



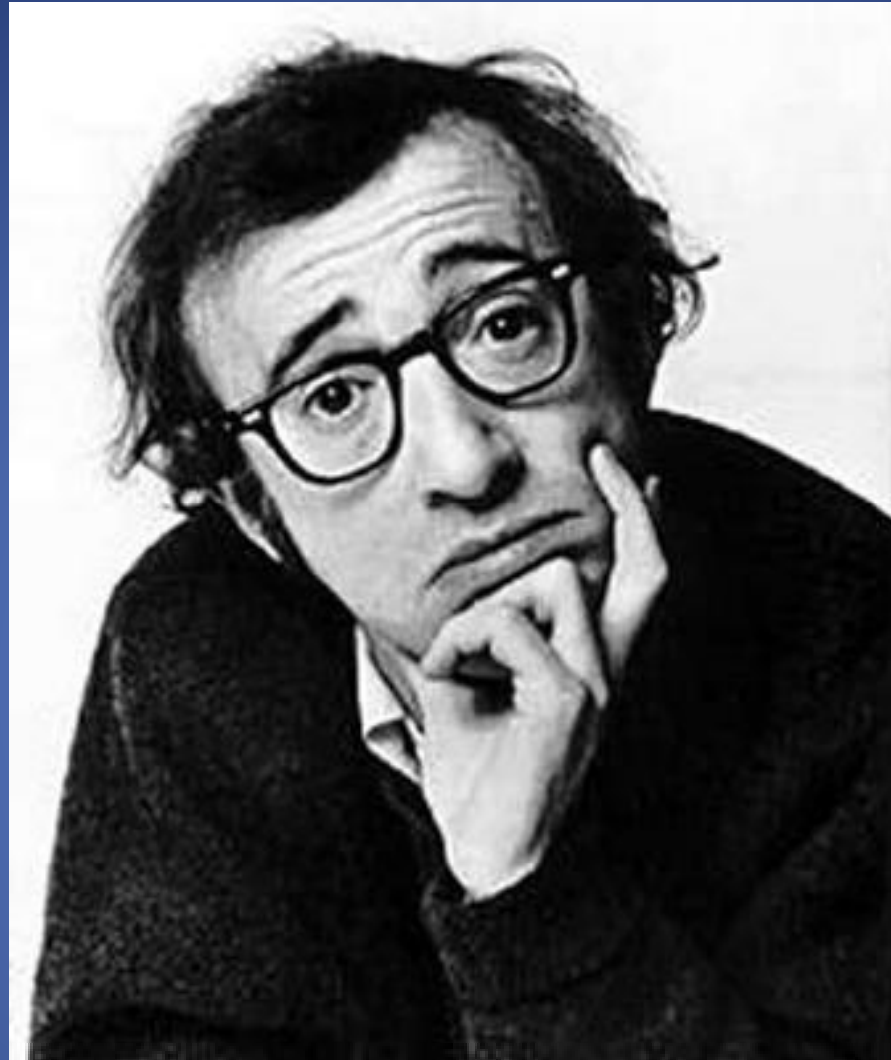
"L'uso degli inibitori di pompa protonica Nel trattamento del tumore"

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MEDICINE EVALUATION
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ROME - ITALY

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

Quotidiano Londra

Diffusione: n.d.

Lettori: n.d.

FINANCIAL TIMES

Direttore: Lionel Barber

30-LUG-2008

da pag. 9

Drug research needs serendipity

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 pharmaceuticals executives overestimated 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 ham-

disease areas, shelving 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 intent are managers on maintaining focus that important opportunities for novel discovery are lost, as is the intellectual space for tinkering and capitalising on the chance observations

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)

Big pharma companies must learn to resist the false comfort of revenue predictions and valuation spreadsheets

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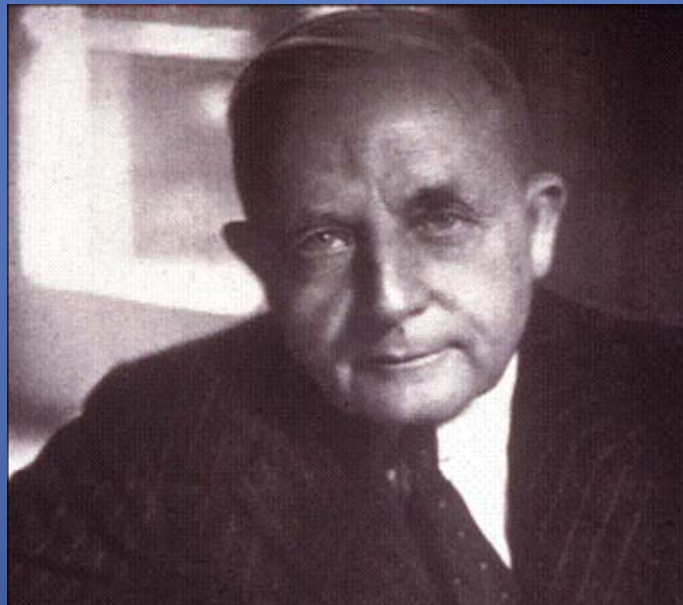
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For all the breathless headlines proclaiming breakthrough discoveries, the truth is that we still do not understand what causes most disease. Even when we can identify a responsible gene or implicate an important mutation, we have made only limited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

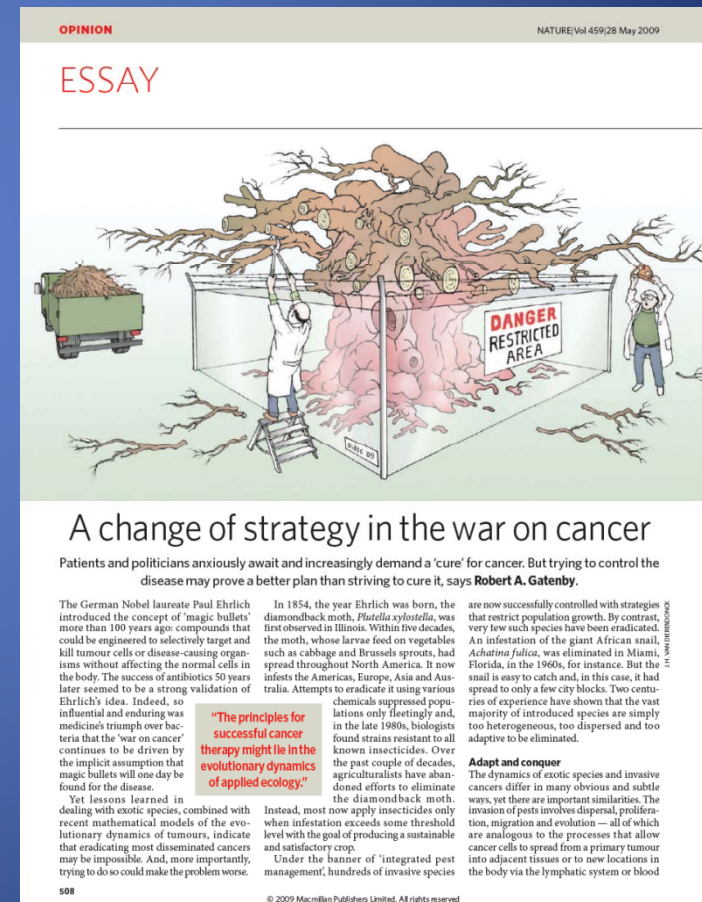


HOW CAN WE CURE?

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



Otto Heinrich Warburg
1883-1970
Nobel Prize 1931

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

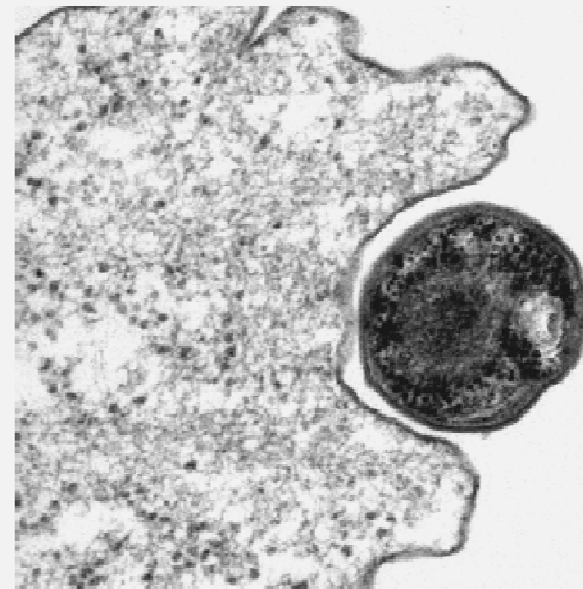
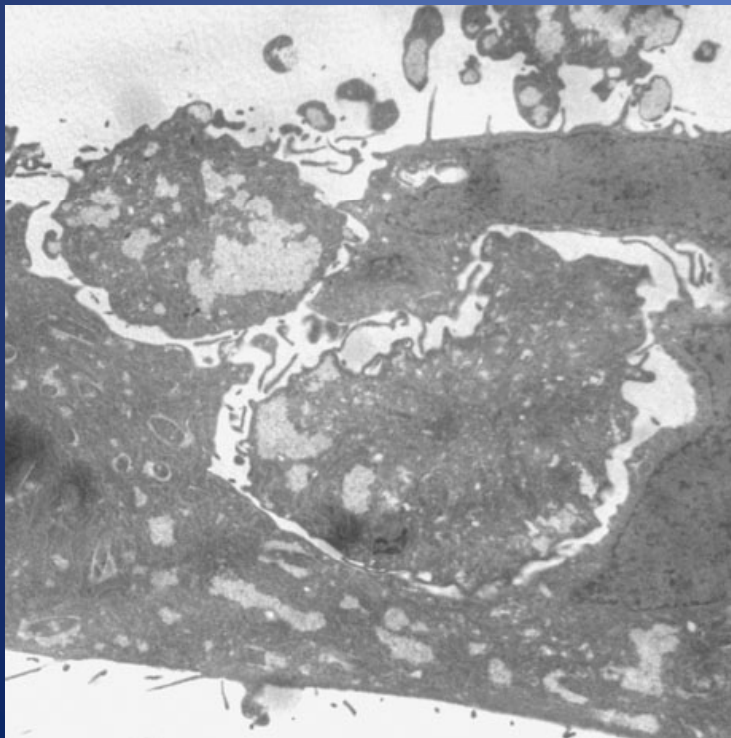
THEY ARE CELLS WHOSE LIFESTYLE IS
IN NO WAY COMPARABLE TO THAT OF
NORMAL CELLS

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)

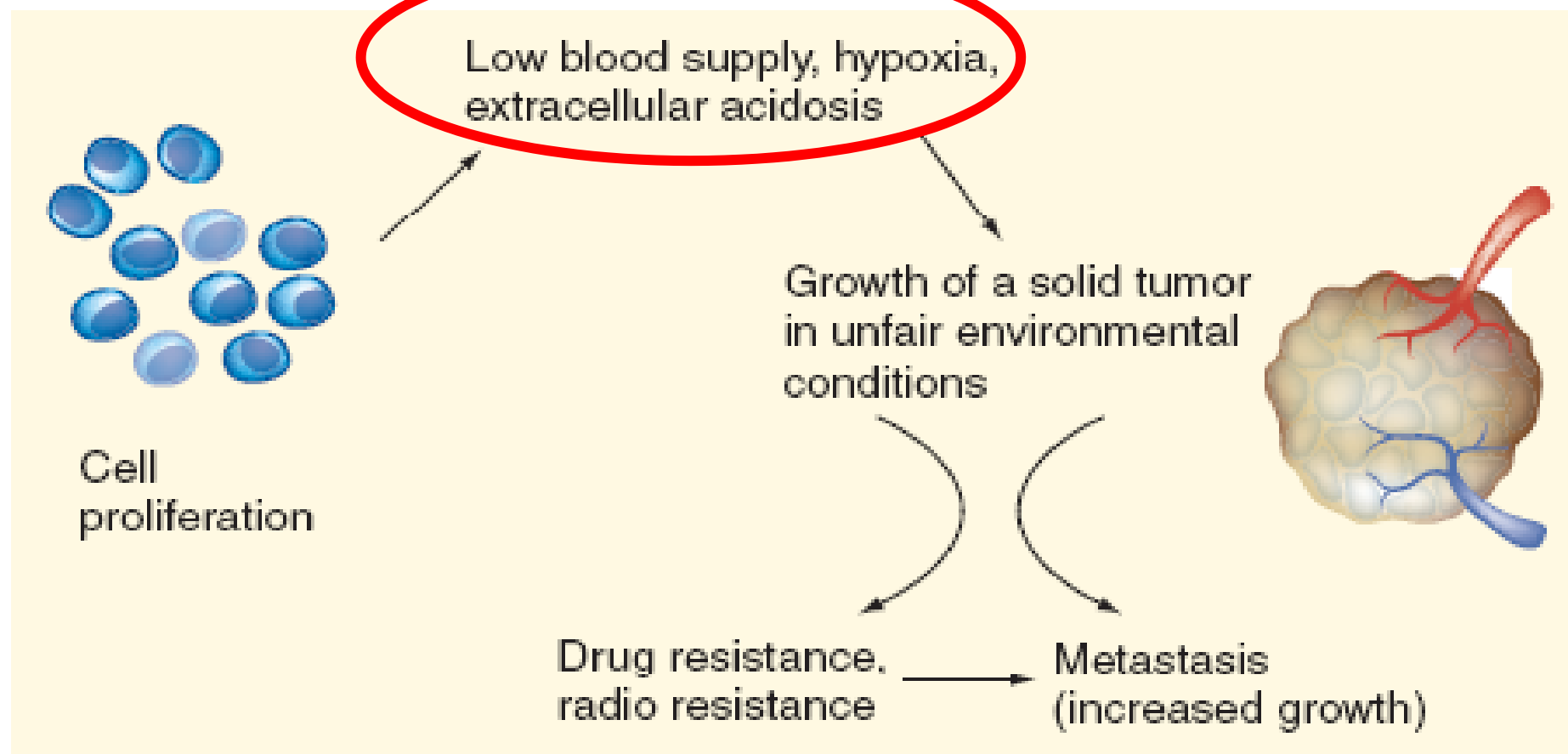
2.SIMILAR MECHANISMS TO FACE OFF LOW LEVEL OF NUTRIENTS
SUPPLY (CELL CANNIBALISM)



Phagocytosis of a Chlamydia-related bacteria by the Acanthamoeba amoeba, as seen by electron microscopy (Picture by G. Greub, Lausanne, Switzerland)

TUMOR MICROENVIRONMENTAL FACTORS

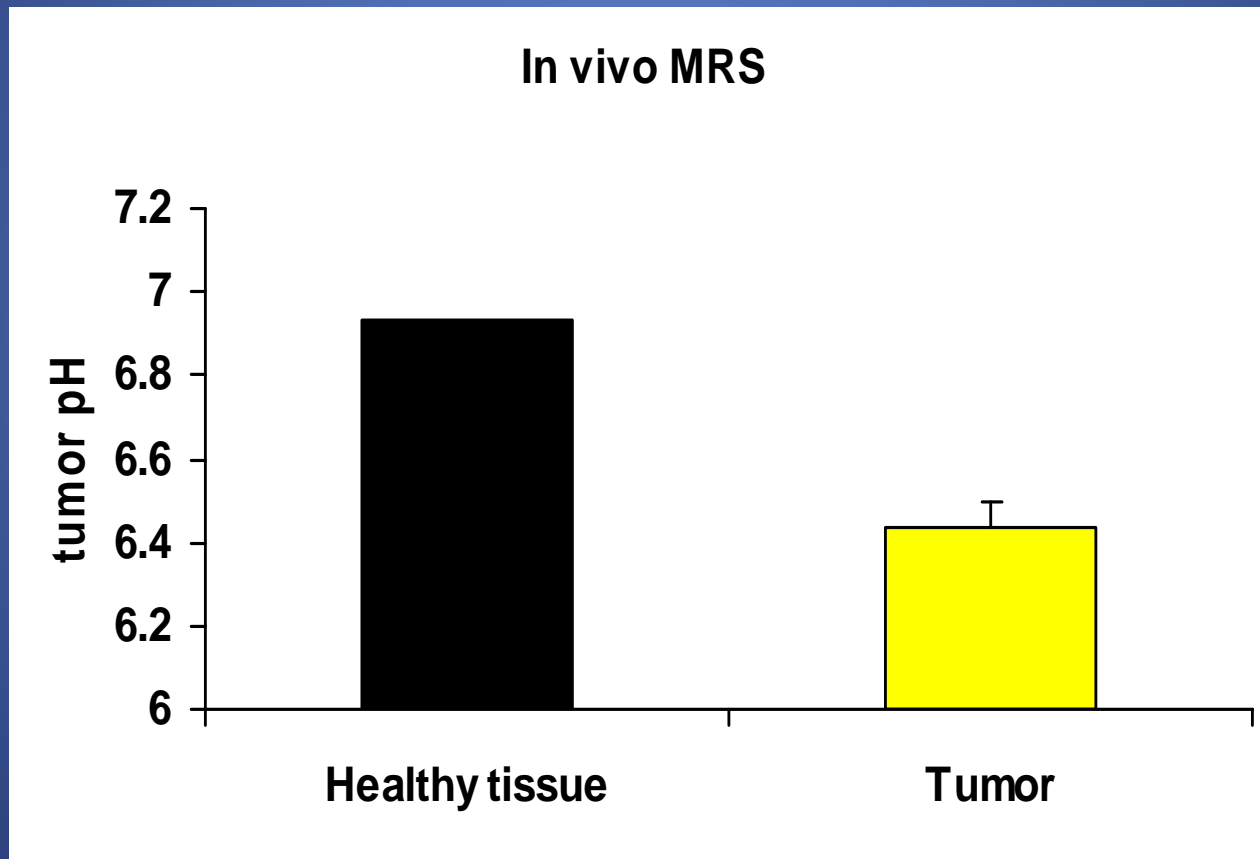
Figure 1. Schematic representation of tumor growth.



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 tumours 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)

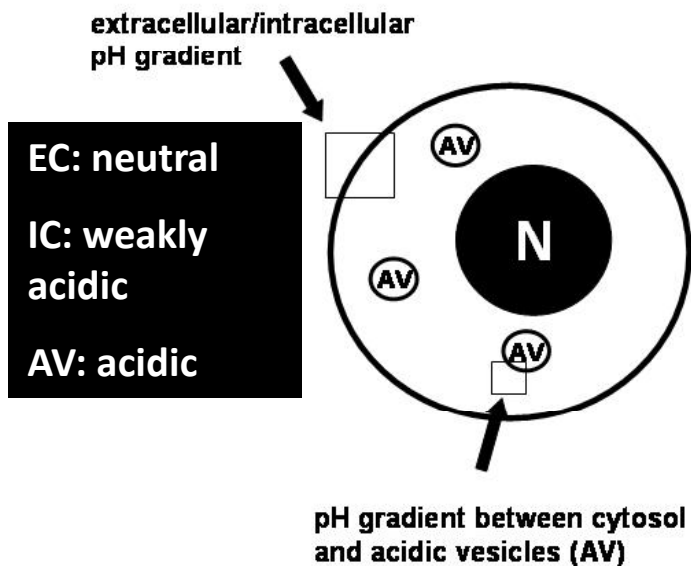
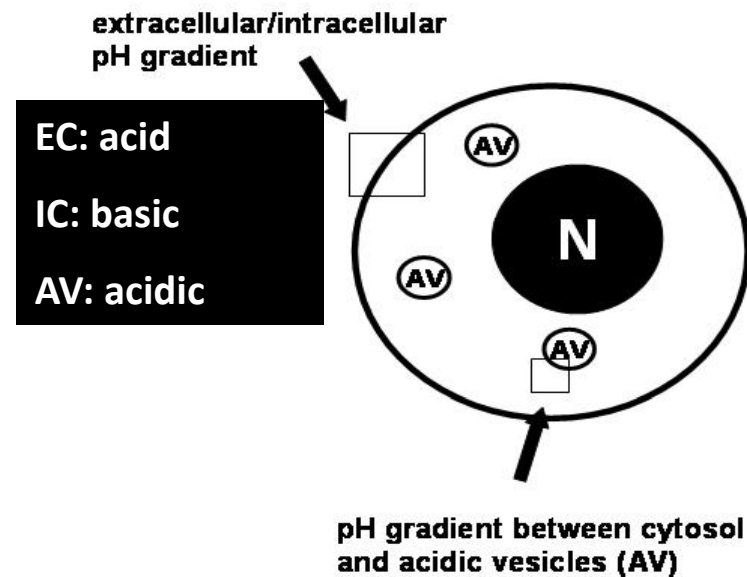


Figure 1B. pH gradient in tumor cells (acidic/unbuffered condition)



REVERSED pH GRADIENTS AN HALLMARK OF MALIGNANT CELLS



ACID OUTSIDE

The diagram consists of a large yellow rectangular border. Inside this border, there are two horizontal rectangular boxes. The top box is red and contains the text 'ACID OUTSIDE' in yellow. The bottom box is blue and contains the text 'ALKALINE INSIDE' in white.

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

CHEMORESISTANCE

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

WHY PROTON PUMPS

ION EFFLUX PUMPS



PUMPING H^+ OUTSIDE THE CELLS

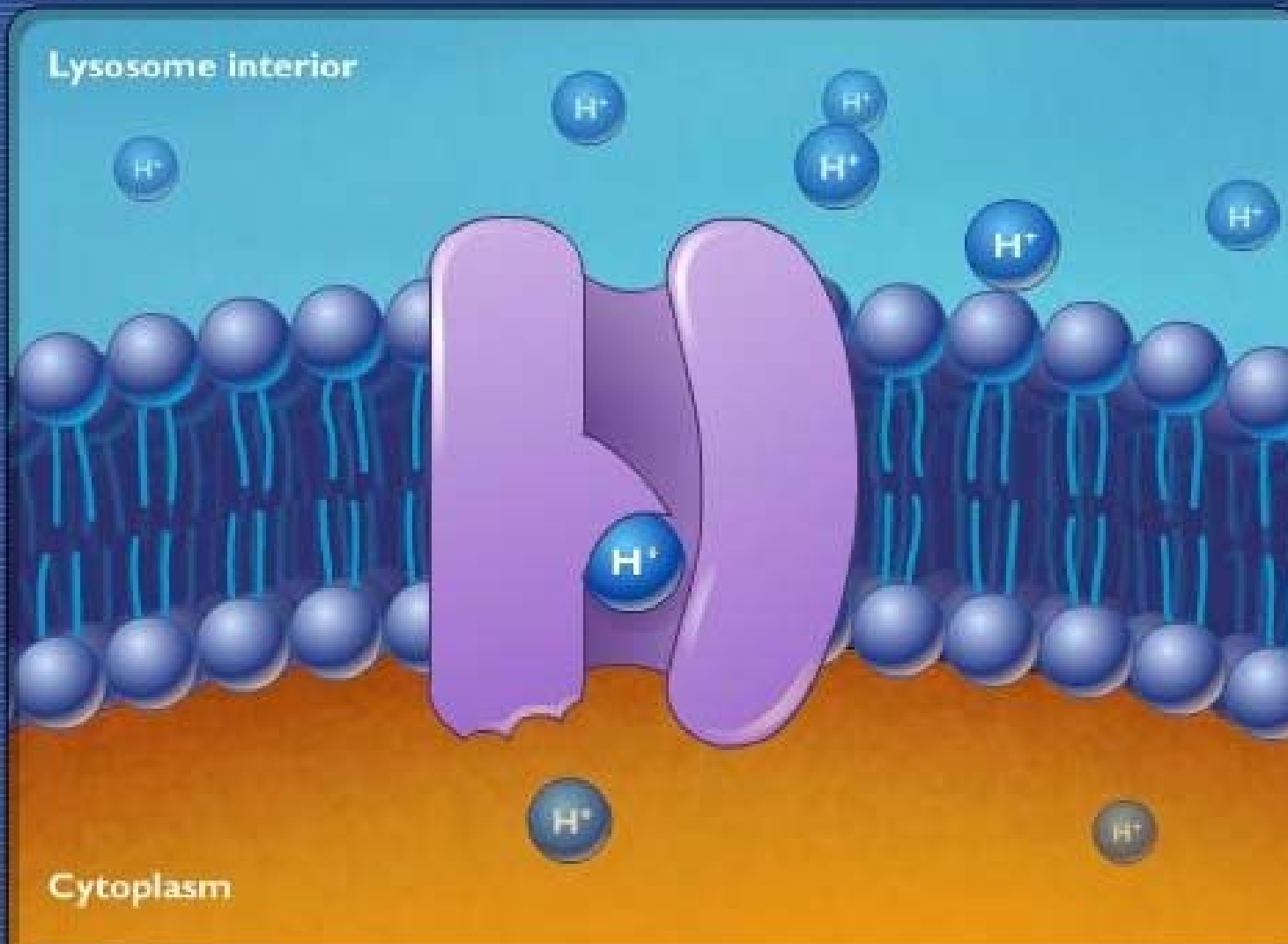
- ❑ Na^+/H^+ -exchanger
- ❑ vacuolar H^+ -ATPases
- ❑ Na^+/K^+ -ATPase pump
- ❑ H^+/Cl^- symporter
- ❑ monocarboxylate transporters (H^+ /lactate)
- ❑ ABC transporters

THEY ALL COOPERATE IN MAINTAINING
DERANGED pH GRADIENTS IN HUMAN TUMORS

WHY V-ATPASES ?

Mc
Graw
Hill

Proton Pump



▶
Play

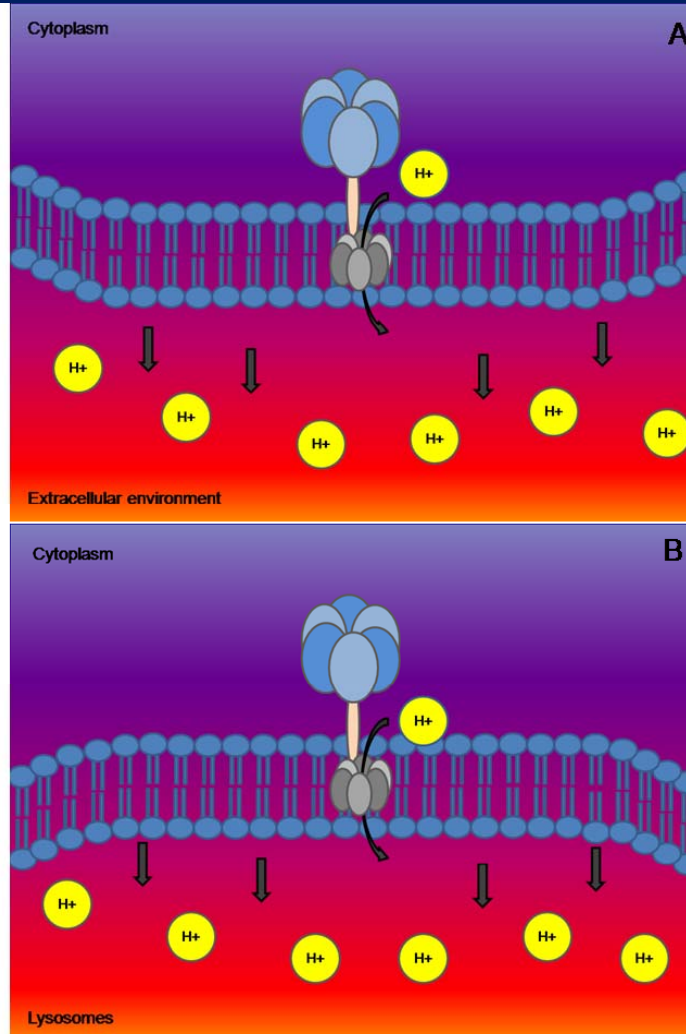
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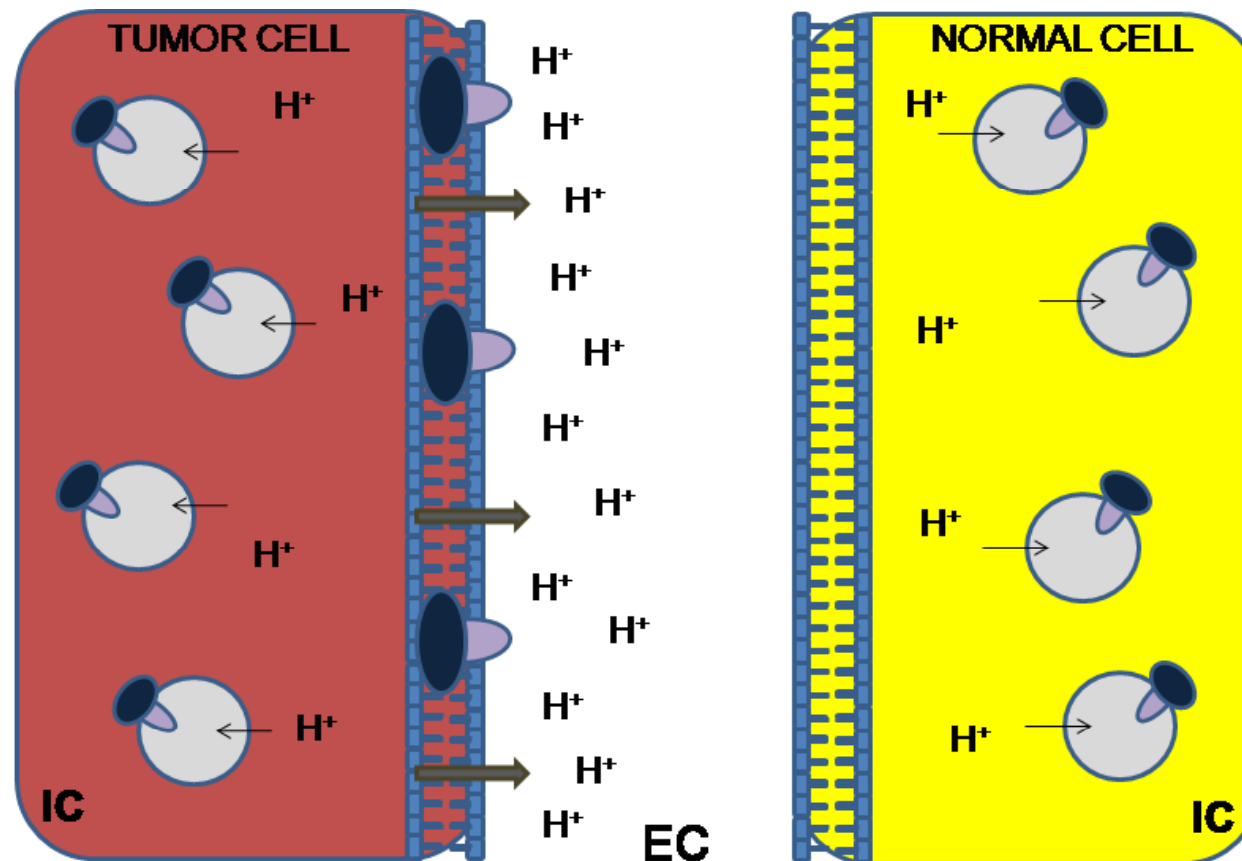
WHY V-ATPASES ?

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



IC Intracellular compartment
EC Extracellular compartment

 Plasmatic Membrane
 Lysosome
 H⁺ V-ATPase

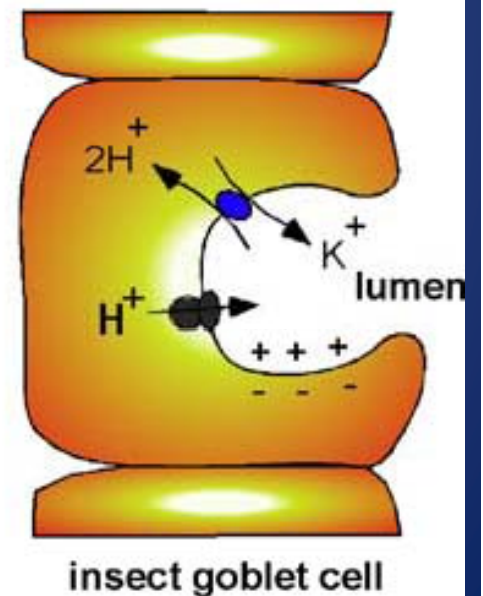
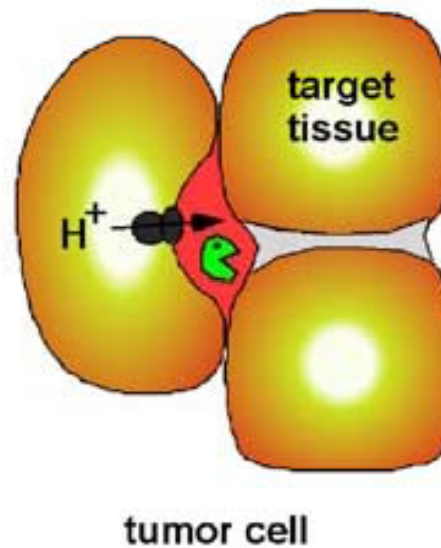
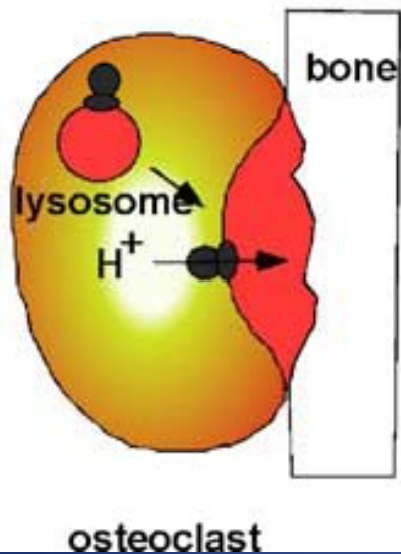
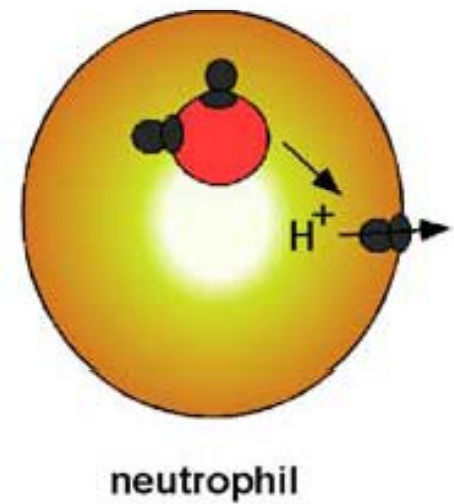
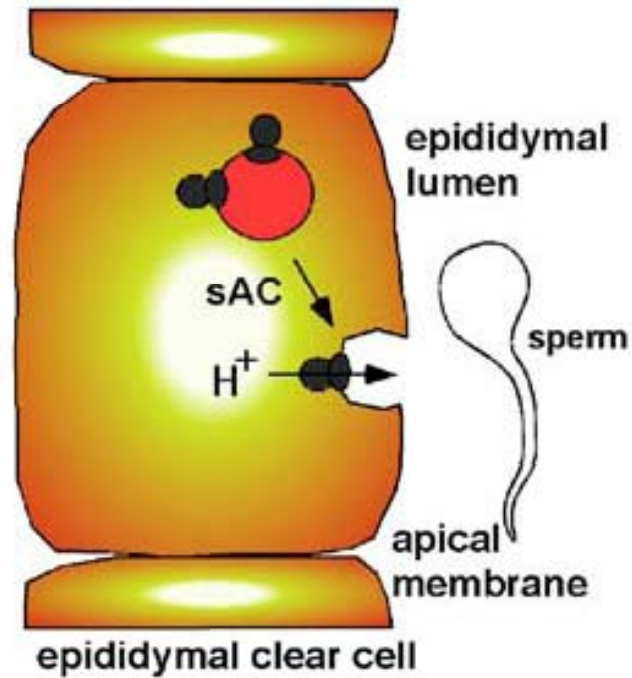
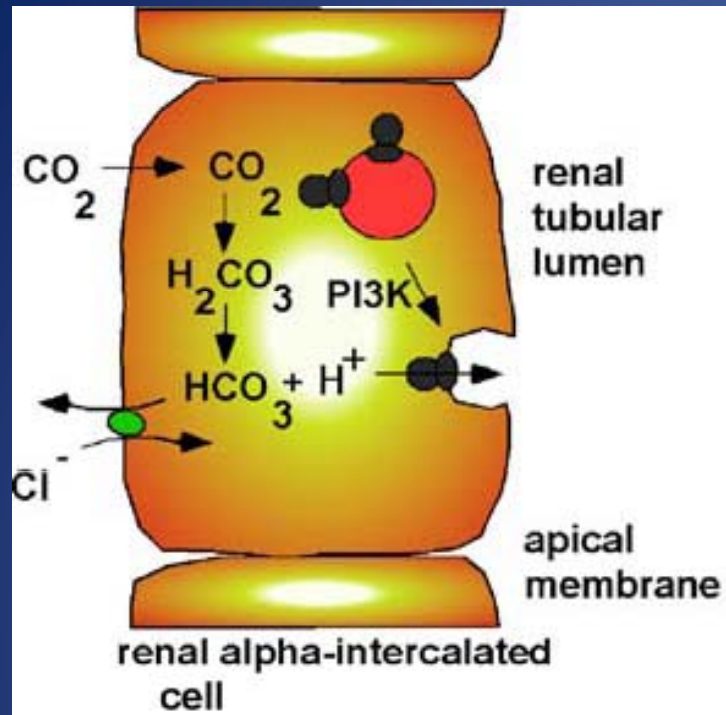
H⁺-ATPases inhibitors

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

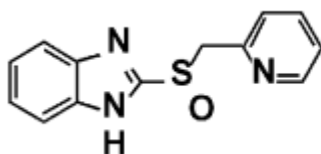
Plasma membrane V-ATPases are
especially important in human disease,
with genetic defects in V-ATPases
expressed in osteoclasts and
intercalated cells leading to the diseases

**osteopetrosis and renal tubule
acidosis, respectively.**

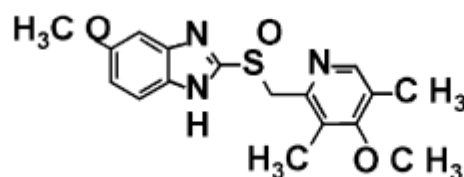
Anti-acid treatment and PPI

PROTON PUMP INHIBITORS

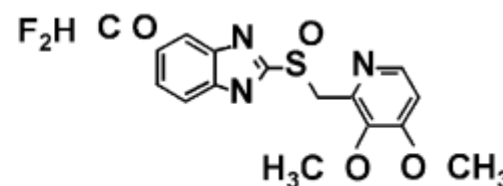
Benzimidazoles



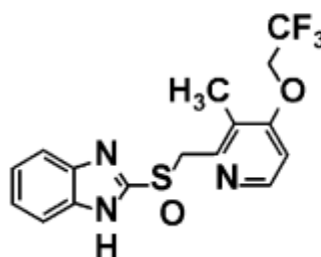
Timoprazole



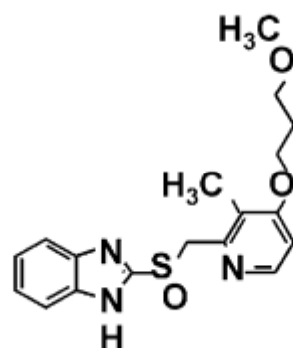
Omeprazole



Pantoprazole

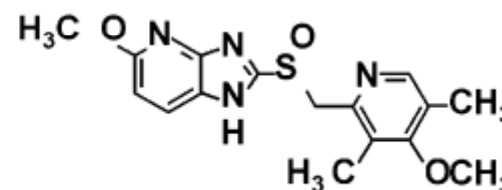


Lansoprazole



Rabeprazole

Imidazopyridine



Tenatoprazole

ANTI-ACID TREATMENT AND PPI

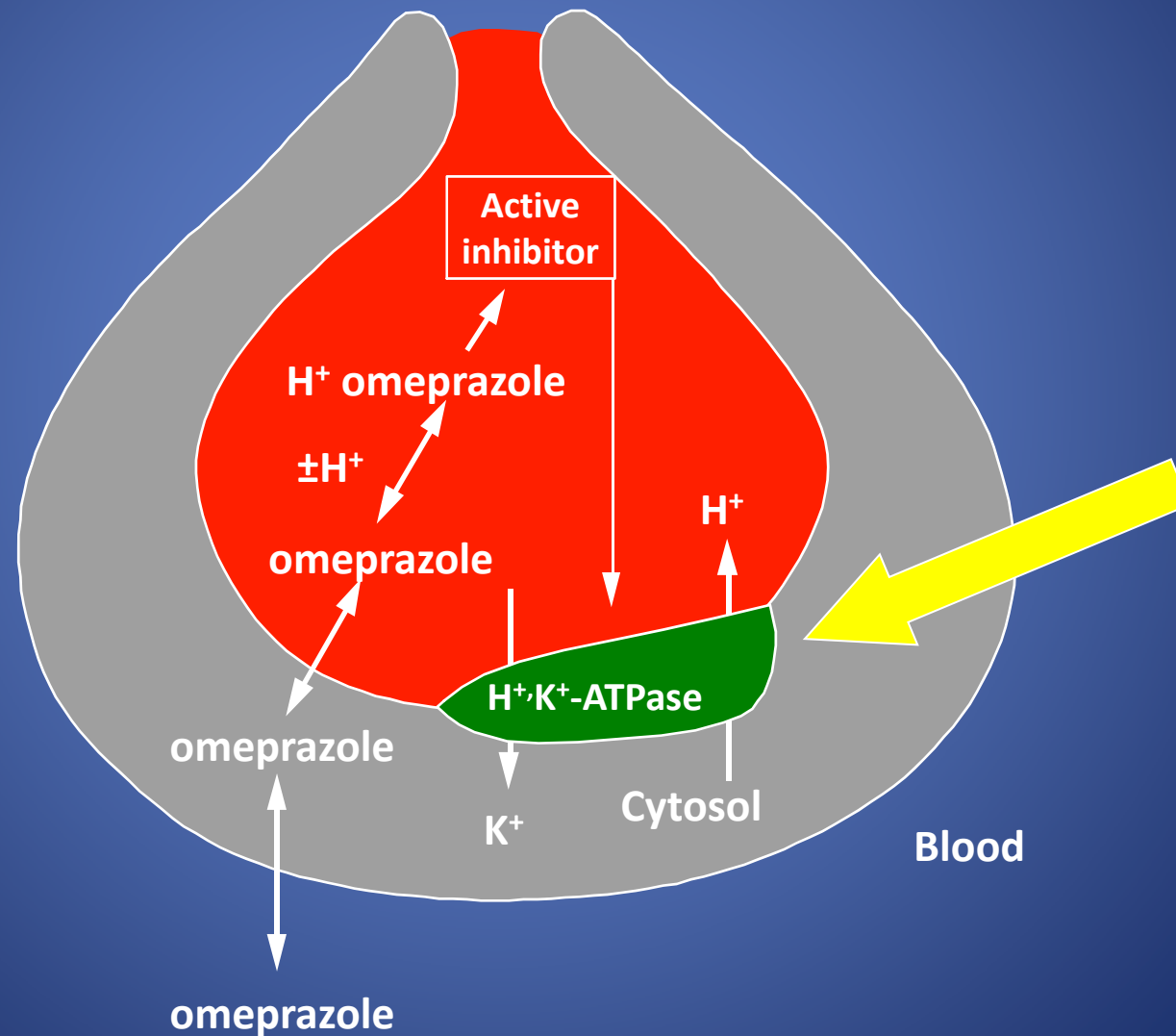
Although they have been included generic drugs, PPI have different bioavailability

PKA VALUES OF PPI

PPI	pKa1	pKa2
Omeprazole	4.06	0.79
Lansoprazole	3.83	0.62
Pantoprazole	3.83	0.11
Rabeprazole	4.53	0.62
Tenatoprazole	4.04	-0.12

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 TARGETTED 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:

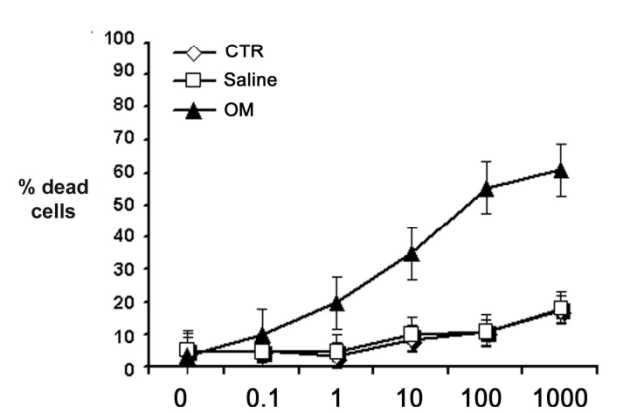
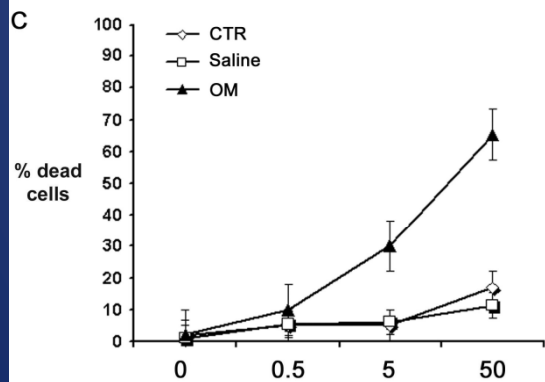
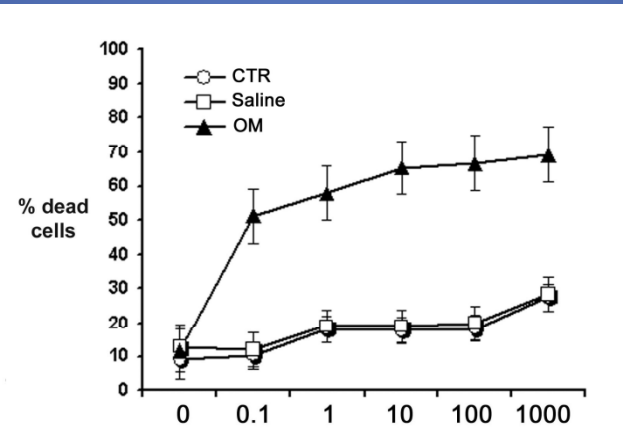
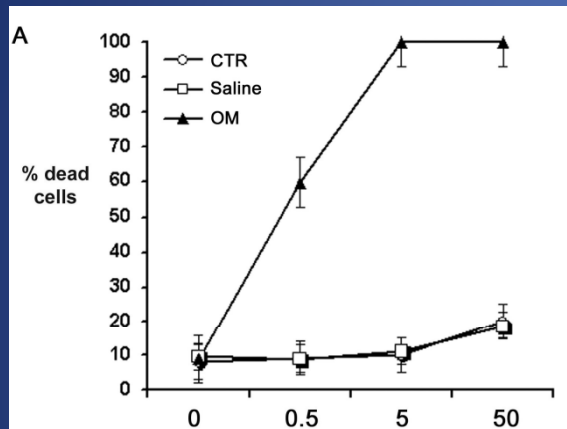
- OMEPRAZOLE AND ESOMEPRAZOLE (ASTRA-ZENECA)
sodium salts were resuspended in normal saline at the concentration of 1 mg/ml immediately before use
- PANTOPRAZOLE (BYK GULDEN)

4. CYTOTOXICITY ASSAYS

- Trypan blue exclusion
- Live/Dead Viability/Cytotoxicity Assay ®

Effects of omeprazole treatment on cisplatin and 5-fluorouracile sensitivity of human melanoma

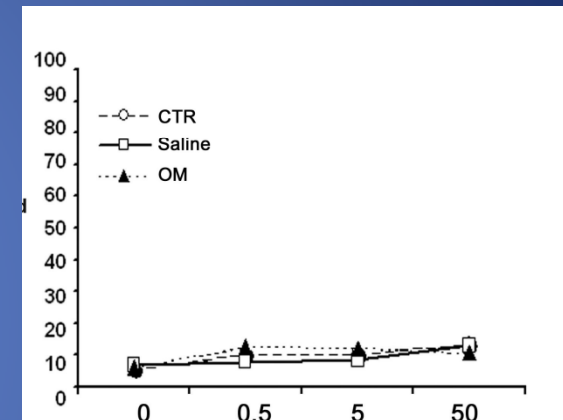
pre-treatment



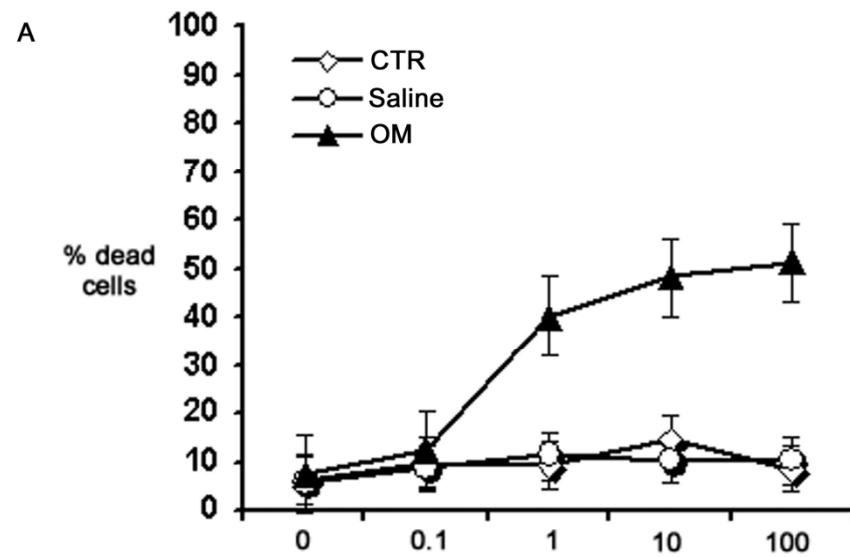
cisplatin (mM)

5-FU (mg/ml)

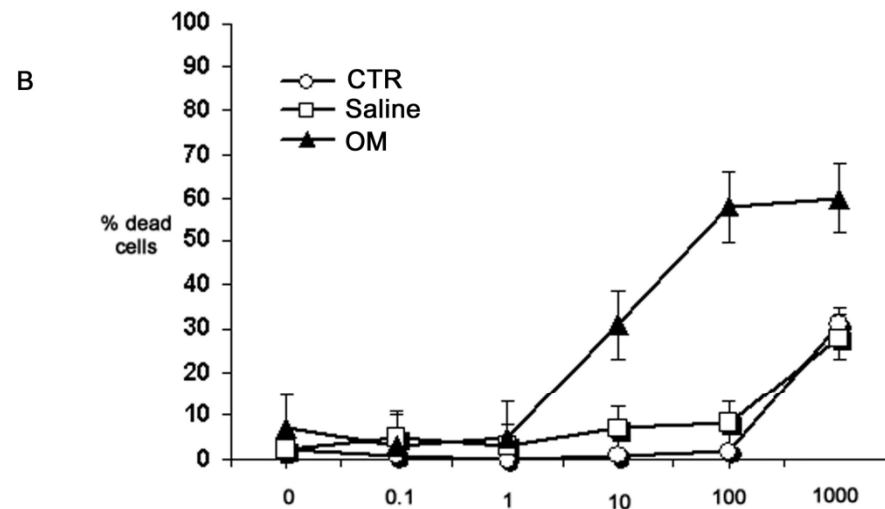
co-treatment



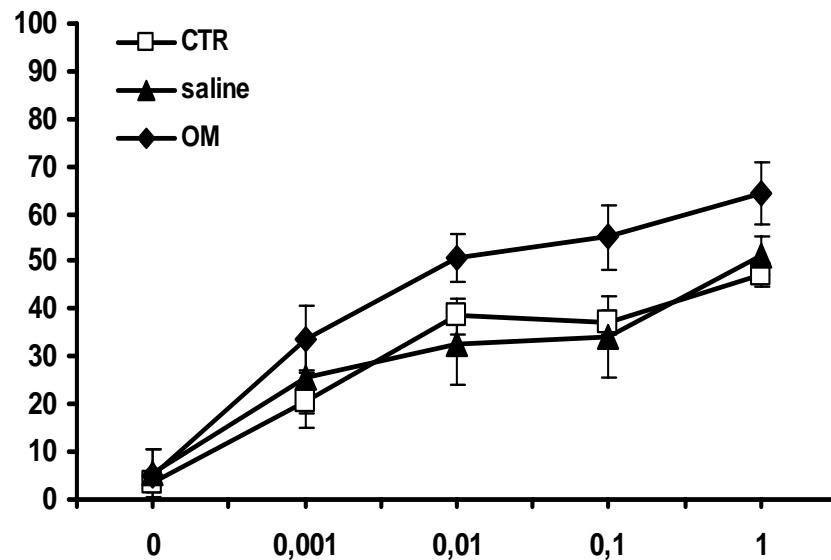
cisplatin (mM)



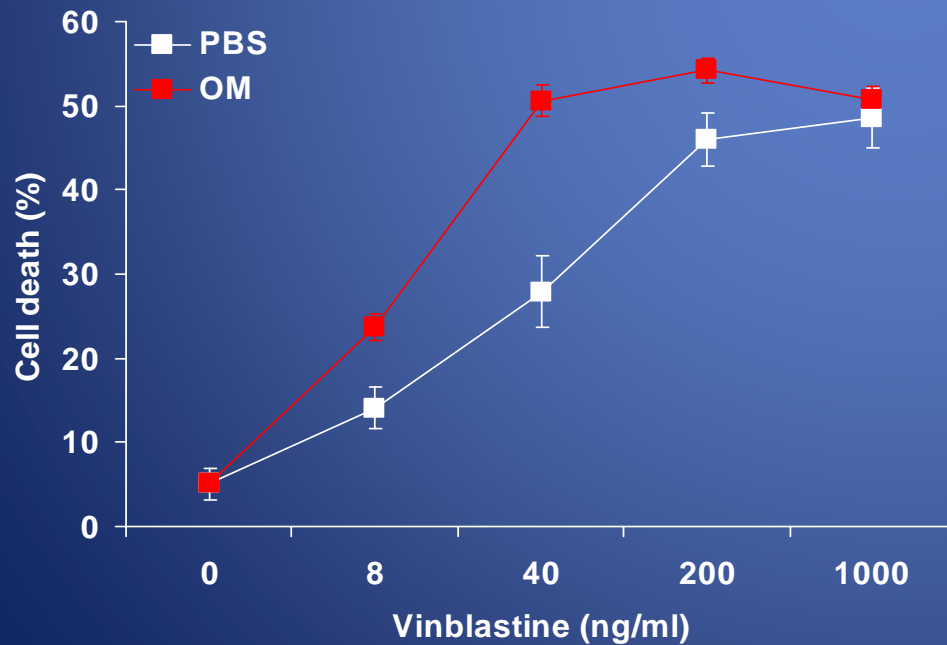
PPI REVERT MDR



PPI INCREASE SENSIBILITY OF SENSIBLE CELLS

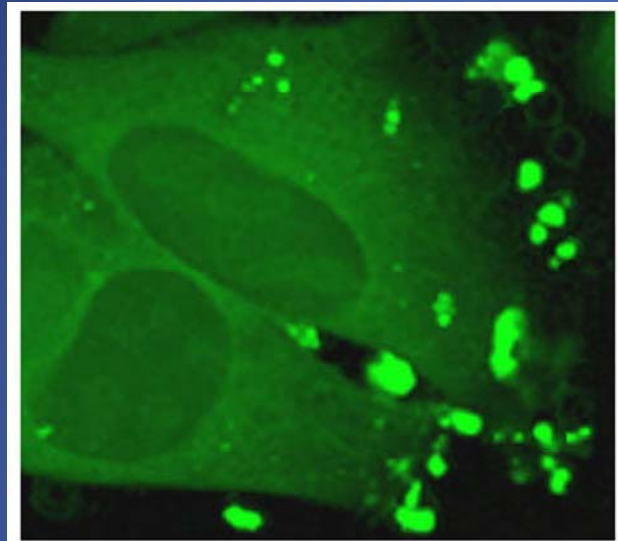


HUMAN LYMPHOBLASTOID
CELLS

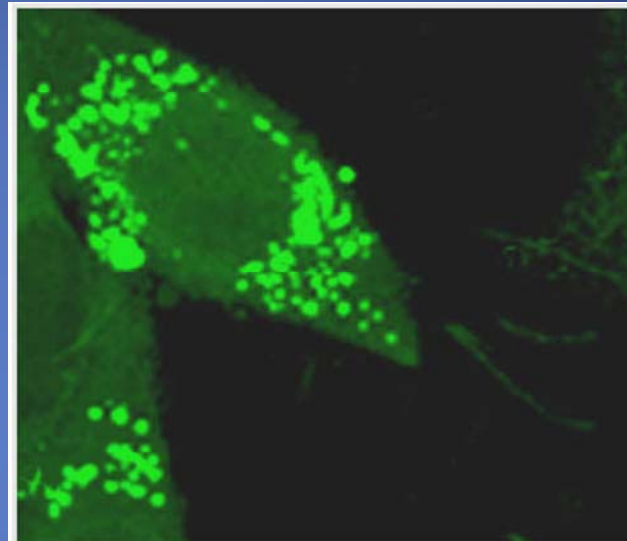


HUMAN ALL CELLS

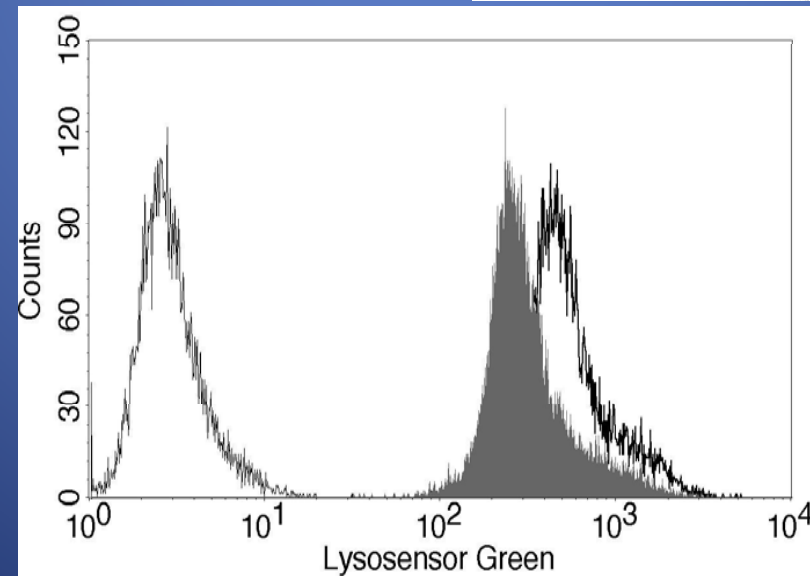
PPI alter pH and traffic of acidic vesicles



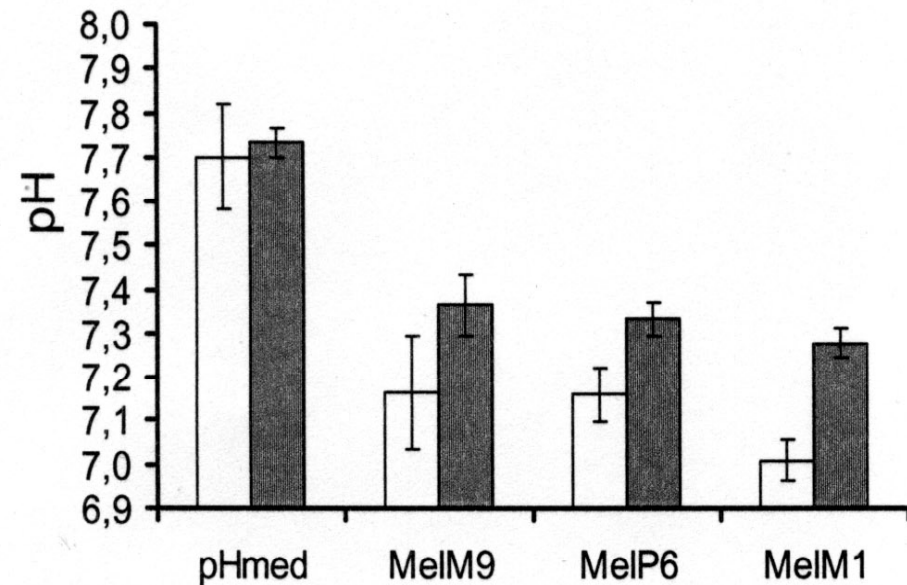
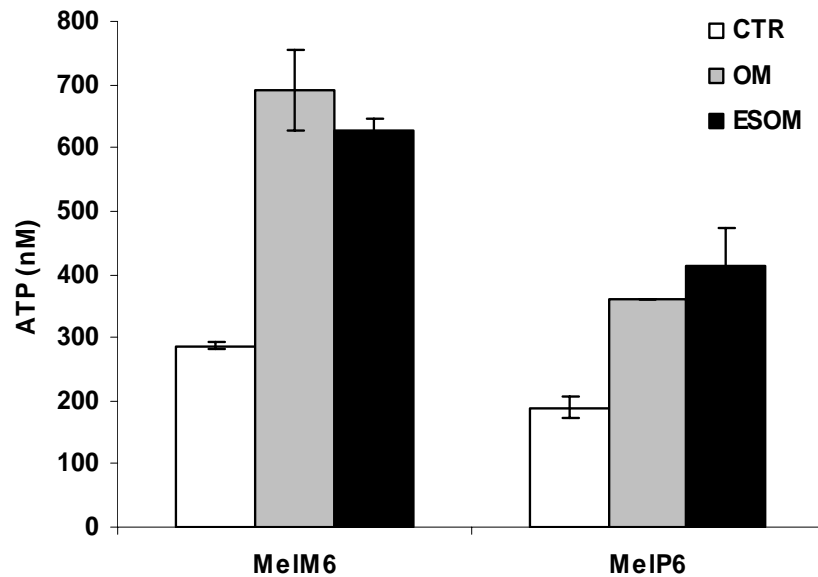
untreated



+PPI

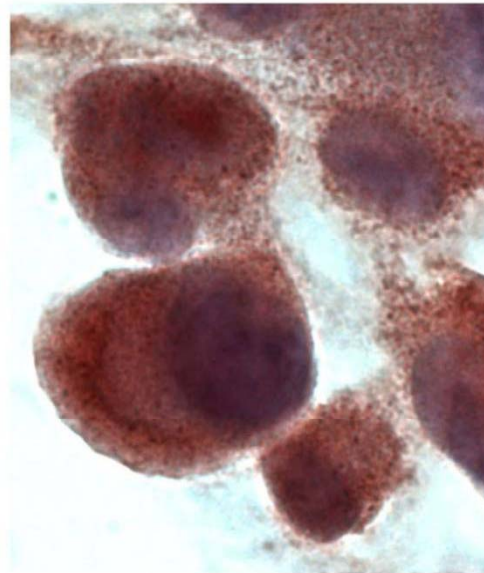


PPI treatment affect pH of human tumor cells in vitro through reduction of ATP consuming

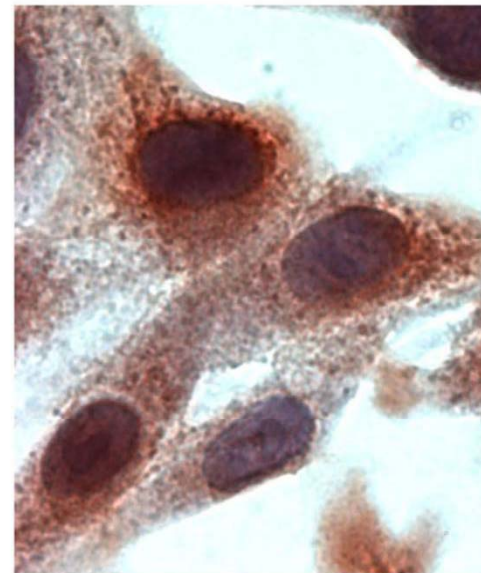


V-ATPASES CELLULAR DISTRIBUTION

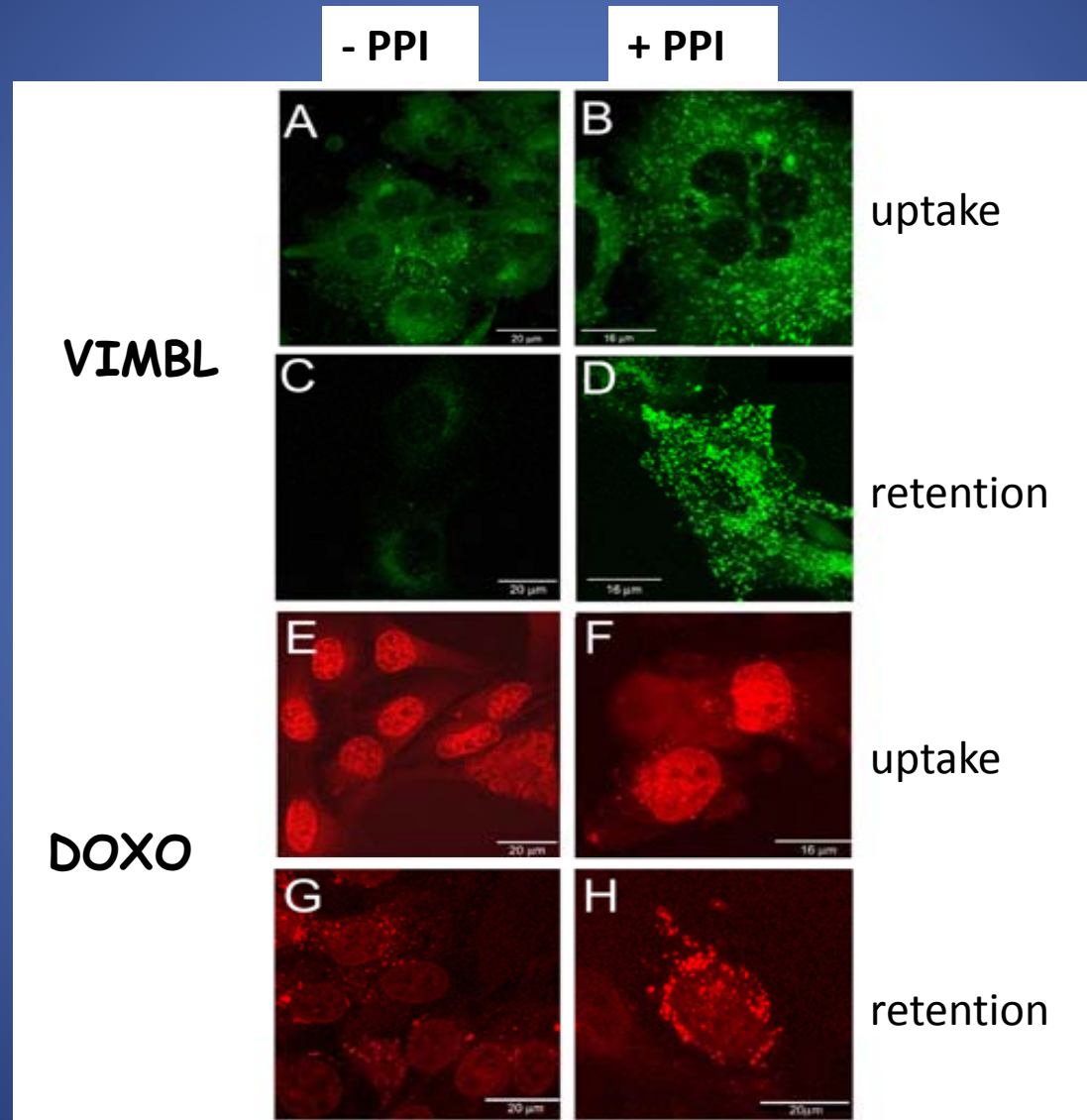
UNTREATED



WITH PPI



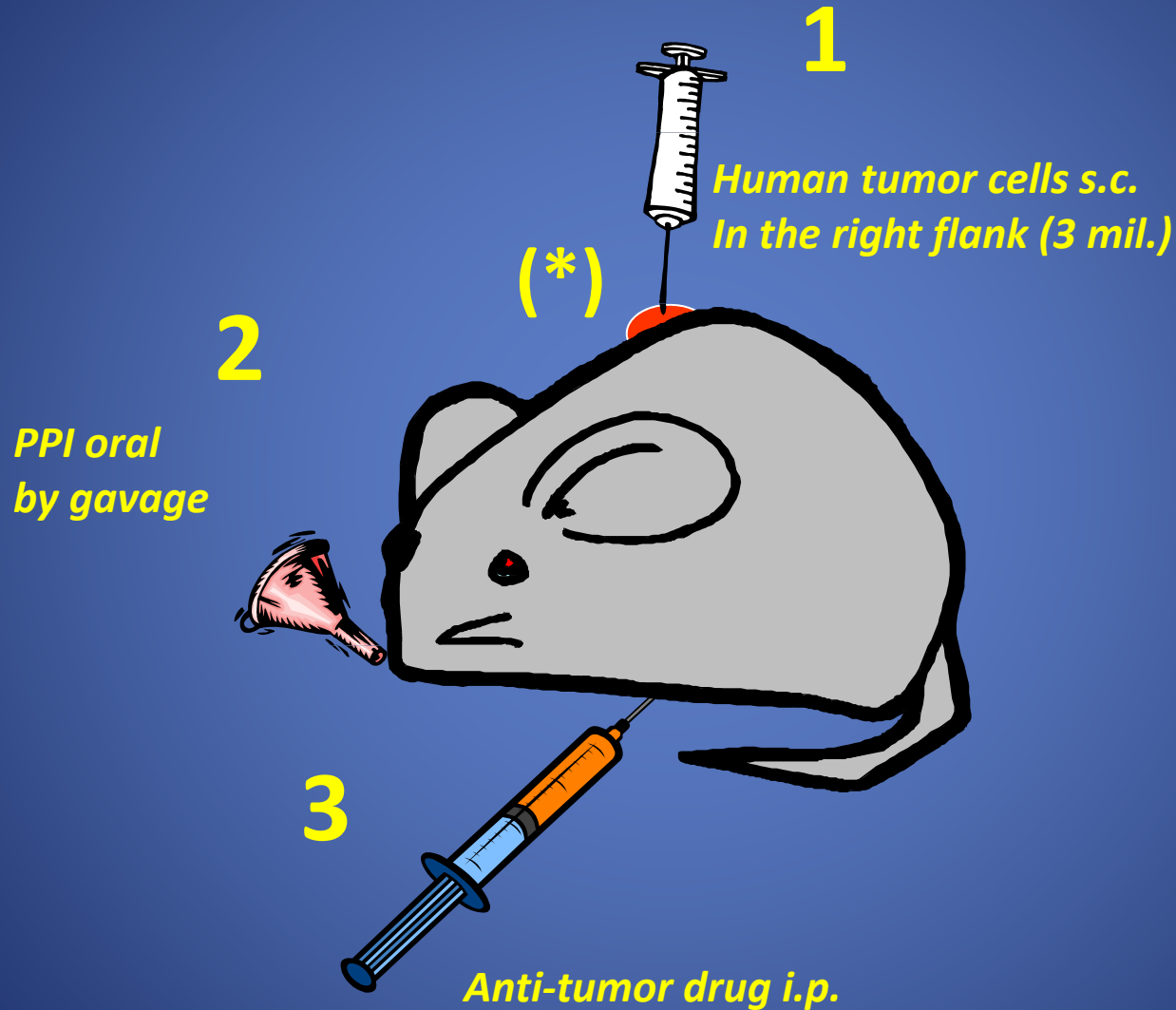
Effects of omeprazole on drug efflux



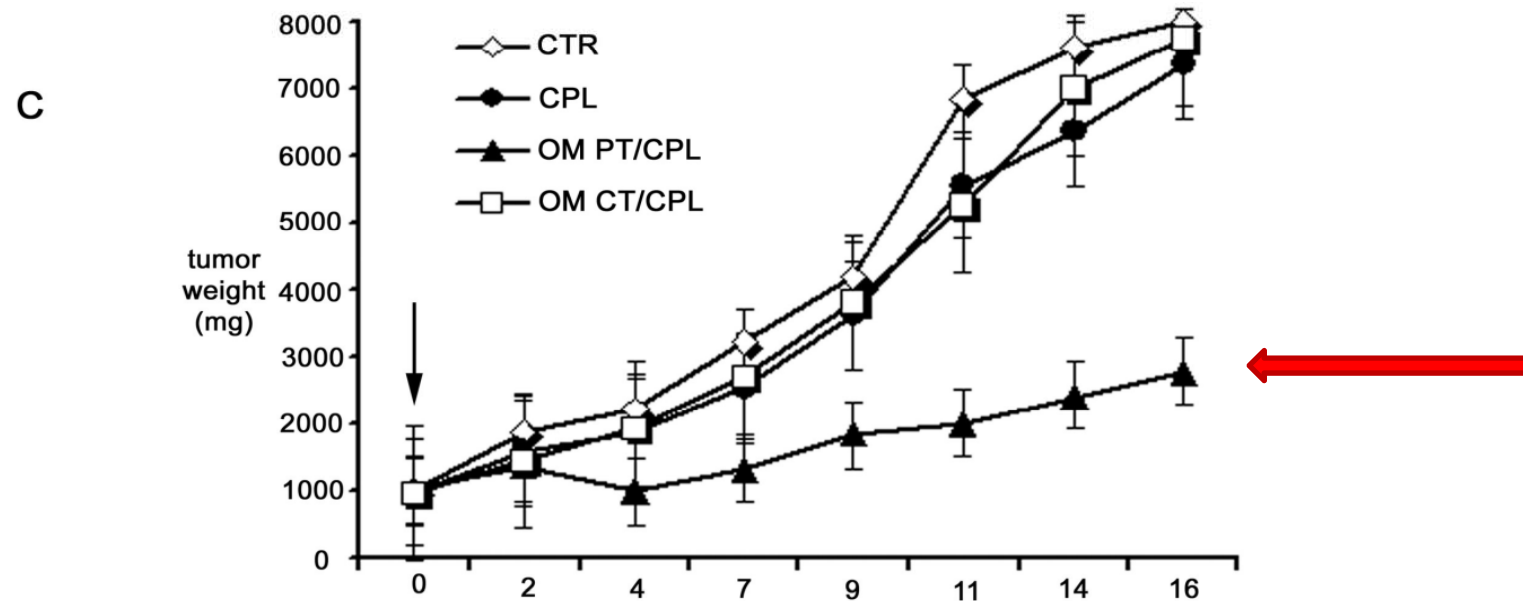
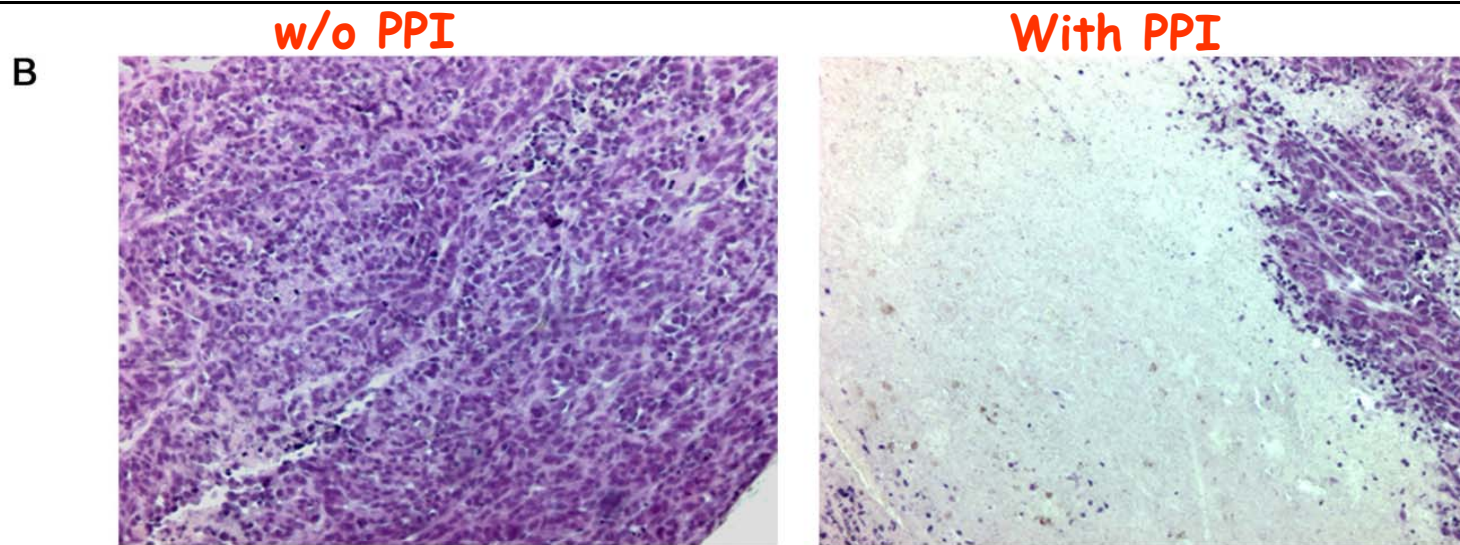
PPI pretreatment reverses chemoresistance of human tumor cells

	Cisplatin (μM)			5-Fluorouracil ($\mu\text{g/ml}$)			Vinblastine (ng/ml)		
	None	OM	ES	None	OM	ES	None	OM	ES
Melanoma (n=22)	443 \pm 86	49 \pm 36	46 \pm 36	894 \pm 168	29 \pm 32	20 \pm 21	>1000	28 \pm 16	20 \pm 12
Colon (n=2)	400 \pm 16	14 \pm 6	17 \pm 3	844 \pm 217	50 \pm 0.5	29 \pm 9	nd	nd	nd
Breast (n=2)	493 \pm 10	51 \pm 55	39 \pm 39	980 \pm 28	14 \pm 11	11 \pm 11	nd	nd	nd
OVCA (n=2)	450 \pm 70	77 \pm 23	71 \pm 21	925 \pm 106	16 \pm 7	12 \pm 5	nd	nd	nd

IN VIVO PRE-TREATMENT IN HUMANIZED SCID MICE

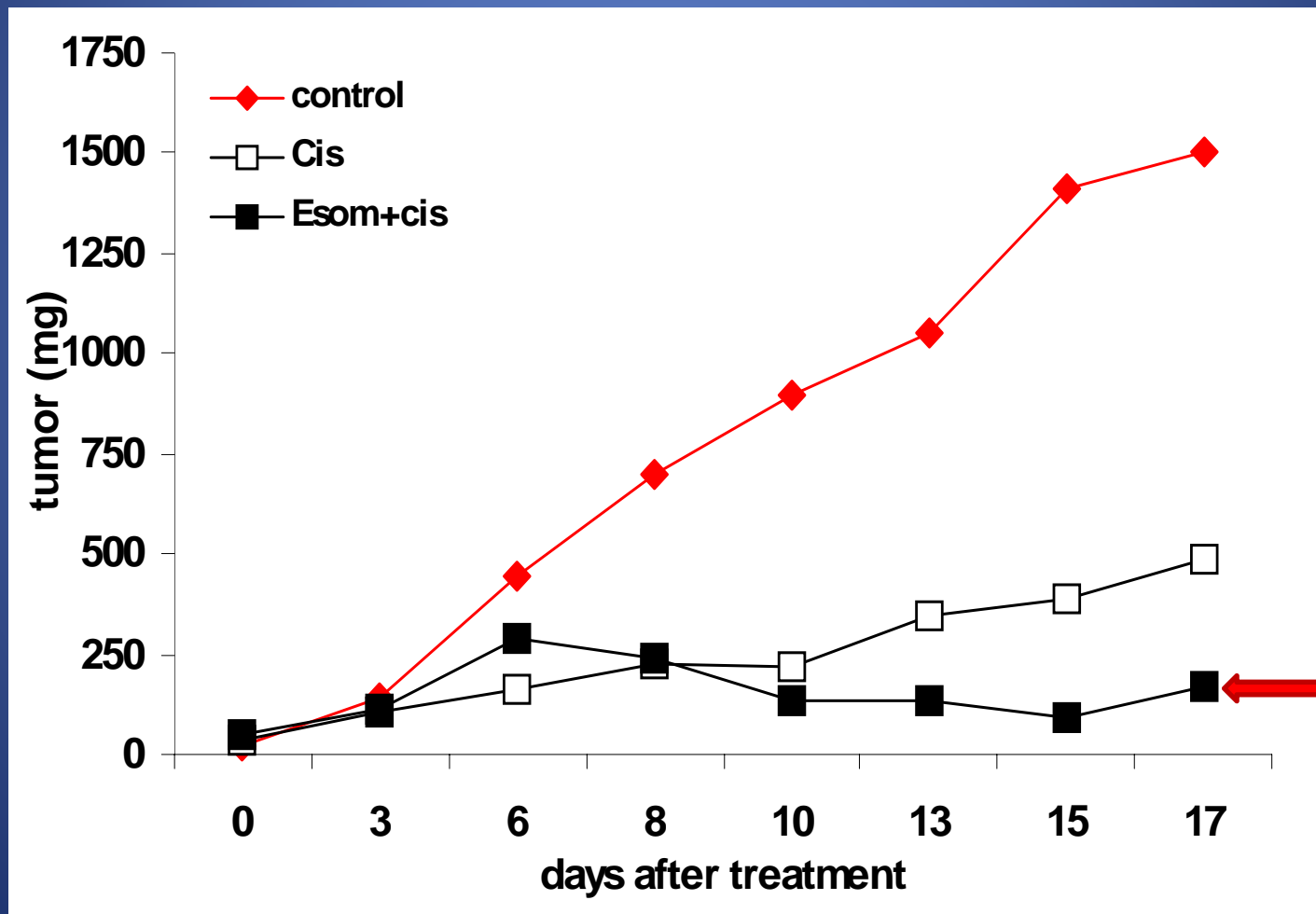


(*) Tumor weight (mg) = lenght (mm) x width² (mm)/2.



oral PPI pre-treatment sensitize 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

•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)

1

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

2

•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)

3

*Some results on the use of PPI as
chemosensitizers in patients
with
melanoma and osteosarcoma*

MELANOMA

CLINICAL PROTOCOL

3 ARMS:

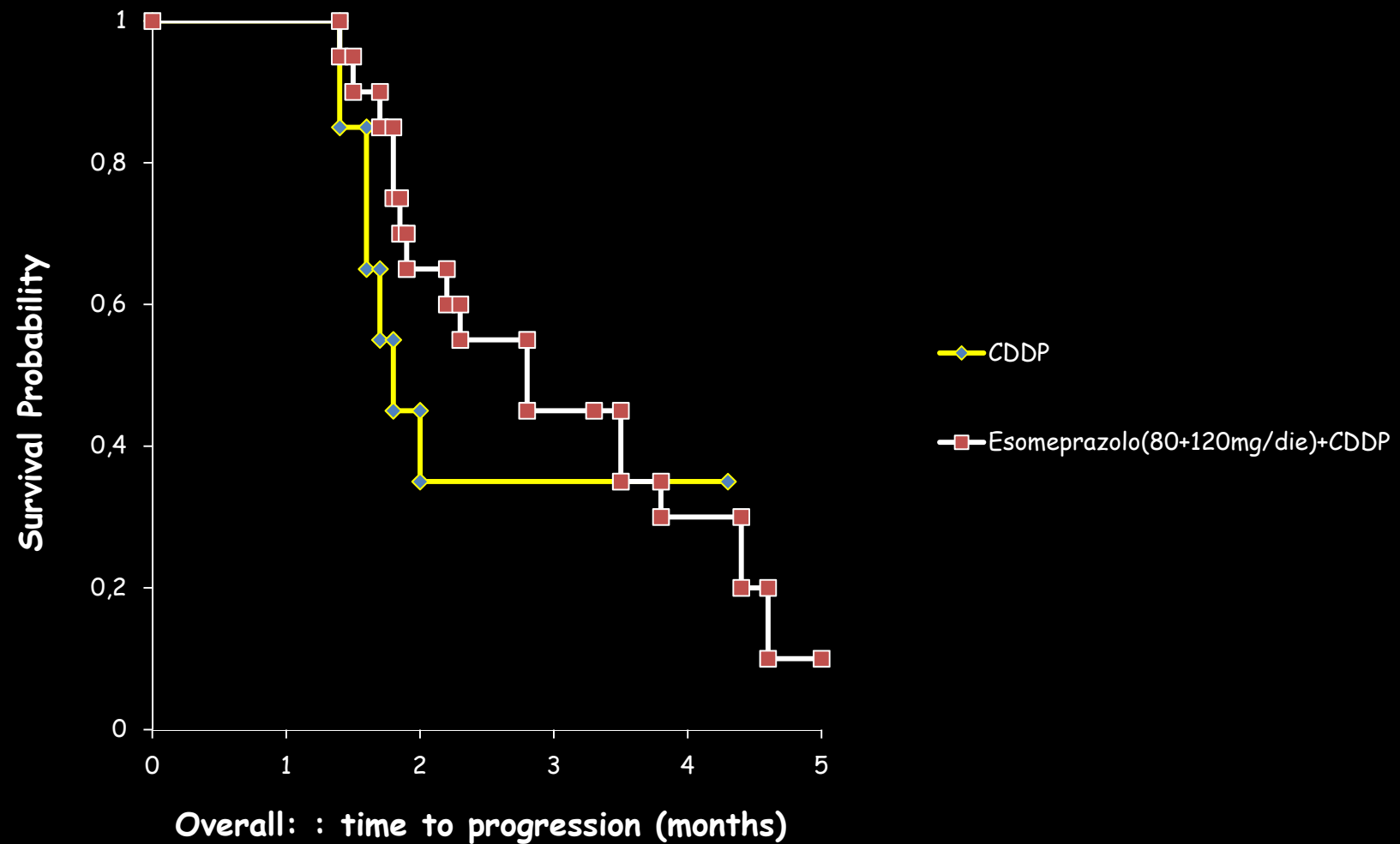
1. CISPLATIN ONLY (10 PATIENTS)
2. ESOMEPRAZOLE 80 MG THE TWO DAYS BEFORE CISPLATIN TREATMENT (10 PATIENTS)
3. ESOMEPRAZOLE 120 MG THE TWO DAYS BEFORE CISPLATIN TREATMENT (10 PATIENTS)

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



OSTEOSARCOMA

PPI - OSTEOSARCOMA STUDY PROTOCOL

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 ≤ 70 ml/min

Left ventricular ejection fraction $\leq 55\%$ or fractional shortening rate of the left ventricle $\leq 28\%$

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

Alkalinization: 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 alkalinization 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

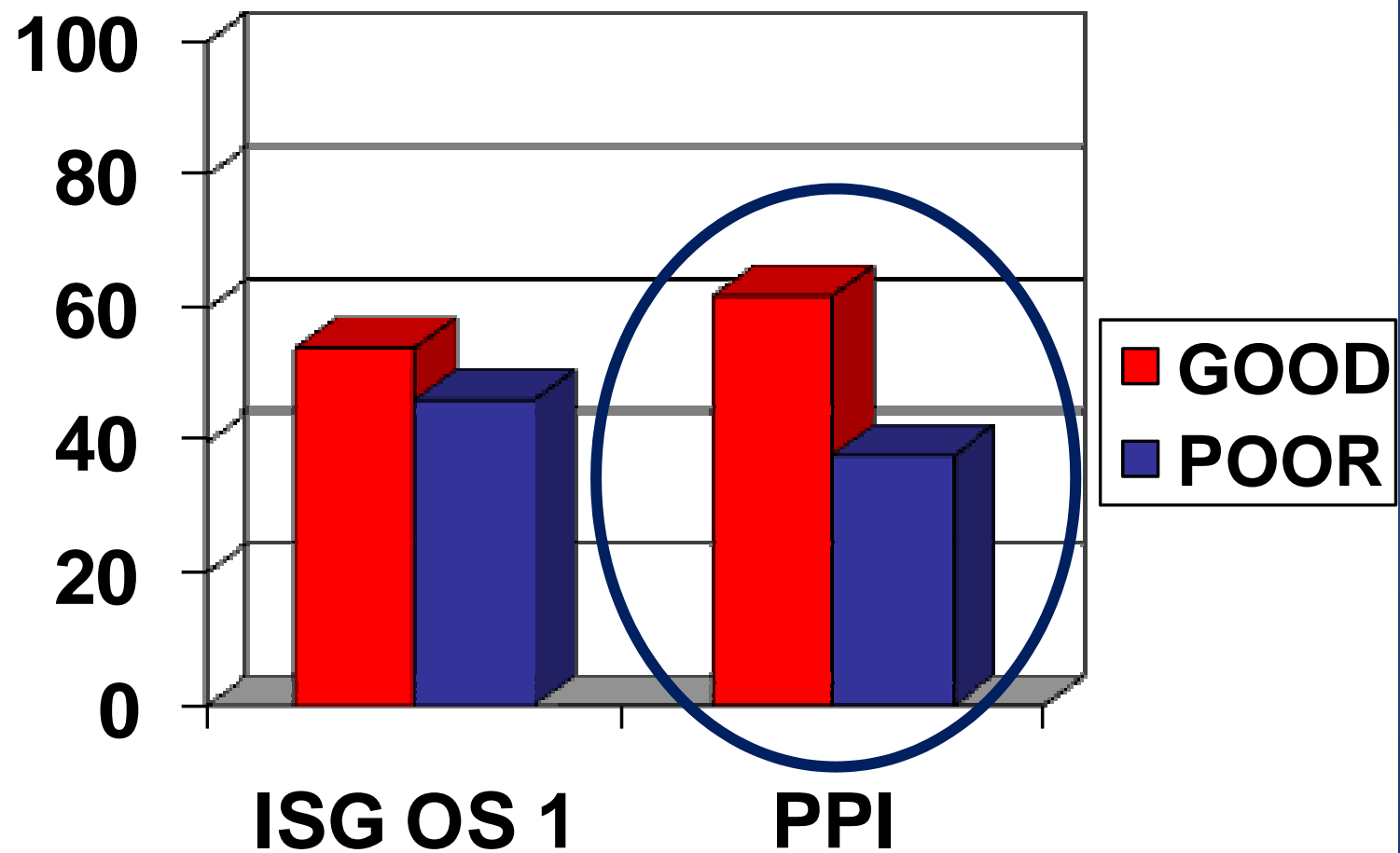
CDP is given before the administration of ADM

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



HISTOLOGY	NECROSIS	ISG OS 1	PPI
Osteoblastic	GR PR	38 (50%) 38 (50%)	33 (57%) 25 (43%)
Condroblastic	GR PR	3 (25%) 9 (75%)	6 (85%) 1(14,3%)
Haemorrhagic/ Fibroblastic	GR PR	14 (67%) 7 (33%)	7 (64%) 4 (36%)

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 Fudan
provided by: University

ClinicalTrials.gov
v 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**

**Spugnini E, Baldi A, Buglioni S, Carocci F, Milesi de Bazzichini
G, Betti G, Pantaleo I, Menicagli F, Citro G, Fais S**

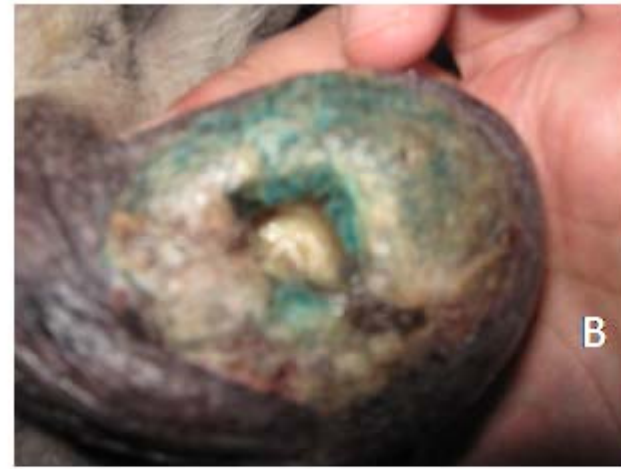
Modified Karnofsky's performance criteria - Grade Criteria

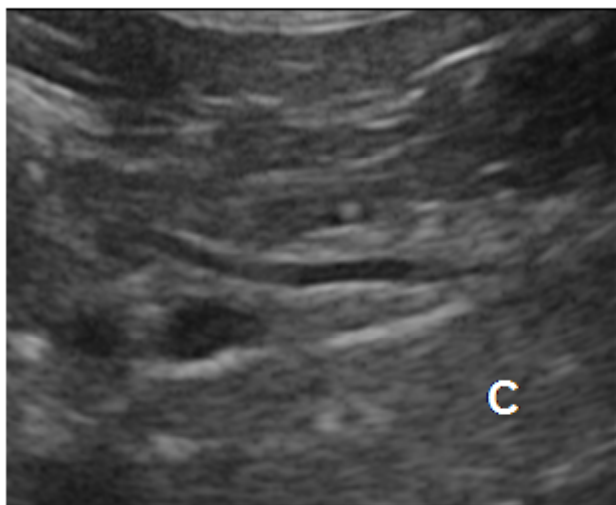
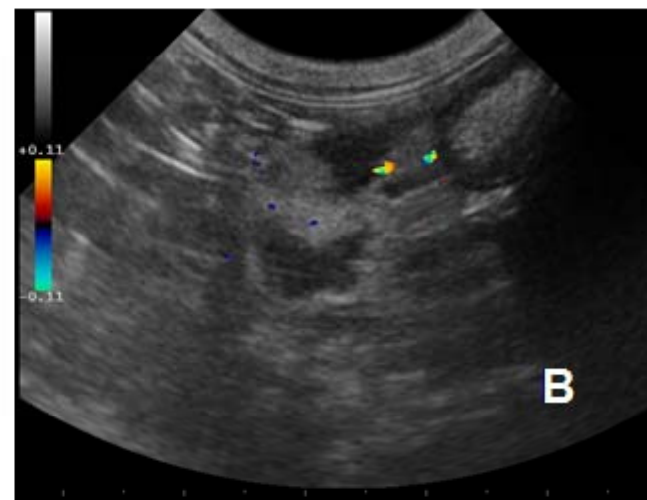
- 0 Fully active, performs at predisease level
- 1 Activity less than predisease level; able to function as acceptable pet
- 2 Severely compromised activity; ambulatory only to point of eating, sleeping, and consistently eliminating in acceptable areas.
- 3 Completely disabled; must be force fed; unable to defecate or urinate in acceptable areas
- 4 Dead

PATIENT	AGE	TUMOR	PREV. TREATMENT	THERAPY	OUTCOME (MONTHS)
GREAT DANE	10	HSA	SURGERY	METRONOMIC	CR 3
WHWT	8	LSA	MADISON	MOPP	CR 12
BULL MAST.	11	ALL	MADISON	MOPP	CR 3
WHWT	8	LSA	MADISON	MOPP	CR 8
ROTTW.	6	LSA	MADISON	MOPP	CR 3
BOXER	8	LSA	MADISON	MOPP	CR 7
BOXER	9	LSA	MADISON	MOPP	CR 5
DOGO	10	LSA	MADISON	MOPP	PR 3
WHWT	10	LSA	MADISON	MOPP	PD
BOXER	10	LSA	COP	MOPP	CR5+
LABRADOR	8	LSA	COP	MOPP	CR 5+
BULL DOG	10	SKIN LSA	MADISON	MITOXANTRONE	CR 5+
POODLE	12	LSA	COP	MOPP	DISCONTINUED
SETTER	14	ORAL SCC	MITOXANTRONE	MITOXANTRONE	DISCONTINUED
MIXED	11	NASAL CA	MITOXANTRONE	MITOXANTRONE	PR 3+
MIXED	10	OSA	BIOPSY	CLODRONATE	PR 7
MIXED	11	OSA	BIOPSY	CLODRONATE	PR 11
SCHNAUTZER	9	OSA	BIOPSY	CLODRONATE	DISCONTINUED
MIXED	15	TCC BLADDER	PIROXICAM	PIROXICAM	SD 7+
BEAGLE	12	TCC BLADDER	SURGERY	MITOXANTRONE	CR 2+
BOXER	10	MELANOMA	NA	CARBOPLATIN	CR 6
ROTTWEILER	5	MELANOMA	SURGERY	CARBOPLATIN	NED 5+
MIXED	10	MAMMARY CA	SURGERY	MITOXANTRONE	NED 5+
MIXED	10	ANAL SAC CA	CARBOPLATIN DOXORUBICIN	MITOXANTRONE	PR 9+
MIXED	11	FSA	SURGERY, ECT	MITOXANTRONE	NED 3+
MIXED	12	MAMMARY CA	SURGERY	MITOXANTRONE	NED 4+
HUSKY	13	MAMMARY CA.SA	SURGERY	MITOXANTRONE	NED 5+

Characteristics and outcome of **feline** patients treated with pump inhibitors and chemotherapy

PATIENT	AGE	TUMOR	PREV. TREATMENT	THERAPY	OUTCOME (MONTHS)
DSH	10	BREAST CARCINOMA	SURGERY	MITOXANTHRONE	PR 4
DSH	12	ORAL SCC	NA	MITOXANTHRONE	SD 2
DSH	10	ORAL SCC	SURGERY	MITOXANTHRONE	NED 5+
DSH	12	ORAL SCC	BIOPSY	MITOXANTHRONE	SD 6
DSH	12	LSA	MOPP	MOPP	CR 4+
DSH	7	FSA	SURGERY, ECT, CARBOPLATIN	MITOXANTHRONE	PD





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

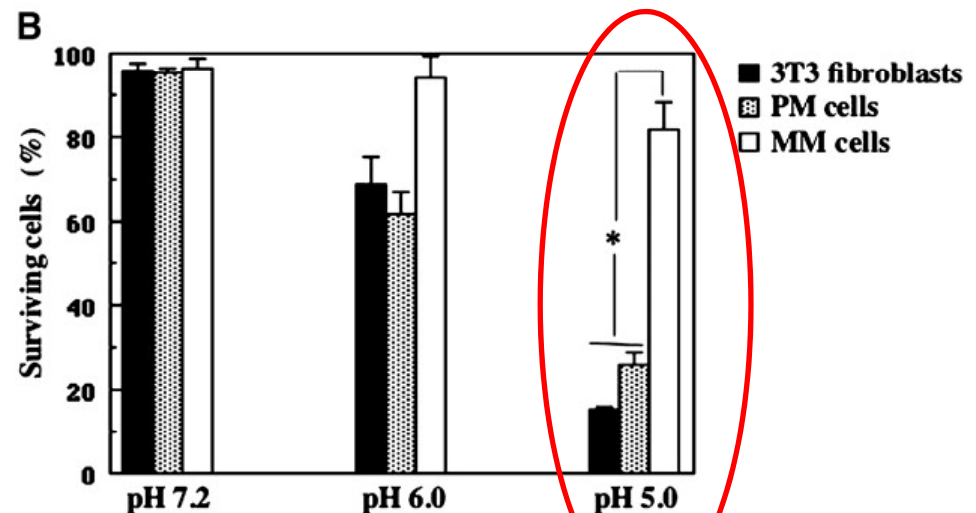
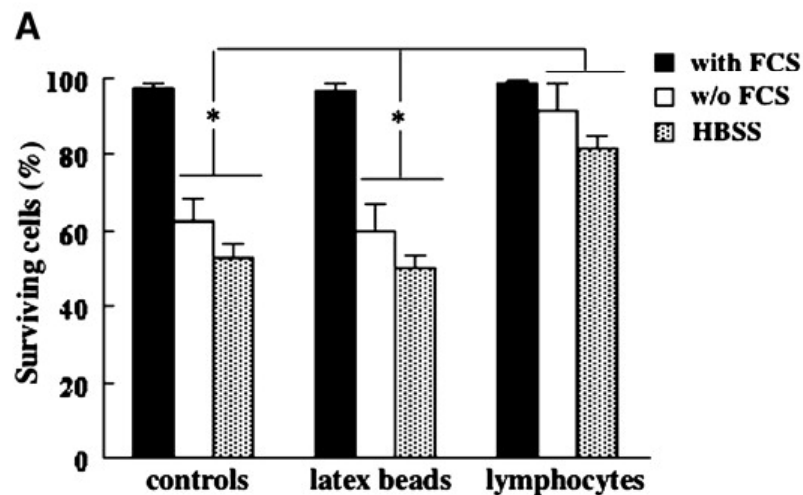
WE HAD A SUITABLE MODEL

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

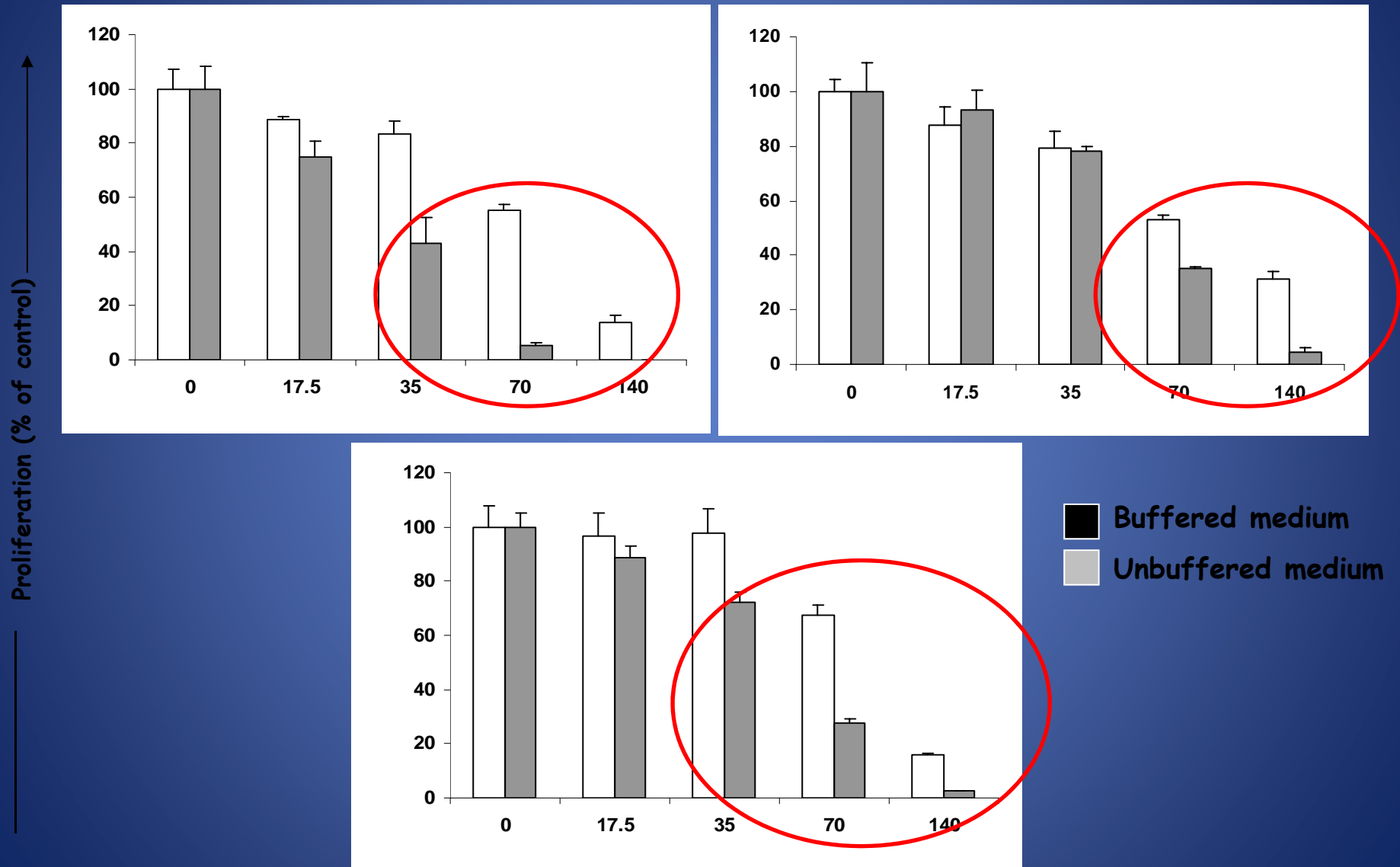
2. We knew also that malignant cells may remain alive in culture even at pH of 5 (Lugini et al Cancer Res 2006)

THIS CONDITION ALLOWS TO TEST PPI ACTIVATION IN A pH-DEPENDENT MANNER

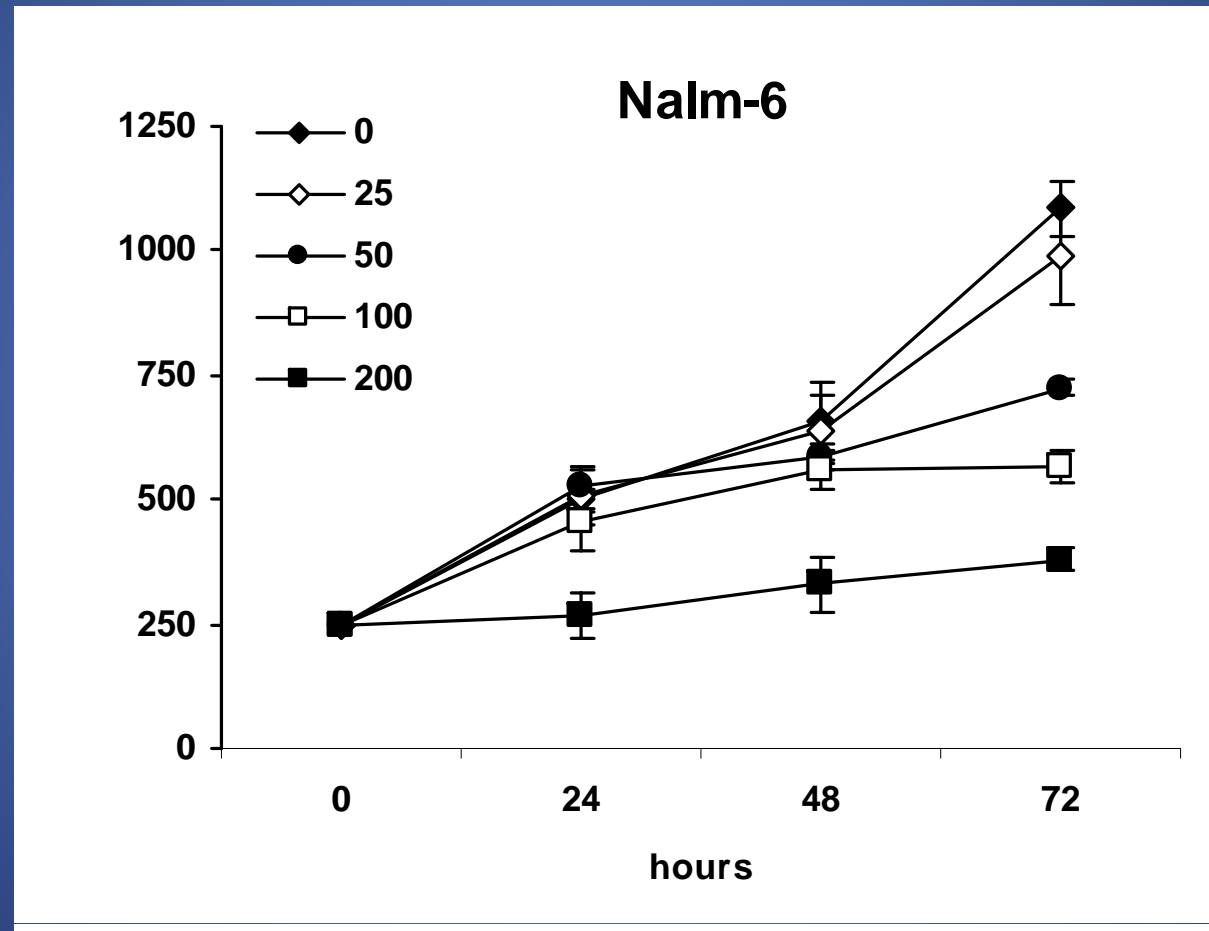


B CELL TUMORS

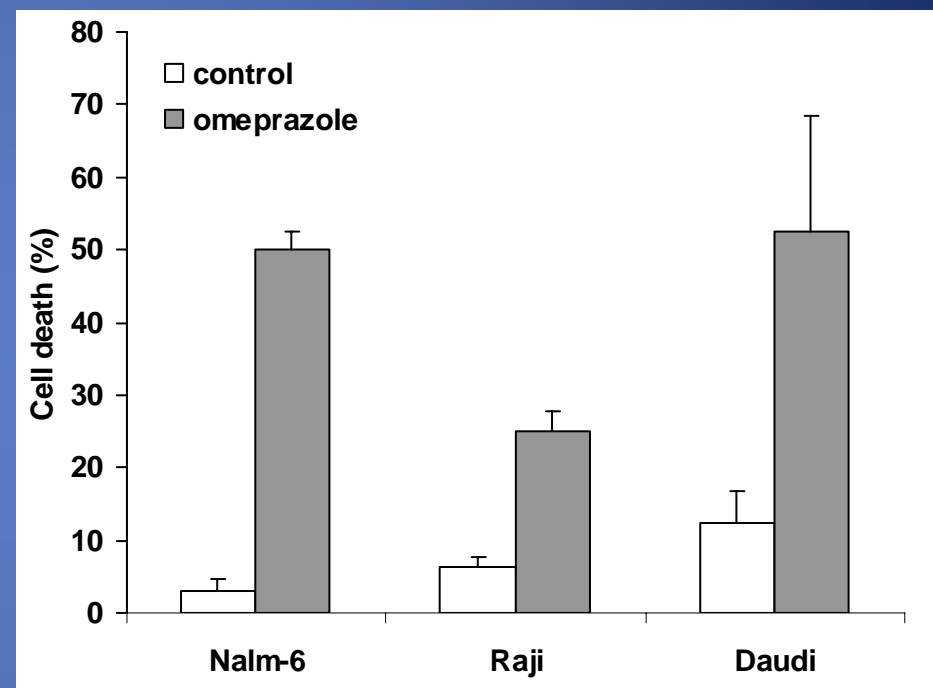
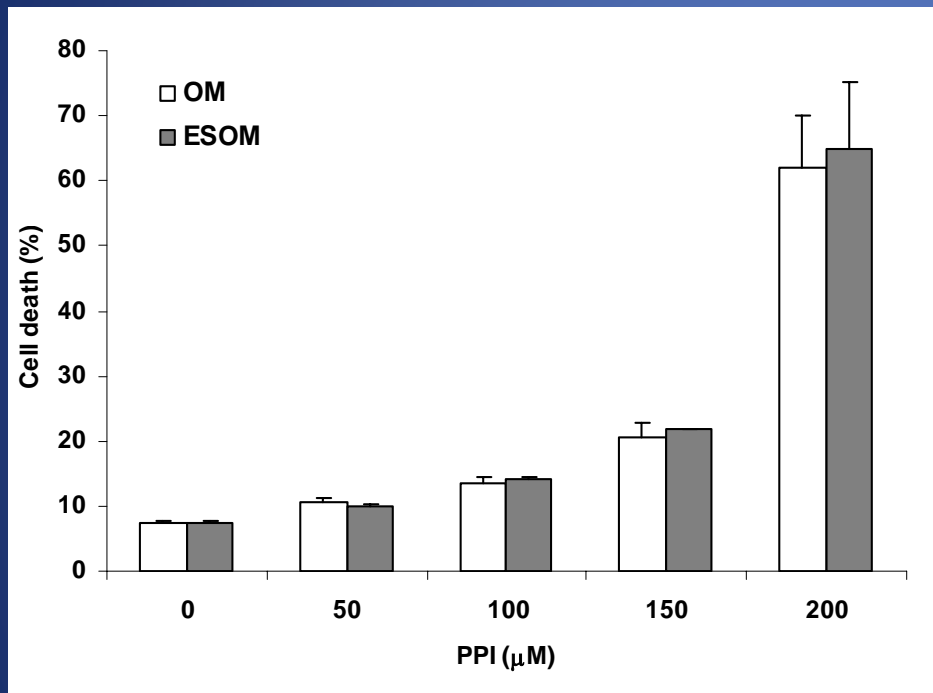
PPI inhibit proliferation of human B cell tumors



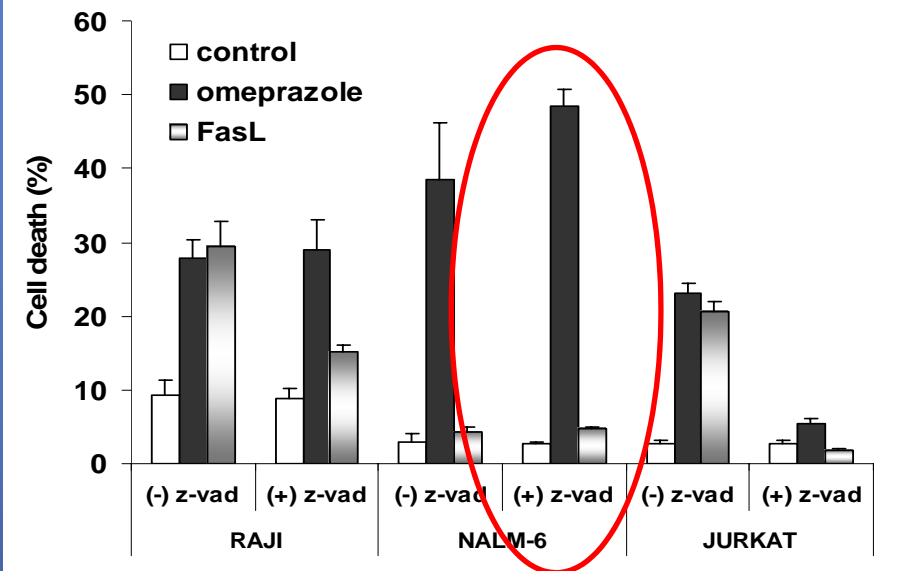
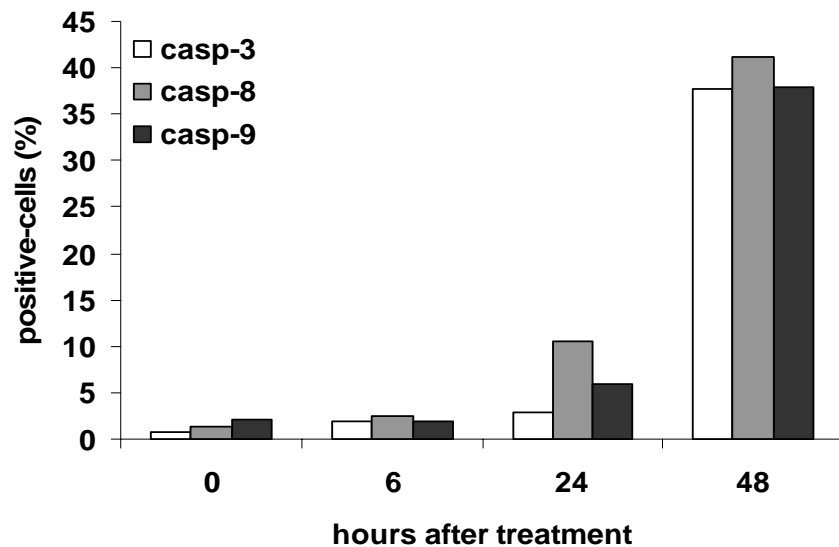
PPI (mM) decrease cell viability in a dose-dependent manner

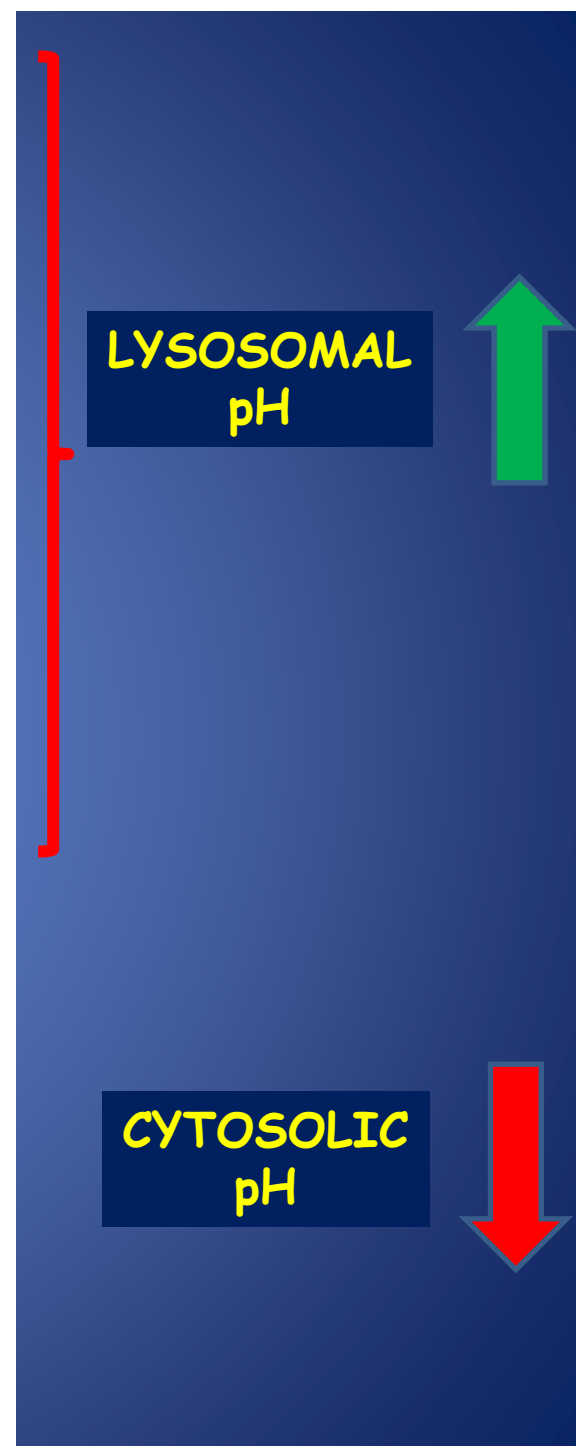
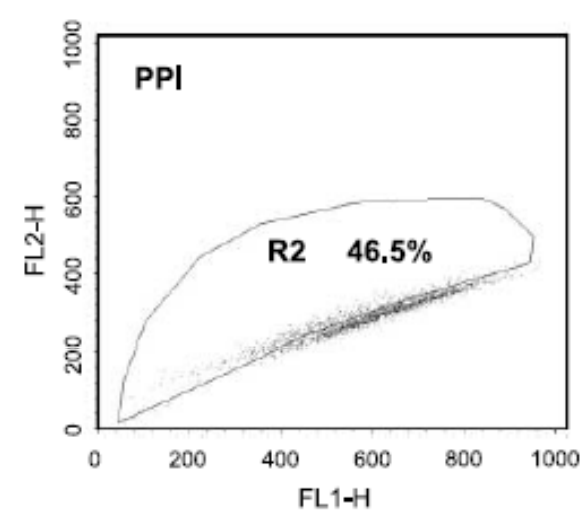
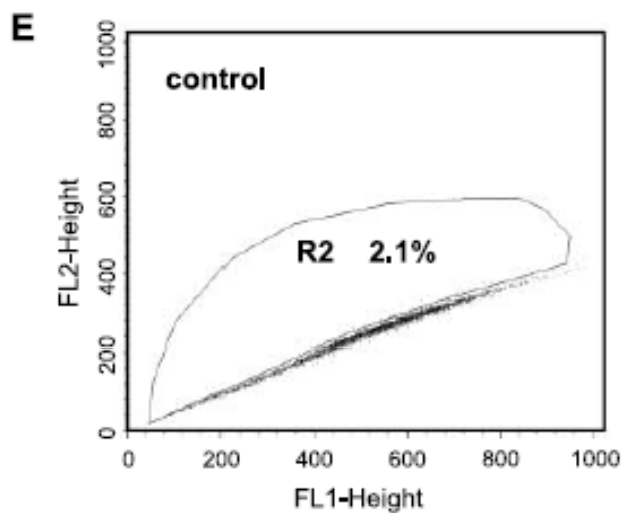
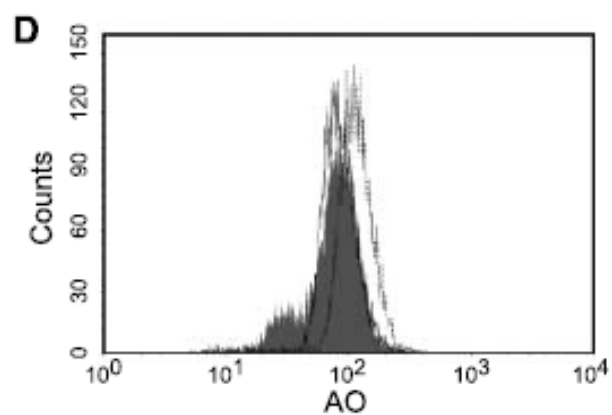
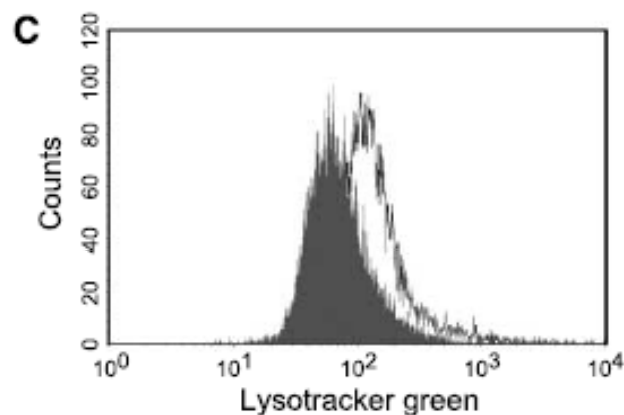
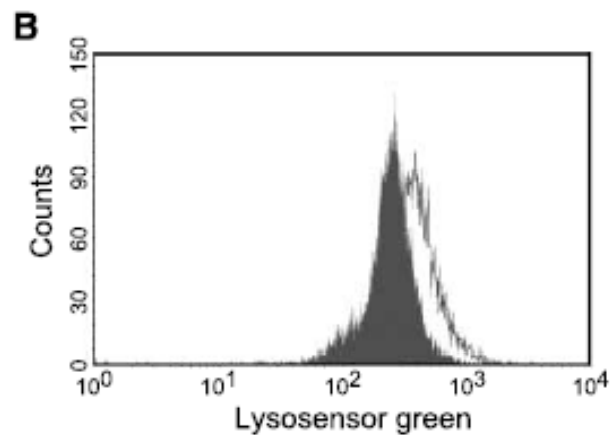
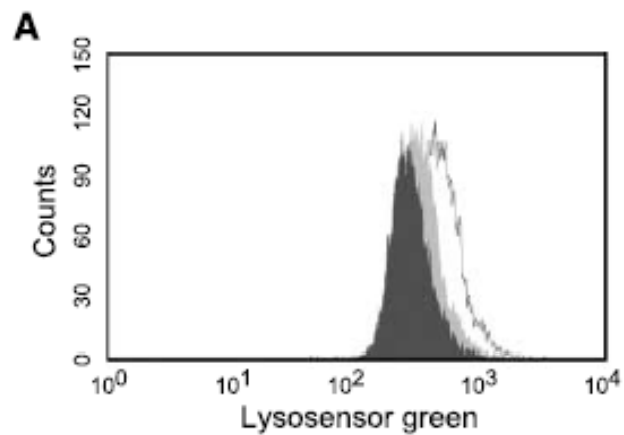


PPI induce cell death



PPI induce activation of caspases but it is not instrumental to cell death





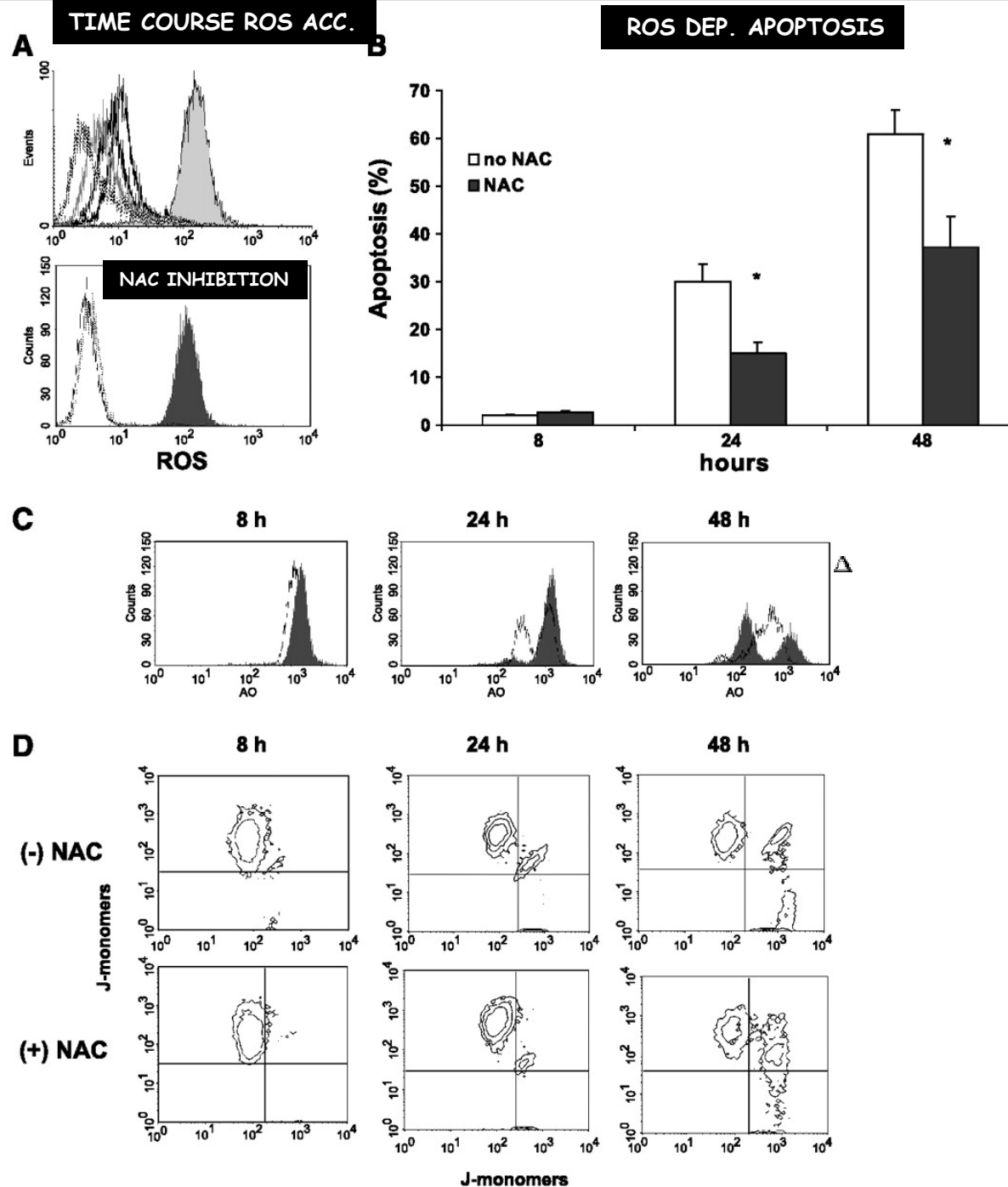
ROS

A ROLE OF ROS

Ros accumulation and Apoptosis

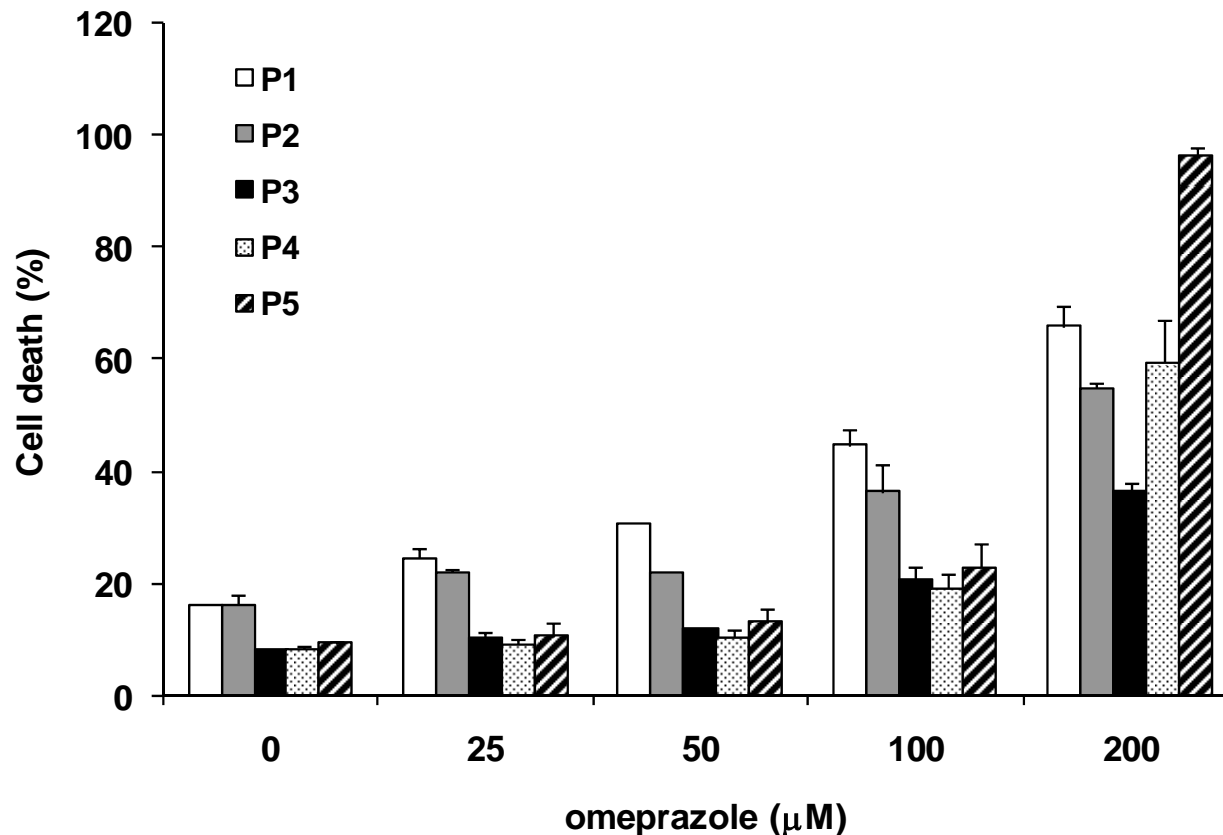
ROS-dependent Lysosomal membrane permeabilization

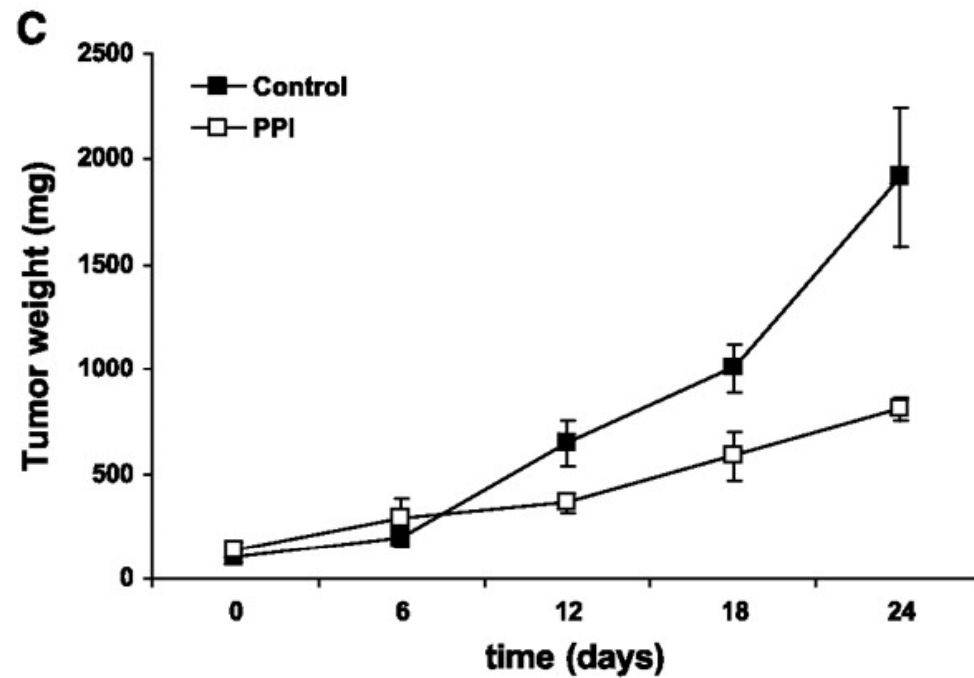
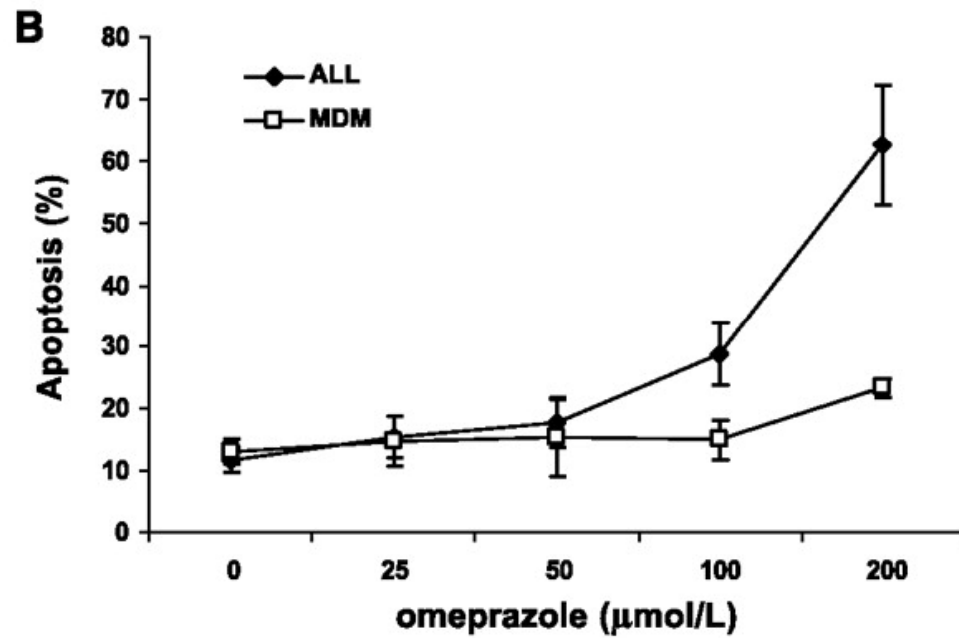
ROS-dependent Mitochondrial membrane depolarization



THE PROOF OF CONCEPT

PPI induce cell death in human
pre-B ALL BM BLASTS





TUMOR SPECIFIC

IN VIVO

CONCLUSIONS

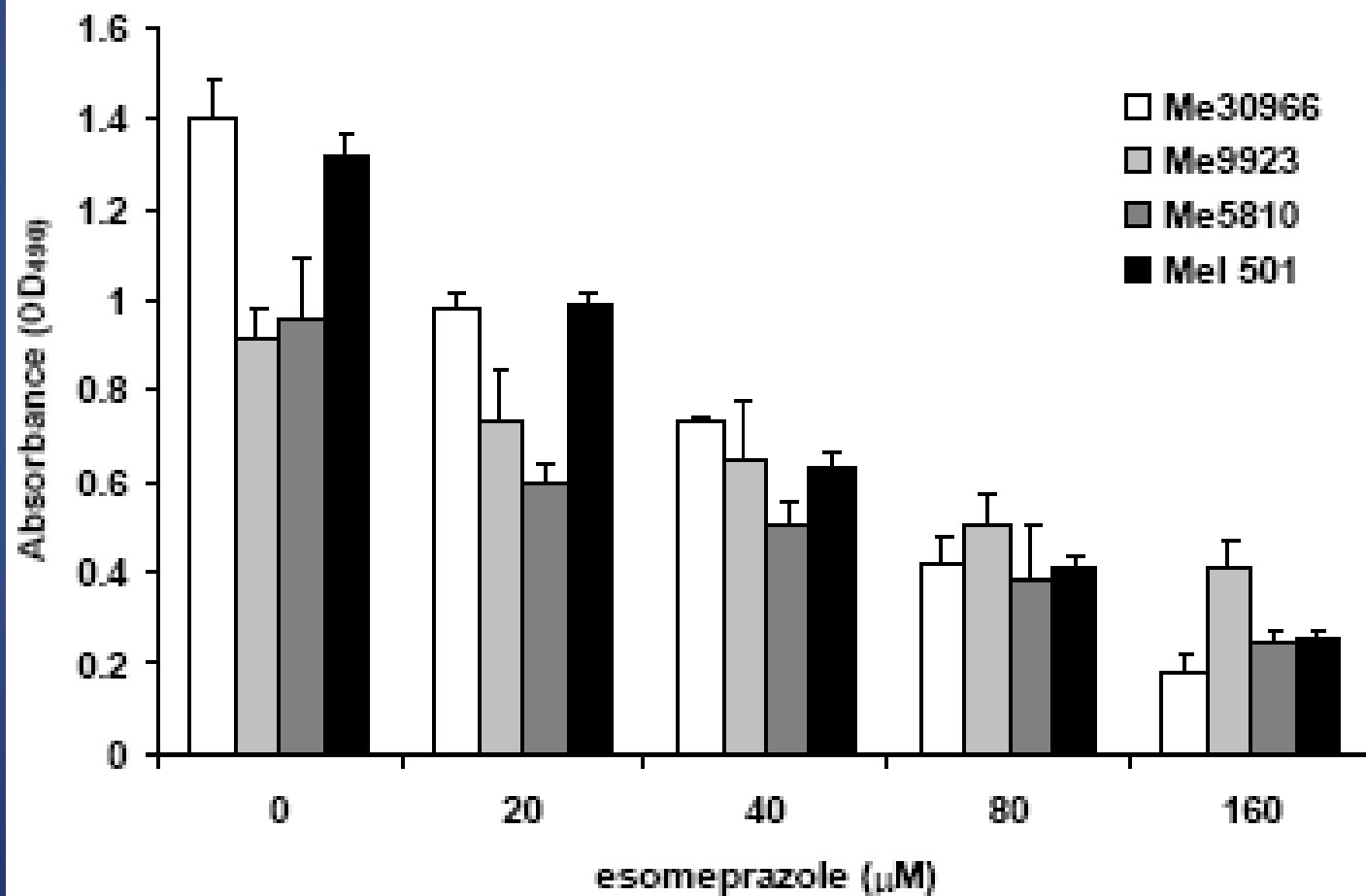
- ❑ PPI inhibit proliferation of human B cell lines
- ❑ PPI are cytotoxic to human B cell lines and pre-B ALL blasts
- ❑ PPI effect is potentiated by acidic conditions (tumor pH is acidic in vivo)
- ❑ PPI cause early destabilization of the lysosomal compartment and depolarization of MMP and cyt C accumulation
- ❑ 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

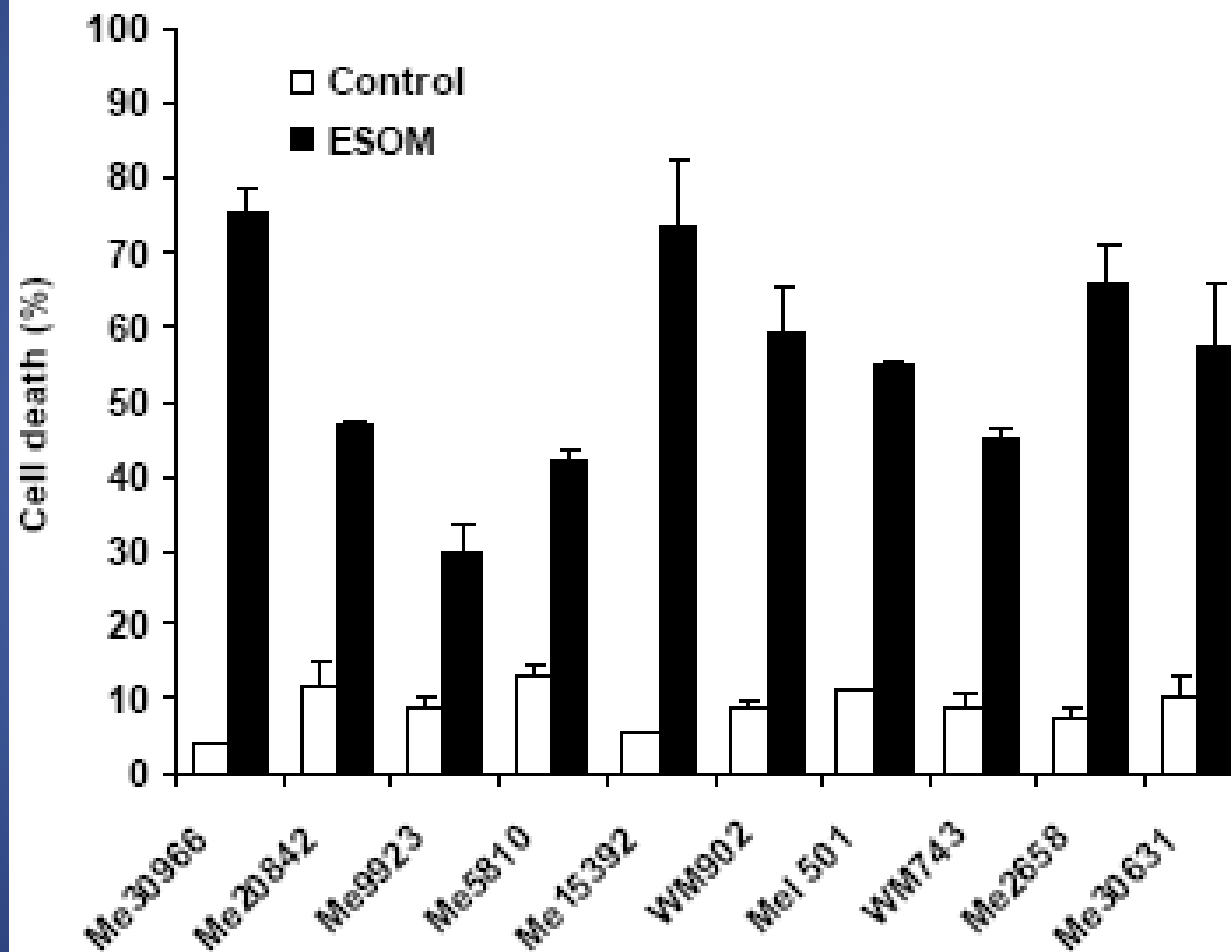
The expression of V-ATPase subunit a was significantly higher in metastatic cells (111±13 MFI) as compared to primary cells (72±10 MFI, P < 0.05)

Cell line	IC ₅₀ (μM) pH 7.4	IC ₅₀ (μM) Unbuffered	IC ₅₀ (μM) pH 6.0	V-ATPase Sub A	V-ATPase Sub a
Me30966 (M)	112±13	55±4	21±2	33±2	84±4
Me20842 (P)	130±10	95±9	62±6	27±2	41±3
Me9923 (P)	85±9	71±8	63±6	31±2	60±3
Me5810 (P)	142±12	95±8	39±6	38±3	91±5
Me15392 (M)	117±12	83±7	30±4	74±5	145±9
WM902 (P)	124±15	103±8	95±11	35±2	79±4
Me1501 (M)	74±8	50±4	28±3	58±3	140±9
WM793 (P)	173±18	106±11	95±9	28±1	91±4
Me2658 (M)	52±4	25±2	14±2	23±1	90±5
Me30631 (M)	57±6	50±8	31±4	43±3	95±4
Mean IC ₅₀	107±12	73±8	48±9	39±5	92±10

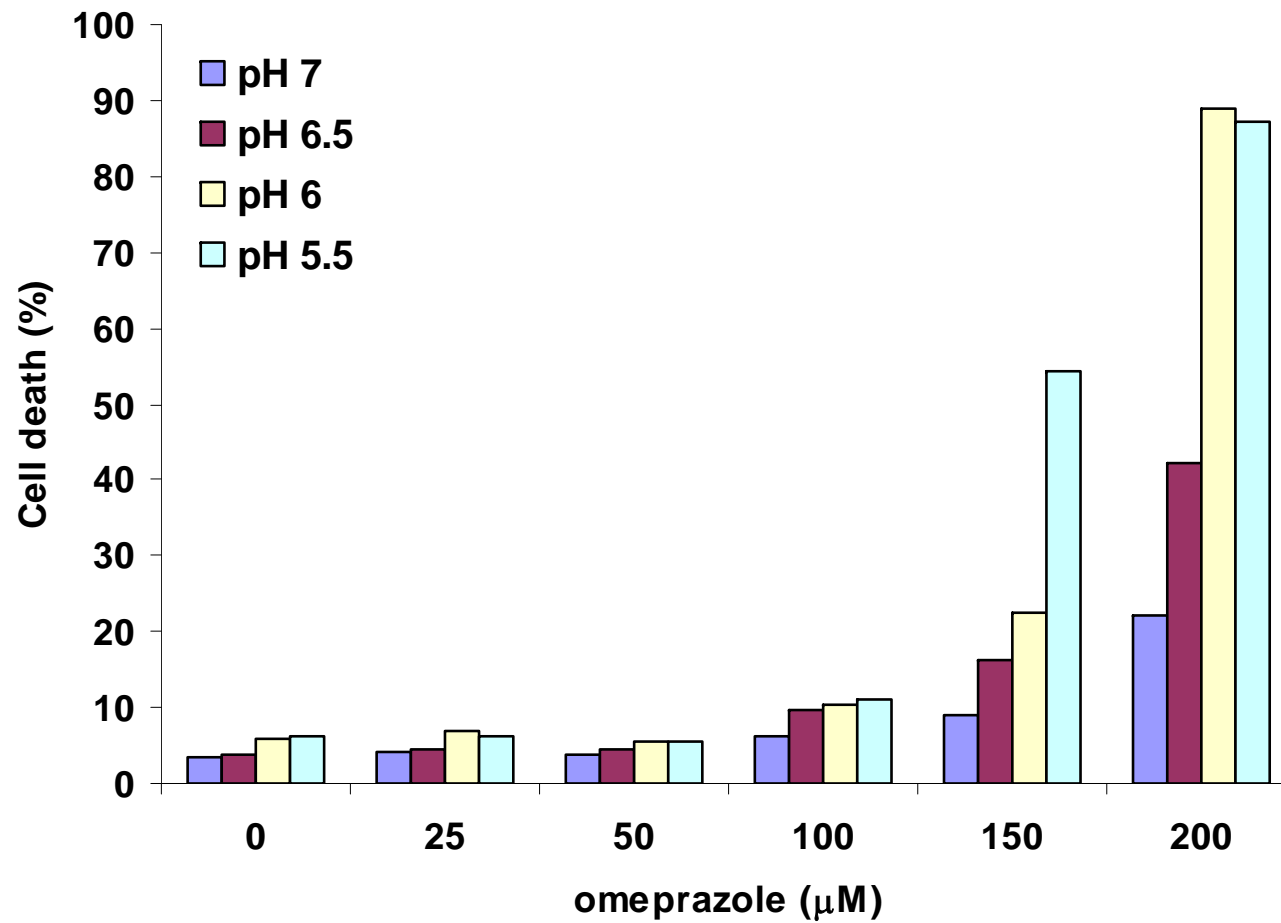
CELL VIABILITY



EXTREMELY CYTOTOXIC FOR METASTATIC MELANOMA

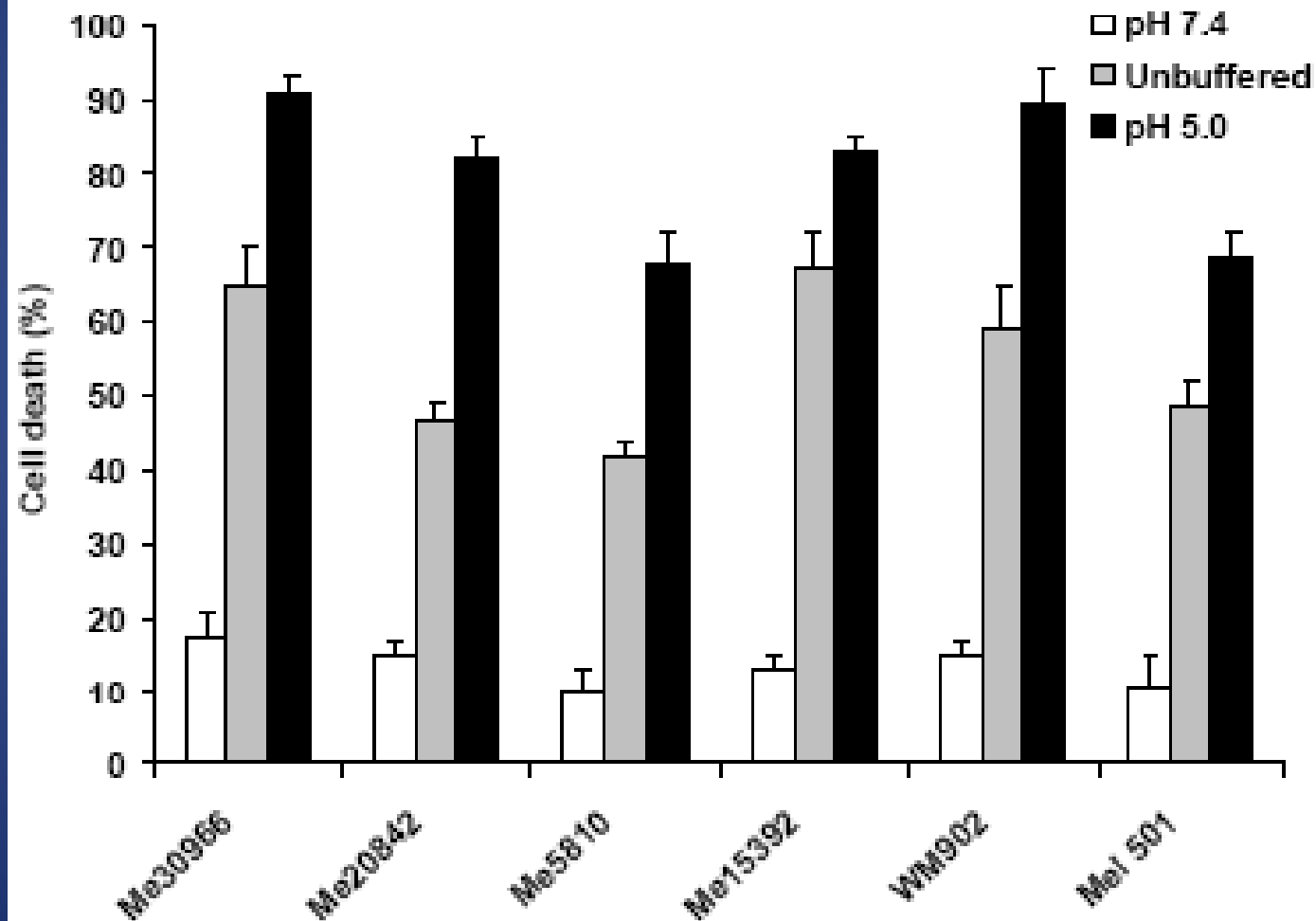


pH DEPENDENCY

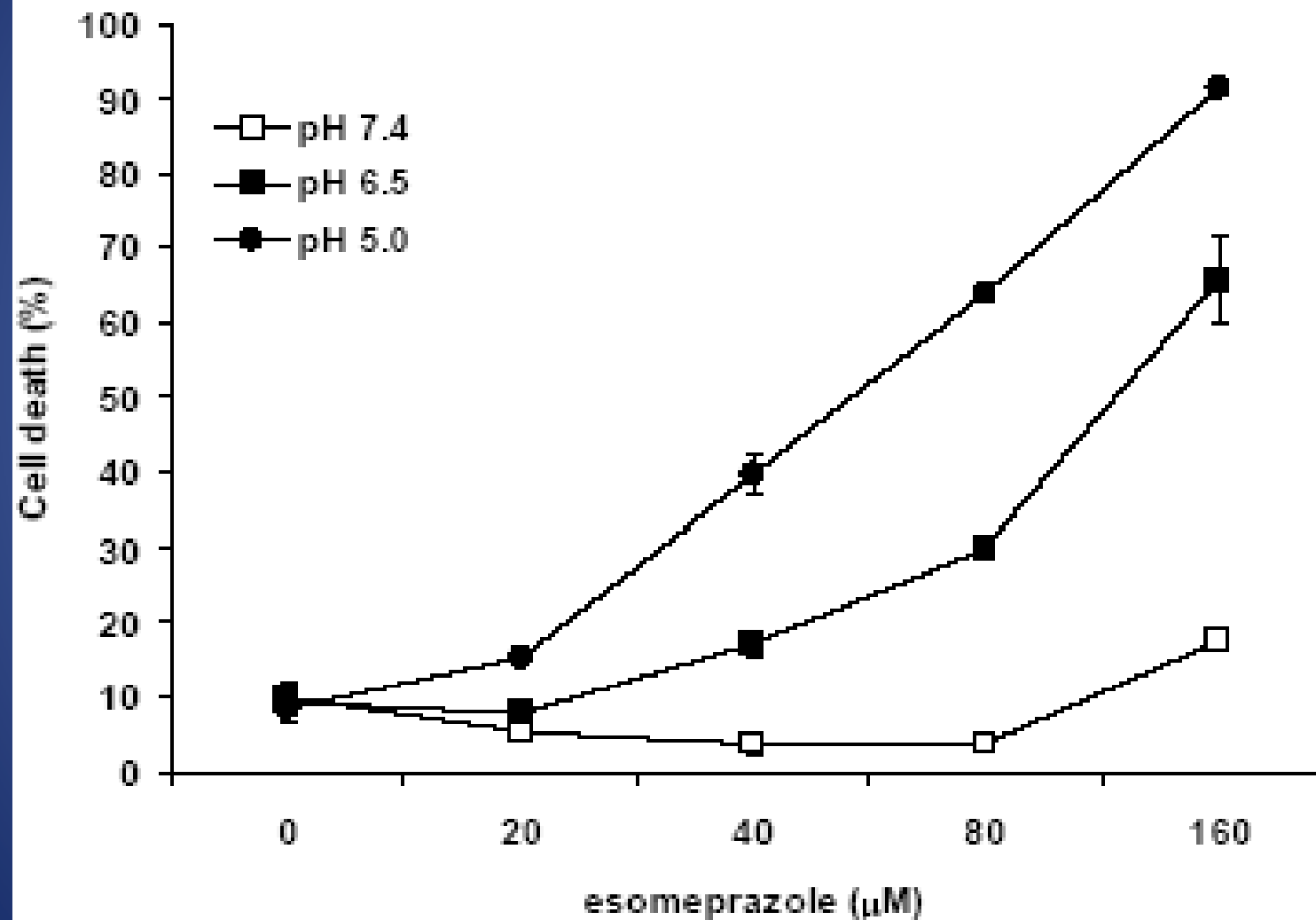


Melanoma M1

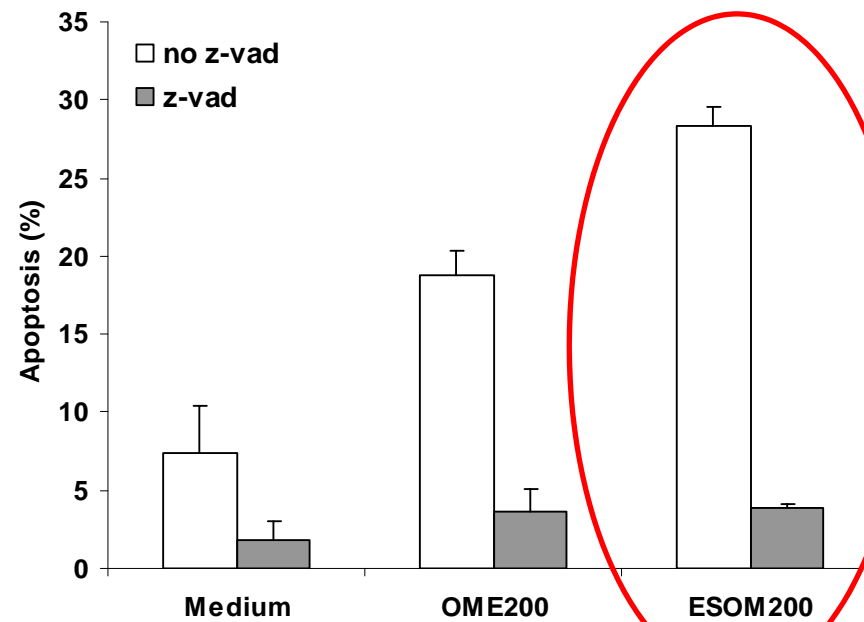
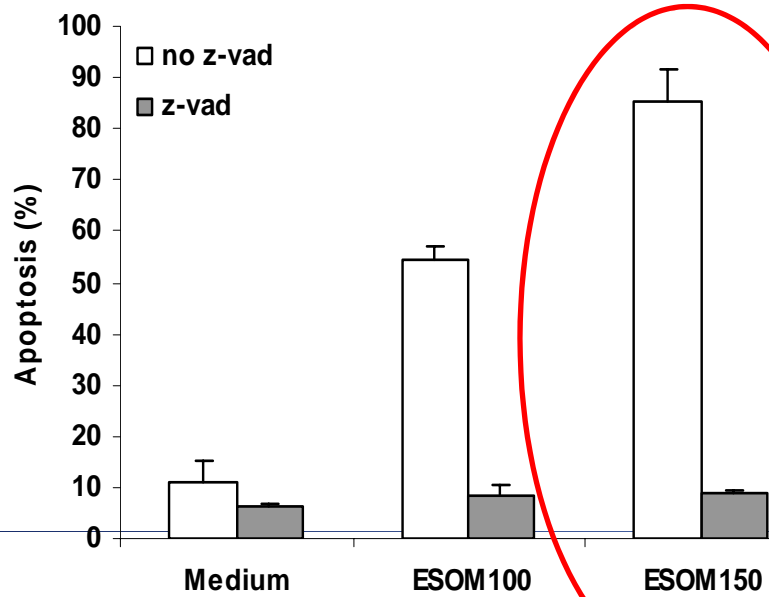
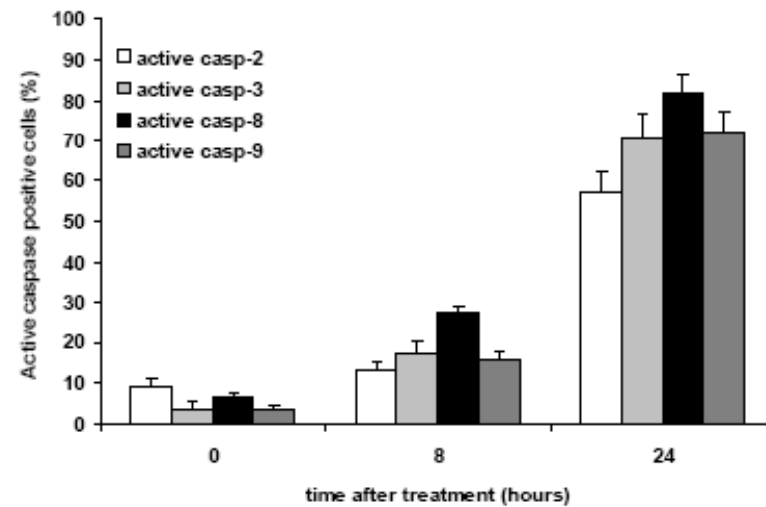
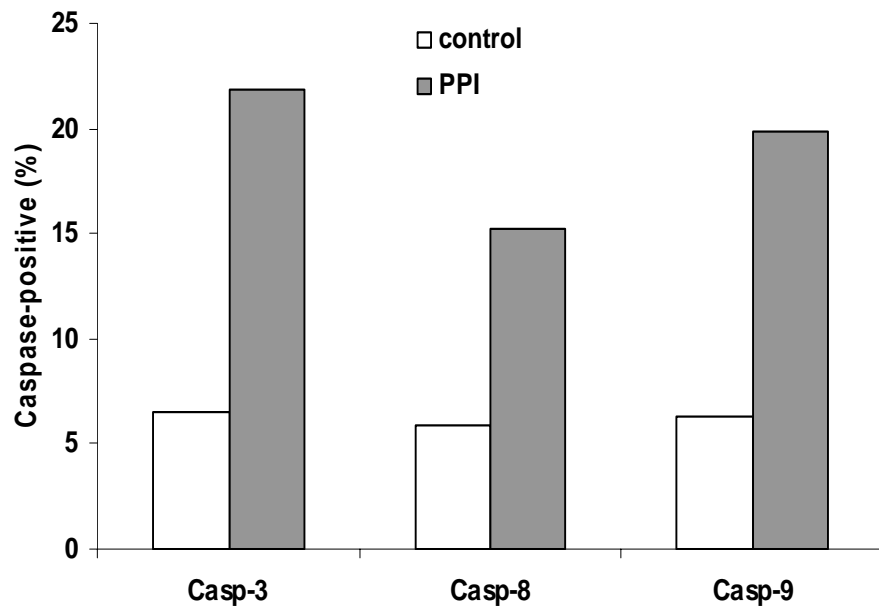
pH DEPENDENCY



DOSE AND pH DEPENDENCY



CASPASES

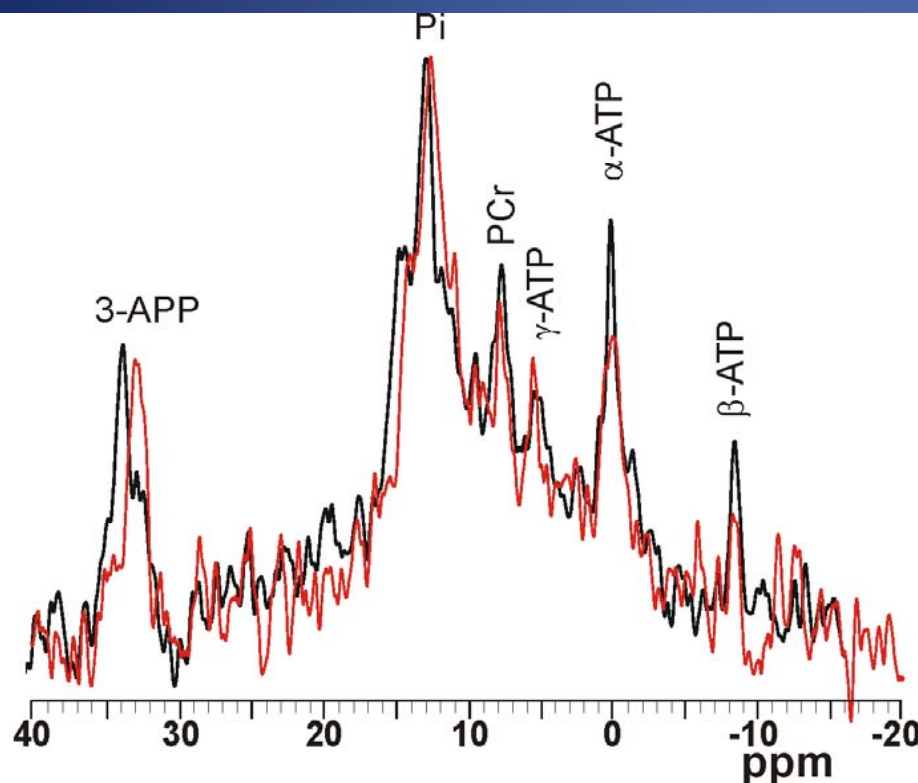


IN VIVO

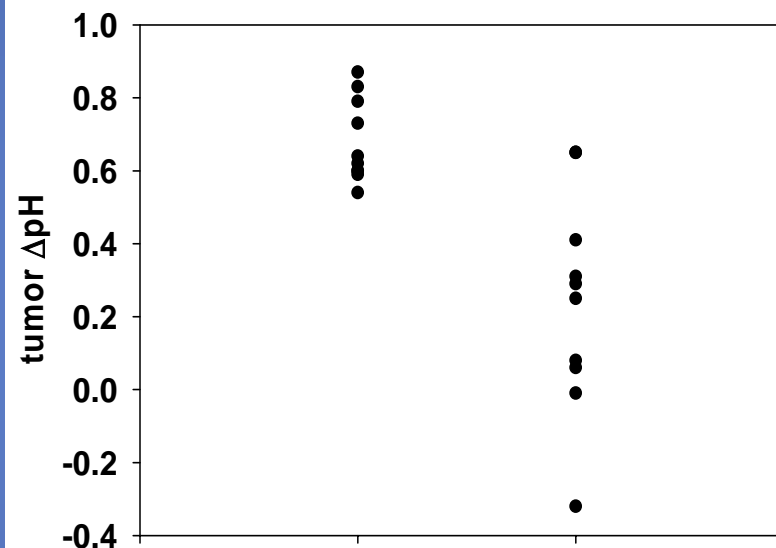
In vivo magnetic resonance spectroscopy (MRS)

- 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



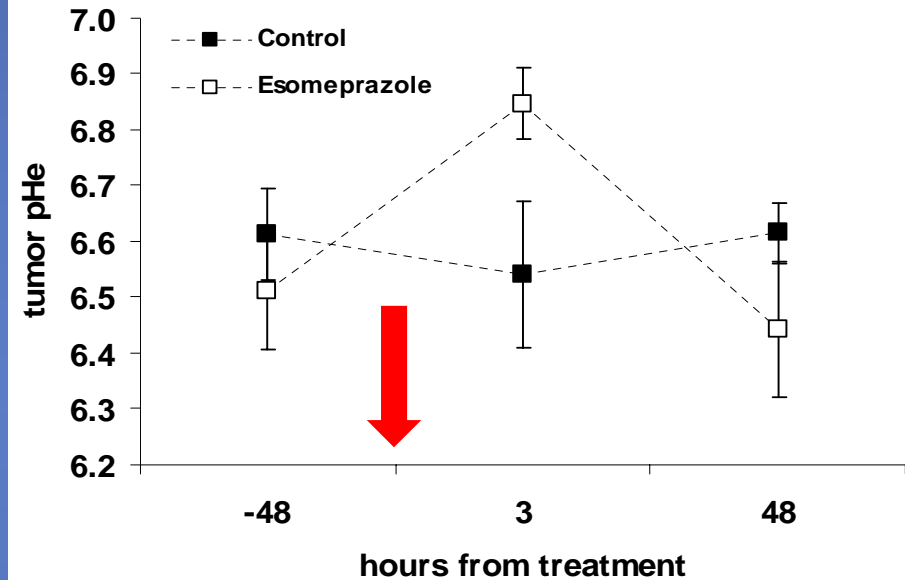
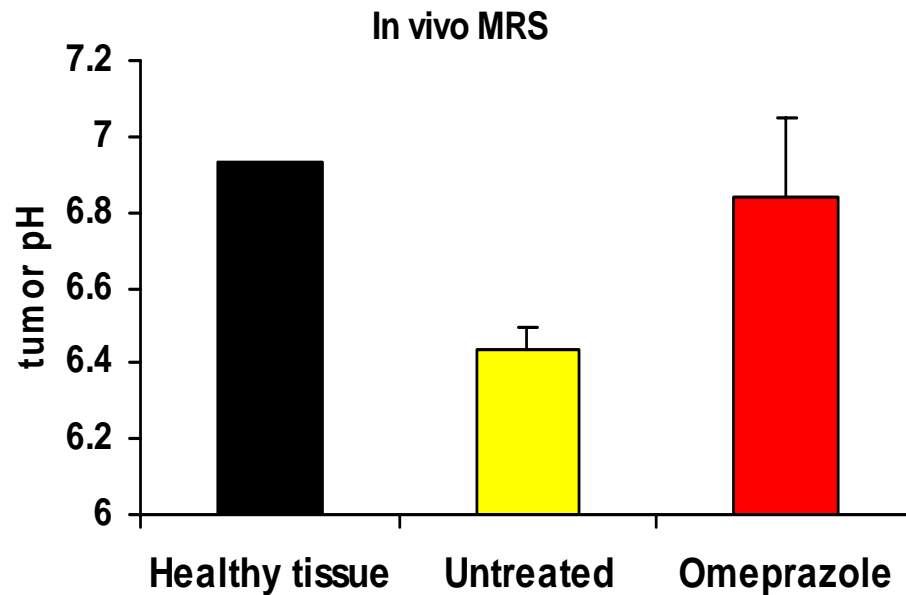
pH gradient



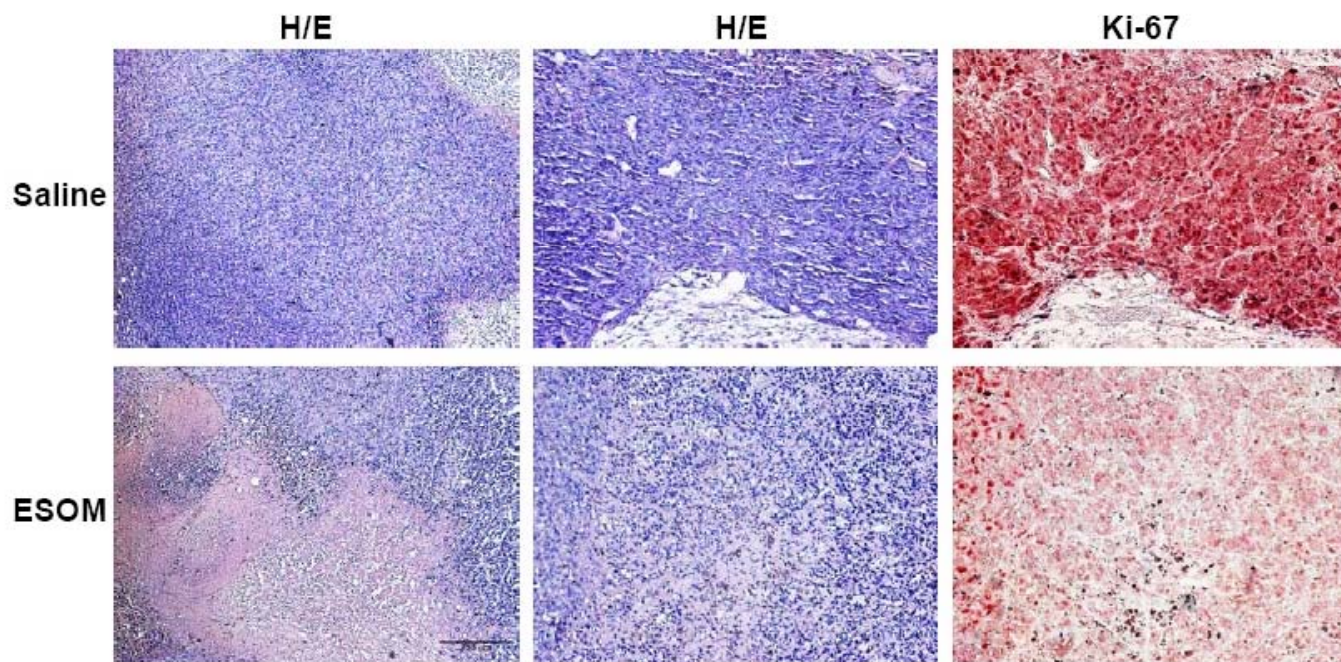
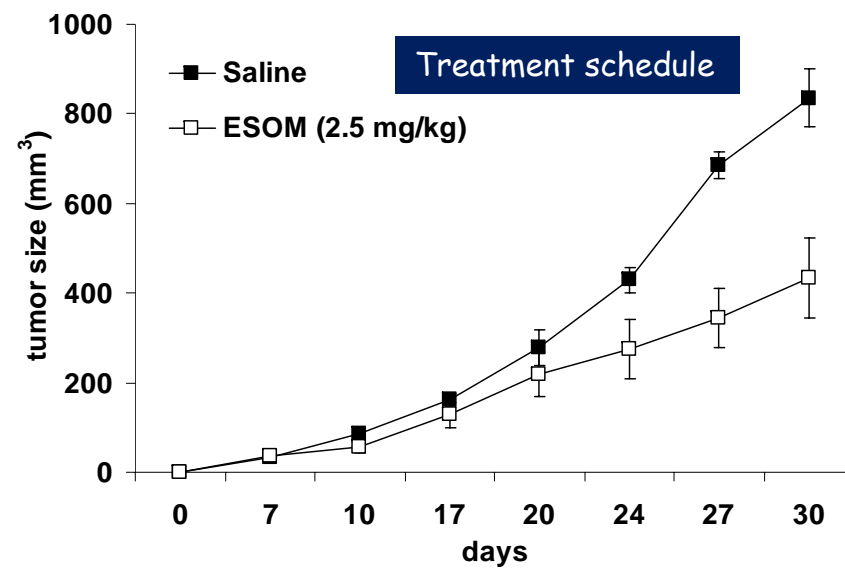
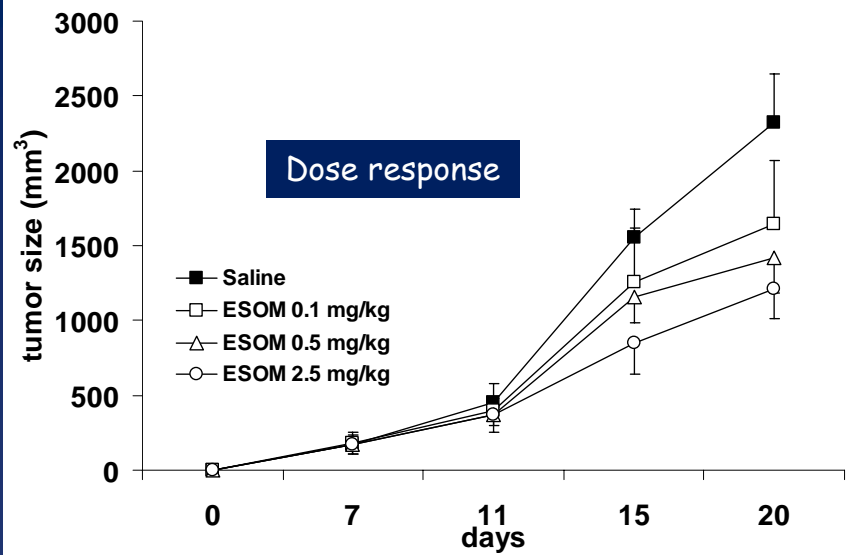
Acidic → Alkaline

	Controls (n=10)	PPI 2.5 mg/kg (n=5)	PPI 62.5 mg/kg (n=4)
pHe	6.42 ± 0.20	6.67 ± 0.20	6.78 ± 0.23
pHi	7.10 ± 0.23	6.91 ± 0.21	6.80 ± 0.30
DpH	0.68 ± 0.11	0.24 ± 0.28	0.03 ± 0.31

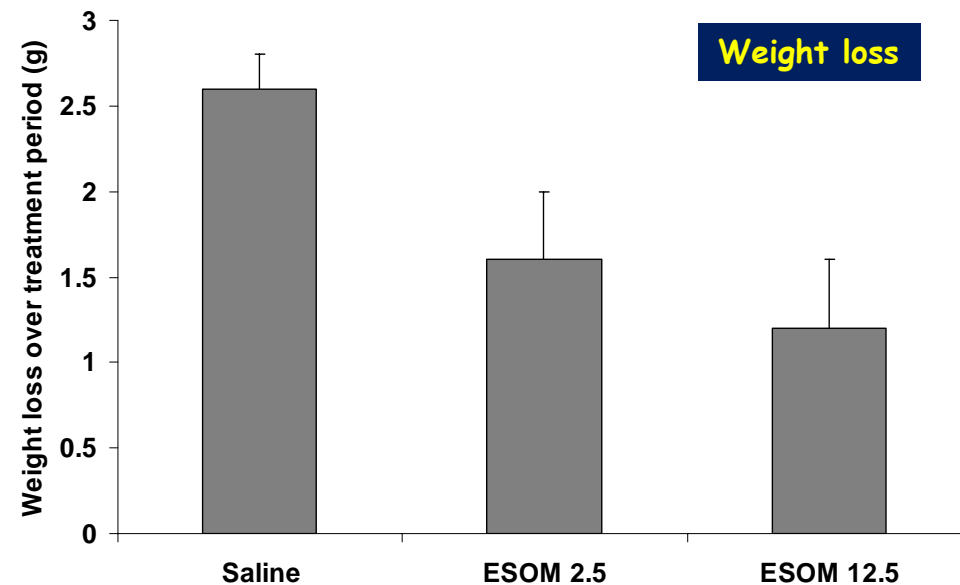
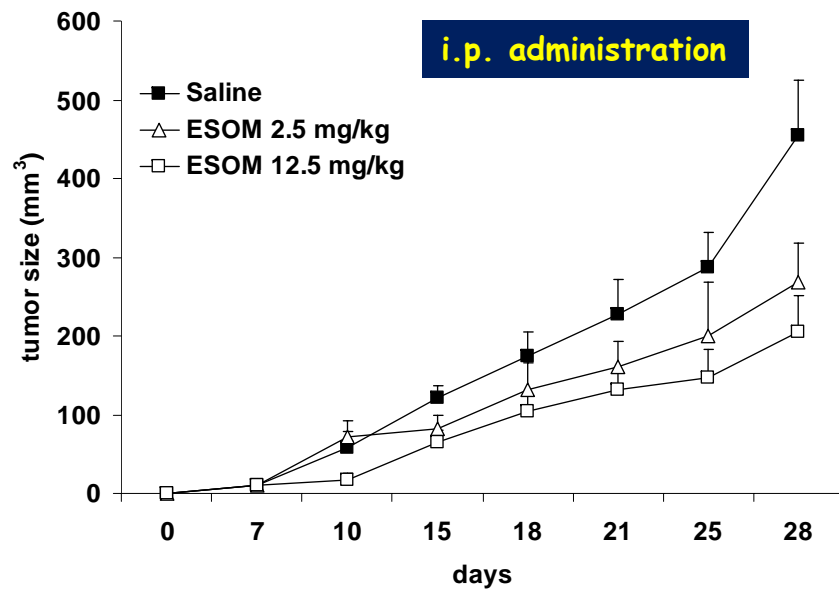
PPI alter tumor pH in vivo



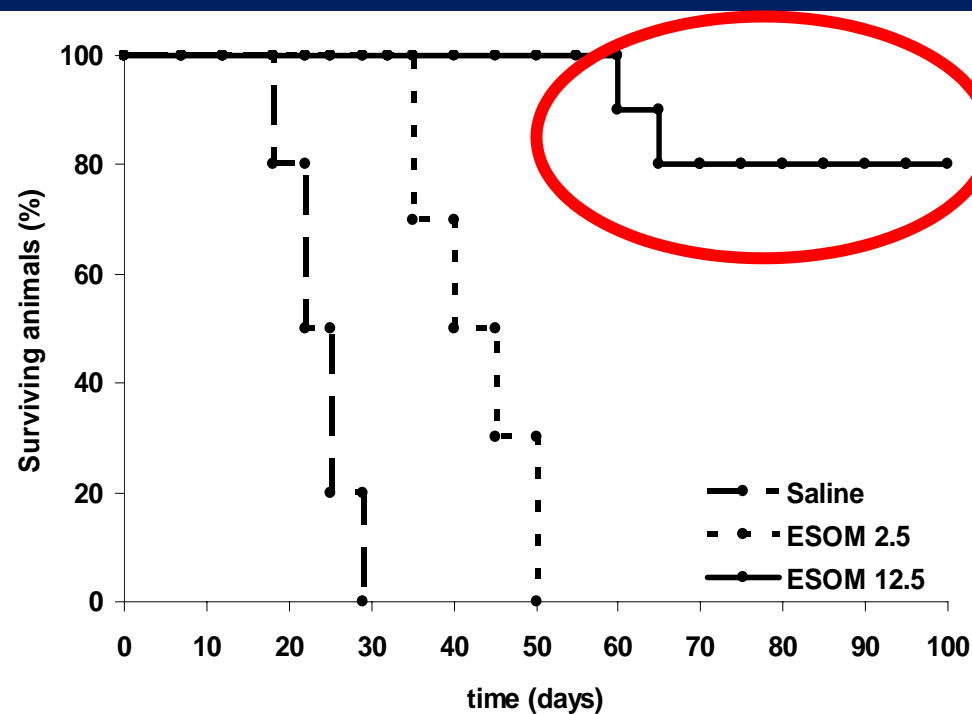
A REVERSIBLE EFFECT



NECROTIC MASS



dramatically improves animal survival



INDEPENDENT OF MAJOR MUTATIONS

Cell line	Protein expression						Mutational analysis					
	pAKT	AKT	pERK	ERK	PTEN	p53	BRAF	NRAS	PTEN	p16	p14	p53
Me30966	++	+	++	+	-	++	V600E	wt	C105fsX112	HD (exon 1 α , 2, 3)	HD (exon 2)	R175H
Me20842	-	+	+	+	+	+	L597S	wt	wt	wt/+	wt/+	G187S
Me9923	+	+	+	+	+	++	wt	G12S	wt	wt/- *	wt/+	wt
Me5810	+	+	++	+	-	+	wt	Q61R	wt	wt/-	wt/- *	wt
Me15392	-	+	-	+	+	++	V600E	wt	wt	HD (exon 1 α , 2, 3)	HD (exon 1 β , 2)	wt
Me1501	-	+	++	+	-	+	wt	G10D ^a	ND	HD (exon 1 α , 2, 3)	HD (exon 1 β , 2)	wt
Me30631	+	+	+	+	-	+	wt	wt	wt	HD (exon 1 α , 2, 3)	HD (exon 1 α , 2, 3)	wt
WM902	+	+	+	+	+	++	V600E	wt	ND	A60fs	G75fs	Y220C
WM793	-	+	++	+	+	+	V600E	wt	ND	wt	wt	wt
Me2658	-	+	++	+	+	++	V600E	ND	ND	HD (exon 1 α , 2, 3)	HD (exon 1 β , 2)	wt

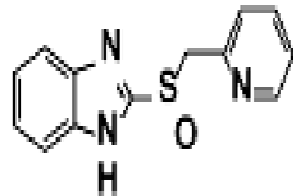
HD, homozygosis deletion; wt, wild-type gene; ND, not done; -, absence of protein; +, presence of protein; ++, protein overexpression; *, absence of mRNA.

PPI AS ANTITUMORAL AGENTS

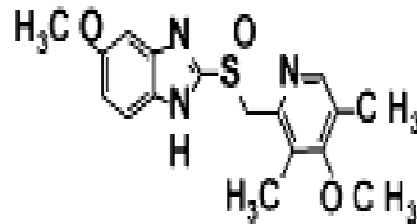
- PPI show a cytotoxic effect towards a variety of human tumor cells
- PPI induce a non-conventional cell death, with different mechanism depending on the tumor histology
- PPI induce marked inhibition of human tumor growth and increase of survival
- PPI target the tumor site in vivo and alter tumor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

PPI ARE THEY EQUIVALENT
COMPOUNDS

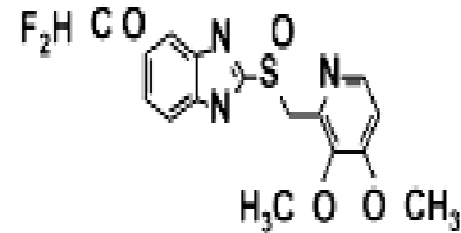
Benzimidazoles



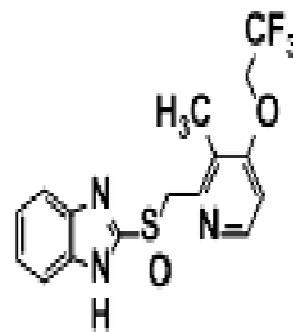
Timoprazole



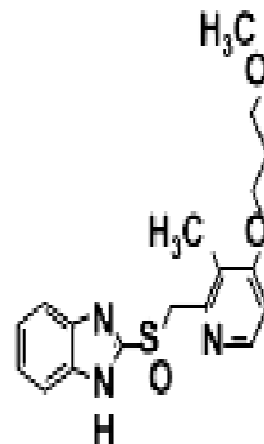
Omeprazole



Pantoprazole

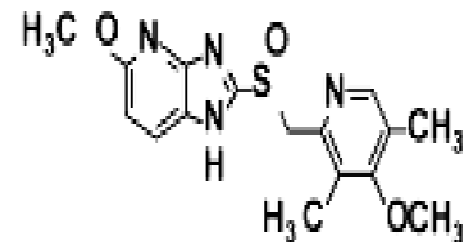


Lansoprazole



Rabeprazole

Imidazopyridine



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

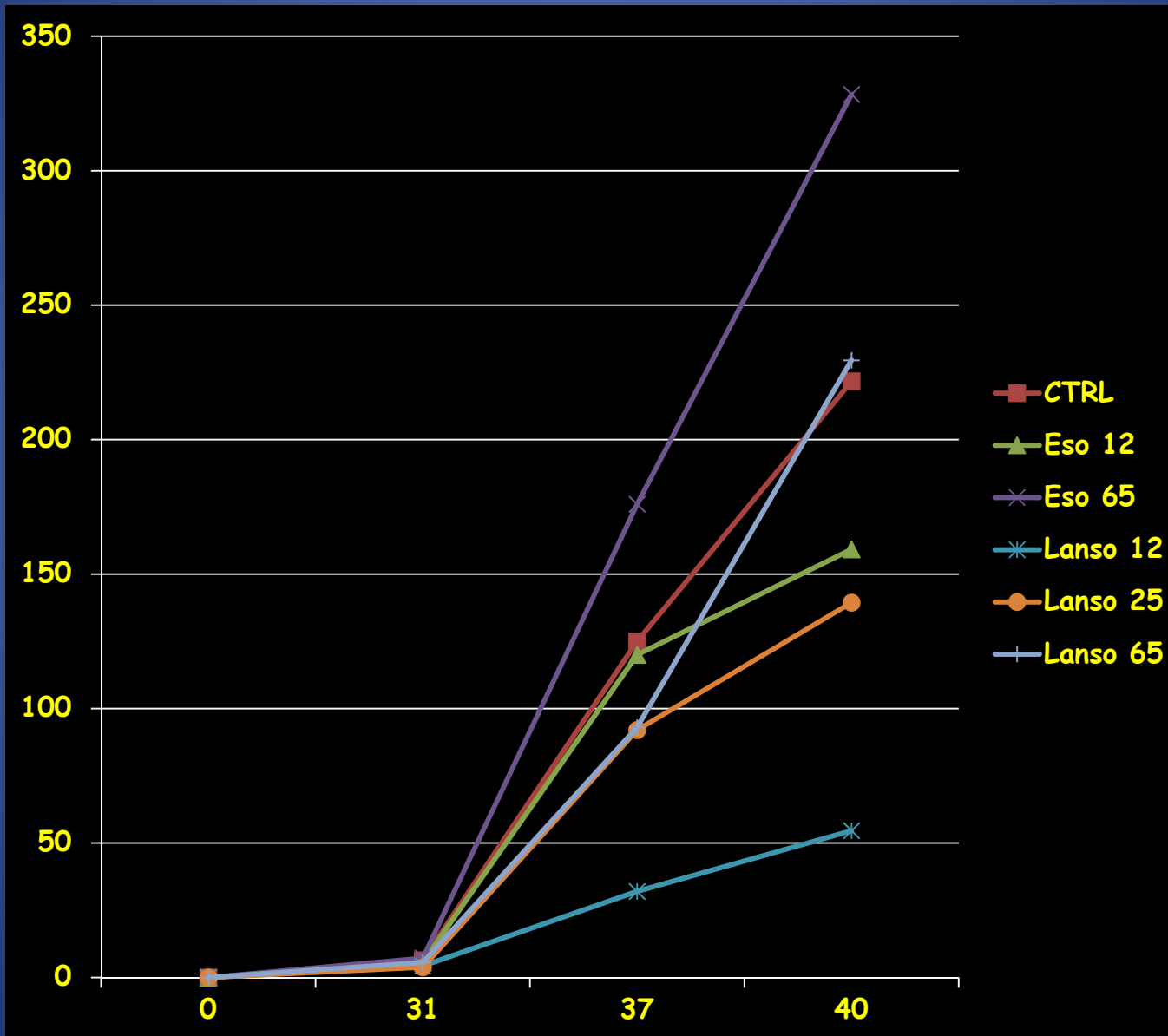
different bioavailability

PKA VALUES OF PPI

PPI	pKa1	pKa2
Omeprazole	4.06	0.79
Lansoprazole	3.83	0.62
Pantoprazole	3.83	0.11
Rabeprazole	4.53	0.62
Tenatoprazole	4.04	-0.12

PPI, proton pump inhibitor.

Lanzoprazole better than esomeprazole (i.p. inoculation)



HIGH DOSAGE PPI IN PATIENTS WITH ZOLLINGER-ELLISON SYNDROM

1. Ramdani A, Mignon M, Samoyeau R. Effect of pantoprazole versus other proton pump inhibitors on 24-hour intragastric pH and basal acid output in Zollinger-Ellison syndrome. *Gastroenterol Clin Biol.* 2002 Apr;26(4):355-9.
2. Metz DC, Forsmark C, Lew EA, Starr JA, Soffer EF, Bochenek W, Pisegna JR. Replacement of oral proton pump inhibitors with intravenous pantoprazole to effectively control gastric acid hypersecretion in patients with Zollinger-Ellison syndrome. *Am J Gastroenterol.* 2001 Dec;96(12):3274-80.
3. Désir B, Poitras P. Oral pantoprazole for acid suppression in the treatment of patients with Zollinger-Ellison syndrome. *Can J Gastroenterol.* 2001 Dec;15(12):795-8.

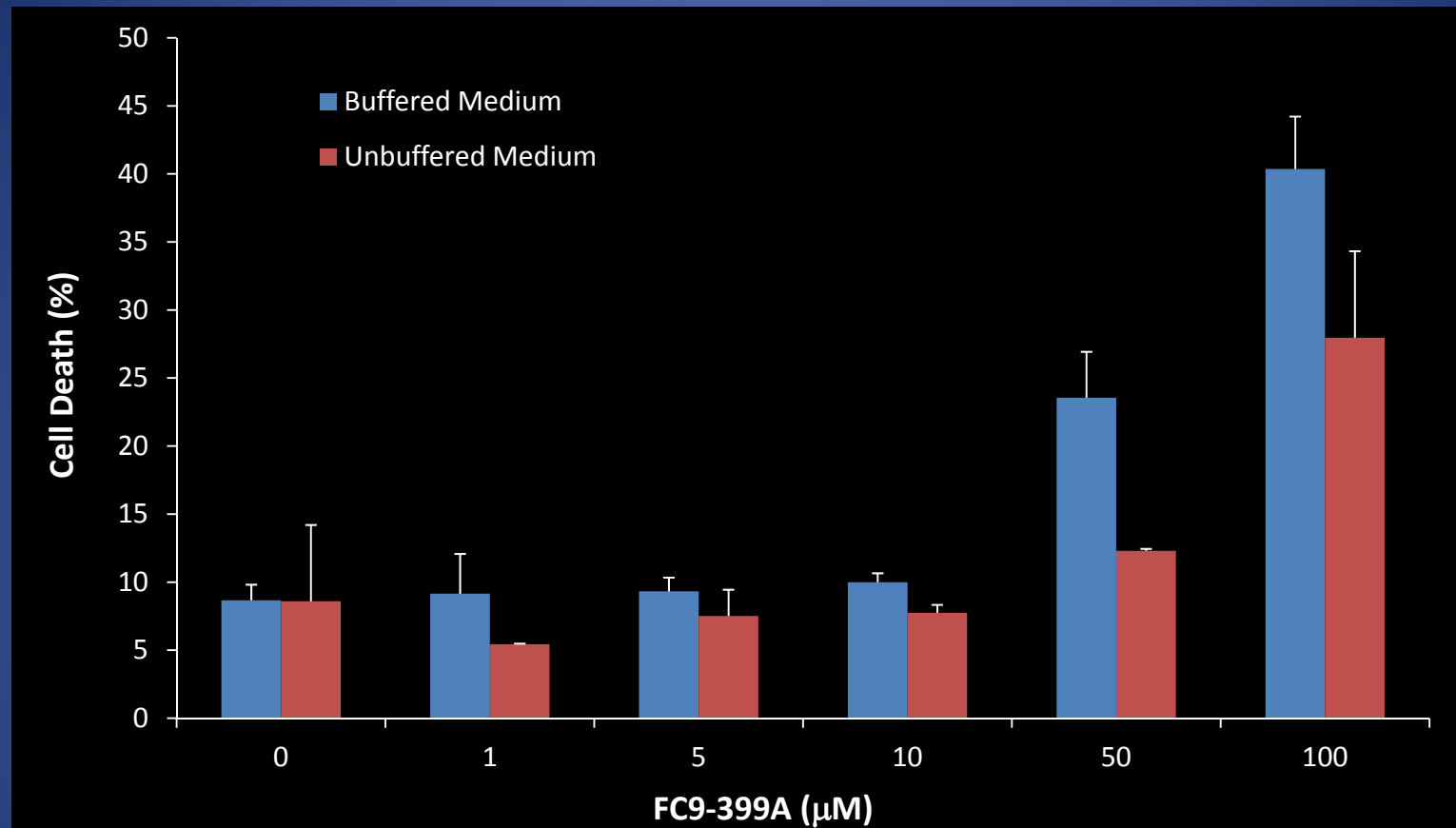
CRITERIA FOR TREATMENT OF TUMOR PATIENTS WITH HIGH DOSAGE PPI

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?**

FC9-399A Toxicity curve



ACIDIFICATION REDUCE THE EFFECTIVENESS OF CA INHIBITORS

Cell Line: Me 30966 (25.000/well)

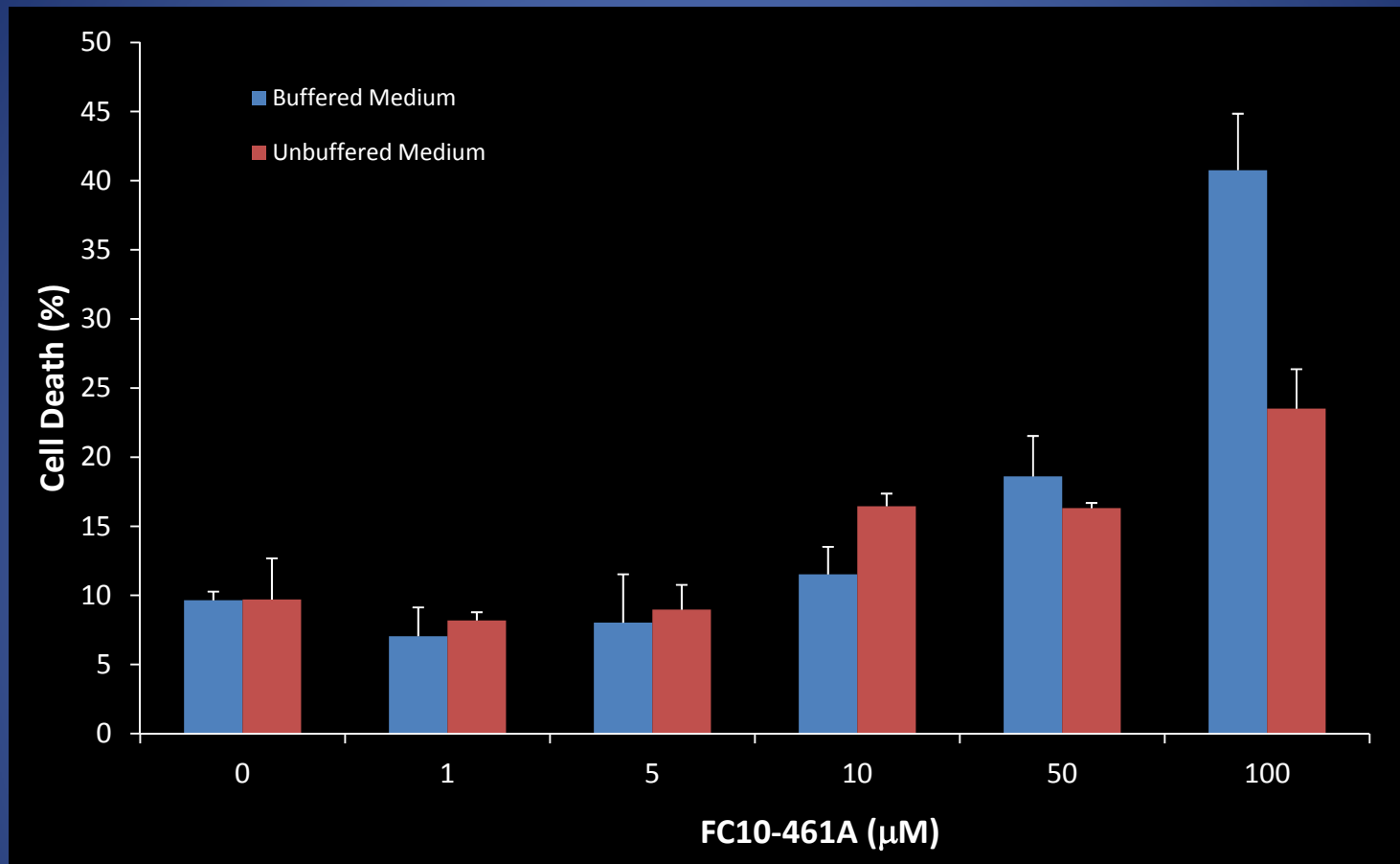
Culture Medium: RPMI 1640 pH 7.4 Buffered or Unbuffered

FC9-399A CAIX Inhibitors Concentration: 1- 5 - 10 – 50 100 μM for 24h

Control: DMSO 1%

Cell death evaluation: Trypan blue

FC10-461A Toxicity curve



ACIDIFICATION REDUCE THE EFFECTIVENESS OF CA INHIBITORS

Cell Line: Me 30966 (25.000/well)

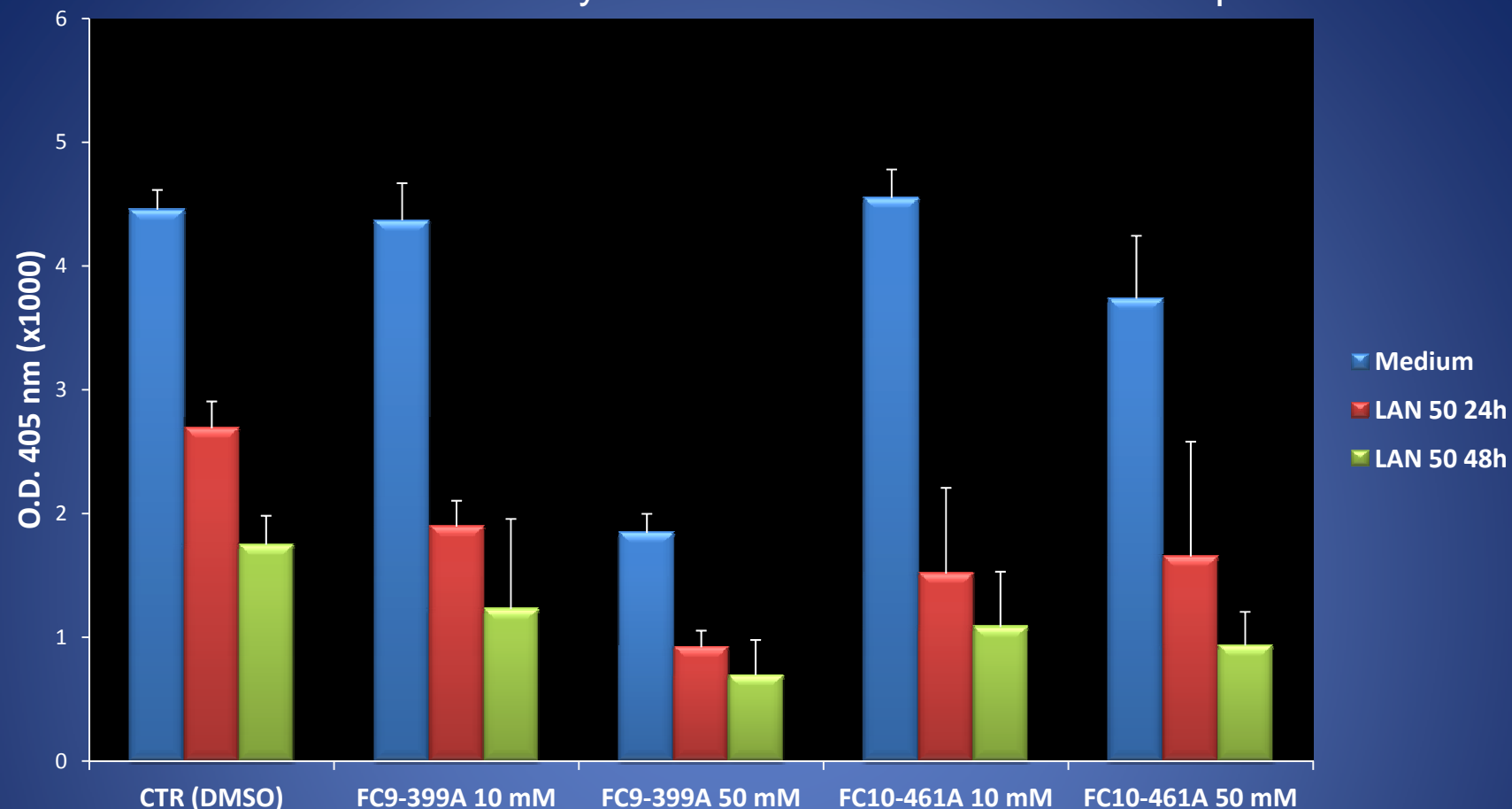
Culture Medium: RPMI 1640 pH 7.4 Buffered or Unbuffered

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 μ M for 24h (pre-treatment) and 48h (combined treatment)

CAIX Inhibitors Concentration: 10 – 50 μ M 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, pHi 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. Pouyssegur

<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

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Franca Podo

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