA/HCA combination reduces tumor development rate and doubles survival time as compared to untreated mice.
LLC tumor model
# Well-known molecules

<table>
<thead>
<tr>
<th></th>
<th>ALA</th>
<th>HCA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td>Lipoic acid</td>
<td>Calcium hydroxycitrate</td>
</tr>
<tr>
<td><strong>Indications</strong></td>
<td>• Anti-oxidant</td>
<td>Weight loss agent</td>
</tr>
<tr>
<td></td>
<td>• Dietary supplement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Diabetic neuropathy</td>
<td></td>
</tr>
<tr>
<td><strong>Toxicity</strong></td>
<td>• No toxicity at 1200mg/day during 2 years</td>
<td>• No toxicity at 5g/day during 8 weeks</td>
</tr>
<tr>
<td></td>
<td>• No mutagenic or genotoxic activity</td>
<td>• No mutagenic or genotoxic activity</td>
</tr>
</tbody>
</table>
Enhanced efficacy of chemotherapy

Addition of ALA/HCA enhanced the efficiency of cisplatin treatment (LLC model).
## In vivo screenings

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-diazo-5-oxo-L-norleucine</td>
<td>Glutaminase inhibition</td>
</tr>
<tr>
<td>Agmatine</td>
<td>Polyamine synthesis inhibition</td>
</tr>
<tr>
<td>Alpha cetoglutarate</td>
<td>Citrate synthase inhibition</td>
</tr>
<tr>
<td>Amiloride</td>
<td>Na+/H+ antiport inhibition</td>
</tr>
<tr>
<td>Apigenine</td>
<td>IGFBP activation</td>
</tr>
<tr>
<td>Bicalutamide</td>
<td>IGFBP activation</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>Hypothalamic D2 receptor agonist</td>
</tr>
<tr>
<td>Butyrate sodium</td>
<td>HDAC inhibition</td>
</tr>
<tr>
<td>Chitosan</td>
<td>PK-M2 inhibition</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>lipotropic factor</td>
</tr>
<tr>
<td>Citrate</td>
<td>Citrate synthase inhibition</td>
</tr>
<tr>
<td>Cryogenine</td>
<td>PEP carboxykinase inhibition</td>
</tr>
<tr>
<td>Curcumin</td>
<td>AID inhibition</td>
</tr>
<tr>
<td>D-alanine</td>
<td>Alanine transaminase inhibition</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>PK-M1/M2 splicing regulation</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Serotoninine reabsorption inhibition</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>NSAIDS</td>
</tr>
<tr>
<td>Indole 3 carbinol</td>
<td>Triglycerides reduction</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>Cyt. P450 demethylase inhibition</td>
</tr>
<tr>
<td>Lactoferrine</td>
<td>Oxidative stress reduction</td>
</tr>
<tr>
<td>Letrozole</td>
<td>Aromatase inhibition</td>
</tr>
<tr>
<td>L-norvaline</td>
<td>Arginase inhibition</td>
</tr>
<tr>
<td>Melatoinine</td>
<td>anti-oxydant, anti-proliferative</td>
</tr>
<tr>
<td>Menadione</td>
<td>Tyr kinase receptor inhibition</td>
</tr>
<tr>
<td>Omépazolé</td>
<td>IGFBP activation</td>
</tr>
<tr>
<td>Oxythiamine</td>
<td>Transketolase inhibition</td>
</tr>
<tr>
<td>PEG8000</td>
<td>PK activation</td>
</tr>
<tr>
<td>Pegvisomant</td>
<td>GH receptor inhibition</td>
</tr>
<tr>
<td>Pralidoxime</td>
<td>Alanine transaminase inhibition</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>Cellular differentiation activation</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>GH secretion inhibition</td>
</tr>
<tr>
<td>Suramine</td>
<td>Citrate synthase inhibition</td>
</tr>
<tr>
<td>Valproate sodium</td>
<td>HDAC inhibition</td>
</tr>
<tr>
<td>Vitamine B12</td>
<td>lipotropic factor</td>
</tr>
</tbody>
</table>
Screening of 19 combinations

Tumor volume (LLC).

Treatment

Time since implantation (days)

Vehicles

ALA/HCA

Cisplatin

ALA/HCA/OCT

Vt (mm$^3$)
Combination of ALA/ HCA/ OCT/ CAP

First report of tumor regression with a metabolic treatment
Schwartz L. Invest New Drugs. 2013 Apr;31(2):256-64.
First patient

7/2001: Adenocarcinoma of the colon pT4 N0

10/ 2009 : Peritoneal metastasis

12/2009 : Chemotherapy at Institut Gustave Roussy (Villejuif)
5-FU, Cisplat, Avastin

12/2009  oral ALA and HCA

3/ 2010 : Avastin discontinued because of poor tolerance
7/ 2010 : abdominal radiotherapy followed by Gemcitabine

7/ 2011: discontinuation of chemotherapy
10/2014: death
CT of the perirectal mass at the date of discovery (Nov. 2009)
CT of the same region, on June 2012
First Italian patient

Glioblastoma multiforme started Metabloc in early 2008

Multiple relapses but alive and reasonably well in 5/2015
In the mean time

- Dr Burt Berkson (New Mexico)

- 10 cases of incurable advanced cancer:
  - Long term stabilization with IV Lipoic acid and low dose naltrexone
  - Number of patients treated not stated

*Integrative Cancer Therapies 2009 (4) 416–422*
Compassionate use in desperate patients
Targeting the Central Carbon Metabolism

- Anaerobic glycolysis
- Oxidative phosphorylation

**Glucose**
- Naltrexone
- Nucleic Acids
- Fatty Acids
- Glutamine

**ALA**
- HCA
Inclusion criteria

- Metastatic carcinoma
- Standard chemotherapy failed
- Offered only palliative care
- Karnofsky status between fifty and eighty
- Life expectancy estimated to be between 2 and 6 months
Chemoresistant advanced metastatic cancer:

- lipoic acid 600 mg IV (Thioctacid® (Meda Pharma))
- hydroxycitrate 500 mg t.i.d (Solgar)
- low-dose naltrexone 5 mg (Revia) at bed time
Clinic results for the first 11 patients
Advanced metastatic cancer:

- lipoic acid 1, 8gr (Tiobec®)
- hydroxycitrate 500 mg t.i.d (Solgar)
- low-dose naltrexone 5 mg (Revia) at bed time

- In combination with standard chemotherapy
Second Series of Clinical treatment

- Group I: Metabolic treatment only (n=17)
- Group II: Metabolic treatment and chemotherapy (n=27)
- 12 months treatment
Florentine

- 3/2006 Sarcoma of the uterus: surgery + RT + chemotherapy (TEC)
- 2008 Radiation induced enteritis
- 1/2013 multiple brain metastasis (N sup 15)
  - Palliative radiatiotherapy (30gy in 10 fractions)
  - Sent home to die
- 3/2013 start metabloc
- 5/2015 NED
Yun

56 year old lady

- 3/13 Squamous cell carcinoma of the lung with bone and brain metastasis (N sup 20)
- Treatment gifetinib, partial response
- 2/14 because of tumor progression, continue gifetinib and start metabolic treatment partial response
- 6/14 brain irradiation 30Gy/10 fractions. During radiation acute psychosis, weight loss.
- 8/14 The husband is told that she is going to die
- 9/14 starts Alimta for palliative care and metabolic treatment
- 5/15 She lives an almost normal life
Marina

- 2/12 Cholangiocarcinoma partial hepatectomy
- 5/12 Lung metastasis
- 5 FU and cisplatin ineffective
  - Stutent ineffective
  - Other treatment option sent home to die
- 2/13 start LDN, LA, HCA
  - Tumor stabilisation for 16 months
- 2/15 Death
PSA in hormone resistant prostate cancer

Start metabolic treatment

PSA concentration (ng/mL)

Dates

PSA concentration (ng/mL)

Dates
Metastatic colon cancer (3 different lines of chemotherapy)
Decrease level of tumor markers followed by regrowth
Targets for a combined anti-tumoral therapy
How to improve METABLOC?

A) Decrease glucose uptake

B) Decrease intracellular pH
PET scan

Colon carcinoma with liver metastasis

Regular administration of Metabloc Digoxin and 5 FU (2 months later)
As of September 2015, it is probable that:

1) Cancer is the simple consequence of mitochondrial inactivation.
2) Alzheimer’s disease has a lot of common features with cancer.
3) It is a matter of time before effective treatment will reach the market.
4) There will be economical/political changes.