Romulus and *Remus* the twins of Cancer

25 July 2013

John A. Hackett
Director Cancer Research JON Pty Ltd Australia. Email: johnahackett@yahoo.com.au

Introduction
Twins are associated with Cancer for promotion of proliferation, drug and radiation resistance. The first twin *Romulus* is Lactic Acid and the second twin *Remus* is Glutathione. Together they are jointly necessary for supporting the increased rate of Cancer cell metabolism, progression, drug and radiation resistance.

Lactic Acid
Cancer malignancy is defined by an increase in the cellular metabolic rate with increased generation of Lactic acid which is transported outside the cells into the microenvironment. (11, 12, 14, 15, 18).

Glutathione
Cancer cells have elevated levels of Glutathione. (1,19, 20, 21, 22 ,23 ,25) One of the main functions of Glutathione in biological systems is to maintain the balance of the Reduction-Oxidation system (REDOX)

$$4\ G\text{-}SH + O_2 \rightleftharpoons 2\ G\text{-}S\text{-}S\text{-}G + 2H_2O$$

$$2\ G\text{-}SH + H_2O_2 \rightleftharpoons G\text{-}S\text{-}S\text{-}G + H_2O$$

As Cancer cell metabolic rates increase there are proportionally more Reactive Oxygen Species (ROS) generated which needs to be balanced by higher levels of the antioxidant Glutathione in the Cancer cells. Excessive ROS levels can cause cellular damage and cell death but low levels of ROS are important for biological system operation and viability. Maintaining this balance is critical for cell survival and proliferation. (1, 3, 23, 25, 29)

Cancer Cell Glutathione Elevation and Drug-Radiation Resistance
Cancer cells as they become more malignant have increased Glutathione levels which contribute to increased drug and radiation resistance. (1, 16, 19, 20, 21, 22)
Increased resistance to drugs such as Cisplatin and Arsenic Oxide has been shown to be directly proportional to the Cancer cell Gutathione level. (1, 16, 19, 20, 21, 22).

The Glutathione can have multiple modes of action. Glutathione like most compounds bearing a Thiol group can act as a simple antioxidant, metal chelator or bind to any active enzyme or chemical with a Thiol group. (1, 4, 19, 20, 21, 22).
Glutathione also forms numerous conjugates in biological systems by other binding mechanisms (29) which can lead to Multi Drug Resistance.

Glutathione Decline in the Cancer Microenvironment.

Increasing levels of Glutathione in the Cancer cells accompanies a lowering of Glutathione in the microenvironment. Cancer patients typically have declining Blood Plasma Glutathione levels. (23, 24, 25)

Proton Pump Inhibitor Effects on Lactic Acid levels, Glutathione Levels and Cancer Progression

Inhibition of the Cancer cell Proton Pumps will lower Lactic acid production in a dose dependent manner. This lowering of the Lactic acid production will eventually lower Glutathione levels in the cancer cell. The Cancer cell metabolic rate will drop resulting in a decline in the Cancer growth rate.

Proton Pump Inhibitor Thiol Interactions and Di-Sulphide Formation

The PPIs are Pro Drugs and oxidants which can be reduced by other biological Thiol based compounds to form Di-Sulphide compounds. (4, 26, 27). These Di-Sulphide compounds can be involved with Thiol – Di-Sulphide reversible exchanges which are a regular feature in biological systems. The Thiol group of the Cancer cell Proton Pump will be only one that PPIs can react with in competition with other Thiol based compounds. The PPI Thiol group can also bind to drugs such as Cisplatin which mitigates against co-administration.

The Link between Proton Pump Inhibitor Transport, Stabilization and Plasma Glutathione Levels

PPIs are acid labile. In the presence of acid and absence of Thiol compounds PPIs break down to inactive compounds. PPIs transported through Cancer acidic microenvironments before reaching the Cancer cells can suffer acidic degradation if there is insufficient Glutathione present to form a stable Glutathione--PPI Di–Sulphide compound.

$$4\ \text{G-SH} + 2\text{PPI-S=O} \underset{\leftarrow}{\rightarrow} 2\text{G-S-S- PPI} + \text{G-S-S-G} + 2H_2O \qquad \text{REDOX Reaction}$$

Glutathione and other low molecular weight Thiol compounds in Plasma can stabilize and increase the transport efficiency of the PPI through the acidic Cancer cell microenvironment. (4, 6, 10, 13).
Once transported through the acidic microenvironment the Glutathione-PPI Di-Sulphide is free to undergo Di-Sulphide exchange with the Cancer cell proton pumps (10). This Di-Sulphide exchange is an equilibria reversible reaction.

Based on Cancer cell culture trials stabilizing the PPI as a Di-Sulphide shows better PPI transport and response with a range of Cancer cell Types. (10,13)

$$\text{G-S-S- PPI} + H^+\text{ATPase --SH} \underset{\leftarrow}{\rightarrow} H^+\text{ATPase -S-S-PPI} + \text{G-SH} \qquad \text{Di-Sulphide Exchange}$$

Metabolic Approaches to Treatment of Melanoma *Clin Cancer Res November 1, 2009* **15**; *6490*
Peter Hersey, Ralph Neal Watts, Xu Dong Zhang and John Hackett

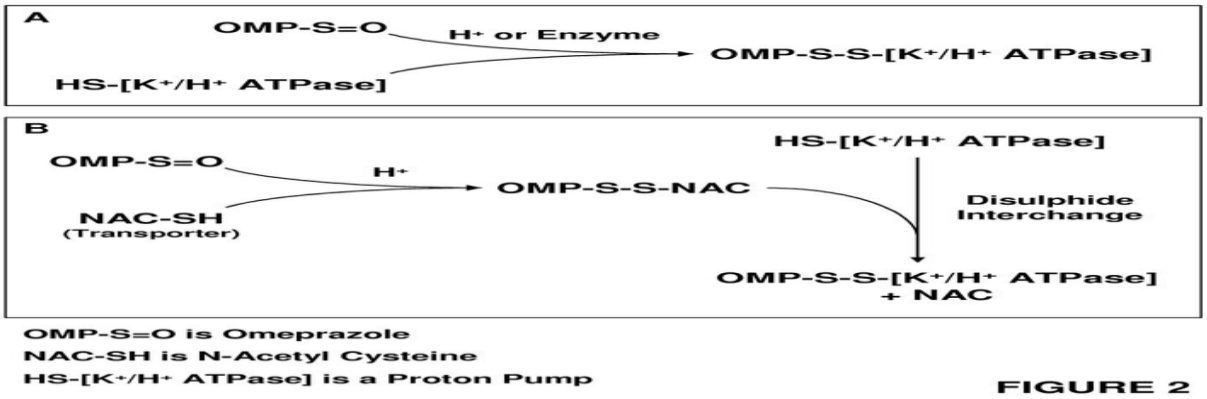

FIGURE 2

A Omeprazole binding to sulfydryl groups on the proton pumps.
B. Role of N-Acetyl Cysteine in acting as a transporter molecule for Omeprazole to proton pumps in cancer cells.

The reverse reaction of the PPIs being displaced from the Gastric Proton Pump by Glutathione has been noted and measured. (28). Lansoprazole showed a Half Life of less than 15 hours, and both Omeprazole and Rabeprazole showed less than 30 Hours, for Pantoprazole the Half Life was approximately 46 hours . The longer Half Life of Pantoprazole is attributed to the inability of Glutathione to reach the less exposed Thiol sites targeted by Pantoprazole.

If Cancer cell Proton Pumps responded in a similar way one might expect Pantoprazole to be a better choice for the treatment of cancers but not necessarily so if a particular type of Cancer Proton Pump has a different design to that of the Gastric Proton Pump.

Glutathione Levels in Laboratory Models compared with Clinical Patients.

Most laboratory Cancer cell culture and animal models invariably have higher Glutathione levels in the Cancer microenvironment compared with Cancer patients. Healthy rats as an example have about fifteen times the plasma Glutathione level than humans . (23, 24, 25). PPI Cancer Clinical trials involving subjects with depressed plasma Glutathione levels will not translate to the better results obtained in laboratory studies with higher plasma Glutathione levels. (7, 2)

Elderly subjects both human and animal have a lower Plasma Glutathione level (24). They are more prone to cancers and refractory to treatment. Less efficient PPI transport in these patients will compromise treatment. Variation in the Plasma Glutathione level in the general population and Cancer patients is also an important factor in the effectiveness of treatments using PPIs for individual patients.

Multifunction Role of Proton Pump Inhibitors

The PPIs do not only inhibit Proton pumps but can inhibit other metabolic pathways in a dose dependent manner (6).

(a) Proton Pumps H^+K^+ATPase, H+Na+ATPase , H+Ca2+ATPase .
(b) Carbonic Anhydrase
(c) Pyruvate Decarboxylase
(d) Transketolase
(e) Histone Deacetylase
(f) Superoxide Dismutase SOD
(g) Zinc Metallo Hydrolase Family
(h) Zinc and Copper based enzymes

. At higher dose rates than those required for Proton Pump inhibition other metabolic pathways can be inhibited to cause cancer cell death.

Increasing PPI Transport in Patient Clinical Trials

It has proven difficult to increase Cancer Patient Plasma Glutathione level which would improve PPI transport.

An alternate approach is to utilize the N-Acetyl Cysteine–PPI Di-Sulphide which has performed better in Cancer cell culture studies by a factor of three. (6,13). It has a lower molecular weight than the equivalent Glutathione–PPI Di-Sulphide with improved drug transport and is capable of passing the blood-brain barrier. (4, 10, 13)

Conclusion.

Proton Pump inhibitors have an important role to play in the treatment of Cancers. Clinical trials to date have been hampered by inefficient Proton Pump Inhibitor transport and insufficient recognition of the joint Lactic acid and Glutathione roles in Cancer progression and treatment.

Foot Note;

Apologies are extended to those Researchers whose references could not be included in this brief Review.

References…

No	Authors	Title	Reference
1	Traverse et al	Role of Glutathione in Cancer Progression and Chemo resistance	Oxidative Medicine and Cellular Longevity Vol 2013 Article ID 972913
2	Xichun Hu et al	Intermittent High Dose Proton Pump Inhibitor improves Progression Free Survival as Compared to Standard Chemotherapy in the First Line Treatment of Patients with Metastatic Breast Cancer	Cancer Research December 15, 2012 Abstract Thirty-Fifth Annual CTRC-AACR San Antonio Breast Cancer Symposium Dec 2012
3	Dang	Links between Metabolism and Cancer	Genes & Development 26:877-890 2012
4	Hackett	Pharmacologically Active Disulfide Compounds	UK Patent GB2447572 June 2011
5	Udelnow et al	Omeprazole Inhibits Proliferation and Modulates Autophagy in Pancreatic Cancer Cells	PLoS ONE May 2011
6	Hackett	The Thermodynamics_Kinetics of Cancer Progression and the Evolutionary role of Proton Expression	ISPDC Conference Nice 18-19 November 2011
7	Spugnini et al	Lanzoprazole as a rescue agent in Chemoresistant tumors a Phase I/II study in companion animals with spontaneously occurring tumors.	Journal of Translational Medicine 2011 9;221
8	De Milito et al	pH-dependent antitumor activity of proton pump inhibitors against human melanoma is mediated by inhibition of tumor acidity	International Journal of Cancer , 127,207-219 (2010)
9	Huber et al	Proton Dynamics in Cancer	Journal of Translational Medicine 2010, 8:57
10	Hersey et al	Metabolic Approaches to Treatment of Melanoma	Clinical Cancer Research 2009:15(21) November 2009
11	De Milito	Proton Pump Inhibitors Induce Apoptosis of Human B-Cell Tumors through a Caspase-Independent Mechanism Involving Reactive Oxygen Species	Cancer Res. 2007;67:5408-5417
12	Fais et al	Targeting H^+-ATPases as a New Strategy against Cancer	Cancer Res. 2007;67:10627-10630
13	Parsons	Cancer Cell Culture Trials Omeprazole and N-Acetyl Cysteine Omeprazole Di-Sulphide	Professor Peter Parsons Queensland Institute of Medical Research Communications November 2007
14	Gatenby et al	Acid-mediated tumor invasion: a multidisciplinary study.	Cancer Res 2006;66: 5216–23.
15	De Milito	Tumor acidity, chemo resistance and Proton Pump Inhibitors	Future Oncol. 2005 Dec; 1(6):779-86
16	Seo et al	Arsenic Trioxide Circumvents Multidrug Resistance Based on Different Mechanisms in Human Leukemia Cell Lines	Anticancer Research 25:991-998 (2005)
17	Luciani et al	Effect of Proton Pump Inhibitor Pretreatment on Resistance of Solid Tumors to Catatonic Drugs	Journal of the National Cancer Institute. Vol.96 No.22 Nov 2004
18	Gatenby et al	Why do cancers have high aerobic glycolysis?	.Nat Recanter 2004;4: 891–9.
19	Ortega et al	Glutathione in Cancer Cell Death	Cancers 2011, 3, 1285-1310
20	Dai et al	Malignant Cells can be Sensitized to Undergo Growth Inhibition and Apoptosis by Arsenic Trioxide Through Modulation of the Glutathione Redox System.	Blood. Vol 93. No.1 pp268-277 January 1999
21	Yang et al	Arsenic Trioxide sensitivity is associated with low levels of Glutathione in Cancer Cells	British Journal of Cancer (1999) 81(5), 796-799
22	Godwin et al	High Resistance to Cisplatin in Human Ovarian Cancer Cell Lines is associated with marked increase of Glutathione synthesis	Proc.Natl. Acad.Sci. USA Vol 89 pp[3070-3074 April 1992
23	Beutler et al	Plasma Glutathione in Health and in Patients with Malignant disease	J Lab Clin Med 1985 May
24	Michelet et al	Blood and Plasma Glutathione Measured in Healthy Subjects by HPLC Relative to Sex, Aging, Biological Variable and Life Habits.	Clinical Chemistry Vol 41 No. 10 1995
25	Navarro et al	Changes in Glutathione Status and the antioxidant System in Blood and in Cancer Cells associated with Tumor Growth.	Free Radical Biology and Medicine Vol. 26, Issues 3-4, February 1999 Pages 410-418
26	Weidolof et al	A Metabolic route of Omeprazole involving conjugation with Glutathione identified in the rat	Drug Metab Dispos 1992 Mar-Apr 20(2) 262-7
27	Zhong et al	Metabolism of Pantoprazole involving conjugation with glutathione in rats.	J Pharm Pharmacol 2005 Mar;57(3):341-9.
28	Sachs et al	Synthesis or Rupture: duration of acid inhibition by proton pump inhibitors.	Drugs Today (Barc) 2003 Mar ; 39 Suppl A : 11-4
29	Wei Wang et al	Endogenous Glutathione Conjugates: Occurrence and Biological Functions.	Pharmaceutical Reviews Vol. 50, No. 3 1998