“L’uso degli inibitori di pompa protonica nel trattamento del tumore”

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After more than 60 years from the introduction of chemotherapy in human beings, the gold standard tumor strategies offered to cancer patients are still based on chemotherapy, surgery, and radiotherapy, which physically try to destroy cancer with brutal force rather than selectively interacting with cancer cells’ unique biological characteristics. Actually, cancer represents an area with significant unmet medical needs, with more than 20 million people worldwide being diagnosed annually and, in spite of the current available therapy, more than a million patients die from this disease every year (INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, IARC, LYON 2010)
There is a continuous need for safe and effective new treatments resulting in durable disease remissions and increased overall survival. In the last decades, the war against cancer has been based on the principle of Paul Ehrlich 'magic bullets', introduced more than 100 years ago, and leading to the success of antibiotics 50 years later. The successful use of antibiotics against infectious agents gave a strong support to the use of the same approach against malignant tumors: to set up new drugs that selectively target and kill tumour cells.
After so many years we are still waiting for this magic bullet against malignant tumors and, of course, this is generating the idea that something went wrong along the way or from the very beginning.
God is dead, Marx is dead, Freud is dead... and I'm not feeling too well myself.
We can cure what we can understand first

a possible new approach:

to understand more on the

MECHANISMS ALLOWING TUMOR CELLS TO SURVIVE

IN THE HOSTILE MICROENVIRONMENT

created during tumor growth
THERE IS AWARENESS ON A GENERAL FAILURE IN DRUG DISCOVERY

David Shaywitz and Nassim Taleb

The molecular revolution was supposed to enable drug discovery to evolve from chance observation into rational design, yet dwindling pipelines threaten the survival of the pharmaceutical industry. What went wrong?

The answer, we suggest, is the mis-measure of uncertainty. As academic researchers underestimated the fragility of their scientific knowledge while pharmaceutical executives over-estimated their ability to domesticate scientific research.

For all the breathless headlines proclaiming breakthrough discoveries, the truth is that we still do not understand what causes most disease. Even when we can identify a responsible gene or implicate an important mutation, we have made only limited progress in turning these results into treatments.

Medical research is particularly hamstrung in disease areas, shying safe but ineffective compounds without fully exploring their scientific potential and trying to ensure that each project the company is working on is carried out with a clearly defined market segment in mind. Unfortunately, for new medicines in particular, this strategy often fails significantly to reduce exposure to negative uncertainty — all the bad things that can happen during drug development — and eliminates much of the exposure to positive uncertainty (serendipity) that remains so vital.

So instead of managers maintaining focus that important opportunities for novel discovery are lost, as is the intellectual space for tinkering and capitalising on the chance observations that is the essence of science.

Big pharma companies must learn to resist the false comfort of revenue predictions and valuation spreadsheets and embrace the opportunity it represents.

David Shaywitz, a physician-scientist, is a management consultant in New Jersey. Nassim Nicholas Taleb is author of The Black Swan: The Impact of the Highly Improbable (Penguin, 2007)
For all the breathless headlines proclaiming breakthrough discoveries, the truth is that we still do not understand what causes most disease. Even when we can identify a responsible gene or implicate an important mutation, we have made only limited progress in turning these results into treatments.

Medical research is particularly hampered by the scarcity of good animal models for most human disease, as well as by the tendency of academic science to focus on the “bits and pieces” of life – DNA, proteins, cultured cells – rather than on the integrative analysis of entire organisms, which can be more difficult to study.
WE STILL DON’T KNOW WHAT ARE THE CAUSES OF THE VAST MAJORITY OF THE UNCURABLE DISEASES

HOW CAN WE CURE?
A CHANGE OF STRATEGY IN THE WAR ON CANCER
Patients and politicians anxiously await and increasingly demand a ‘cure’ for cancer. But trying to control the disease may prove a better plan than striving to cure it, says Robert A. Gatenby. Nature, 459: 508-9, 28 May 2009

therapeutic strategies aimed at controlling cancer could prove more effective than trying to cure it
it is possible to approach new anti-cancer therapies by trying to know more on the mechanism/s through which cancers avoid growth control.

Highly proliferative cancer cells produce a large amount of protons (H+) generated by glycolysis, glucose utilization and lactic acid production. These protons are released outside the cells as a means to avoid intracellular acidification, thus contributing to acidify the tumor microenvironment.
Warburg's hypothesis

It claims that cancer is caused by the fact that TUMOR CELLS MAINLY GENERATE ENERGY BY NON-OXIDATIVE BREAKDOWN OF GLUCOSE

"HEALTHY" CELLS MAINLY GENERATE ENERGY FROM OXIDATIVE BREAKDOWN OF PYRUVATE.

Put in his own words, "the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar."
THE TAKEHOME MESSAGE WAS:
CANCER CELLS ARE NOT MERELY MUTATED OR MODIFIED
NORMAL CELLS SELECTED OR INDUCED TO CHANGES BY EXTERNAL STIMULI

THEY ARE CELLS WHOSE LIFESTYLE IS IN NO WAY COMPARABLE TO THAT OF NORMAL CELLS

MORE RECENT EVIDENCE SUGGEST IF BY ANY CHANCE TUMOR CELLS BEHAVE RATHER AS MICROORGANISMS USING COMPARABLE STRATEGIES TO REMAIN ALIVE IN HOSTILE CONDITION
SIMILARITIES BETWEEN TUMOR CELLS AND MICROORGANISMS

1. SIMILAR MECHANISMS TO AVOID CELL POISONING (DE-TOXIFICATION)

2. SIMILAR MECHANISMS TO FACE OFF LOW LEVEL OF NUTRIENTS SUPPLY (CELL CANNIBALISM)
Tumor microenvironment represents a key factor regulating tumor growth and the survival of the “best fitted” cells which, in turn, will develop malignancy, chemoresistance and metastatic behavior.
TUMOR ACIDITY
Tumor microenvironment is acidic as compared to normal tissues.
DETERMINANTS OF TUMOR ACIDITY

AEROBIC GLYCOLYSIS

LACTIC ACID PRODUCTION

ION EFFLUX PUMPS

PUMPING H+ OUTSIDE THE CELLS

- Tumors in nude mice derived from cells lacking lactate dehydrogenase are fully able to acidify their microenvironment (Yamagata, Br J Cancer 1998)

- Restriction of blood flow to murine tumors by vascular clamping stops lactate production/release but the microenvironment continues to acidify (Parkins, Br J Cancer 1997)

- Na+/H+-exchanger
- vacuolar H+-ATPases
- Na+/K+-ATPase pump
- H+/Cl− symporter
- monocarboxylate transporters (H+/lactate)
- ABC transporters
pH gradients in normal and tumor cells

**Figure 1A. pH gradient in normal cells (neutral/buffered condition)**
- EC: neutral
- IC: weakly acidic
- AV: acidic
- pH gradient between cytosol and acidic vesicles (AV)

**Figure 1B. pH gradient in tumor cells (acidic/unbuffered condition)**
- EC: acid
- IC: basic
- AV: acidic
- pH gradient between cytosol and acidic vesicles (AV)
REVERSED pH GRADIENTS
AN HALLMARK OF MALIGNANT CELLS

ACID OUTSIDE
ALKALINE INSIDE
Reversed pH gradient is involved in many tumor advantages

1. Drug-resistance and unresponsiveness to antineoplastic agents
2. Cell proliferation
3. Invasion and metastasis
Tumor resistance to drugs

- **MULTIDRUG RESISTANCE**: A tumor responds initially but later it develops drug-resistance (ABC transporters, e.g. P-gp). To date after an initial enthusiasm this phenomenon seems exclusively to occur in CML patients.

- **UNRESPONSIVENESS TO CYTOTOXIC DRUGS**

  - The majority of human tumors are not responsive.
HOW A DRUG CAN ENTER INTO A CELL

CONCENTRATION GRADIENTS

pH GRADIENTS

THE MAJORITY OF ANTICANCER DRUGS ARE WEAK BASE COMPOUNDS NEEDING OPTIMAL pH GRADIENTS TO PASS FROM OUTSIDE TO INSIDE A CELL
### MECHANISMS OF DRUG RESISTANCE MEDIATED BY ACIDITY

1. **Extracellular protonation** of the weak base drugs due to the high $H^+$ concentration, leading to drug neutralization outside the cells.

2. **Sequestration of the drugs within acidic vesicles** with two possible fates:
   - 2.A. Protonation and neutralization of the drug within the acidic vesicles
   - 2.B. Elimination of the drug through exocytosis

Of course these mechanisms may **co-exist** within the resistant cells.
TO ATTACK TUMOR ACIDITY

A NEW STRATEGY TO CHEMOSENSITIZE MALIGNANT TUMORS
ION EFFLUX PUMPS

PUMPING H+ OUTSIDE THE CELLS

- Na+/H+-exchanger
- vacuolar H+-ATPases
- Na+/K+-ATPase pump
- H+/Cl- symporter
- monocarboxylate transporters (H+/lactate)
- ABC transporters

THEY ALL COOPERATE IN MAINTAINING DERANGED pH GRADIENTS IN HUMAN TUMORS
WHY V-ATPASES?
WHY V-ATPASES?

They represent a major mechanism for the regulation of cellular pH and operate not only to acidify a wide array of intracellular compartments, but also to pump protons across the plasma membrane.
WHY V-ATPASES?

Tumor cells exhibit enhanced V-ATPase membrane expression and activity.
H+-ATPases inhibitors

THEY ARE TOXIC FOR NORMAL CELLS AS WELL

THIS IDEA HAS BECOME OBSOLETE CAUSE
THE HIGH LEVEL OF SYSTEMIC TOXICITY

We knew that some V-ATPases inhibitors, such as bafilomycin A1, are toxic for normal cells as well, however being active on tumor cells.
THEIR NON SPECIFIC INHIBITION MAY LEAD TO TOXICITY AGAINST A VARIETY OF NORMAL CELLS BUT ALSO TO LOSS OF FUNCTION DISEASES

SOME EXAMPLES

Plasma membrane V-ATPases are especially important in human disease, with genetic defects in V-ATPases expressed in osteoclasts and intercalated cells leading to the diseases osteopetrosis and renal tubule acidosis, respectively.
Anti-acid treatment and PPI

PROTON PUMP INHIBITORS

Benzimidazoles

<table>
<thead>
<tr>
<th>Timoprazole</th>
<th>Omeprazole</th>
<th>Pantoprazole</th>
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Imidazopyridine

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<tr>
<th>Lansoprazole</th>
<th>Rabeprazole</th>
<th>Tenatoprazole</th>
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Although they have been included generic drugs, PPI have different bioavailability.

<table>
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<tr>
<th>PPI</th>
<th>pKa1</th>
<th>pKa2</th>
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<td>Omeprazole</td>
<td>4.06</td>
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<td>Lansoprazole</td>
<td>3.83</td>
<td>0.62</td>
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<tr>
<td>Pantoprazole</td>
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<tr>
<td>Rabeprazole</td>
<td>4.53</td>
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</tr>
<tr>
<td>Tenatoprazole</td>
<td>4.04</td>
<td>-0.12</td>
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PPI, proton pump inhibitor.
PPI bind irreversibly to proton pump and inhibit acid secretion.
PPI ARE INTELLIGENT PRO-DRUGS

- PPI are protonable weak bases with PKA value of ~4

- They accumulate selectively in acidic spaces with a pH of <4. In a such acidic environment they are protonated with the formation of a tetracyclic sulfenamide, which represents the active drug

They are targeted to acidic compartments

Gastric parietal cells

Tumors

?
PPI need protonation in acidic environment to exert their primary function.
CO-TREATMENT MAY REDUCE ACTIVATION OF PPI

1. PPI may compete for sequestration in acidic microenvironment with weak base drugs. The majority of anti-cancer drugs are weak base drugs that may be recruited in the acidic compartments equally as PPI.
2. Tumor drugs may enhance the activity of V-ATPase (e.g. cisplatin)
3. Tumor patients are currently treated with simultaneous administration of low doses PPI (as gastroprotective drugs) and chemotherapeutics

PRE-TREATMENT
IN VITRO MODEL

1. HUMAN TUMOR CELL LINES SHOWING INTRINSIC RESISTANCE TO ANTICANCER DRUGS

2. DIFFERENT CLASSES OF ANTICANCER DRUGS:
   • CISPLATIN (AVENTIS) STOCK CONCENTRATION OF 1 MG/ML
   • 5-FLUOROURACIL (TEVA PHARMA) 50 MG/ML
   • VINBLASTINE SULFATE (ELI LILLY) 0.1 MG/ML

3. 24 HRS PRE-TREATMENT VS CO-TREATMENT USING SODIUM SALT PREPARATIONS OF PPI:
   • OMEPRAZOLE AND ESOMEPRAZOLE (ASTRA-ZENECA)
     sodium salts were resuspended in normal saline at the concentration of 1 mg/ml immediately before use
   • PANTOPRAZOLE (BYK GULDEN)

4. CYTOTOXICITY ASSAYS
   • Trypan blue exclusion
   • Live/Dead Viability/Cytotoxicity Assay ®
Effects of omeprazole treatment on cisplatin and 5-fluorouracile sensitivity of human melanoma

pre-treatment

co-treatment

cisplatin (mM) 5-FU (mg/ml)
**A**

![Graph](image)

- **CTR**
- **Saline**
- **OM**

**% dead cells**

![Graph](image)

**B**

![Graph](image)

**PPI REVERT MDR**
PPI INCREASE SENSIBILITY OF SENSIBLE CELLS

HUMAN LYMPHOBLASTOID CELLS

HUMAN ALL CELLS
PPI alter pH and traffic of acidic vesicles

untreated

+PPI

Counts

Lysosensor Green
PPI treatment affect pH of human tumor cells in vitro through reduction of ATP consuming.
Effects of omeprazole on drug efflux

- PPI  + PPI

**VIMBL**
- A: uptake
- B: retention
- C: uptake
- D: retention

**DOXO**
- E: uptake
- F: retention
- G: uptake
- H: retention
### PPI pretreatment reverses chemoresistance of human tumor cells

<table>
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<tr>
<th></th>
<th>Cisplatin (µM)</th>
<th>5-Fluorouracil (µg/ml)</th>
<th>Vinblastine (ng/ml)</th>
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<tr>
<td></td>
<td>None</td>
<td>OM</td>
<td>ES</td>
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<tr>
<td><strong>Melanoma (n=22)</strong></td>
<td>443±86</td>
<td>49±36</td>
<td>46±36</td>
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<td><strong>Colon (n=2)</strong></td>
<td>400±16</td>
<td>14±6</td>
<td>17±3</td>
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<td><strong>Breast (n=2)</strong></td>
<td>493±10</td>
<td>51±55</td>
<td>39±39</td>
</tr>
<tr>
<td><strong>OVCA (n=2)</strong></td>
<td>450±70</td>
<td>77±23</td>
<td>71±21</td>
</tr>
</tbody>
</table>
IN VIVO PRE-TREATMENT IN HUMANIZED SCID MICE

1. Human tumor cells s.c. In the right flank (3 mil.)

2. PPI oral by gavage


(*) Tumor weight (mg) = length (mm) x width² (mm)/2.
oral PPI pre-treatment sensitizes melanoma to cisplatin cytotoxicity in vivo
PPI increases sensitivity of osteosarcoma to cisplatin cytotoxicity in vivo
PRE-CLINICAL STUDIES HAVE SHOWN THAT PPI MAY BE USED AS CHEMOSENSITIZERS IN MANY TUMOR HISTOLOGIES. WE NEEDED THE CLINICAL BREAKTHROUGH.
A TRANSLATIONAL PROJECT

PHASE II CLINICAL TRIALS ON THE USE OF PPI AS CHEMOSENSITIZERS

1. Phase I trial with a fixed dose of Cisplatin/Vindesine/Dacarbazine (CVD) in combination with a dose-escalation of esomeprazole as first-line therapy of metastatic MELANOMA PATIENTS
   INT MILAN (2007)

2. Phase II clinical study on efficacy of proton pump inhibitors pre-treatment in OSTEOSARCOMA PATIENTS undergoing chemotherapy
   ITALIAN SARCOMA GROUP (2006)

3. Randomized phase II clinical trial of omeprazole/esomeprazole followed by the combination of taxotere and cisplatin versus chemotherapy in patients with METASTATIC AND RELAPSING BREAST CANCER
   SHANGHAI CANCER HOSPITAL FUDAN UNIVERSITY
   SHANGHAI CHINA (2009)
Some results on the use of PPI as chemosensitizers in patients with melanoma and osteosarcoma
MELANOMA
CLINICAL PROTOCOL

3 ARMS:

1. CISPLATIN ONLY (10 PATIENTS)
2. ESOMEPRAZOLE 80 MG THE TWO DAYS BEFORE CISPLATIN TREATMENT (10 PATIENTS)
3. ESOMEPRAZOLE 120 MG THE TWO DAYS BEFORE CISPLATIN TREATMENT (10 PATIENTS)
UNFORTUNATELY TOO MANY DROP OUTS AND ONLY 20 PATIENTS WERE INCLUDED INTO THE FINAL EVALUATION WITHOUT ALLOWING A RELIABLE STATISTICAL ANALYSIS
HOWEVER

PROMISING RESULTS
Production-limit Survival Function Estimates

Overall: time to progression (months)
Phase II study
To explore the percentage of patients with osteosarcoma having a good histologic response to a primary chemotherapy treatment based on the use of proton pump inhibitors as chemosensitizer and methotrexate, cisplatin and doxorubicin.

Patients will be enrolled over a three-year period. About 60% of the patients are expected to be enrolled at the Istituto Ortopedico Rizzoli.

Percentage of good responders to primary chemotherapy treatment based on the use of methotrexate, cisplatin and doxorubicin: 50% (historical control from Chemotherapy Department of Istituto Ortopedico Rizzoli and from ISG (Italian Sarcoma Group) Osteosarcoma Committee data center).

Sample size: 85 patients. Study power 80%, alfa 0.05.
PPI - OSTEOSARCOMA STUDY PROTOCOL

CRITERIA FOR ELIGIBILITY
Histologically proven diagnosis of high-grade osteosarcoma of bone
Age: 4 - 40
Normal bone marrow, hepatic, cardiac and renal function
Absence of contraindications to the use of methotrexate, cisplatin, doxorubicin
Written informed consent

CRITERIA FOR EXCLUSION
Previous chemotherapy treatment and or medical contraindication to the use of one or more drugs, included in the present protocol
Previous chemotherapy treatment for the current tumor
White blood count $\leq 3.0 \times 10^9/L$, and platelets $\leq 100 \times 10^9/L$
Creatinine clearance $\leq 70$ ml/min
Left ventricular ejection fraction $< 55\%$ or fractional shortening rate of the left ventricle $< 28\%$
Serum transaminases and bilirubin $> 2$ times the normal values
ECOG performance status $> 2$
**PPI - OSTEOSARCOMA STUDY PROTOCOL**

**Esomeprazole**: 60 -120 mg once a day, the day before administration of each chemotherapy cycle

**Methotrexate**
- **Dose**: 12 mg/m² (Top dose 24g)
- **Infusion**: intravenously over 4 hours (T0-T4), dissolved in basal solution
- **Basal solution**: 1 L of basal solution contain 0.9% NaCl with KCl 20 mEq and Bicarbonate 60 mEq
- **Prehydration**: Basal solution 500 mL/m² over 2 hours
- **Hydration**: T0-T24 Basal solution 2.5 L/m²
- **Alkalization**: T0-T24 bicarbonate 4 mEq/kg
- **In the following 24 hours (T25-T-48) hydration with IV basal solution 2 L/m²**
- **Adequate hydration and alkalization according to the Groups standards are allowed**
- **Lederfolin**: Starting from T24 8 mg/m² every 6 hours for 11 administration (up to T84)
- **Leukovorin**: 15 mg/m² x 12 times are allowed.
- **Serum MTX measurement**: suggested at T4, mandatory at T24 -T48 and up to serum levels <0.2 µM.

**Cisplatin** (CDP)
- **Dose**: 120 mg/m²
- **Infusion**: intravenously over 48-72 hours, dissolved in basal solution
- **Basal solution**: 0.9% NaCl with KCl 15 mEq/L and Mg 3mEq/L
- **Prehydration**: Basal solution 500 mL/m² over 2 hours
- **Hydration**: Basal solution 2 L/m²/24hours
- **Posthydration**: Basal solution 500 mL/m² over 2 hours
- **CDP is given before the administration of ADM**

**Doxorubicin** (ADM)
- **Dose**: 75 mg/m²
- **Infusion**: intravenously over 24 hours, dissolved in 2,000 mL 0.9% NaCl
- **ADM starts after the 48-72 hour infusion of CDP**
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<th>NECROSIS</th>
<th>ISG OS 1</th>
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<tr>
<td></td>
<td>GR  PR</td>
<td>38 (50%)</td>
<td>33 (57%)</td>
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<tr>
<td>Osteoblastic</td>
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<td></td>
<td></td>
<td>38 (50%)</td>
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<tr>
<td>Condroblastic</td>
<td>GR  PR</td>
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<td></td>
<td>9 (75%)</td>
<td>1 (14.3%)</td>
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<tr>
<td>Haemorrhagic/Fibroblastic</td>
<td>GR  PR</td>
<td>14 (67%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 (33%)</td>
<td>4 (36%)</td>
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REGISTRATION
Docetaxel and Cisplatin Chemotherapy With or Without High Dose Proton Pump Inhibitor in Metastatic Breast Cancer

This study is currently recruiting participants.
Verified by Fudan University, September 2010
First Received: February 10, 2010  Last Updated: September 13, 2010

Sponsor: Fudan University
Collaborator: Istituto Superiore di Sanita

Information provided by: University

ClinicalTrials.gov Identifier: NCT01069081

Purpose
The objectives of this study are to evaluate the efficacy and tolerability of high dose proton pump inhibitor combined with chemotherapy in metastatic breast cancer.
Proton pump inhibitor lansoprazole as a rescue agent in chemoresistant tumors: a preclinical study in companion animals with spontaneously occurring tumors

## Modified Karnofsky's performance criteria - Grade Criteria

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<td>Fully active, performs at predisease level</td>
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<tr>
<td>1</td>
<td>Activity less than predisease level; able to function as acceptable pet</td>
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<tr>
<td>2</td>
<td>Severely compromised activity; ambulatory only to point of eating, sleeping, and consistently eliminating in acceptable areas</td>
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<tr>
<td>3</td>
<td>Completely disabled; must be force fed; unable to defecate or urinate in acceptable areas</td>
</tr>
<tr>
<td>4</td>
<td>Dead</td>
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<tr>
<td>PATIENT</td>
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## Characteristics and outcome of feline patients treated with pump inhibitors and chemotherapy

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</table>
PPI CAN BE CYTOTOXIC FOR TUMORS
PPI are pro-drugs which are converted into the active drug after protonation in acidic conditions.

2

PPI use acidity as a specific delivery.

3

They need acidity for full activation.
1. Culturing cells in unbuffered medium might simulate in vivo conditions and create the acidic environment optimal for PPI protonation. This condition allows cells to spontaneously acidify their microenvironment.

2. We knew also that malignant cells may remain alive in culture even at pH of 5 (Lugini et al Cancer Res 2006). This condition allows to test PPI activation in a pH-dependent manner.
B CELL TUMORS
PPI inhibit proliferation of human B cell tumors

![Graph showing the inhibition of proliferation of human B cell tumors by PPI. The x-axis represents time in days (0, 17.5, 35, 70, 140), and the y-axis represents proliferation (% of control). The graph compares proliferation under buffered medium and unbuffered medium conditions. The data indicates a significant reduction in proliferation with increasing concentrations of PPI.](image-url)
PPI (mM) decrease cell viability in a dose-dependent manner
PPI induce cell death

- OM
- ESOM

Cell death (%)

- Control
- Omeprazole

PPI (µM)

Cell death (%)

Nalm-6
Raji
Daudi
PPI induce activation of caspases but it is not instrumental to cell death.
ROS
A ROLE OF ROS

Ros accumulation and Apoptosis

ROS-dependent Lysosomal membrane permeabilization

ROS-dependent Mitochondrial membrane depolarization
PPI induce cell death in human pre-B ALL BM BLASTS
CONCLUSIONS

- PPI inhibit proliferation of human B cell lines
- PPI are cytotoxic to human B cell lines and pre-B ALL blasts
- PPI effect is potentiated by acidic conditions (tumor pH is acidic in vivo)
- PPI cause early destabilization of the lysosomal compartment and depolarization of MMP and cyt C accumulation
- PPI induce activation of caspases 3, 8, 9 which are not instrumental for the execution of the cell death process
- It passes through a clear lysosomal membrane permeabilization (LMP)
- It occurs through an early ROS accumulation
MELANOMA
The expression of V-ATPase subunit a was significantly higher in metastatic cells (111±13 MFI) as compared to primary cells (72±10 MFI, P < 0.05)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC$_{50}$ (µM) pH 7.4</th>
<th>IC$_{50}$ (µM) Unbuffered</th>
<th>IC$_{50}$ (µM) pH 6.0</th>
<th>V-ATPase Sub A</th>
<th>V-ATPase Sub a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me30966 (M)</td>
<td>112±13</td>
<td>55±4</td>
<td>21±2</td>
<td>33±2</td>
<td>84±4</td>
</tr>
<tr>
<td>Me20842 (P)</td>
<td>130±10</td>
<td>95±9</td>
<td>62±6</td>
<td>27±2</td>
<td>41±3</td>
</tr>
<tr>
<td>Me9923 (P)</td>
<td>85±9</td>
<td>71±8</td>
<td>63±6</td>
<td>31±2</td>
<td>60±3</td>
</tr>
<tr>
<td>Me5810 (P)</td>
<td>142±12</td>
<td>95±8</td>
<td>39±6</td>
<td>38±3</td>
<td>91±5</td>
</tr>
<tr>
<td>Me15392 (M)</td>
<td>117±12</td>
<td>83±7</td>
<td>30±4</td>
<td>74±5</td>
<td>145±9</td>
</tr>
<tr>
<td>WM902 (P)</td>
<td>124±15</td>
<td>103±8</td>
<td>95±11</td>
<td>35±2</td>
<td>79±4</td>
</tr>
<tr>
<td>Mel501 (M)</td>
<td>74±8</td>
<td>50±4</td>
<td>28±3</td>
<td>58±3</td>
<td>140±9</td>
</tr>
<tr>
<td>WM793 (P)</td>
<td>173±18</td>
<td>106±11</td>
<td>95±9</td>
<td>28±1</td>
<td>91±4</td>
</tr>
<tr>
<td>Me2658 (M)</td>
<td>52±4</td>
<td>25±2</td>
<td>14±2</td>
<td>23±1</td>
<td>90±5</td>
</tr>
<tr>
<td>Me30631 (M)</td>
<td>57±6</td>
<td>50±8</td>
<td>31±4</td>
<td>43±3</td>
<td>95±4</td>
</tr>
<tr>
<td>Mean IC$_{50}$</td>
<td>107±12</td>
<td>73±8</td>
<td>48±9</td>
<td>39±5</td>
<td>92±10</td>
</tr>
</tbody>
</table>
CELL VIABILITY

![Graph showing cell viability with absorbance (OD490) on the y-axis and esomeprazole (µM) on the x-axis. The graph includes bars for Me30966, Me9923, Me5810, and Mel 501 at different concentrations of esomeprazole.]
EXTREMELY CYTOTOXIC FOR METASTATIC MELANOMA
pH DEPENDENCY

Cell death (%)

omeprazole (µM)

0 25 50 100 150 200

0 10 20 30 40 50 60 70 80 90 100

pH 7  pH 6.5  pH 6  pH 5.5

Melanoma M1
pH DEPENDENCY
DOSE AND pH DEPENDENCY
CASPASES
IN VIVO
In vivo magnetic resonance spectroscopy (MRS)

- SCID mice engrafted with human melanoma cells
- After PPI treatment, mice injected with cell-impermeant $^{31}$P reporter 3-aminopropyl phosphonate (3-APP) via i.p. and anaesthetised
- Chemical shift imaging between 3-APP and α-ATP
PPI alter tumor pH in vivo

**pH gradient**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=10)</th>
<th>PPI 2.5 mg/kg (n=5)</th>
<th>PPI 62.5 mg/kg (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.42 ± 0.20</td>
<td>6.67 ± 0.20</td>
<td>6.78 ± 0.23</td>
</tr>
<tr>
<td>pH</td>
<td>7.10 ± 0.23</td>
<td>6.91 ± 0.21</td>
<td>6.80 ± 0.30</td>
</tr>
<tr>
<td>ΔpH</td>
<td>0.68 ± 0.11</td>
<td>0.24 ± 0.28</td>
<td>0.03 ± 0.31</td>
</tr>
</tbody>
</table>

Acidic ——> Alkaline
PPI alter tumor pH in vivo

A REVERSIBLE EFFECT
Saline ESOM (2.5 mg/kg)

Saline
ESOM 0.1 mg/kg
ESOM 0.5 mg/kg
ESOM 2.5 mg/kg

Dose response

Treatment schedule

NECROTIC MASS
ESOM 2.5 mg/kg and ESOM 12.5 mg/kg dramatically improves animal survival. Weight loss over treatment period (g) shows significant differences among groups. Tumor size (mm²) progression is also suppressed by ESOM treatment.
**INDEPENDENT OF MAJOR MUTATIONS**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Protein expression</th>
<th>Mutational analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pAKT</td>
<td>AK T</td>
</tr>
<tr>
<td>Me30966</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Me20842</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Me9923</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Me5810</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Me15392</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mel501</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Me30631</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WM902</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WM793</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Me2658</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

HD, homozygous deletion; wt, wild-type gene; ND, not done; -, absence of protein; +, presence of protein; ++, protein overexpression; *, absence of mRNA.
PPI AS ANTITUMORAL AGENTS

- PPI show a cytotoxic effect towards a variety of human tumor cells
- PPI induce a non-conventional cell death, with different mechanism depending on the tumor histology
- PPI induce marked inhibition of human tumor growth and increase of survival
- PPI target the tumor site in vivo and alter tumor pH
PPI ARE THEY EQUIVALENT COMPOUNDS
Benzimidazoles

Timoprazole

Omeprazole

Pantoprazole

Imidazopyridine

Lansoprazole

Rabeprazole

Tenatoprazole
WHAT THEY HAVE IN COMMON

• PPI ARE PROTONABLE WEAK BASES WITH PKA VALUE OF ~4

• THEY ACCUMULATE SELECTIVELY IN ACIDIC SPACES WITH A pH OF <4. IN A SUCH ACIDIC ENVIRONMENT THEY ARE PROTONATED WITH THE FORMATION OF A TETRACYCLIC SULFENAMIDE, WHICH REPRESENTS THE ACTIVE DRUG
different bioavailability

<table>
<thead>
<tr>
<th>PPI</th>
<th>pKa1</th>
<th>pKa2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>4.06</td>
<td>0.79</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>3.83</td>
<td>0.62</td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>3.83</td>
<td>0.11</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>4.53</td>
<td>0.62</td>
</tr>
<tr>
<td>Tenatoprazole</td>
<td>4.04</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

PPI, proton pump inhibitor.
Lanzoprazole better than esomeprazole (i.p. inoculation)
HIGH DOSAGE PPI IN PATIENTS WITH ZOLLINGER-ELLISON SYNDROME


CRITERIA FOR TREATMENT OF TUMOR PATIENTS WITH HIGH DOSAGE PPI

1. The dosage: 2,5mg/kg, but for rabeprazole (1,5mg/kg) inasmuch as it is extremely effective as antiacidic compound.
2. The schedule: intermittent, inasmuch as PPI are pro-drugs needing low pH for a full activation (e.g. 3 days/week).
3. Which PPI: now we don’t know but they have different bioavailability and we are using to alternate monthly 3 different PPI (i.e. rabeprazole, esomeprazole and lansoprazole).

HOW TO OVERCOME THE OFF-LABEL USE OF PPI
WHY DON'T COMBINE INHIBITORS OF PROTEINS INVOLVED IN pH REGULATION OF TUMOR CELLS?
ACIDIFICATION REDUCE THE EFFECTIVENESS OF CA INHIBITORS

Cell Line: Me 30966 (25,000/well)
Culture Medium: RPMI 1640 pH 7.4  Buffered or Unbuffered
FC9-399A CAIX Inhibitors Concentration: 1 - 5 - 10 – 50 – 100 µM for 24h
Control: DMSO 1%
Cell death evaluation: Trypan blue
ACIDIFICATION REDUCE THE EFFECTIVENESS OF CA INHIBITORS

Cell Line: Me 30966 (25,000/well)
Culture Medium: RPMI 1640 pH 7.4 Buffered or Unbuffered
FC10-461A CAIX Inhibitors Concentration: 1 - 5 - 10 – 50 100 μM for 24h
Control: DMSO 1%
Cell death evaluation: Trypan Blue
FC9-399A and FC10-461A Toxicity curve in combination with Lansoprazole treatment

**COMBINATION WITH PPI INCREASE EFFECTIVENESS OF CA INHIBITORS**

Cell Line: Me 30966 (7,000/well in 96 multiwell)
Culture Medium: RPMI 1640 pH 7.4 Unbuffered
Lansoprazole Concentration (LAN) 50 µM for 24h (pre-treatment) and 48h (combined treatment)
CAIX Inhibitors Concentration: 10 – 50 µM for 24h
Control: DMSO 1%
Cytotoxicity Assay: Acid Phosphatase Viability Assay
The International Society for Proton Dynamics in Cancer (ISPDC)
a new network of investigators focusing on pH-related aspects of cancer, from etiopathogenesis to treatment, and hope to attract junior and senior researchers to join us in this venture!

1° Symposium of the International Society for Proton Dynamics in Cancer
September 27-28, 2010 - Rome, Italy
2nd Symposium of the International Society for Proton Dynamics in Cancer (ISPDC)
Nice, France, November 18-19, 2011

From
Basic Research to Cancer Treatment

Topics
Acid-Base homeostasis, pH control, carbonic anhydrases, H+ pumps, Lactic acid transporters,
Pharmacology of pH-regulating systems, Preclinical cancer approaches, Cancer clinical trials &
pH-regulating systems inhibitors

Speakers (preliminary list)
Walter Boron (USA) Plenary Lecture
Shoukat Dedhar (Canada)
Dario Neri (Switzerland)
Silvia Pastorekova (Slovakia)
Scott Parks (France)
Ian Tannock (Canada)
Stefano Fais (IT)
Robert Gatenby (USA)
Salvador Harguindeguy (SP)
Stine Pedersen (DK)
Stephan Reshkin (IT)
Licia Rivoltini (IT)
Pierre Sonveaux (BE)
Pawell Swietach (UK)

Organizing committee
J. Chiche, L. Counillon, S. Parks, J. Pouysségur

http://www.ispdc.net/
“Si pudiera vivir nuevamente mi vida en la próxima trataría de cometer más errores”

(If I could live my life again, in the next life I’ll try to make more mistakes)

ISTANTES

Jorge Luis Borges
I DON'T GIVE UP
# Acknowledgements

## Preclinical Setting

<table>
<thead>
<tr>
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<th>Contributors</th>
</tr>
</thead>
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Michele Maio |
| UNIVERSITY OF SIENA |  |
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