UNIVERSITI PUTRA MALAYSIA

CARCINOGENIC EFFECTS OF N-METHYL-N-NITROSOUREA ADMINISTERED VIA ORAL AND INTRAPERITONEAL ROUTES IN FEMALE SPRAGUE DAWLEY RATS

MUHAMMAD HAKIMI BIN MOHD KASSIM

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By

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June 2014

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N-methyl-*N*-nitrosourea (MNU) is able to induce multiple tumours via different routes of administration. The development of tumours is well associated with failure of apoptosis process. In cancer studies, real time quantitative reverse transcription polymerase chain reaction (qRT-PCR) is widely used to measure changes of the messenger ribonucleic acid (mRNA) level of targeted genes. To date, there is no comparative study of the carcinogenic effects of MNU between administration via oral and intraperitoneal (IP) routes. Besides comparing the apoptotic genes normalization using single and multiple housekeeping (HK) genes, changes of the expression of the targeted apoptotic gene during the carcinogenesis in whole blood and spleen between all groups and their correlation to the lesion scoring in the selected organs remained undetermined. The main objective of this study was to compare the carcinogenic effects of MNU post administration via oral and IP routes through blood profiles, urinalysis, gross and histopathological examination. Comparison of apoptotic genes normalization using single and multiple HK genes, the levels of Bcl-2 to Bax fold expression ratios in whole blood and spleen and their correlations to the lymphoma lesion scores served as the second, third and fourth objectives in this study. A total of 27 female Sprague Dawley (SD) rats was separated equally into three groups which were control (group A), MNU-treated orally (group B) and MNU-treated intraperitoneally (group C). Rats in MNU-treated groups received 60 mg/kg of body weight MNU twice a week for two consecutive weeks which equal to a total dose of 240 mg/kg of body weight. Rats in control group received normal saline following the same procedure. All rats were humanely sacrificed after 6 months of animal study. In addition to IP route, oral administration of MNU successfully induced leukaemia in all rats and emerged as a new promising route to induce this cancer. Administration of MNU regardless of routes contributed to the elevation of Bcl-2 to Bax fold expression ratios leading to overproduction of neoplastic cells observed in the blood smear with a prominent occurrence of lymphocytosis in rats administering MNU intraperitoneally. Introduction of MNU into the intraperitoneum cavity led to rapid absorption and higher bioavailability of this carcinogen which in turn, significantly increased serum urea, AST, LDH and reduced TP concentrations respectively. Regardless of administration routes, significant reduction of creatinine clearance (CrCl) and elevation of urine protein to creatinine (UPC) ratios post administration of MNU reflecting the renal insufficiency of rats even though the renal lymphoma severity averagely mild. The

incidences of splenomegaly with higher prevalence, hepatomegaly and stomach mass were related to the enteral process conferred by oral route of administration. Intraperitoneal route of MNU administration inferred as a reliable route to promote the development of mammary gland tumours of multiple types as there was no incidence of this type of tumour in rats administered the carcinogen orally. Administration of MNU regardless of routes contributed to no significant differences in terms of the incidence of splenomegaly, percentage of splenic lymphoma and lesion scores and these findings were further confirmed by the real time qRT-PCR as a more sensitive method showing nearly similar splenic fold expression ratios of Bcl-2 to Bax. Lungs emerged as a non-haematopoietic target organ for leukaemia dissemination due to increased weight of lungs, high incidence of pulmonary lymphoma and severity of lymphoma lesion were insignificantly difference in rats administered MNU via both routes. The leukaemia induced post administration of MNU regardless of routes played a prominent role as a systemic disease as it was able to metastasise to all selected organs with higher affinity to lungs and heart observed in rats having intraperitoneal administration as compared to oral administration of the carcinogen. Administration of MNU regardless of routes could impart nearly similar lymphoma lesion severity reflected by lesion scores. The application of both glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and β-actin for normalisation purpose produced more reliable and precise expression data as outlier or error associated with any individual set of data was averaged out leading to reduced data dispersion. At the molecular level, Bcl-2 to Bax fold expression ratios in whole blood and spleen post administration of MNU regardless to routes were significantly increased at nearly similar expression patterns promoting cell proliferation of neoplastic cells showed in the blood smears and also massive proliferation of neoplastic cells in spleen. It was further inferred that measuring Bcl-2 to Bax fold expression ratios in whole blood was suffice to detect the presence and severity of lymphoma in the selected organs. Conclusively, the application of real time qRT-PCR using Bcl-2 to Bax fold expression ratio in whole blood as the panel to detect and decipher the severity of lymphoma lesion was reliable and more accurate rather than the invasive method.

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KESAN KARSINOGENIK *N*-METHYL-*N*-NITROSOUREA MELALUI ORAL DAN INTRAPERITONEUM TERHADAP TIKUS SPRAGUE DAWLEY BETINA

Oleh

MUHAMMAD HAKIMI BIN MOHD KASSIM

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N-methyl-*N*-nitrosourea (MNU) dapat mengaruh pertumbuhan pelbagai tumor melalui laluan administrasi yang berbeza. Pertumbuhan tumor adalah berkait rapat dengan kegagalan proses apoptosis. Dalam pelbagai kajian kanser, 'quantitative reverse transcription polymerase chain reaction' (qRT-PCR) masa nyata adalah teknik yang sering digunakan untuk mengira perubahan level 'messenger ribonucleic acid' (mRNA) gen-gen. Sehingga kini, tiada perbandingan kajian dijalankan mengenai kesan-kesan karsinogenik MNU antara administrasi secara oral dan juga intraperitoneum. Selain perbandingan normalisasi gen-gen apoptotic dengan menggunakan satu dan pelbagai gen 'housekeeping' (HK), perubahan pengekspresian gen-gen apoptotik dalam darah dan limpa tikus-tikus dalam setiap kumpulan semasa proses karsinogenesis dan hubungkait dengan mata luka limfoma masih belum ditentukan. Objektif utama kajian ini adalah untuk membandingkan kesan-kesan karsinogenik MNU selepas administrasi melalui oral dan intraperitoneum berdasarkan profil darah, urinalisis dan pemeriksaan kasar dan histopatologi. Perbandingan normalisasi gen-gen apoptotic dengan menggunakan satu dan juga pelbagai gen-gen HK, paras nisbah expresi gandaan Bcl-2 kepada Bax dalam darah dan juga limpa dan hubungkait dengan mata luka limfoma adalah objektif kajian yang kedua, ketiga dan keempat. Sejumlah 27 ekor tikus betina Sprague Dawley (SD) telah diagihkan sama rata kepada tiga kumpulan iaitu kawalan (kumpulan A), rawatan MNU melaui oral (kumpulan B) dan rawatan melalui intraperitoneum (kumpulan C). Tikus-tikus kumpulan rawatan MNU menerima MNU sebanyak 60 mg/kg berat badan dua kali seminggu selama dua minggu berturut-turut yang bersamaan dengan dos total sebanyak 240 mg/kg berat badan. Tikus-tikus dalam kumpulan kawanlan menerima larutan garam fisiologis melalui prosedur yang sama. Semua tikus dikorbankan secara berperikemanusiaan selepas 6 bulan kajian haiwan. Tambahan kepada laluan administrasi intraperitoneum, laluan administration MNU secara oral telah berjaya mengaruh leukaemia pada semua tikus dan menjadi satu laluan administrasi baharu untuk mengaruh kanser ini. Penerimaan MNU tanpa mengira laluan administrasi menyebabkan peningkatan nisbah expresi gandaan Bcl-2 kepada Bax dan seterusnya membawa kepada penghasilan sel neoplastik secara berlebihan yang dapat dilihat pada smer darah dengan kejadian limfositosis yang ketara pada tikus yang menerima MNU melalui intraperitoneum. Introduksi MNU ke dalam intraperitoneum kaviti menjurus kepada penyerapan karsinogen yang pantas dan tinggi bioavailabiliti dan seterusnya menyebabkan peningkatan kepekatan urea, AST dan LDH dalam serum dan

penurunan TP yang signifikan. Penurunan 'creatinine clearance' (CrCl) dan peningkatan nisbah 'urine protein to creatinine' (UPC) secara signifikan selepas administrasi MNU tanpa mengira laluan penerimaan menunjukkan penurunan fungsi ginjal walaupun luka limfoma secara purata adalah pada tahap kurang serius. Insiden splenomegaly yang lebih kerap, hepatomegaly dan ketumbuhan pada hati adalah berkaitan dengan proses enteral selepas administrasi secara oral. Administrasi MNU secara intraperitoneum dapat disimpulkan sebagai laluan yang berkesan untuk mengaruh pertumbuhan tumor kelenjar mamari yang pelbagai jenis kerana tiada kejadian tumor jenis ini pada tikus yang menerima karsinogen secara oral. Administrasi MNU tanpa mengira laluan penerimaan menyumbang kepada perubahan yang tidak signifikan dalam konteks insiden splenomegaly, peratusan dan mata luka limfoma limpa dan seterusnya penemuan ini telah disahkan dengan qRT-PCR masa nyata yang merupakan teknik yang lebih sensitif dan menunjukkan nisbah ekspresi gandaan Bcl-2 kepada Bax yang hampir sama. Paru-paru merupakan organ bukan hematopoietik sasaran bagi sel leukaemia disebabkan oleh kenaikan berat paru-paru, insiden dan luka limfoma pulmonari yang tidak signifikan antara pada tikus-tikus yang menerima MNU melalui kedua-dua laluan penerimaan. Leukemia yang diaruh selepas administrasi MNU tanpa mengira laluan penerimaan memainkan peranan yang ketara sebagai penyakit sistemik disebabkan dapat metastasis ke semua organ pilihan dengan kecenderungan yang lebih tinggi ke paru-paru dan jantung tikus yang menerima karsinogen ini secara laluan intraperitoneum berbanding dengan oral. Administrasi MNU tanpa mengira laluan penerimaan juga dapat menyebabkan keseriusan luka limfoma yang hampir sama pada semua organ pilihan. Penggunaan 'glyceraldenhyde-3-phosphate dehydrogenase' (GAPDH) dan β-actin untuk tujuan normalisasi menghasilkan data ekspresi yang lebih tepat dan jitu kerana unsur luaran dan ralat yang berkaitan dengan set data individu dibahagi seterusnya menghasilkan penurunan penyerakan data. Pada paras molekul, nisbah ekspresi gandaan Bcl-2 kepada Bax dalam darah dan juga limpa selepas administrasi MNU tanpa mengira laluan penerimaan adalah meningkat secara signifikan dan mempunyai nilai ekspresi yang hampir sama seterusnya menggalakkan pembahagian sel neoplastik yang dapat dilihat pada smer darah dan jugapembahagian sel secara massif pada limpa. Pengukuran nisbah ekspresi gandaan Bcl-2 kepada Bax dalam darah adalah memadai untuk mengesan kehadiran dan keseriusan luka limfoma diseabkan korelasi yang signifikan antara kedua-dua pembolehubah. Secara kesimpulan, aplikasi qRT-PCR masa nyata dengan menggunakan nisbah ekspresi gandaan Bcl-2 kepada Bax dalam darah sebagai panel untuk mengesan dan mengetahui keseriusan luka limfoma adalah boleh diterimapakai dan lebih tepat berbanding kaedah invasif.

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I certify that an Examination Committee has met on 25/06/14 to conduct the final examination of Muhammad Hakimi Bin Mohd Kassim on his degree thesis entitled "Carcinogenic effects of *N*-methyl-*N*-nitrosourea administered via oral and intraperitoneal routes in female Sprague Dawley rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

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The thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ºC	Degree Celsius
%	Percentage
aCGH	Array-based comparative genomic hybridisation
AIF	Apoptosis inducing factor
Alb	Albumin
ALL	Acute lymphocytic leukaemia
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AML	Acute myeloid leukaemia
APAF-1	Apoptotic protease activating factor 1
AST	Aspartate transpeptidase
Bag	Bcl-2 associated athanogene
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma extra large
BH	Bcl-2 homology
Bid	BH3 interacting-domain death agonist
BTL	B- and T-cell lymphoma
Ca	Calcium
CAD	Caspase activated DNase
Caspase	Cysteine aspartic protease
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CK	Creatinine kinase
CLL	Chronic lymphocytic leukaemia
CNS	Central nervous system
Cr	Creatinine
CrCl	Creatinine clearance
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dGTP	Deoxyguanosine triphosphate
DISC	Death-inducing signalling complex
DNA	Deoxyribonucleic acid
dTTP	Deoxythymidine triphosphate
EDTA	Ethylene diamine tetra acetic acid
EL	Erythrocyte lysis
FADD	Fas-associated death domain
FAS	TNF receptor superfamily member 6
FRGS	Fundamental Research Grant Scheme
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GFR	Glomerular filtration rate
Glb	Globulin
GOI	Gene of interest
HIF	Hypoxia-inducing factor
HK	Housekeeping
H&E	Haematoxylin and eosin

IACUC	Institutional Animal Care and Use Committee
IBS	Institute of Bioscience
II	Icteric index
IL-3	Interleukin 3
IP	Intraperitoneal
IV	Intravenous
K	Potassium
L	Litre
LDH	Lactate dehydrogenase
LHS	Lympho-haematopoietic system
MARDI	Malaysian Agriculture Research and Development Institute
MCH	Mean cell haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean cell volume
$MgCl_2$	Magnesium chloride
mg/L	Milligram per litre
µL	Microlitre
µmol	Micromolar
mmol	Millimolar
MNNG	*N*-methyl-*N*-nitro-*N*-nitrosoguanine
MNU	*N*-methyl-*N*-nitrosourea
MPT	Mitochondrial permeability transition
MRD	Minimal residual disease
mRNA	Messenger ribonucleic acid
n	Number of reference target
Na	Sodium
NOC	*N*-nitroso compound
NTC	Non template control
O^6-meG	O^6-methylguanine
PARP	Poly (ADP-ribose) polymerase
PCV	Packed cell volume
PP	Plasma protein
ppm	Part per million
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RBC	Red blood cell
RCBD	Randomised complete block design
RNA	Ribonucleic acid
rpm	Revolution per minute
RQ	Relative quantity
SCC	Squamous cell carcinoma
SD	Sprague Dawley
SEM	Standard error of mean
TNF	Tumour necrosis factor
TP	Total protein
UA	Uric acid
UPC	Urine protein to creatinine ratio
UPM	Universiti Putra Malaysia
WBC	White blood cell

CHAPTER 1

INTRODUCTION

N-methyl-*N*-nitrosourea (MNU) is a potent direct-acting carcinogen that is able to cause damages to cellular genetic materials which leads to tumour formation. Previous findings unveil that MNU is cancerous in a wide array of organs including those derived from haematopoietic system and able to induce various types of cancer in approximately 40 different animal species with regards to different dosage, age, treatment period and route of administration (Alexandrov, 1965; Lee *et al*., 2000; Gal *et al*., 2012).

In previous studies, prostate cancer (Liao *et al*., 2002) and thymic lymphoma (Franchi *et al*., 2003) were developed post administration of MNU via intraperitoneal (IP) route in male Winstar rats. Besides, T-cell lymphoma was successfully induced in male Sprague Dawley (SD) rat following IP administration of MNU (Hutheyfa *et al*., 2011). Apart from lymphomas, a study showed that acute pro-myelocytic leukaemia was also developed after receiving MNU via intravenous (IV) route (Chang *et al*., 2012). Moreover, tumourigenic effect of MNU on the development of mammary gland tumour was also observed in female Sprague Dawley rats administrating this carcinogen intraperitoneally (Barathidasan *et al*., 2013). Colon cancer was another type of solid tumours developed in male Winstar rats post administration of MNU via intrarectal route (Tuncel *et al*., 2013). Furthermore, female Balb/C mice developed hepatocellular carcinoma post IP administration of MNU (Verma *et al*., 2013).

As the tumourigenesis is a multi-step process, a pre-cancerous cell must be able to evade the cell death programme in order to transform the genetically abnormal cell to neoplastic state (Hanahan and Weinberg, 2011). The development of tumour is very well associated with the failure of apoptosis process. Apoptosis can be activated via extrinsic and intrinsic pathways (Elmore, 2007). The intrinsic pathway of apoptosis is regulated by pro-apoptotic and anti-apoptotic proteins. B-cell lymphoma 2 (Bcl-2) and Bcl-2 associated protein X (Bax) are anti- and pro-apoptotic proteins, respectively. The ratios of anti-apoptotic to pro-apoptotic gene expressions determine cell fate (Jaafar *et al*., 2012). The increased of Bcl-2 to Bax expression ratios have been observed in solid tumours (Matsumoto *et al*, 2004; Yoon and Roh, 2012; Sar *et al*., 2012; Tano *et al*., 2013) and also haematological malignancies (Savli *et al*., 2011; Kaparou *et al*., 2013; Sharawat *et al*., 2013).

The accuracy of cancer diagnosis is of importance in determining good therapeutic treatment (Mulrow, 1994). Haematological analyses (Bain, 2005; Ceballos, 2007) and serum biochemical analyses (Srivinis *et al*., 2001; O'connell *et al*., 2005) are two important cancer diagnostic methods. Besides, urinalysis is also a method used to evaluate renal function especially in renal carcinoma patients (Ahmad *et al*., 2013). Apart from non-invasive methods, the information gained from clinical presentation and histopathological data are vital to classify cancers (Ramaswamy *et al*., 2001). However, histopathological examination is unable to define specific morphological signatures of cancer cells for differential diagnosis and also the underlying genetic aberrancies that contribute to the development of tumour (Khan *et al*., 2001).

Molecular diagnostic method is a method of choice in oncology because of its accuracy, efficiency and reproducibility (Bernard and Wittwer, 2002). Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) approach is the benchmark in detecting and quantifying ribonucleic acid (RNA) targets and being widely used in clinical diagnostic assays (Bustin, 2000). Being an efficient, sensitive, and reliable method, qRT-PCR is usually referenced to appropriate housekeeping genes in order to avoid bias and produce accurate data (Nicot *et al*., 2005). One such concern continues to be linked with the selection and the appropriate number of reliable housekeeping (HK) gene for normalisation. It is suggested by a number of authors that normalisation of gene expression should be carried out using more than one HK gene to produce valid data and also prevent misled conclusion (Vandesompele *et al*., 2002; Radonic *et al*., 2004; Nicot *et al*., 2005).

Problem statements

Inducing any types of tumour in animal model by carcinogen requires a consideration on the route of administration (Hoffman *et al*., 1983). Animals can develop leukaemia due to the exposure to internal and external leukaemogenesis factors (Chang *et al*., 2012). The induction of leukaemia-lymphoma in rats via IP route of administration was successful in a few studies (Franchi *et al*., 2003; Hazilawati *et al*., 2010). In a broad array of species including human, the exposure of carcinogenic effect of MNU is possibly gained through its generation in the gastrointestinal tract (Budan *et al*., 2008). To date, there is no single study conducted to compare the carcinogenic effects of administered MNU via oral and IP routes in animal model.

Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) is a method of choice in quantifying the level of mRNA and requires normalisation by HK genes (Vandesompele *et al*., 2002). Even though this method confers many positive attributes, the issue of using a single HK gene pursues to grab major concerns (Radonic *et al*., 2004). The normalisation of targeted genes by multiple HK genes eliminates biases in gene expression data (Watson *et al*., 2007). Normalisation of apoptotic genes using a single HK gene via real-time qRT-PCR is still documented in several recent publications (Alshatwi *et al*., 2011; Song *et al*., 2013). However, the comparison of normalising the expression of apoptotic genes by single and multiple HK genes in any animal model is inconclusive.

Administration of carcinogen to laboratory animals is often a critical component of experimental design and types of administration route determine the bioavailability of the tested agent (Turner *et al*., 2011). To date, there is no comparative study carried out regarding the consequence of difference routes of carcinogen administration on the expression of apoptotic genes involved in carcinogenesis and correlation between gene expressions and lesion scoring.

Objectives of study

In the current study, it was hypothesised that administration of MNU at a total dose of 240 mg/kg of body weight via oral and IP routes of administration induced multiple types of tumours. Based on blood profiles, urinalysis, gross and histopathological analyses, it was also hypothesised that manifestations of carcinogenesis were more apparent in rats administered MNU intraperitoneally as compared to rats that received MNU orally. It was further hypothesised that normalisation of Bcl-2 to Bax fold expression ratios by more than one HK genes produced more reliable results. Another hypothesis in this experiment was Bcl-2 to Bax fold expression ratios in whole blood and spleen of rats administered MNU via IP and oral routes were increased higher than rats received MNU orally.

The objectives of this study were as followed.

1. to compare the carcinogenic effects of MNU between oral and IP routes of administration in female SD rats through blood profiles, urinalysis, gross and histopathological examination.
2. to compare the patterns of Bcl-2 to Bax fold expression ratios between normalisation using a single and multiple housekeeping genes.
3. to compare the pattern of Bcl-2 to Bax fold expression ratios between administration of MNU through oral and IP routes in whole blood and spleen via real time-qRT-PCR.
4. to correlate Bcl-2 to Bax fold expression ratios normalised by both GAPDH and β-actin in whole blood and spleen of rats administered MNU via oral and IP routes to the lymphoma lesion in organs of interest.

REFERENCES

Abbasalipourkabir, R., Dehghan, A., Salehzadeh, A., Shamsabadi, F. and Abdullah, R. (2010). Induction of mammary gland tumour in female Sprague Dawley rats with LA7 cells. *African Journal of Biotechnology*, **9**: 4491-4498.

Ahmad, A., Wu, K. and Tan, W. (2013). Renal cell carcinoma with skin metastasis: a case report and literature review. *Cancer and Clinical Oncology*, **2**: 80-86.

Al-Joudi, F.S. (2005). Prognostic value of an index for serum globulin compensation in colon and breast cancers. *Singapore Medical Journal*, **46**: 710-713.

Alberts, B. (2010). Model organism and human health. *Science*, **330**: 1724.

Alexandrov, V.A. (1965). Uterine, vaginal and mammary tumours induced by nitroso-ureas in pregnant rats. *Nature*, **222**: 1064-1065.

Alshatwi, A.A., Shafi, G., Hassan, T.N., Al-Hazzani, A.A., Alsaif, M.A., Alfawaz, M.A., Lei, K.Y. and Munshi, A. (2011). Apoptosis-mediated inhibition of human breast cancer cell proliferation by lemon citrus extract. *Asian Pacific Journal of Cancer Prevention*, **12**: 1555-1559.

Anvari, K., Toussi, M.S., Kalantari, M., Naseri, S., Shahri, M.K., Ahmadnia, H., Katebi, M., Pashaki, A.S., Dayani, M., Broumand M. (2012). Expression of Bcl-2 and Bax in advanced or metastatic prostate carcinoma. *Urological Oncology*, **9**: 381-388.

Antone, P.D. (2012). Energy metabolism in cancer cells: how to explain the Warburg and Crabtree effects. *Medical Hypotheses*, **79**: 388-392.

Antonsson, B., Conti, F., Ciavatta, A., Montessuit, S., Lewis, S., Martinou, I., Bernasconi, L., Bernard, A., Mermod, J-J., Mazzeni, G., Maundrell, K., Gambale, F., Sadoul, R. and Martinou J-C. (1997). Inhibition of Bax channel-forming activity by Bcl-2. *Science*, **277**: 370.

Avery, A.C. and Avery, P.R. (2007). Determining of significance of persistent lymphocytosis. *Veterinary Clinics of North America: Small Animal Practice*, **37**: 267-282.

Azoulay, E., Fieux, F., Moreau, D., Thiery, G., Rousselot, P., Parrot, A., Gall, J-R.L., Dombret, H. and Schlemmer, B. (2003). Acute monocytic leukemia presenting as acute respiratory failure. *American Journal of Respiratory and Critical Care Medicine*, **167**: 1329-1333.

Bailey, S.A., Robert, H.Z. and Perry, R.W. (2004). Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint? *Toxicologic Pathology*, **32**: 448-466.

Bain, B.J. (2005). Diagnosis from the blood smear. *The New England Journal of Medicine*, **353**: 498-507.

Barathidasan, R., Pawaiya, R.S., Rai, R.B. and Dhama, K. (2013). Upregulated Myc expression in N-methyl Nitrosourea (MNU)-induced rat mammary tumours. *Asian Pacific Journal of Cancer Prevention*, **14**: 4883-4889.

Barcos, M., Lane, W. and Gomez, G.A. (1987). An autopsy of 1206 acute and chronic leukemia (1958-1982). *Cancer*, **60**: 827-837.

Barth, R.F. and Kaur, B. (2009). Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas. *Journal of Neuro-Oncology*, **94**: 299-312.

Bartsch, H. and Montesano, R. (1984). Relevance of nitrosamines to human cancer. *Carcinogenesis*, **5**: 1381-1393.

Baumhoer, D., Tzankov, A., Dirnhofer, S., Tornillo, L. and Terracciano, L.M. (2008). Patterns of liver infiltration in lymphoproliferative disease. *Histopathology*, **53**: 81-90.

Behl, C. (2000). Apoptosis and Alzheimer's disease. *Journal of Neural Transmission*, **107**: 1325-1344.

Benda, P., Someda, K. and Messer, J. (1971). Morphological and immunochemical studies of rat glial tumors and clonal strains propagated in culture. *Journal of Neurosurgery*, **34**: 310-323.

Berge, T. (1974). Splenic metastasis: frequencies pattern. *Acta Pathologica Microbiologica Scandinavic*, **82**: 499-506.

Bernard, P.S. and Wittwer, C.T. (2002). Real-time PCR technology in cancer diagnostic. *Chemical Chemistry*, **48**: 1178-1185.

Blank, N. and Castellino, R.A. (1980). The intrathroracic manifestations of the malignant lymphomas and the leukemias. *Seminars in Roentgenology*, **15**: 227-245.

Bleasdale, C., Golding, B.T., McGinnis, J., Muller, S. and Watson, W.P. (1991). The mechanism of decomposition of N-methyl-N-nitrosourea in aqueous solution according to 13C and 15N NMR studies: quantitative fragmentation to cyanate. *Journal of the Chemical Society, Chemical Communications*, **24**: 1726-1728.

Bogovski, P. and Bogovski, S. (1981). Animal species in which *N*-nitroso compounds induce cancer. *International Journal of Cancer*, **27**: 471-474.

Bose, P., Klimowicz, A.C., Kornaga, E., Petrillo, S.K., Matthews, T.W., Chandarana, S., Magliocco, A.M., Brockton, N.T. and Dort, J.C. (2012). Bax expression measured by AQUAnalysis is an independent prognostic marker in oral squamous cell carcinoma. *BMC Cancer*, **12**: 332-343.

Broughton, B.R.S., Reutens, D.C. and Sobey, C.G. (2009). Apoptosis mechanisms after cerebral ischemia. *Stroke*, **40**: 331-339.

Brown, K., Buchmann, A., and Balmain, A (1990). Carcinogen-induced mutations in the mouse c-Ha-ras gene provide evidence of multiple pathways for tumour-progression. *Proceedings of the National Academy of Sciences of the United Sate of America*, **87**: 538-542.

Brown. A.P., Dinger, N. and Levine, B.S. (2000). Stress produced by gavage administration in the rat. *Contemporary Topics in Laboratory Animal Science*, **39**: 17-21.

Buchem, M.A.V., Te Velde, J., Willemze, R. and Spaander, P.J. (1988). Leukostasis, an underestimated cause of death in leukemia. *Blut*, **56**: 39-44.

Budan, F., Varjas, T., Nowrasteh, G., Varga, Z., Boncz, I., Cseh, J., Prantner, I., Antal, T., Pazsit, E., Gobel, G., Miklos, B., Gracza, T., Perjesi, P., Ember, I. And Gyongyi, Z. (2008). Early modification of c-myc, Ha-ras and p53 expressions by *N*-methyl-*N*-nitrosourea. *In Vivo*, **22**: 793-798.

Burch, M., Misra, M. and Phillips, E. (2005). Splenic malignancy: a minimally invasive approach. *The Cancer Journal*, **11**: 36-42.

Bustin, S.A. (2000). Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular Endocrinology*, **25**: 169-193.

Bustin, S.A. (2002). Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems.*Journal of Molecular Endocrinology*, **29**: 23-39.

Bustin, S.A., Benes, V., Nolan, T. and Pfaffl, M.W. (2005). Quantitative real-time RT-PCR – a perspective. *Journal of Molecular Endocrinology*, **34**: 597-601.

Cairns, R.A., Harris, I.S. and Mak, T.W. (2011). Regulation of cancer cell metabolism. *Nature Reviews Cancer*, **8**: 705-713.

Cavill, I. (1997). Diagnosis of cobalamin deficiency: the old and the new. *British Journal of Haematology*, **99**: 238-239.

Ceballos, C. (2007). Adopting molecular tools for diagnosis and monitoring malignant haematological disease. *Cancerologia*, **2**: 121-136.

Centeno, B.A. (2006). Pathology of liver metastases. *Cancer Control*, **13**: 13-26.

Chang, Y-C., Hsu, J-D., Lin, W-L., Lee, Y-J. and Wang, C-J. (2012). High incidence of acute promyelocytic leukemia specifically induced by *N*-nitroso-*N*-methylurea (NMU) in *Sprague Dawley rats*. *Archives of Toxicology*, **86**: 315-327.

Cheung, H.M., Mok, G.C.F., Lee, V., Shing, M.M.K. and Li, C.K. (2009). A rare presentation of acute lymphocytic leukaemia in a teenage girl: heart failure. *Hong Kong Journal of Paediatrics*, **14**: 126-128.

Cho, I.C., Chung, H.S. and Cho, K.S. (2010). Bcl-2 as a predictive factor for biochemical recurrence after radical prostatectomy: an interim analysis. *Cancer Research and Treatment*, **42**: 157-162.

Cory, S. and Adams, J.M. (2002). The Bcl2 family: regulators of the cellular life-or-death switch. *Nature Reviews Cancer*, **2**: 647-656.

Crawford, S.L. (2006). Correlation and Regression. *Circulation*,**114**: 2083-2088.

Crew, K.D. and Neugut, A.I. (2004). Epidemiology of upper gastrointestinal malignancies. *Seminars in Oncology*, **31**: 450-464.

Cross, S.S., Benes, K., Stephenson, T.J., Harrison, R.F. (2011). Grading in histopathology. *Diagnostic Histopathology*, **17**: 263-267.

Da'as, N., Polliack, A., Cohen, Y., Amir, G., Darmon, D., Kleinman, Y., Goldfarb, A.W., Ben-Yehuda, D. (2002). Kidney involvement and renal manifestations in non-Hodgkin's lymphoma and lymphocytic leukemia: a restrospective study in 700 patients. *European Journal of Haematology*, **67**: 158-164.

Dang, C.V. (2012). Links between metabolism and cancer. *Genes &Development*, **26**: 877-890.

Dean, J.D., Geodwin, P.H. and Hsiang, T. (2002). Comparative of relative RT-PCR and northern blot analyses to measure expression of β-1, 3-glucanase in *Nicotiana Benthamiana* infected with *Colletotrichumdestructivum*. *Plant Molecular Biology Reporter*, **20**: 347-356.

Denko, N.C. (2008). Hypoxia, HIF1 and glucose metabolism in the solid tumour. *Nature Reviews Cancer*, **8**: 705-713.

Dheda, K., Huggett, J.F., Chang, J.S., Kim, L.U., Bustin, S.A., Johnson, M.A., Rook, G.A.W. and Zumla, A. (2005). The implications of using an inappropriate reference gene for real-time reverse transcription PCR data normalisation. *Analytical Biochemistry*, **344**: 141-143.

DiGiuseppe, J.A. and Kastan, M.B. (1997). Apoptosis in haematological malignancies. *Journal of Clinical Pathology*, **50**: 361-364.

Dimopoulus, M.A., Barlogie, B., Smith, T. and Alexanian, R. (1989). High serum levels of lactic dehydrogenase identify a high-grade lymphoma-like myeloma. *Annals of Internal Medicine*, **110**: 521-525.

Draoui, N and Feron, O. (2011). Lactate shuttles at a glance: from physiological paradigms to anti-cancer treatments. *Disease Models and Mechanisms*, **4**: 727-732.

Druckrey, H., Preussmann, R., Ivankovic, S. and Schmahl, D. (1967). Organotropic carcinogenic effects of 65 various N-nitroso-compounds on BD rats (in German). *Z. Krebsforch*, **69**: 103-201.

Dufay, C., Abdelli, A., Pennec, V.L. and Chiche, L. (2012). Mesenteric tumors. *Journal of Visceral Surgery*, **149**: 239-251.

Durgawale, P., Sontakke, A., Shukla, P.S., Durgawale, P. and Chougule, P. (2011). Alteration in total sialic acid (TSA), total proteins (TP) and TSA/TP ratio in cancer patients. *Biomedical Research*, **22**: 492-494.

Eagen, J.W. and Lewis, E.J. (1977). Glomerulopathies of neoplasia. *Kidney International*, **11**: 297-306.

Eddy, J.A., sung, J.S., Geman, D. and Proce, N.D. (2010). Relative expression analysis for molecular cancer diagnosis and prognosis. *Technology in Cancer Research and Treatment*, **9**: 149-159.

Eisenberger, C.F., Shoenberg, M., Enger, C., Hortopan, S., Shah, S., Chow, N-H., Marshall, F.F. and Sidransky, D. (1999). Diagnosis of renal cancer by molecular urinalysis. *Journal of National Cancer Institute*, **91**: 2028-2032.

Elmore, S.A. (2006). Histopathology of lymph nodes. (2006). *Toxicologic Pathology*, **34**: 425-454.

Elmore, S. A. (2007). Apoptosis: a review of programmed cell death. *Toxicologic Pathology*, **35**: 495-516.

Erten, N., Saka, B., Caliskan, Y.K., Besisik, S., Karan, M.A. and Tascioglu, C. (2005). Acute renal failure due to leukemic infiltration in chronic lymphocytic leukemia: case report. *International Journal of Clinical Practice*, **59**: 53-55.

Esposti, M.D. (2002). The roles of Bid. *Apoptosis*, **7**: 433-440.

Fadok, V.A., de Cathelineau, A., Daleka, D.L., Henson, P.M. and Bratton D.L. (2001). Loss of phospholipid asymmetry and surface exposure of phosphotidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. *Journal of Biological Chemistry*, **276**: 1071-1077.

Fantin, V.R., St-Pierre, J. and Leder, P. (2006). Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology and tumor maintenance. *Cancer Cell*, **9**: 425-434.

Ferris, R.L., Xi, L., Seethala, R.R. (2011). Intraoperative qRT-PCR for detection of lymph node metastasis in head and neck cancer. *Clinical Cancer Research*, **17**: 1858-1866.

Franchi, D.S.C.A., Bacchi M.M., Padovani C.R. and de Camargo J.L. (2003).Thymic lymphomaas in *Winstar* rats exposed to *N*-methyl-*N*-nitrosourea (MNU). *Cancer Science*, **94**: 240-243.

Fulda, S. and Debatin, K-M. (2006). Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapgy. *Oncogene*, **25**: 4798-4811.

Gal, A.F., Andrei, S., Cernea, C., Taulescu, M. and Catoi, C. (2012). Effects of astaxanthin supplementation on chemically induced tumourigenesis in Winstar rats. *Acta Veterinaria Scandinavica*, **54**: 50-55.

Gangolli, S.D., van den Brandt, P.A., Feron, V.J. (1994). Nitrate, nitrite and *N*-nitroso compounds. *European Journal of Pharmacology*, **292**: 1-38.

Garcia-Gonzalez, M.A., Morandeira, M.J., Ucar, A. and Morandiera, J.R. (2000). A new model for the induction of tumours in the forestomach of rats by *N*-methyl-*N*-nitrosourea. *European Surgical Research*, **32**: 315-321.

Gates, K.S. (2009). An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation and reactions with radicals. *Chemical Research in Toxicology*, **22**: 1747-1760.

Gatenby, R.A. and Gilles, R.J. (2008). A microenvironment model of carcinogenesis. *Nature Reviews Cancer*, **8**: 56-61.

Godfrey, T.E., Raja, S., Finkelstein, S.D., Gooding, W.E., Kelly, L.A. and Luketich, J.D. (2001). Prognostic value of quantitative reverse transcription-polymerase chain reaction in lymph node-negative esophageal cancer patients. *Clinical Cancer Research*, **7**: 4041-4048.

Goldman, J.E. (2000). Glial differentiation and lineages. *Journal of Neuroscience Research*, **59**: 410-412.

Green, R.A., Nicholas, N.J. (1959). Pulmonary involvement in leukemia. *The American Review of Respiratory Disease*, **80**: 833-844.

Gross, A., McDonnell, J.M. and Korsmeyer, S.J. (1999). Bcl-2 family members and the mitochondria in apoptosis. *Genes & Development*, **13**: 1899-1911.

Gullino, P.M., Pettigrew, H.M. and Grantham, F.H. (1975). *N*-nitrosomethylurea as mammary gland carcinogen in rats. *Journal of National Cancer Institute*, **54**: 401-414.

Hanahan D. and Weinberg R.A. (2000). The hallmarks of cancer. *Cell*, **100**: 57-70.

Hanahan D. and Weinberg R.A. (2011). Hallmarks of cancer: the next generation. *Cell*, **144**: 646-674.

Hanby, A.M. (2005). The pathology of breast cancer and the role of the histopathology laboratory. *Clinical Oncology*, **17**: 234-239.

Hazilawati, H., Hutheyfa, A.H., Rosly, S.M., Jasni, S., Noordin, M.M. and Shanmugavelu, S. (2010). Haematological parameters of leukaemic rats supplemented with Morinda Citrifolia. *Medical Journal of Malaysia*, **65**: 125-126.

Hecht, S.S. (1999). DNA adduct formation from tobacco-specific *N*-nitrosamines. *Mutation Research*, **424**: 127-142.

Heid, C.A, Stevens, J., Livak, K.J. and Williams P.M. (1996). Real time quantitative PCR. *Genome Research*, **6**: 986-994.

Hengartner, M.O. (1998). Death cycle and Swiss army knives. *Nature*, **391**: 441.

Heywood, R. (1983). Long term toxicity. In M. Balls, R.J. Riddel and A.N. Worden (Eds), *Animals and alternatives in toxicity testing* (pp. 79-89). London: Academic Press.

Hill, M.M., Adrain, C., Duriez, P.J., Creagh, E.M. and Martin, S.J. (2004). Analysis of the composition, assembly kinetics and activity of native Apaf-1 apoptosomes. *Embo Journal*, **23**: 2134-2145.

Hinuma, Y., Nagata, K. and Hanaoka, M. (1981). Adult T cell leukemia: antigen in an ALT cell line and detection of antibodies to the antigen in human sera. *Proceedings of National Academy of Sciences of the United States of America*, **78**: 6476-6480.

Hoffman, D., Rivenson, A., Adams, J.D., Juchatz, A., Vinchkoshi, N. and Hecth, S.S. (1983). Effects of route of administration and dose on the carcinogenicity of *N*-nitrosodiethanolamine in the Syrian Golden Hamster. *Cancer Research*, **43**: 2521-2524.

Huang, D.C.S., Adams J.M. and Cory, S. (1998). The conserved N-terminal BH4 domain of Bcl-2 homologues is essential for inhibition of apoptosis and interaction with CED-4. *European Molecular Biology Organisation Journal*, **17**: 1029.

Huang, H. and Lu, P. (2012). Classical Hodgkin's lymphoma infiltrated both lungs. *Quantitative Imaging in Medicine and Surgery*, **2**: 288-290.

Hubscher, S.G., Lumley, M.A. and Elias, E. (1993).Vanishing bile duct syndrome: a possible mechanism for intrahepatic cholestasis in Hodgkin's lymphoma. *Hepatology*, **17**: 70-77.

Huggins, C.B., Grand, L. and Ueda, N. (1982). Specific induction of erythroleuekemia and myelogenous leukemia in Sprague Dawley rats. *Proceedings of National Academy of Sciences of United States of America*, **17**: 5411-5414.

Huggins, C.B. and Ueda, N. (1984). Regression of myelocytic leukemia in rats after hypophysectomy. *Proceedings of National Academy of Sciences of United States of America*, **81**: 598-601.

Huh, Y.O., Medeiros, L.J., Ravandi, F., Konoplev, S., Jorgensen, J.L. and Mranda, R.N. (2009). T-cell large granular lymphocyte leukemia associated with myelodysplastic syndrome. *Hematopathology*, **131**: 347-356.

Huhn, D., von Schiling, C., Wilhelm, M., Ho, A.D., Hallek, M., Kuse, R., Knauf, W., Riedel, U., Hinke, A., Srock, S., Serke, S., Peschel, C. and Emmerich, B. (2001). Rutuximab therapy of patients with B-cell chronic lymphocytic leukemia. *Blood*, **98**: 1326-1331.

Hunter, P. (2008). The paradox of model organisms. *European Molecular Biology Organisation Reports*, **9**: 717-720.

Hutheyfa, A.H., Hazilawati, H., Rosly, S.M., Jasni, S., Noordin, M.M. and Shanmugavelu, S. (2011). Histopathological features of peripheral T-cell lymphoma in *Sprague Dawley* rats induced with *N*-methyl-*N*-nitrosourea. *Pertanika Journal of Tropical Agricultural Science*, **34**: 351-361.

Ichinose, M., Nakanishi, H., Fujino, S. and Tatematsu, M. (1998). Establishment and characterization of two cell lines from N-methyl-N-nitrosourea-induced mouse glandular stomach carcinomas. *Japanese Journal of Cancer Research*, **89**: 516-524.

Igney, F.H. and Krammer, P.H. (2002). Death and anti-death: tumours resistance to apoptosis. *Nature Reviews Cancer*, **2**: 277-288.

Ilaria, R.L.Jr. (2004). Animal models of chronic myelogenous leukemia. *Hematology/Oncology Clinics of North America*, **18**: 525-543.

Inui, A. (2002). Cancer anorexia-cachexia syndrome: current issues in research and management. *A Cancer Journal for Clinicians*, **52**: 72-91.

Jaafar, H., Abdullah, S., Murtey, M.D., Idris, F.M. (2012). Expression of Bax and Bcl-2 in tumour cells and blood vessels of breast cancer and their association with angiogenesis and hormonal receptors. *Asian Pacific Journal of Cancer Prevention*, **13**: 3857-3862.

Jaffe, E.S. (1987). Malignant lymphomas: pathology of hepatic involvement. *Seminars in Liver Disease*, **7**: 257-268.

Jones, B.H. (1848). On a new substance occurring in the urine of a patient with mollities ossium. *Philosophical Transaction of the Royal Society of London*, **138**: 55-62.

Joza, N., Susin, S.A., Daugas, E., Stanford, W.L., Cho, S.K., Li, C.Y., Sasaki, T., Elia, A.J., Cheng, H.Y., Ravagnan, L., Ferri, K.F., Zamzami, N., Wakeham, A., Hakem, R., Yoshida, H., Kong, Y.Y., Mak, T.W., Zuniga-Pflucker, J.C., Kroemer, G. and Penninger, J.M. (2001). Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature*, **410**: 549-554.

Jurgensmeier, J.M., Xie, Z., Devereaux, Q., Ellerby, L., Bredesden, D. and Reed, J.C. (1998). Bax directly induces release of cytochrome c from isolated mitochondria. *Proceedings of National Academy of Sciences of United States of America*, **95**: 4997.

Kanduc, D. (1995). *N*-methyl-*N*-nitrosourea evidences cell cycle associated transient sequences in hepatic replicating DNA. *Biochemical and Biophysical Research Commuications*, **208**: 506-510.

Kaparou, M., Choumerianou, D., Chrysoula, P., Martimianaki, G., Kalmanti, M. and Stiakaki, Eftichia. (2013). Enhanced levels of the apoptotic Bax/Bcl-2 ratio in children with acute lymphocytic leukaemia and high-risk features. *Genetics and Molecular Biology*, **36**: 7-11.

Karp, J.E. and Smith, M.A. (1997). The molecular pathogenesis of treatment-induced (secondary) leukemias: foundations for treatment and prevention. *Seminar in Oncology*, **24**: 103-113.

Kazianis, S., Gimenez-Conti, I., Setlow, R.B., Woodhead, A.D., Harshbarger, J.C., Trono, D., Ledesma, M., Nairn, R.S., Walter, R.B. (2001). MNU induction of neoplasia in a platyfish model. *Laboratory Investigation*, **81**: 1191-1198.

Kenderian, S.S. and Litzow, M.R. (2013). Acute lymphoblastic leukemia in adolescents and young adults-from genomics to the clinics. *Clinical Oncology in Adolescents and Young Adults*, **3**: 49-52.

Khan, J., Wei, J.S., Ringner, M., Saal, L.H., Ladanyi, M., Westermann, F., Berthold, F., Schwab, M., Antonesco, C.R., Peterson, C. and Meltzer, P.S. (2001). Classification and diagnostic prediction of cancer using gene expression profiling and artificial neural networks. *Nature Medicine*, **7**: 673-679.

Kim, K.E., Onesti, G., Ramirez, O., Brest, A.N. and Swartz, C. (1969). Creatinine clearance in renal disease. A reappraisal. *British Medical Journal*, **4**: 11-14.

Knudson, C.M. and Korsmeyer, S.J. (1997). Bcl-2 and Bax function independently to regulate cell death. *Nature* Genetics, **16**: 358.

Kobayashi, T., Sawa, H., Morikawa, J., Zhang, W. and Shiku, H. (2000). Bax induction activates apoptotic cascade via mitochondria cytochrome c release and Bax of the expression enhances apoptosis induced cells. *Japanese Journal of Cancer Research*, **91**: 1264-1268.

Koestner, A.W., Ruecker, F.A. and Koestner, A. (1977). Morphology and pathogenesis of tumors of the thymus and stomach in Sprague Dawley rats following intragastric administration of methylnitrosourea (MNU). *International Journal of Cancer*, **20**: 418-426.

Kokkinakis, D.M., Watson, M.L., Honig, L.S., Rushing, E.J., Mickey, B.E. and Schold, S.C.Jr. (2001). Characterization of initiated cells in *N*-methylnitrosourea-induced carcinogenesis of the CNS in the adult rat. *Neuro-Oncology*, **3**: 99-112.

Krakstad, C. and Chekenya, M. (2010). Survival signalling and apoptosis resistance in glioblastomas: opportunities for targeted therapeutics. *Molecular Cancer*, **9**:135-148.

Kraus, M.D., Fleming, M.D. and Vonderheide, R.H. (2001). The spleen as a diagnostic specimen: a review of 10 years' experience at two tertiary care institutions. *Cancer*, **91**: 2001-2009.

Kroemer, G. and Pouyssegur, J. (2008). Tumor cell metabolism: cancer Achilles' heel. *Cancer Cell*, **13**: 472-482.

Lam, K.Y., Dicken, P. and Chan, A.C. (1993). Tumors of the heart. A 20-year experience with a review of 12, 485 consecutive autopsies. *Archieves of Pthology & Laboratory Medicine*, **117**: 1027-1031.

Lannitto, E. and Tripodo, C. (2011). How I diagnose and treat splenic lymphoma. *Blood*, **117**: 2585-2595.

Lee, J-S., Park, E.H., Choe, J. and Chipman, J.K. (2000). *N*-methyl-*N*-nitrosourea (MNU) induces papillary thyroid tumors which lack ras gene mutations in the hermaphroditic fish *Rivulus marmoratus*. *Teratogen Carcinogen Mutagen*, **20**: 1-9.

Lewis-Wambi, J.S. and Jordan, V.C. (2009). Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit? *Breast Cancer Research*, **11**: 206-217.

Liao, Z., Boileau, T.W.M., Erdman, J.W. and Clinton, S.K. (2002). Interrelationships among angiogenesis, proliferation and apoptosis in the tumor microenvironment during N-methyl-N-nitrosourea androgen-induced prostate carcinogenesis in rats. *Carcinogenesis*, **23**: 1701-1711.

Lijinsky, W., Garcia, H., Keefer, L., Loo, J. and Rose, A.E. (1972). Carcinogenesis and alkylation of rat liver nucleic acids by nitrosomethylurea and nitrosoethylurea administered intrapotal injection. *Cancer Research*, **32**: 893-897.

Limdi, J.K. and Hyde, G.M. (2003). Evaluation of abnormal liver function tests. *Postgraduate Medical Journal*, **79**: 307-312.

Lin, Y-W. and Aplan, P.D. (2006). Animal models of T-cell lymphoma. *Haematologica Reports*, **2**: 79-82.

Liska, J., Galbavy, S., Macejova, D., Zlatos, J. and Brtko, J. (2000). Histopathology of mammary tumours in female rats treated with 1-methyl-nitrosourea. *Endocrine Regulations*, **34**: 91-96.

Luciano, R.L. and Brewster, U.C. (2014). Kidneys involvement in leukemia and lymphoma. *Advances in Chronis Kidney Disease*, **21**: 27-35.

Ludwig, H. and Fritz, E. (1998). Anemia in cancer patients. *Seminars in Oncology*, **25**: 2-6.

Ludwig, H. and Strasser, K. (2001). Symptomatology of anemia. *Seminars in* Oncology, **28**: 7-14.

Maile, C.W., Moore, A.V., Ulreich, S. and Putman C.E. (1983). Chest radiographic-pathologic correlation in adult leukemia patients. *Investigative Radiology*, **18**: 495-499.

Mallet, S., Deeks, J.J., Halligan, S., Hopewell, S, Cornelius, V. and Altman, D.G. (2006). Systematic reviews of diagnostic tests in cancer: review of methods and reporting. *British Medical Journal*, **10**: 1136-1142.

Maloukh, L., Matousek, J., Bockstaele, E.V. and Roldan-Ruiz, I. (2009). Housekeeping gene selection for real time-PCR normalization in female Hop (*Humulus lupulus* L) tissues. *Journal of Plant Biochemistry & Biotechnology*, **18**: 53-58.

Mandal, D., Mazumder, A., Das, P., Kundu, M. and Basu, J. (2005). Fas-, caspase-8- and caspse-3-dependent signalling regulates the activity of the human erythrocytes. *Journal of Biological Chemistry*, **280**: 39460-39467.

Margison, G.P., Koref, M.F.S. and Povey, A.C. (2002). Mechanisms of carcinogenicity/chemotherapy by O^6-methylguanine. *Mutagenesis*, **17**: 483-487.

Marnett, L.J. and Burcham, P.C. (1993). Endogenous DNA adducts: potential and paradox. *Chemical Research in Toxicology*, **6**: 771-785.

Masui, T., Tezuka, N., Nakanishi, H., Inada, K., Miyashita, N. and Tatematsu, M. (1997). Induction of invasive squamous cell carcinomas in the forestomach of (C3H x MSM)F1, MSM and C3H mice by N-methyl-N-nitrosourea and mutational analysis of the H-ras and p53genes. *Cancer Letter*, **111**: 97-104.

Matsumoto, H., Wada, T., Fukunaga, K., Yoshihiro, S., Matsuyama, H. and Nalto, K. (2004). Bax to Bcl-2 ratio and Ki-67 index are useful predictors of neoadjuvant chemoradiation therapy in bladder cancer. *Japanese Journal of Clinical Oncology*, **34**: 124-130.

Matsushima, K., Yamakawa, S., Edamoto, H., Yamaguchi, Y., Nagatani, M. and Tamura, K. (2010). Spontaneous Malignant T-cell lymphoma in a young adult Crl:CD (SD) rat. *Journal of Toxicological Pathology*, **23**: 49-52.

Matutes, E., Oscier, D. and Montalban, C. (2008). Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. *Leukemia*, **22**: 487-495.

Maung, Z.T., MacLean, F.R. and Reid, M.M. (1994). The relationship between Bcl-2 expression and response to chemotherapy in acute leukaemia. *British Journal of Haematology*, **88**: 105-109.

McCaffrey, J. and Hamilton, J.W. (2006). Comparison of effects of direct-acting DNA methylating and ethylating agents on inducible gene expression *in vivo*. *Environmental and Molecular Mutagenesis*, **23**: 164-170.

Mercadante, S., Gebbia, V. and Marrazzo, A. (2000). Anaemia in cancer: pathophysiology and treatment. *Cancer Treatment Reviews*, **26**: 303-311.

Merkin, J., Russell, C., Chen, P., Burge, C.B. (2012). Evolutionary dynamics of gene and isoform regulation in mammalian tissues. *Science*, **338**: 1593-1599.

Miclaus, G.A.F.V., Taulescu, M., Bolfa, P., Tabaran, F., Bouari, C. and Catoi, C. (2012). Concurrent mammary adenocarcinoma and lymphoma in MNU-induced tumourigenesis in a Sprague Dawley female rat. *Bulletin of University of Agricultural Sciences and Veterinary Medicine*, **69**: 98-100.

Minn, A.J., Velez, P., Schendel, S.L., Liang, H., Muchmore, S.W., Fesik, S.W., Fill, M. and Thompson, C.B. (1997). Bcl-XL forms an ion channel in synthetic lipid membranes. *Nature*, **385**: 353.

Mishra, S., Sharma, D.C. and Sharma, P. (2004). Studies of biochemical parameter in breast cancer with and without metastasis. *Indian Journal of Clinical Biochemistry*, **19**: 71-75.

Mitas, M., Cole, D.J., Hoover, L., Fraig, M.M., Mikhitarian, K. and Block, M.I. (2003). Real-time reverse-transcription-PCR detects KS1/4 mRNA in mediastinal lymph nodes from patients with non-small cell lung cancer. *Clinical Chemistry*, **49**: 312-315.

Mitra, S. and Kaina, B. (1993). Regulation of repair of alkylation damage in mammalian genomes. *Progress in Nucleic Acid Research and Molecular Biology*, **44**: 109-142.

Mizoguchi, M., Naito, H., Kurata, Y., Shibata, M-A., Tsuda, H., Wild, C.P., Montesano, R. and Fukushima, S. (1993). Influence of aging on multi-organ carcinogenesis in rats induced by N-methyl-N-nitrosourea. *Japanese Journal of Cancer Research*, **84**: 139-146.

Mocellin, S., Rossi, C.R., Pilati, P., Nitti, D. and Marincola, F.M. (2003). Quantitative real-time PCR: a powerful ally in cancer research. *Trends in Molecular Medicine*, **9**: 189-195.

Molica, S. (1991). Progression and survival studies in early chronic lymphocytic leukemia. *Blood*, **78**: 895-899.

Mori, M., Mimori, K., Ueo, H., Karimine, N., Barnard, G.F. and Sugimachi, K. (1996). Molecular detection of circulating solid carcinoma cells in the peripheral blood: the concept of early systemic disease. *International Journal of Cancer*, **68**: 739-743.

Morton, D.B., Jennings, M., Buckwell, A., Ewbank, R., Godfrey, C., Holgate, B., Inglis, I., James, R., Page, C., Sharman, I., Verschoyle, R., Westall, L. and Wilson, A.B. (2001). Refining procedures for the administration of substances. *Laboratory Animals*, **35**: 1-41.

Moullet, I., Salles, G. and Ketterer, N. (1998). Frequency and significance of anemia in non-Hodgkin's lymphoma patients. *Annals in Oncology*, **9**: 1109-1115.

Mulrow, C.D. (1994). Rationale for systemic reviews. *British Medical Journal*, **309**: 597-595.

Nicot, N., Hausman, J.F., Hoffman, L., Evers, D. (2005). Housekeeping gene selection for real-time RT-PCR normalisation in potato during biotic and abiotic stress. *Journal of Experimental Botany*, **56**: 2907-2914.

Noronha, V., Shafi, N.Q., Obando, J.A. and Kummar, S. (2005). Primary non-Hodgkin's lymphoma of the liver. *Critical Reviews in Oncology/Hematology*, **53**: 199-207.

Norris, S.H. (1993). Paraneoplastic glomerulopathies. *Seminars in Nephrology*, **13**: 258-272.

Nursyuhada, H., Hazilawati, H., Hutheyfa, A.H., Rosly, S.M., Shanmugavellu, S., Noordin, M.M. and Jasni, M. (2011). Detection of Bcl-2 gene in leukaemic rats using Evagreen real-time RT-PCR assay. *Pertanika Journal of Tropical Agricultural Sciences*, **34**: 373-380.

O'Connell, T.X., Horita, T.J. and Kasravi, B. (2005). Understanding and interpreting serum protein electrophoresis. *American Family Physician*, **71**: 105-112.

O'Mahony, D., Piekarz, R.L., Bandettini, P., Arai, A.E., Wilson, W.H. and Bates, S.E. (2008). Cardiac involvement with lymphoma: a review of the literature. *Clinical Lymphoma, Myeloma & Leukemia*, **8**: 249-252.

Ohshima, H and Bartsch, H. (1994). Chronic infection and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Molecular Mechanisms of Mutagenesis*, **305**: 253-264.

Oltvai, Z.N. and Korsmeyer, S.J. (1994). Check points of duelling dimmers foil death wishes. *Cell*, **79**: 189.

Oppezzo, P. and Dighiero, G. (2013). Role of the B-cell receptor and the microenvironment in chronic lymphocytic leukaemia. *Blood Cancer Journal*, **3**: 149-157.

Osbourne, M.R. and Phillips, D.H. (2000). Preparation of a methylated DNA standard and its stability on storage. *Chemical Research in Toxicology*, **13**: 257-261.

Pascal, R.R., Iannaccone, P.M., Rollwagen, F.M., Harding, T.A. and Bennette, S.J. (1976). Electron microscopy and immunofluorescence of glomerulus immune complex deposits in cancer patients. *Cancer Research*, **36**: 43-47.

Paster, E.V., Vilines, K.A. and Hickman, D.L. (2009). Endpoint for mouse abdominal tumor models: refinement of current criteria. *Comparative Medicine*, **48**: 234-241.

Paul-Samojedny, M., Kokocińska, D., Samojedny, A., Mazurek, U., Partyka, R., Lorenz, Z. and Wilczok, T. (2005). Expression of cell survival/death genes: Bcl-2 and Bax at the rate of colon cancer prognosis. *Biochimica etBiophysicaActa*, **1741**: 25-29.

Perrone, R.D., Madias, N.E. and Levey, A.S. (1992). Serum creatinine as an index of renal function: new insights into old concepts. *Clinical Chemistry*, **38**: 1933-1953.

Piao, Y., Liu, Y. and Xie, X. (2013). Change trends of organ weight background data in Sprague Dawley rats at different ages. *Journal of Toxicologic Pathology*, **26**: 29-34.

Placzek, W.J., Wei, J., Kitada, S., Zhai, D., Reed, J.C. and Pellecchia, M. (2010). A survey of the anti-apoptotic Bcl-2 subfamily expression in cancer types provides a platform to predict the efficacy of Bcl-2 antagonist in cancer therapy. *Cell Death and Disease*, **1**: 1-9.

Poeta, G.D., Venditti, A., Principe, M.I.D., Maurillo, L., Buccisano, F., Tamburini, A., Cox, M.C., Franchi, A., Bruno, A., Mazzone, C., Panetta, P., Suppo, G., Masi, M and Amadori, S. (2003). Amount of spontaneous apoptosis detected by Bax/Bcl-2 ratio predicts outcome in acute myeloid leukaemia (AML): presented in part at the 42nd annual meeting of the American Society of Haematology, San Francisco, CA, December 1-5, 2000.46. *Blood*, **101**: 2125-2131.

Poiesz, B.J., Ruscetti, F.W., Gazder, A.F., Bunn, P.A., Minna, J.D. and Gallo, R.C. (1980). Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T cell lymphoma. *Proceedings of National Academy of Science of United States of America*, **77**: 7415-7419.

Poletti, V., Salvucci, M., Zanchini, R., Molinari, A.L., Zuffa, E., Poletti, G., Zaccaria, A. (2000). The lung as a target organ in patients with hematologic disorders. *Haematologica*, **85**: 855-864.

Porcu, P., Farag, S., Marcucci, G., Cataland, S.R., Kennedy, M.S. and Bissell, M. (2002). Leukocytoreduction for acute leukemia. *Therapeutic Apheresis*, **6**: 15-23.

Portt, L., Norman, G., Clapp, C., Greenwood, M. and Greenwood, M.T. (2011). Anti-apoptosis and cell survival: a review. *Biochimica et Biophysica Acta*, **1813**: 238-259.

Povey, A.C. (2000). DNA adducts: endogenous and induced. *Toxicologic Pathology*, **28**: 405-414.

Pratt, D.S. and Kaplan, M.M. (2000). Evaluation of abnormal liver enzyme results in asymptotic patients. *New England Journal of Medicine*, **342**: 1266-1271.

Prokop, A., Wieder, T., Sturm, I., Essman, F., Seeger, K., Wuchter, C., Ludwig, W.D., Henze, G., Dorken, B. and Daniel P.T. (2000). Relapse in childhood acute lymphoblastic leukaemia in associated with a decrease of the Bax/Bcl-2 ratio and loss of spontaneous caspase-3 processing in vivo. *Leukemia*, **14**: 1606-1613.

Radonic, A., Thulke, S., Mackay, I.M., Landt, O., Siegert, W. and Nitsche, A. (2004). Guideline to reference gene selection for quantitative real-time PCR. *Biochemical and Biophysical Research Communications*, **313**: 856-862.

Ramaswamy, S., Tamayo, P., Rifki. R., Mukherjee, S., Yeang, C-H., Angelo, M., Ladd, C., Reich, M., Latulippe, E., Mesirov, J.P., Tomaso, P., Gerald, W., Loda, M., Lander, E.S. and Golub, T.R. (2001). Multiclass cancer diagnosis using tumor gene expression signatures. *Proceedings of the National Academy of Sciences*, **98**: 15149-15154.

Rasouli, M., Okhovatio, A. and Enderami, A. (2005). Serum proteins profile as an indicator of malignancy: multivariate logistic regression and ROC analyses. *Clinical Chemistry and Laboratory Medicine*, **43**: 913-918.

Rawstron, A.C., Bennette, F.L., O'Connor, S.J.M., Kwok, M., Fenton, J.A.L., Plummer, M., de Tute, R., Owen, R.G. and Richards, S.J. (2008). *New England Journal of Medicine*, **359**: 575-583.

Reed, J.C. (2000). Mechanism of apoptosis. *American Journal of Pathology*, **157**: 1415-1426.

Rivera, E.S., Andrade, N., Martin, G., Melito, G., Cricco, G., Mohamad, N., Davio, C., Caro, R and Bergoc, R.M. (1994). Induction of mammary tumours in rat by intraperitoneal injection of NMU: histopathology and estral cycle influence. *Cancer Letter*, **86**: 223-228.

Rockx, M.A., Rizkalla, K. and Clark, W.F. (2008). Acute renal failure and chronic lymphocytic leukemia. *Nephrology Dialysis Transplantation*, **23**: 770-771.

Rose, D.P., Pruitt, B., Stauber, P., Erturk, E. and Bryan, G.T. (1980). Influence of dosage schedule on the biological characteristics of N-nitrosomethylurea-induced rat mammary tumors. *Cancer Research*, **40**: 235-239.

Russo, J. Wilgus, G. and Russo, I.H. (1992). Differentiation of mammary gland and susceptibility to carcinogenesis. *Breast Cancer Research Treatment*, **2**: 5-73.

Russo, J. and Russo, I.H. (2000). Atlas and histological classification of tumors of the mammary gland. *Journal of Mammary Gland Biology*, **5**: 187-188.

Rushing, E.J., Watson, M.L., Schold, S.C., Land, K.J. and Kokkinakis, D.M. (1998). Glial tumors in the MNU rat model: induction of pure and mixed gliomas that do not require typical missense mutations of p53. *Journal of Neuropathology and Experimental Neurology*, **57**: 1053-1060.

Saboo, S.S., Krajewski, K.M., O'regan, K.N., Giardino, A., Brown, J.R., Ramaiya, N. and Jagannathan, J.P. (2012). Spleen in haematological malignancies: spectrum of imaging findings. *The British Journal of Radiology*, **85**: 81-92.

Saelens, X., Festjens, N., VandeWalle, L., van Gurp, M., van Loo G. and Vandenabeele, P. (2004). Toxic proteins released from mitochondria in cell death. *Oncogene*, **23**: 2861-2874.

Sajjarattul, N.N.A, Hazilawati, H, Rosly, S.M., Hakimi, M.K., Nursyuhada, H., Yusnaini, M.Y., Hutheyfa, A.H., Nurul Huda, M.Z., Shanmugavelu, S., Noordin, M.M., Jasni, S. and Tan, S.W. (2014). Detection of vascular endothelial growth factor mRNA transcript in rat spleen exposed to n-methyl-n-nitrosourea via oral and intraperitoneal routes. *Online Journal of Veterinary Research*, **18**: 756-766.

Sakumi, K., Shiraisha, S., Tsuzuki, T., Ishikawa, T. and Sekiguchi, M. (1997). Methylnitrosourea-induced tumorigenesis in MGMT gene knockout mice. *Cancer Research*, **57**: 2415-2418.

Sar, P., Bhargava, D.K., Sengupta, D., rath, B., Chaudhary, S. and Mishra, S.K. (2012). In human breast cancer cells TR β competes with ER α for altering Bcl-2/bax ratio through SMP30-mediated p53 induction. *Journal of Cancer Science and Therapy*, **4**: 227-234.

Sato, E., Hasui, K. and Tokunaga, M. (1982). Autopsy findings of adult T cell lymphoma-leukemia. *Gann Monographs of Cancer Research*, **39**: 69-80.

Sattler, M., Liang, H., Nettesheim, D., Meadows, R.P., Harlen, J.E., Eberstadt, M., Yoon, H.S., Shuker, S.B., Chang, B.S., Minn, A.J., Thompson, C.B. and Fesik, S.W. (1997). Structure of bcl-XL-Bak peptide complex: recognition between regulators of apoptosis. *Science*, **275**: 983.

Savli, H., Gluzman, D.F., Sunnetci, D., Zavelevich, M.P., Sklyarenko, L.M., Nadgornaya, V.A. and Koval, S.V. (2011). Quantitative real time PCR analysis of apoptosis-related gene expression in leukemias in Ukrainian patient. *Experimental Oncology*, **33**: 104-106.

Saxena, A., Viswanathan, S., Moshynska, O., Tandon, P., Sankaran, K. and Sheridan, D.P. (2004). Mcl-1 and Bcl-2/Bax ratio are associated with treatment response but not with Rai stage in B-cell chronic lymphocytic leukaemia. *American Journal of Hematology*, **75**: 22-33.

Scarfo, L. and Ghia, P. (2013). Reprogramming cell death: Bcl-2 family inhibition in haematological malignancies. *Immunology Letters*, **155**: 36-39.

Scheider, E., Cowan, K.H. and Bader, H. (1995). Increased expression of the multidrug resistance associated protein gene in relapsed acute leukaemia. *Blood*, **85**: 186-193.

Schimmer, A.D., Munk-Pedersen, I., Minden, M.D. and Reed, J.C. (2003). Bcl-2 and apoptosis in chronic lymphocytic leukemia. *Current Treatment Options in Oncology*, **4**: 211-218.

Sedgwick, B. (1997). Nitrosated peptides and polyamines as endogenous mutagens in O^6-alkylguanine-DNA alkyltransferase deficient cells. *Carcinogenesis*, **18**: 1561-1567.

Sen, N.P., Seaman, S.W., Burgess, C., Baddoo, P.A. and Weber, D. (2000). Investigation on the possible formation of N-nitroso-N-methylurea by nitrosation of creatinine in model sytems and in cured meats at gastric pH. *Journal of Agricultural and Food Chemistry*, **48**: 5088-5096.

Serebryanyi, A.M., Sal'nikova, L.E., Bakhitova, L.M. and Paschin, Y.V. (1990). Role of carbamoylation reaction in the biological activity of methyl nitrosourea. *Mutation Research*, **231**: 195-203.

Sharawat, S.K., Bakhshi, R., Vishnubhatla, S., and Gupta, R and Bakhshi, S. (2013). BAX/BCL2 RMF1 ratio predicts better induction responses in pediatric patients with acute myeloid leukemia. *Pediatric Blood & Cancer*, **60**: 63-66.

Shayan, R., Achen, M.G. and Stacker, S.A. (2006). Lymphatic vessels in cancer metastasis: bridging the gaps. *Carcinogenesis*, **27**: 1729-1738.

Shemesh, O., Golbertz, H., Kriss, J.P. and Myers, B.D. (1985). Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney International*, **28**: 830-838.

Simonian, P.L., Grillot, D.A.M., Andrews, D.W., Leber, B. and Nunez, G. (1996). Bax homodimerization is not required for bax to accelerate chemotherapy-induced cell death. *Journal of Biological Chemistry*, **271**: 32073.

Sintara, K., Thong-Ngam, D., Patumraj, S. and Klaikeaw, N. (2012). Curcumin attenuates gastric cancer induced by *N*-methyl-*N*-nitrosourea and saturated sodium chloride in rats. *Journal of Biomedicine and Biotechnology*, **2012**: 1-8.

Slee, E.A., Adrain, C. and Martin, S.J. (2001). Executioner caspase 3, 6 and 7 perform distinct, non-redundant roles during the demolition phase of apoptosis. *Journal of Biological Chemistry*, **276**: 7320-7326.

Soares, F.A., Landell, G.A. and Cardoso, M.C. (1992). Pulmonary leukostasis without hyperleukocytosis: a clinicopathologic study of 16 cases. *American Journal of Hematology*, **40**: 28-32.

Song, M., Ou, X., Xiao, C., Xiao, Z., Wei, F. and Hong, Y. (2013). Hedgehog signalling inhibitor cyclopamine induces apoptosis by decreasing Gli2 and *Reports*, **1**: 325-329.

Srinivas, P.R., Kramer, B.S. and Srivastava S. (2001). Trends in biomarker research for cancer detection. *Lancet Oncology*, **2**: 698-704.

Sugimura, T. and Fujimura, S. (1967). Tumor production in glandular stomach of rat by *N*-methyl-*N'*-nitro-*N*-nitrosoguanine. *Nature*, **216**: 943-944.

Susin, S.A., Daugas, E., Ravagnan, L., Samejima, K., Zamzami, N., Loeffler, M., Costantini, P., Ferri, K.F., Irinopoulus, T., Prevost, M.C. and Kroemer, G. (2000). Two distinct pathways leading to nuclear apoptosis. *Journal of Experimental Medicine*, **192**: 571-580.

Suzuki, T., Higgins, P.J. and Crawford, D.R. (2000). Control selection for RNA quantitation. *Biotechniques*, **29**: 332-337.

Swenberg, J.A., Koestner, A., Wechsler, W., Brunden, M.N. and Abe, H. (1975). Differential oncogenic effects of methylnitrosourea. *Journal of the National Cancer Institute*, **54**: 86-95.

Tano, T., Okamoto, M., Kan, S., Nakashiro, K., Shimodaira, S., Koido, S., Homma, S., Sato, M., Fujita, T., Kawakami, Y. and Hamakawa, H. (2013). Prognostic impact of expression of Bcl-2 and Bax genes in circulating immune cells derived from patients with head and neck carcinoma. *Neoplasia*, **15**: 305-314.

Tatematsu, M., Ogawa, K., Hoshiya, T., Shichino, Y., Kato, T., Imaida, K. and Ho, N. (1992). Induction of adenocarcinomas in the glandular stomach of BALB/c mice treated with *N*-methyl-*N*-nitrosourea. *Cancer Science*, **83**: 915-918.

Tatematsu, M., Yamamoto, M., Hitoshi, I., Fukami, H., Yuasa, H., Tezuka, N., Tsuneo, M. and Nakanishi, H. (1993). Induction of glandular stomach cancers in CH3 mice treated with *N*-methyl-*N*-nitrosourea in drinking water. *Cancer Science*, **84**: 1258-1264.

Thannickal, V.J. and Famburg, B.C. (2000). Reactive oxygen species in cell signaling. *American Journal of Physiology*, **279**: 1005-1028.

Thompson, H.J. and Meeker, L.D. (1983). Induction of mammary gland carcinomas by the subcutaneous injection of 1-methyl-1-nitrosourea. *Cancer Research*, **43**: 1628-1629.

Thompson, H.J. and Adlakha, H. (1991). Dose-responsive induction of mammary gland carcinomas by the intraperitoneal injection of 1-methyl-1-nitrosourea. *Cancer Research*, **51**: 3411-3415.

Thompson, H.J., McGinley, J.N., Rothhammer, K. and Singh, M. (1995). Rapid induction of mammary intraductal proliferation, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-nitrosourea. *Carcinogenesis*, **16**: 2407-2411.

Thong-Ngam, D., Sintara, K., Chayanupatkul, M., Klaikaew, N. and Chatsuwan, T. (2010). The rat models of gastric cancer using Helicobactor pylori infection, N-methyl-N-nitrosourea and high salt induced carcinogenesis. *Thai Journal of Gastroenterology*, **11**: 41-48.

Tolonen, T.T., Tommola, S., Jokinen, S., Parviainen, T., Martikainen, P.M. (2007). Bax and Bcl-2 are focally overexpressed in the normal epithelium of cancerous prostates. *Scandinavian Journal of Urology and Nephrology*, **41**: 85-90.

Toth, L.A. (2000). Defining the moribund condition as an experimental endpoint for animal research. *Institute of Laboratory Animal Resources Journal*, **41**: 72-79.

Tronov, V.A., Kramarenko, I.I., Smirnova, T.D. and Terekhnov, S.M. (2006). Comparison of geno- and cytotoxicity of methylnitrosourea on MMR-proficient and MMR-deficient human tumor cell lines. *Tsitologiia*, **48**: 19-27.

Tronov, V.A., Loginova, M.J. and Kramarenko, I.I. (2008). Methylnitrosourea as challenge mutagen in assessment of the DNA mismatch repair (MMR) activity: association with some types of cancer. *Russian Journal of Genetics*, **44**: 595-600.

Tsubura, A., Lai, Y-C., Miki, H., Sasaki, T., Uehara, N., Yuri, T. and Yosshizawa, K. (2011). Animal model of *N*-methyl-*N*-nitrosourea-induced mammary cancer and retinal degeneration with special emphasis on therapeutic trial. *In Vivo*, **25**: 11-22.

Tsuda, H., Fukushima, S., Imaida, K., Kurata, Y. and Ito, N. (1983). Organ specific promoting effect of Phenobarbital and saccharin in induction of thyroid, liver and urinary bladder tumors in rats after initiation with *N*-nitrosomethylurea. *Cancer Research*, **43**: 3292-3296.

Tsukamoto, T., Tsutomu, M. and Tatematsu, M. (2007). Animal model for stomach carcinogenesis. *Toxicologic Pathology*, **35**: 636-648.

Tuncel, H., Shimamoto, F., Cirakoglu, A., Korpinar, M.A. and Kalkan, T. (2013). P-selectin expression in a colon tumor model exposed by sinusoidal electromagnetic fields. *Biomedical Reports*, **1**: 389-392.

Turner, P.V., Brabb, T., Pekow, C. And Vasbinder, M.A. (2011). Administration of substances to laboratory animals: routes of administration and factors to consider. *Journal of the American Association for Laboratory Animal Science,* **50**: 600-613.

Vasconcelos, Y., Davi, F., Levy, V., Oppezzo, P., Magnac, C. and Michel, A. (2003). Binet's staging system and VH genes are independent but complementary prognostic indicators in chronic lymphocytic leukaemia. *Journal of Clinical Oncology*, **21**: 3928-3932.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. and Speleman F. (2002). Accurate normalisation of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, **3**: 1-11.

Vakkala, M., Pääkkö, P and Soini, Y. (1999). Expression of caspases 3, 6 and 8 is increased in parallel with apoptosis and histological aggressive of breast lesion. *British Journal of Cancer*, **81**: 592-599.

Vaux, D.L., Cory, S. and Adams, J.M. (1988). Bcl-2 gene promotes haematopoietic cells survival and cooperates with c-myc to immortalize pre-B cells. *Nature*, **335**: 440.

Verma, S., Bahorun, T., Singh, R.K., Aruoma, O.I. and Kumar, A. (2013). Effect of Aegle marmelos leaf extract on *N*-methyl-*N*-nitrosourea-induced hepatocarcinogenesis in Balb/c mice. *Pharmaceutical Biology,* 51: 1272-1281.

Vergis, R., Corbishley, C.M. and Thomas, K. (2010). Expression of Bcl-2, p53 and MDM2 in localised prostate cancer with respect to the outcome of radical radiotherapy dose escalation. *International Journal of Radiation Oncology Biology and Physics*, **78**: 35-41.

Watson, S., Mercier, S., Bye, C., Wilkinson, J., Cunningham, A.L. and Harman, A.N. (2007). Determination of suitable housekeeping genes for normalisation of quantitative real-time PCR analysis of cells infected with human immunodeficiency virus and herpes viruses. *Virology Journal*, **4**: 1-5.

Wingard, J.R., Hiemenz, J.W. and Jantz, M.A. (2012). How I manage pulmonary nodular lesions and nodular infiltrates in patients with hematologic malignancies or undergoing hematopoietic cell transplantation. *Blood*, **120**: 1791-1800.

Wirth-Dzieciolowska, E., Karaszewska, J., Sadowski, T., Pyśniak, K. And Gajewska, M. (2009). Selected blood serum biochemical indicators in twelve inbred strains of laboratory mice. *Animal Science Papers and Reports*, **27**: 159-167.

Wogan, G.N., Hecht, S.S., Felton, J.S., Conney, A.H. and Loeb, L.A. (2004).Environmental and chemical carcinogenesis. *Seminars in Cancer Biology*, **14**: 473-486.

Wolter, K.G., Hsu, Y.T., Smith, C.L., Nechushtan, A., Xi, X.G. and Youle, R.J. (1997). Movement of Bax from the cytosol to mitochondria during apoptosis. *Journal of Cell Biology*, **139**: 1281-1292.

Wyatt, M.D. and Pittman, D.L. (2006). Methylating agents and DNA repair responses: methylated bases and sources of strand breaks.

Wyss, M. and Kaddurah-Daouk, R. (2000). Creatine and creatinine metabolism. *Physiological Reviews*, **80**: 1107-1213.

Yamamoto, S., Mitsumori, K., Kodama, Y., Matsunuma, N. and Manabe, S. (1996). Rapid induction of more malignant tumors by various genotoxic carcinogens in transgenic mice harbouring a human protype c-Ha-ras gene than in control non-transgenic mice. *Carcinogenesis*, **17**: 2455-2461.

Yamamoto, M., Tsukamoto, T., Sakai, H., Shirai, N., Ohgaki, H., Furihata, C., Donehower, L.A., Yoshida, K. and Tatematsu, M. (2000). p53 knockout mice (-/-) are more susceptible than (+/-) or (+/+) mice to *N*-methyl-*N*-nitrosourea stomach carcinogenesis. *Carcinogenesis*, **21**: 1891-1897.

Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D. and Somero, G.N. (1982). Living with water stress: evolution of osmolyte systems. *Science*, **217**: 1214-1222.

Yang, E. and Korsmeyer, S.J. (1996). Molecular thanatopsis: a discourse on the Bcl-2 family. *Blood*, **88**: 386.

Yang, J., Liu, X., Bhalla, K., Kim, C.N., Ibrado, A.M., Cai, J., Peng, T.I., Jones, D.P. and Wang, X. (1997). Prevention of apoptosis by Bcl-2: release of cytochrome c from the mitochondrial blocked. *Science*, **275**: 1129-1132.

Yano, K., Sonoda, M., Sakagishi, Y., Sakamoto, Y. and Uyemura, K. (1988). Reactions of ultimate carcinogens with cell membranes: importance of carbomylation of phosphatidylethanolamine by the carcinogens. *Carcinogenesis*, **9**: 1085-1090.

Yilmaz, I.A. (2003). Relation between bladder cancer and protein oxidation. *International Urology and Nephrology*, **35**: 345-350.

Yoon, O. and Roh, J. (2012). Downregulation of KLF4 and the Bcl-2/Bax ratio in advanced epithelial ovarian cancer. *Oncology Letters*, **4**: 1033-1036.

Young, J.M. and Goldman, I.R. (1954). Tumour metastasis to the heart. *Circulation*, **9**: 220-229.

Zhang, Z., Yang, X.Y. and Cohen, D.M. (1999). Urea associated oxidative stress and Gadd153/CHOP induction. *American Journal of Physiology*, **276**: 786-793.

Zhang, Z., Dmitrieva, N.I., Park, J-H., Levine, R.L. and Burg, M.B. (2004). High urea and NaCl carbonylate proteins in renal cells in culture and *in vivo* and high urea causes 8-oxoguanine lesions in their DNA. *Proceedings of National Academy of Sciences*, **101**: 9491-9496.

Zhang, D. and Loughran, T.P.Jr. (2012). Large granular lymphocytic leukemia: molecular pathogenesis, clinical manifestations and treatment. *Hematology*, **2012**: 652-659.

Zhang, Y. (2013). Gap! Transgenesis, model organisms and human diseases. *Cloning & Transgenesis*, **2**: 103-106.