Free radicals in chemotherapy induced cytotoxicity and oxidative stress in triple negative breast and ovarian cancers under hypoxic and normoxic conditions

Clifford W Fong

Free radicals in chemotherapy induced cytotoxicity and oxidative stress in triple negative breast and ovarian cancers under hypoxic and normoxic conditions

Clifford W. Fong
Eigenenergy, Adelaide, South Australia, Australia.

Email: cwfong@internode.on.net

Keywords: triple negative breast cancer, ovarian cancer, cytotoxicity, hypoxia, anoxia, normoxia, free radicals, oxidative stress, electron affinity, BRCA1, HIF-1α, VEGF, quantum mechanics

Abbreviations

TNBC triple negative breast cancer, BRCA1 human tumour suppressor gene and protein, oxidative stress, MnSOD manganese superoxide dismutase, HIF-1 hypoxia-inducible factor-1, VEGF vascular endothelial growth factor family, $\Delta G_{desolv,CDS}$ free energy of water desolvation, $\Delta G_{lipo,CDS}$ lipophilicity free energy, CDS cavity dispersion solvent structure of the first solvation shell, AEA adiabatic electron affinity, HOMO highest occupied molecular orbital, LUMO lowest unoccupied molecular orbital , R^2 multiple correlation coefficient, the F test of significance, SEE standards errors for the estimate, standard errors of the variables SE($\Delta G_{desolCDS}$), SE($\Delta G_{lipoCDS}$), SE(Dipole Moment), SE (Molecular Volume, ROS reactive oxygen species, ET electron transfer, OS oxidative stress

Abstract

An analysis of the cytotoxicity data for a wide range of anti-cancer drugs applied to triple negative breast cancer (TNBC) cells and ovarian cancer cells under normoxic, hypoxic and anoxic conditions has shown that free radical forms of the drugs are involved. This finding is consistent with the known involvement of oxidative stress in the TNBC and ovarian cancer cells. A mechanism to explain the formation of these drug free radicals and how they can be involved in oxidative stress is proposed.

A hypothesis that might explain the known link between TNBC and ovarian cancer is proposed which involves involvement of free radicals interacting with the BRCA1/HIF-1α complex or VEGF under hypoxic or anoxic conditions.

Introduction

There are two main approaches towards targeting hypoxia in cancer chemotherapy: bioreductive prodrugs (such as tirapazamine, and quinones) and inhibitors of molecular targets upon which hypoxic cell survival depends, such as the hypoxia-inducible factor-1 (HIF-1) or Src tyrosine kinase or the vascular endothelial growth factor family VEGF. [1-4][Wilson 2011, Kappus 1986, Brown 2004, Denny 2004]

However a third approach which is similar to the bioreduction of prodrugs, can involve bioreduction of many anti-cancer drugs followed by direct cytotoxicity or by the formation of reactive oxygen species (ROS) which subsequently cause oxidative stress (OS) leading to cellular damage. Extensive evidence supports involvement of electron transfer (ET), ROS and OS in the mechanism of many anticancer drugs. These free radical ET agents function catalytically in redox cycling with formation of ROS from oxygen. These ET agents include

aliphatic and aromatic N-oxides, quinones (or phenolic precursors), metal complexes, aromatic nitro compounds (or reduced hydroxylamine and nitroso derivatives), and conjugated imines (or iminium species). It is known that cancer cells may be characterized by a reduced intracellular environment and high levels of antioxidants with weakly bound electrons. [1,5-7][Wilson 2011,Kovacic 2007, Sainz 2012, Neese 2016]

OS is a normal function in all cells. ROS are produced in a controlled manner in normal cells and regulate various cellular functions including transcriptional regulation and signal transduction [reviewed in [8] Moreira 2016, [9] Muz 2015, [10] Valko 2006, [11] Kwee 2014, [12] Kovacic 2013]. Uncontrolled production of ROS causes OS that can result in DNA damage and impaired cellular functions leading to various human diseases including cancer. OS occurs when excessive production of ROS overwhelms the antioxidant defence system of the body. Cellular targets of OS include DNA, lipids, proteins, damage and modulation of kinase signalling.

Bioreduction of anti-cancer drugs can be induced by a number of enzymic systems. [1-4][Wilson 2011, Kappus 1986, Brown 2004, Denny 2004,] with mitochondrial processes having a particularly important role. It is thought that most of the one-electron reductases responsible for the redox cycling and hypoxic selectivity of drugs are the NAD(P)H-dependent flavoproteins which have low substrate affinities. Another group of two-electron reductases which are not inhibited by oxygen are believed to catalyse hydride (H^-) transfer from NAD(P)H. [1,2][Wilson 2011, Kappus 1986]

A recent study comparing the redox properties of cancer and normal cells is pertinent to the bioreduction of anti-cancer drugs: the average ATP concentration over the cell cycle is higher and the intracellular acidity pH_i is globally more acidic in normal proliferating cells than in cancer cells. The NAD^+/NADH and $NADP^+$/NADPH redox ratios are, respectively, five times and ten times higher in cancer cells compared to the normal cell population. A drastic decrease of the mitochondrial membrane potential of human and mice normal and cancer cell lines is reported in cancer cell lines compared to their normal counterparts. [8][Moreira 2016] It is known that the oxygen level in hypoxic tumour tissues is poorer than the oxygenation of the respective normal tissues and on average it is between 1%–2% O_2 and below. [9][Muz 2015]

Drug-induced oxidative stress is implicated as a mechanism of toxicity in numerous tissues and organ systems. The metabolism of a drug may generate a reactive intermediate that can reduce molecular oxygen directly to generate ROS. There is extensive evidence of chemotherapy induced oxidative stress during anti-neoplastic processes. [13-16][Deavall 2012, Chen 2007, Conklin 2004, Banerjee 2016] Patients with breast cancer treated with Doxorubicin and Cyclophosphamide chemotherapy presented with oxidative stress before, during, and after treatment, as well as showing increased genotoxic damage in all stages of treatment. [17][Junior 2015]

Many anti-cancer drugs have multiple mechanisms of action. For example Doxorubicin has three modes of action: intercalation into the DNA to prevent replication, interference with topoisomerase II which is involved in DNA replication, or by the formation of a free radical form which can generate oxidative stress in cancer cells. [14,18,19] [Chen 2007,Marcillat 1989, Sinha 2015] It has been shown that patients with advanced breast cancer subjected to chemotherapy with doxorubicin or paclitaxel presented immediate (within 1 hour) systemic

oxidative stress and red blood cell oxidative injury with anemia development. Reduced antioxidant status, ie reduced glutathione levels was also observed. It has been shown that oxidative stress is an ultimate participant in the harmful systemic processes in advanced cancer patients, and treatment with chemotherapy drugs sustains these injuries. [20][Panis 2011]

Cisplatin, a known radiosensitizer, has been shown to undergo a reductive DNA damage mechanism termed dissociative electron transfer (DET) where ultrashort-lived high energy cis-$Pt(NH_3)_2Cl$• or cis-$Pt(NH_3)_2$• radicals leading to the formation of transient anions at cisplatin's binding site of DNA with subsequent DNA damage and cell death. The DET mechanism also occurs with oxaliplatin and certain halogenated aminobenzene compounds as well. [21,22][Fong 2016]

Apoptosis of cancer cells induced by the aquated forms of cisplatin crosslinking with DNA is considered the major mechanism of cisplatin's anti-cancer effect, but it has also been shown that apoptosis is more likely to be due to an 'off-target' effect, likely to involve cytoplasmic generation of ROS, than being induced by DNA damage. [23][Berndtsson 2006] Exposure to cisplatin induces a mitochondrial-dependent ROS response that significantly enhances the cytotoxic effect caused by DNA damage. ROS generation is independent of the amount of cisplatin-induced DNA damage and occurs in mitochondria as a consequence of protein synthesis impairment. The cytotoxic effect of cisplatin-induced mitochondrial dysfunction varies among cells and depends on mitochondrial redox status, mitochondrial DNA integrity and bioenergetic function. [24][Marullo 2013] Cisplatin has been shown to be active by both a free radical and non free radical mechanism. [25][Uslu 1995] Cisplatin induced almost complete growth inhibition of BRCA1-defectivehuman breast cancer xenografts, while BRCA1-reconstituted xenografts were only partially inhibited. A significant S- and G2/M cell cycle blockade in BRCA1-defective was found as compared with parental BRCA1-reconstituted cells. [26][Tassone 2009]

OS plays a pivotal role in dasatinib-mediated hepatotoxicity. Dasatinib greatly increased the level of ROS in hepatocytes, reduced the intracellular glutathione content, attenuated the activity of superoxide dismutase, generated malondialdehyde, a product of lipid peroxidation, decreased the mitochondrial membrane potential, and activated nuclear factor erythroid 2-related factor 2 and mitogen-activated protein kinases related to oxidative stress and survival. [27][Xue 2012]

In cancer chemotherapy, tyrosine kinases are an important target since they play an important role in the modulation of tumour growth factor signalling, cell proliferation and migration. Tyrosine kinase inhibitors (TKI) are a class of targeted therapy that interfere with specific cell signalling pathways and hence allow target specific therapy for selected malignancies. [28-30][Arora 2005, Gotnik 2010, Siemann 2012]

Src is a non-receptor tyrosine kinase that is frequently over-expressed in malignancies, and increased Src activity is well known to contribute to the metastatic phenotype of tumour cells. Dasatinib is a Src inhibitor. Dasatinib has been found to significantly inhibit multiple cell functions critical to the successful metastatic dissemination of 4T1 breast cancer cells. Dasatinib treatment inhibited 4T1 tumour cell-induced angiogenesis by up to 60%. The results suggest that Dasatinib may be useful in the reduction of breast cancer metastasis. [31][Saffran 2011]

VEGF is secreted by all almost all solid tumours and tumour-associated stroma in response to hypoxia. It is highly specific for vascular endothelium and regulates both vascular proliferation and permeability. Excessive expression of VEGF levels correlate with increased microvascular density, cancer recurrence, and decreased survival Many TKIs are VEGF inhibitors. [32,33][Parikh 2004, Kerbel 2018].

There is extensive evidence that OS plays a major role in breast cancer. [34-37][Vera-Ramirez 2011, Ríos-Arrabal 2013, Gilmore 2017, Li 2016] OS also has an important role in the pathogenesis, neoangiogenesis, and dissemination of local or distant ovarian cancer, as it induces phenotypic modifications of tumour cells by cross talk between tumour cells and the surrounding stroma. OS has a major role in the pathogenesis and chemotherapy of ovarian cancer. [38-42][Saed 2017, Senthil 2004, Bandebuche 2011, Bukhari 2016, Pylvas 2010]

BRCA1 acts as a tumour suppressor and functions predominantly by maintaining genome integrity. BRCA1 is also involved in oxidative stress regulation. There is extensive evidence that BRCA1 may exert its tumour suppressive functions through oxidative stress regulation. Breast cancer cells are subject to high levels of intracellular and extracellular oxidative stress. [34,35,43][Vera-Ramirez 2011, Ríos-Arrabal 2013, Yi 2014]

It has been shown that Tempol, a stable free radical, increases the apoptotic rates of triple negative breast cancer (TNBC) MDA-MB-231 cells (which have a wild-type BRCA1), but only during co-culture with fibroblasts. BRCA1, the human tumour suppressor gene and its protein, normally functions as an endogenous antioxidant, so helping prevents the onset of hereditary breast cancers. The loss of BRCA1 functionality (common in sporadic breast cancers) increases hydrogen peroxide generation in both epithelial breast cancer cells and neighbouring stromal fibroblasts, and promotes the onset of a reactive glycolytic stroma, with increased monocarboxylate transporter 4 and decreased caveolin-1expression. These glycolytic cancer-associated fibroblasts in turn provide mitochondrial fuels (such as L-lactate) to epithelial cancer cells, to burn via oxidative mitochondrial metabolism. Cancer cells produce high levels of ROS, which promotes cancer mutagenesis. By acting on cells producing ROS in the local microenvironment, Tempol antioxidant therapy appears to be synthetically lethal with a BRCA1-deficiency in TNBC cells.[44,45,46][Martinez-Outschoorn 2012, Fong 2018]

Germline mutations in the breast cancer susceptibility gene (BRCA1) are strongly linked to familial breast and ovarian cancers. It is known that a smaller proportion of BRCA mutations are not hereditary in origin, but are somatic or sporadic in origin. BRCA1 mutations occur in about 18% of ovarian cancers, 13% germline and 5% somatic mutations. [47][Pennington 2014] *BRCA1 mutation carriers are only predisposed to breast and ovarian tumours.* [48, 37, 49][Futreal 1994, Li 2016, Celik 2015] BRCA1 is involved in ROS production and in the response to oxidative stress, exerting antioxidant activity by inducing the expression of antioxidant enzymes. It is a multifunctional protein involved in numerous cell processes, including cell cycle control, maintenance of genetic stability, DNA damage repair, apoptosis, and the transcription of different genes. Cancer cells with BRCA1 mutations suffer from genomic instability and increased DNA lesions. [35,43,49][Rios-Arrabal 2013, Yi 2014, Celik 2015] Modifications to BRCA1 induced by oxidative stress influence nuclear events that can weaken

DNA repair response. It is thought that antioxidant modalities may be a chemo-preventative strategy for familial breast cancers. [36,37,43][Gilmore 2017, Li 2016, Yi 2014]

There is a potential for chemotherapeutic agents to induce somatic changes to the BRCA genes, particularly if highly reactive free radical species are involved in the anti-cancer processes. [Banerjee 2016, Conklin 2004] Some sporadic ovarian cancers have the same pathological traits of BRCA mutation-associated cases, but in the absence of a germline BRCA mutation, described as 'BRCAness'. BRCA1 and BRCA2 germline mutations were identified in 9% and 8% of the cases, whereas somatic BRCA mutations occur in approximately 5%–7% of ovarian cancer cases. [36,50,51][Gilmore 2017, Moschetta 2016, Alsop 2012]

BRCA1 binds and stabilizes the hypoxia-inducible factor-1α (HIF-1α) and activates its target gene vascular endothelial growth factor (VEGF). Under hypoxic condition, BRCA1 stimulates VEGF promoter activity along with HIF-1α. [52][Kang 2016] HIF-1 is a protein with DNA binding activity. It is composed of two subunits: HIF-1α and HIF-1β. Among the first responses at the onset of tumour hypoxia is an increase in the protein levels of HIF-1. HIF-1 helps normal tissues as well as tumors to survive under hypoxic conditions, and severe hypoxia is usually found in solid tumors. HIF-1 is a transcription factor that turns on genes needed for survival under hypoxic conditions. More than 40 target genes have been found to be regulated by HIF-1. Because hypoxia in the tumour microenvironment is a driving force in breast cancer metastasis, it has been found that cytotoxic chemotherapy (such as paclitaxel or gemcitabine) combined with drugs that inhibit hypoxia-inducible factors (such as acriflavine or digoxin) can improve outcomes for women with TNBC. [53][Semenza 2015]

Increased HIF-1α expression is known to contribute to mitochondrial activity and ROS formation during hypoxia. High ROS concentrations generated from the mitochondria have been shown to stabilize HIF-1α. ROS may be also produced in the cytosol, derived from NADPH oxidases. [54,55][Chandel 2000, Chang 2005] HIF-1α is not expressed during normoxia.

Study objectives

(a) To examine whether the free radical species of a series of anti-cancer drugs can be involved in breast and ovarian cancer cell cytotoxicity under hypoxic and normoxic conditions
(b) To examine chemotherapy induced cytotoxicity and oxidative stress in breast and ovarian cancer treatment and mutations of the BRCA genes under hypoxic or anoxic conditions

Results

The drug cytotoxicity data for the human breast cancer cell line MDA-MB-231, under normoxia and hypoxia (0.1% oxygen) conditions (Table 1) [56][Ahmadi 2014] and the human ovarian cancer cell line A2780, under normoxia (20% O_2), and hypoxia (1% O_2) anoxia 0.1% oxygen conditions (Table 2, drugs in the free radical form) [57][Strese 2013] have been analysed using the general equation (1).

Equation 1 has been shown to apply to the transport and anti-cancer and metabolic efficacy of various drugs. The model, equation 1, is based on establishing linear free energy relationships between the four drug properties and various biological processes. Equation 1 has been previously applied to passive and facilitated diffusion of a wide range of drugs crossing the

blood brain barrier, the active competitive transport of tyrosine kinase inhibitors by the hOCT3, OATP1A2 and OCT1 transporters, and cyclin-dependent kinase inhibitors and HIV-1 protease inhibitors. The model also applies to PARP inhibitors, the anti-bacterial and anti-malarial properties of fluoroquinolones, and active organic anion transporter drug membrane transport, and some competitive statin-CYP enzyme binding processes. There is strong independent evidence from the literature that $\Delta G_{desolvation}$, $\Delta G_{lipophilicity}$, the dipole moment and molecular volume are good inherent indicators of the transport or binding ability of drugs. [58-67][Fong 2014-17].

Equation 1:

Transport or Binding = $\Delta G_{desolv,CDS}$ + $\Delta G_{lipo,CDS}$ + Dipole Moment + Molecular Volume or Ionization Potential or Electron Affinity

Equation 1 uses the free energy of water desolvation ($\Delta G_{desolv,CDS}$) and the lipophilicity free energy ($\Delta G_{lipo,CDS}$) where CDS represents the non-electrostatic first solvation shell solvent properties. CDS may be a better approximation of the cybotactic environment around the drug approaching or within the protein receptor pocket, or the cell membrane surface or the surface of a drug transporter, than the bulk water environment outside the receptor pocket or cell membrane surface. The CDS includes dispersion, cavitation, and covalent components of hydrogen bonding, hydrophobic effects. Desolvation of water from the drug ($\Delta G_{desolv,CDS}$) before binding in the receptor pocket is required, and hydrophobic interactions between the drug and protein ($\Delta G_{lipo,CDS}$) is a positive contribution to binding. $\Delta G_{lipo,CDS}$ is calculated from the solvation energy in n-octane. In some biological processes, where oxidation or reduction may be occurring, and the influence of molecular volume is small, the ionization potential or reduction potential (adiabatic electron affinity) has been included in place of the molecular volume. In other processes, the influence of some of the independent variables is small and can be eliminated to focus on the major determinants of biological activity.

We have recently used this model to develop a predictive model of the transport and efficacy of hypoxia specific cytotoxic analogues of tirapazmine and the effect on the extravascular penetration of tirapazamine into tumours. [58][Fong 2017] It was found that the multiparameter model of the diffusion, antiproliferative assays IC_{50} and aerobic and hypoxic clonogenic assays for a wide range of neutral and radical anion forms of tirapazamine (TPZ) analogues showed: (a) extravascular diffusion is governed by the desolvation, lipophilicity, dipole moment and molecular volume, similar to passive and facilitated permeation through the blood brain barrier and other cellular membranes, (b) hypoxic assay properties of the TPZ analogues showed dependencies on the electron affinity, as well as lipophilicity and dipole moment and desolvation, similar to other biological processes involving permeation of cellular membranes, including nuclear membranes, (c) aerobic assay properties were dependent on the almost exclusively on the electron affinity, consistent with electron transfer involving free radicals being the dominant species.

(a) Human Breast Cancer cell line MDA-MB-231, under normoxia (~20% oxygen) and hypoxia 0.1% oxygen conditions [56][Ahmadi 2014]

Where the relevant pKa values indicate that significant concentrations of the protonated species exist at physiological pH, these species have also been included in the analyses along with the neutral species of the drugs. AEA values have been normalised (multiplied by a factor of 5 to allow direct comparison of the magnitude of the various coefficients in the following equations).

Normoxia analysis yields: IC_{50} (μM) Excluding Imatinib as an outlier 18 drugs including 5-Fluorouracil, Doxorubicin, Vinblastin, Dasatinib, DasatinibH^+, Vandetinib, VandetinibH^+, Gefitinib, GefitinibH^+, Masatinib, MasatinibH^+, Nilotinib, NilotinibH^+, Sunitinib, SunitinibH^+, Sorafenib, Tirapazamine, as drug and drugH^+ species (excluded ErlotinibH^+ and ImatinibH^+)

Equation 2(a)

$$\mathbf{IC_{50} = -0.56\Delta G_{desolv,CDS} + 0.99\Delta G_{lipo,CDS} + 0.03 Dipole\ Moment - 0.58 Adiabatic\ Electron\ Affinity + 27.54}$$

Where $R^2 = 0.44$, SEE = 4.27, SE($\Delta G_{desolvCDS}$) = 0.31, SE($\Delta G_{lipoCDS}$) = 0.38, SE(Dipole Moment) = 0.06, SE(AEA) = 0.40, F=2.53, Significance=0.091

Equation 2(b)

$$\mathbf{IC_{50} = -0.55\Delta G_{desolv,CDS} + 0.92\Delta G_{lipo,CDS} - 0.59 Adiabatic\ Electron\ Affinity + 27.67}$$

Where $R^2 = 0.43$, SEE = 4.15, SE($\Delta G_{desolvCDS}$) = 0.30, SE($\Delta G_{lipoCDS}$) = 0.34, SE(AEA) = 0.38, F=3.49, Significance=0.044

Hypoxia analysis yields: IC_{50} (μM) Excluding Imatinib as an outlier 18 drugs

Equation 3(a)

$$\mathbf{IC_{50} = -0.98\Delta G_{desolv,CDS} + 2.24\Delta G_{lipo,CDS} + 0.04 Dipole\ Moment - 1.49 Adiabatic\ Electron\ Affinity + 56.44}$$

Where $R^2 = 0.58$, SEE = 7.22, SE($\Delta G_{desolvCDS}$) = 0.53, SE($\Delta G_{lipoCDS}$) = 0.64, SE(Dipole Moment) = 0.11, SE(AEA) = 0.67, F=4.40, Significance=0.018

Equation 3(b)

$$\mathbf{IC_{50} = -0.97\Delta G_{desolv,CDS} + 2.17\Delta G_{lipo,CDS} - 1.50 Adiabatic\ Electron\ Affinity + 56.58}$$

Where $R^2 = 0.57$, SEE = 6.99, SE($\Delta G_{desolvCDS}$) = 0.41, SE($\Delta G_{lipoCDS}$) = 0.57, SE(AEA) = 0.65, F=6.25, Significance=0.006

(b) Human Ovarian Cancer cell line A2780, under normoxia (20% O_2), and hypoxia (1% O_2) anoxia 0.1% oxygen conditions: 17 drugs in the free radical form with their cytotoxicity ratios (R_{anox} = anoxic IC_{50}/normoxic IC_{50} and R_{hypox} = hypoxic IC_{50}/normoxic IC_{50}) and the normoxia IC_{50} values taken from [57] [Strese 2013]
(Drugs: 5-Fluorouracil, Bortezomib, Cisplatinum, Digitoxin, Digoxin, Docetaxel, Doxorubicin, Etoposide, Irinotecan, Melphalan, Mitomycin C, Rapamycin, Sorafenib, Topotecan, Tirapazamine, Vincristine) AEA values have been normalised (multiplied by a factor of 5 to allow direct comparison of the magnitude of the various coefficients in the following equations).

R_{anox} Anoxia analysis yields: Excluding Vincristine as an outlier

Equation 4(a)

$R_{anox} = -0.037\Delta G_{desolv,CDS} + 0.029\Delta G_{lipo,CDS} + 0.038$Dipole Moment $- 0.079$Adiabatic Electron Affinity $+ 1.142$

Where $R^2 = 0.656$, SEE = 0.690, SE($\Delta G_{desolvCDS}$) = 0.059, SE($\Delta G_{lipoCDS}$) = 0.064, SE(Dipole Moment) = 0.014, SE(AEA) = 0.050, F=5.24, Significance=0.013

Equation 4(b) Excluding Vincristine as an outlier

$R_{anox} = -0.118$Adiabatic Electron Affinity $+ 2.540$

Where $R^2 = 0.321$, SEE = 0.701, SE(AEA) = 0.008, F=6.14, Significance=0.027

R_{hypox} Hypoxia analysis yields: Excluding Vincristine as an outlier

Equation 5(a)

$R_{hypox} = 0.073\Delta G_{desolv,CDS} - 0.063\Delta G_{lipo,CDS} - 0.003$Dipole Moment $- 0.130$Adiabatic Electron Affinity $+ 2.613$

Where $R^2 = 0.649$, SEE = 0.690, SE($\Delta G_{desolvCDS}$) = 0.036, SE($\Delta G_{lipoCDS}$) = 0.040, SE(Dipole Moment) = 0.009, SE(AEA) = 0.031, F=4.63, Significance=0.022

Equation 5(b) Excluding Vincristine as an outlier

$R_{hypox} = -0.111$Adiabatic Electron Affinity $+ 2.165$

Where $R^2 = 0.504$, SEE = 0.450, SE(AEA) = 0.030, F=13.22, Significance=0.003

Normoxia analysis yields:

Equation 6(a) Excluding Fluorouracil and Vincristine as outliers

IC_{50} Normoxia $= 0.489\Delta G_{desolv,CDS} - 2.089\Delta G_{lipo,CDS} - 1.438$Dipole Moment $+ 3.323$Adiabatic Electron Affinity $+ 1.544$

Where $R^2 = 0.443$, SEE = 33.500, SE($\Delta G_{desolvCDS}$) = 2.438, SE($\Delta G_{lipoCDS}$) = 3.167, SE(Dipole Moment) = 0.720, SE(AEA) = 2.438, F=2.00, Significance=0.171

Equation 6(b) Excluding Fluorouracil and Vincristine as outliers

IC_{50} Normoxia $= 4.446$Adiabatic Electron Affinity $- 27.109$

Where $R^2 = 0.214$, SEE = 34.91, SE(AEA) =2.216, F=3.54, Significance=0.082

Discussion

Equations 2(b) and 3(b) for TNBC and 4(b) and 5(b) for ovarian cancer cells show that the free radical forms of the various anti-cancer drugs play a role in cellular cytotoxicity under normoxic conditions but become increasing important under anoxic or hypoxic conditions.

For the TNBC results, under normoxia eq 2(b) & hypoxia 3(b), the coefficients for the ratios $\{\Delta G_{desolv,CDS} : \Delta G_{lipo,CDS}\}$ & $\{\Delta G_{lipo,CDS} : AEA\}$ for eq 2(b) are 1:2 & 2:1. For eq 3(b) the ratios are 1:2.2 & 2.2:1.5. These ratios show that water desolvation and lipophilicity are relatively of equal importance, but the AEA become more important under hypoxia, consistent with a greater free radical mechanism under hypoxic conditions. The partial dependence on desolvation and lipophilicity points to some drug-target binding interaction, and/or some drug-cell membrane transport interaction.

For the ovarian cancer cells, comparison of anoxia eq 4(b) and hypoxia 5(b) show that the AEA is the statistically dominant factor, with water desolvation and lipophilicity being insignificant. It

is noted that eq 4(b) and 5(b) are actually IC_{50} ratios of anoxia or hypoxia relative to normoxia conditions. Eq 6(b) suggests that free radical species also play a role during cytotoxicity under normoxia, as well as anoxia and hypoxia, though much reduced.

The data in the equations described above refer to cellular cytotoxicity but the molecular target(s) in these processes are unknown. However, the available literature on the effect of chemotherapy on TNBC and ovarian cancer, and the known relationship between TNBC and ovarian cancer can be indicative of the likely target(s).

The literature described in the introduction on anti-cancer properties of TNBC and ovarian cancers points to a strong linkage between the two cancers with free radicals most likely being involved, particularly under hypoxic or anoxic conditions.

By acting on cells producing ROS in the local microenvironment, Tempol (a stable free radical) antioxidant therapy appears to be synthetically lethal with a BRCA1-deficiency in TNBC cells. [44,45,46][Martinez-Outschoorn 2012, Fong 2018] BRCA1 mutation carriers are only predisposed to breast and ovarian tumours. [48,37,49][Futreal 1994, Li 2016, Celik 2015] BRCA1 mutations can be hereditary or sporadic or somatic, with the latter two possibly chemotherapy induced. [50][Moschetta 2015]

BRCA1 binds and stabilizes the hypoxia-inducible factor-1α (HIF-1α) and activates its target gene vascular endothelial growth factor (VEGF). VEGF is secreted by all almost all solid tumours and tumour-associated stroma in response to hypoxia. Under hypoxic condition, BRCA1 stimulates VEGF promoter activity along with HIF-1α. [52][Kang 2016] Increased HIF-1α expression is known to contribute to mitochondrial activity and ROS formation during hypoxia. High ROS concentrations generated from the mitochondria have been shown to stabilize HIF-1α. ROS may be also produced in the cytosol, derived from NADPH oxidases. [54,55][Chandel 2000, Chang 2005] HIF-1α is not expressed in normoxia.

The TNBC human breast cancer cell line MDA-MB-231 and the ovarian cancer cell line A2780 used in this study both have a wild type BRCA1 gene. [44,68][Martinez-Outschoorn 2012, Stordahl 2013,] The breast cancer drugs used in this study are very varied and come from quite different drug classes. They include a wide range of tyrosine kinase inhibitors, anti-metabolite pyrimidine analogue (5 Fluorouracil, Gemcitabine), proteasome inhibitor (Bortezomib), platinum compounds (Cisplatin), cardiac glycosides (Digitoxin, Digoxin), anthracyclines and topoisomerase II inhibitors (Doxorubicin, Irinotecan, Topotecan), quinone antibiotics (Mitomycin C), mitosis inhibitors or taxanes (Docetaxel, Etoposide), antiseptic (Acriflavine), alkylating mustard analogue (Melphalan), oral macrolide, m-TOR inhibitor (Rapamycin), bioreductive pro-drug (Tirapazamine), vinca alkaloids (Vinblastin). It is possible that some of these drugs used in this study exhibit multiple mechanisms (such as those known for Doxorubicin, Cisplatin etc) but a common free radical / oxidative stress mechanism can provide a generic mechanism for the observed cytotoxicity, especially since many of the investigated drugs are independently known to use free radicals to exert their anti-cancer effect.

A plausible hypothesis that can explain the results for the TNBC and ovarian cancer cells is that free radicals are involved in an oxidative stress environment where HIF-1α expression occurs in

hypoxic or anoxic conditions, and may involve BRCA1 mutations and VEGF. This hypothesis relies on known literature studies regarding the possible roles of BRCA1, HIF-1α and VEGF.

A proposed mechanism is:

Drug + e• (bioreductase) → Drug• (Rate determining step) **Eq 7**

Drug• + cellular target 1 → {cellular target 1}• → cell death (Anoxia or Hypoxia) **Eq 8**

Drug• + O_2 → O_2•$^-$ (+ other ROS) + Drug (Hypoxia or Normoxia) **Eq 9**

ROS + cellular target 2 → {cellular target 2}• → cell death **Eq 10**

Cellular target 1 or 2 could be BRCA1/HIF-1α or VEGF.

Conclusions

An analysis of the cytotoxicity data for a wide range of anti-cancer drugs applied to triple negative breast cancer (TNBC) cells and ovarian cancer cells under normoxic, hypoxic and anoxic conditions has shown that free radical forms of the drugs are involved. This finding is consistent with the known involvement of oxidative stress in the TNBC and ovarian cancer cells. A mechanism to explain the formation of these drug free radicals and how they can be involved in oxidative stress is proposed.

A hypothesis that might explain the known link between TNBC and ovarian cancer is proposed which involves involvement of free radicals interacting with the BRCA1/HIF-1α complex or VEGF under hypoxic or anoxic conditions.

References

[1] WR Wilson, MP Hay, Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011, 11:393-410
[2] H Kappus, Overview of enzyme systems involved in bio-reduction of drugs and in redox cycling, Biochem Pharmacol. 1986, 35:1-6.
[3] JM Brown, WR Wilson, Exploiting tumour hypoxia in cancer treatment, Nature Rev. Cancer. 2004, 4:437–447.
[4] WA Denny, Prospects for hypoxia-activated anticancer drugs. Curr Med Chem. 2004, 4:395–9.
[5] P Kovacic, Unifying mechanism for anticancer agents involving electron transfer and oxidative stress: clinical implications, Med Hypotheses 2007, 69:510-516.
[6] RM Sainz, F Lombo, JC Mayo, Radical Decisions in Cancer: Redox Control of Cell Growth and Death. Cancers, 2012, 4:442-474
[7] A Neesse, E Hessmann, Electron Transfer-Based Compounds: A Novel Weapon in the Cancer Battlespace? EBioMedicine, 2015, 2:484–485

[8] JV Moreira, M Hamraz, M Abolhassani, E Bigan, S Pérès, L Paulevé, ML Nogueira, JM Steyaert, L Schwartz. The Redox Status of Cancer Cells Supports Mechanisms behind the Warburg Effect, Metabolites, 2016, 6:33-45
[9] B Muz, P de la Puente, F Azab, AK Azab, The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy, Hypoxia, 2015, 3:83–92
[10] M Valko, CJ Rhodes, J Moncol, M Izakovic, M Mazur, Free radicals, metals and antioxidants in oxidative stress-induced cancer, Chemico-Biological Interactions, 2006, 160: 1–40
[11] JK Kwee, A Paradoxical Chemoresistance and Tumor Suppressive Role of Antioxidant in Solid Cancer Cells: A Strange Case of Dr. Jekyll and Mr. Hyde, BioMed Research International Volume 2014, Article ID 209845, http://dx.doi.org/10.1155/2014/209845
[12] P Kovacic, R Somanathan, Broad overview of oxidative stress and its complications in human health, Open J Preventive Medicine, 2013, 3:32-41
[13] Deavall DG, Martin EA, Horner JM, Roberts R. Drug-Induced Oxidative Stress and Toxicity, Journal of Toxicology, Volume 2012:1-13, Article ID 645460, doi:10.1155/2012/645460
[14] Y Chen, P Jungsuwadee, M Vore, DA Butterfield, DK St Clair, Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues, Mol. Interv. 2007, 7: 147-156.
[15] KA Conklin, Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness, Integrative Cancer Therapies 2004, 3:294-300
[16] S Banerjee, J Ghosh, PC Sil, Drug Metabolism and Oxidative Stress: Cellular Mechanism and New Therapeutic Insights, Biochem Anal Biochem 2016, 5:1, DOI: 10.4172/2161-1009.1000255
[17] ALG Junior, MFC Jardim Paz, LIS da Silva, S da Costa, S Carvalho, ALP Sobral, K da Conceição Machado, PMP Ferreira, P Satyal, RM de Freitas, AA de Carvalho Melo Cavalcante. Serum Oxidative Stress Markers and Genotoxic Profile Induced by Chemotherapy in Patients with Breast Cancer. A Pilot Study, Oxidative Medicine and Cellular Longevity, Volume 2015:1-11, Article ID 212964.
[18] O Marcillat, Y Zhang, KJ Davies, Oxidative and non-oxidative mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. Biochem J. 1989, 259: 181-189.
[19] BK Sinha, RP Mason, Is Metabolic Activation of Topoisomerase II Poisons Important In The
Mechanism Of Cytotoxicity? J Drug Metab Toxicol 2015, 6:3, http://dx.doi.org/10.4172/2157-7609.1000186
[20] C Panis, ACSA Herrera, VJ Victorino, FC Campos, LF Freitas, T De Rossi, A N Colado Simao, A L Cecchini, R Cecchini. Oxidative stress and hematological profiles of advanced breast cancer patients subjected to paclitaxel or doxorubicin chemotherapy, Breast Cancer Res Treat 2011
DOI 10.1007/s10549-011-1693-x
[20] CW Fong, Platinum based radiochemotherapies: Free radical mechanisms and radiotherapy sensitizers, Free Rad Biol Medicine, 2016, 99:99–109
[21] CW Fong, Platinum anti-cancer drugs: Free radical mechanism of Pt-DNA adduct formation and anti-neoplastic effect, Free Rad Biol Medicine, 2016, 95:216–229

[22] M Berndtsson, M Hagg, T Panaretakis, AM Havelka, MC Shoshan, S Linder, Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA, Int. J. Cancer 2006, 120:175–180.
[23] R Marullo, E Werner, N Degtyareva, B Moore, G Altavilla, SS Ramalingam, PW Doetsch, Cisplatin Induces a Mitochondrial-ROS Response That Contributes to Cytotoxicity Depending on Mitochondrial Redox Status and Bioenergetic Functions, PLoS ONE 2013, 8(11): e81162. doi:10.1371/journal.pone.0081162
[24] R Uslu, B Bonavida, Free radicals dependent and independent pathways of CDDP-mediated cytotoxicity and apoptosis in ovarian tumour cell lines, Int. J. Oncol. 1995, 6:1003-1009
[25] P Tassone, MT Di Martino, M Ventura, A Pietragalla, I Cucinotto, Loss of BRCA1 function increases the antitumor activity of cisplatin against human breast cancer xenografts in vivo, Cancer Biology Therapy, 2009, 8: Issue 7
[26] T Xue , P Luo , H Zhu , Y Zhao , H Wu , R Gai, Y Wu , B Yang , X Yang , Q He, Oxidative stress is involved in Dasatinib-induced apoptosis in rat primary hepatocytes, Toxicol Appl Pharmacol. 2012; 261:280-91.
[27] A Arora, EM Scholar, Role of Tyrosine Kinase Inhibitors in Cancer Therapy, J Pharmacol. Expt. Ther. 2005, 3:971-979.
[28] KJ Gotink, HMW Verheul. Anti-angiogenic tyrosine kinase inhibitors: what is their mechanism of action? Angiogenesis 2010, 13:1–14
[29] DW Siemann, M Dong, C Pampo, W Shi, 2012 Src Signaling Interference Impairs the Dissemination of Blood-Borne Tumor Cells, Cell Tissue Res. 2012, 349(2): 541–550
[30] V Saffran, DW Siemann, Src inhibitor dasatinib reduces the metastatic phenotype of breast cancer cells, AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; San Francisco, CA. 2011. p. Abstract B151.
[31] AA Parikh, LM Ellis, The vascular endothelial growth factor family and its receptors, Hematol Oncol Clin North Am 2004, 18: 951–971.
[32] RS Kerbel, Targeting the VEGF/VEGFR Pathway: Strategies for Improving Therapeutic Outcomes, 2018 ASCO Annual Meeting, https://am.asco.org/daily-news/targeting-vegfvegfr-pathway-strategies-improving-therapeutic-outcomes
[33] L Vera-Ramirez, P Sanchez-Rovira, MC Ramirez-Tortosa, CL Ramirez-Tortosa, S Granados-Principal, JA Lorente, JL Quiles, Free radicals in breast carcinogenesis, breast cancer progression and cancer stem cells, Biological bases to develop oxidative-based therapies. Crit Revs Oncol Hematol. 2011, 80:347–368
[35] S Ríos-Arrabal, F Artacho-Cordón, J León, E Román-Marinetto, M del Mar Salinas-Asensio, I Calvente, MI Núñez, Involvement of free radicals in breast cancer, SpringerPlus 2013, 2:404-16
[36] BL Gilmore, Y Liang, CE Winton, K Patel, V Karageorge, A C Varano, W Dearnaley, Zhi Sheng, DF Kelly, Molecular Analysis of BRCA1 in Human Breast Cancer Cells Under Oxidative Stress, Sci. Rep. 2017, 7, 43435; doi: 10.1038/srep43435.
[37] M Li, Q Chen, X Yu. Chemopreventive Effects of ROS Targeting in a Murine Model of BRCA1-Deficient Breast Cancer. Cancer Res. 2016, 77:448-458
[38] GM Saed , MP Diamond, NM Fletcher, Updates of the role of oxidative stress in the pathogenesis of ovarian cancer, Gynecol Oncol. 2017; 145:595-602
[39] K Senthil, S Aranganathan, N Nalini, Evidence of oxidative stress in the circulation of ovarian cancer patients, Clin Chim Acta. 2004, 339:27-32

[40] S Bandebuche, RR Melinkeri, Oxidative Stress and Antioxidant Status in Patients of Ovarian Cancer. Biomedical Res. 2011; 22: 196-200
[41] SA Bukhari, K Zafar, MI Rajoka, Z Ibrahim, S Javed, R Sadiq, Oxidative stress-induced DNA damage and homocysteine accumulation may be involved in ovarian cancer progression in both young and old patients, Turk J Med Sci 2016, 46: 583-589
[42] M Pylvas, U Puistola, S Kauppila, Y Soini, P Karihtala, Oxidative stress induced antioxidant enzyme expression is an early phenomenon in ovarian carcinogenesis, Eur J Cancer, 2010, 46: 1661–1667.
[43] YW Yi , HJ Kang, I Bae, BRCA1 and Oxidative Stress, Cancers 2014, 6:771-795
[44] UE Martinez-Outschoorn, R Balliet, Z Lin, D Whitaker-Menezes, RC Birbe, et al, BRCA1 mutations drive oxidative stress and glycolysis in the tumor microenvironment. Implications for breast cancer prevention with antioxidant therapies, Cell Cycle 2012, 11: 4402–4413.
[45] CW Fong, The role of free radicals in the effectiveness of anti-cancer chemotherapy in hypoxic ovarian cells and tumours, HAL Archives, 2018, hal-01659879, https://hal.archives-ouvertes.fr/hal-01659879v2
[46] CW Fong, Role of stable free radicals in conjugated antioxidant and cytotoxicity treatment of triple negative breast cancer, HAL archives 2018, hal-01803297
[47]KP Pennington, T Walsh, MI Harrell, MK Lee, CC Pennil, MH Rendi, A Thornton, BM Norquist, S Casadei, AS Nord, KJ Agnew, CC Pritchard, S Scroggins, RL Garcia, MC King, EM Swisher, Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas, Clin. Cancer Res. 2014, 20:764–75.
[48] PA Futreal, Q Liu, D Shattuck-Eidens, C Cochran, K Harshman, S Tavtigian, LM Bennett, A Haugen-Strano, J Swensen, Y Miki, et al, BRCA1 mutations in primary breast and ovarian carcinomas, Science 1994, 266:120–122.
[49] A Celik, M Acar, C M Erkul, E Gunduz, M Gunduz, Relationship of Breast Cancer with Ovarian Cancer, A Concise Review of Molecular Pathology of Breast Cancer, 2015, InTech Open, http://www.intechopen.com/books/a-concise-review-of-molecularpathology-of-breast-cancer.
[50] M Moschetta, A George, SB Kaye, S Banerjee. BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer, Annals of Oncology, 2016, 27:1449–1455.
[51] K Alsop, S Fereday, C Meldrum, A deFazio, C Emmanuel, J George, A Dobrovic, MJ Birrer, PM Webb, C Stewart, M Friedlander, S Fox, D Bowtell, G Mitchell. BRCA Mutation Frequency and Patterns of Treatment Response in *BRCA* Mutation–Positive Women With Ovarian Cancer: A Report From the Australian Ovarian Cancer Study Group, J Clin Oncol. 2012, 30: 2654–2663.
[52] HJ Kang, H J Kim, JK Rih, TL Mattson, KW Kim, CH Cho, JS Isaacs, I Bae, BRCA1 Plays a Role in the Hypoxic Response by Regulating HIF-1α Stability and by Modulating Vascular Endothelial Growth Factor Expression, J Biol Chem, 2006, 281:13047
[53] GL Semenza, The hypoxic tumor microenvironment: A driving force for breast cancer progression, Biochim. Biophys. Acta 2015, http://dx.doi.org/10.1016/j.bbamcr.2015.05.036
[54] NS Chandel, DS McClintock, CE Feliciano, TM Wood, JA Melendez, AM Rodriguez, PT Schumacker, Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O_2 sensing, J. Biol. Chem. 2000, 275:25130–25138.

[55] TC Chang, CJ Huang, K Tam, SF Chen, KT Tan, MS Tsai, TN Lin, and S. K. Shyue, Stabilization of hypoxia-inducible factor-1α by prostacyclin under prolonged hypoxia via reducing reactive oxygen species level in endothelial cells, J. Biol. Chem. 2005, 280:36567–36574.
[56] M Ahmadi, Z Ahmadihosseini, S J Allison, S Begum, K Rockley, M Sadiq, S Chintamaneni, R Lokwani, N Hughes, R M Phillips, Hypoxia modulates the activity of a series of clinically approved tyrosine kinase inhibitors, Brit J of Pharmacol 2014, 171:224–236
[57] Strese S, Fryknäs M, Larsson R, Gullbo J. Effects of hypoxia on human cancer cell line chemosensitivity. BMC Cancer 2013; 13:331-342
[58] CW Fong, The extravascular penetration of tirapazamine into tumours: a predictive model of the transport and efficacy of hypoxia specific cytotoxic analogues and the potential use of cucurbiturils to facilitate delivery, Int J Comput Biol Drug Design. 2017, 10:343-373
[59] CW Fong, Drug discovery model using molecular orbital computations: tyrosine kinase inhibitors. HAL Archives, 2016, hal-01350862v1
60] CW Fong, Permeability of the Blood–Brain Barrier: Molecular Mechanism of Transport of Drugs and Physiologically Important Compounds. J Membrane Biol. 2015, 248:651-69.
[61] CW Fong, The effect of desolvation on the binding of inhibitors to HIV-1 protease and cyclin-dependent kinases: Causes of resistance. Bioorg Med Chem Lett. 2016, 26:3705–3713.
[62] CW Fong, Predicting PARP inhibitory activity – A novel quantum mechanical based model. HAL Archives 2016, hal-01367894v1.
[63] CW Fong, A novel predictive model for the anti-bacterial, anti-malarial and hERG cardiac QT prolongation properties of fluoroquinolones. HAL Archives, 2016, hal-01363812v1.
[64] CW Fong, Statins in therapy: Cellular transport, side effects, drug-drug interactions and cytotoxicity - the unrecognized role of lactones, HAL Archives, 2016, hal-01185910v1.
[65] CW Fong, Physiology of ionophore transport of potassium and sodium ions across cell membranes: Valinomycin and 18-Crown-6 Ether. Int J Comput Biol Drug Design 2016, 9: 228-246.
[66] CW Fong, Statins in therapy: Understanding their hydrophilicity, lipophilicity, binding to 3-hydroxy-3-methylglutaryl-CoA reductase, ability to cross the blood brain barrier and metabolic stability based on electrostatic molecular orbital studies. Eur J Med Chem. 2014, 85:661-674
[67] CW Fong, The effect of lipophilic modifications to dasatinib on ovarian cancer cytotoxicity proliferation and anti-angiogenesis, HAL Archives, 2018, hal-01767026v1
[68] B Stordal, K Timms, A Farrelly, D Gallagher, S Busschots, et al, BRCA1/2 mutation analysis in 41 ovarian cell lines reveals only one functionally deleterious BRCA1 mutation, Molecular Oncology 2013, 7:567-579

Table 1. Cytotoxicity ratios for MDA MB 231 triple negative breast cancer cells for the free radical form of drugs with their desolvation, lipophilicity, dipole moment, and adiabatic electron affinity (AEA) molecular properties

	Normoxia IC_{50}	Hypoxia IC_{50}	$\Delta G_{desolv,CDS}$ kcal/mol	$\Delta G_{lipo,CDS}$ kcal/mol	Dipole Moment D	AEA eV
5 Fluorouracil	14.61	39.17	5.55	-2.76	7	2.29
Doxorubicin	0.42	0.46	10.96	-10.05	22.8	3.46
Vinblastine	0.55	0.85	15.4	-10.05	6.6	1.35
Sunitinib	8.17	5.08	7.46	-8.72	9.5	2.75
Sorafenib	0.51	2.1	9.93	-9.15	27.2	2.43
Gefitinib	10.62	11.85	5.35	-11.08	20.45	2.17
Masatinib	8.39	12.93	2.7	-12.55	8	2.17
Vandetinib	8.12	5.56	3.14	-11.79	12.7	2.22
Nilotinib	2.81	1.73	8.76	-13.93	19	2.49
Erlotinib	15.7	20.11	9.61	-9.55	15.8	1.93
Tirapazamine	7.84	2.89	5.02	-6.13	4.8	3.65
Dasatinib	0.033	0.006	2.77	-13.52	18.5	2.43
$DasatinibH^+$	0.033	0.006	6.77	-14.08	53.2	2.24
$GefitinibH^+$	10.62	11.85	7.76	-11.28	45.5	2.06
$VandetinibH^+$	8.12	5.56	7.26	-12.14	49.1	2.1
$MasatinibH^+$	8.39	12.93	6.74	-12.75	46.2	2.48
$SunitinibH^+$	8.17	5.08	10.52	-8.82	39.31	2.58
$NilotinibH^+$	2.81	1.73	10.96	-14.48	12.95	2.91
Imatinib	18.28	19.69	3.81	-15.58	11.8	2.36
$ImatinibH^+$	18.28	19.69	7.17	-15.77	11.4	2.28
$ErlotinibH^+$	15.7	20.11	10.77	-9.53	13.1	2.62

Footnotes:
Cytotoxicity ratios from M Ahmadi, Z Ahmadihosseini, S J Allison, S Begum, K Rockley, M Sadiq, S Chintamaneni, R Lokwani, N Hughes, R M Phillips, Hypoxia modulates the activity of a series of clinically approved tyrosine kinase inhibitors, Brit J of Pharmacol 2014, 171:224–236
$DasatinibH^+$ is the protonated form of Dasatinib etc.

Table 2 Cytotoxicity ratios for A2780 ovarian cancer cells for the free radical form of drugs with their desolvation, lipophilicity, dipole moment, molecular volume and adiabatic electron affinity (AEA) molecular properties

	Anoxia ratio	Hypoxia ratio	Normoxia IC_{50}	$\Delta G_{desolv,CDS}$ kcal/mol	$\Delta G_{lipo,CDS}$ kcal/mol	Dipole Moment D	Molec Volume cm^3/mol	AEA eV
5-Fluorouracil	0.29	NA	690	5.55	-2.76	7	97	2.29
Acriflavine	0.56	NA	6.2	3.24	-4.32	23.7	173	2.29
Bortezomib	0.1	0.2	0.011	5.85	-6.29	35.2	278	2.67
CisPt vert	1.6	0.9	9.3	4.57	-0.47	17.08	104	1.82
CisPt AEA	1.6	0.9	9.3	4.44	-0.62	18.8	97	2.8
Digitoxin	3.6	0.94	0.11	14.42	-15.32	64.7	562	2.24
Digoxin	2.4	1.1	0.15	13.88	-14.69	57	529	1.85
Docetaxel	2.3	1	10	16.88	-14.51	36.7	584	2.33
Doxorubicin	1.3	0.5	18	10.96	-10.05	22.8	346	3.46
Etoposide	1.3	0.73	34	13.48	-8.48	27.2	386	1.25

Irinotecan	1.3	1.3	38	6.92	-14.02	33.1	383	2.69
Melphalan	2	2.6	18	5.66	-6.41	10.7	205	0.91
Mitomycin C	0.17	0.22	53	4.54	-3.64	9.9	211	3.61
Rapamycin	1.2	0.34	47	19.92	-14.21	22.7	840	2.3
Sorafenib	0.85	0.87	7.2	9.93	-9.15	27.2	243	2.43
Topotecan	0.023	0.5	15	5.23	-9.56	9.8	297	2.68
Tirapazamine	0.044	0.045	150	5.02	-6.13	4.8	107	3.65
Vincristine	5	0.01	3500	15.04	-13.37	18.5	651	1.53

Footnotes:

Cytotoxicity ratios from S Strese, M Fryknäs, R Larsson, J Gullbo, Effects of hypoxia on human cancer cell line chemosensitivity, BMC Cancer 2013; 13:331-342

Cisplatin shows both AEA and vertical EA values since the AEA value in water show elongation of Pt---Cl bonds when relaxed from the vertical form.