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REVIEW

# Glutathione in cancer progression and chemoresistance: an update

**Giulia Elda Valenti[1], Bruno Tasso[2], Nicola Traverso[1], Cinzia Domenicotti[1] and Barbara Marengo[1]**

[1]Department of Experimental Medicine, University of Genova, Italy
[2]Department of Pharmacy, University of Genova, Italy

**Correspondence should be addressed to C Domenicotti: cinzia.domenicotti@unige.it**

## Abstract

Glutathione (GSH) is one of the most important components of the cellular antioxidant system, and it is able to exert several pleiotropic functions influencing cell growth, proliferation, adaptation and death. It has been demonstrated that changes in GSH levels underlie the pathogenesis of many human diseases, including cancer. In detail, although on one hand GSH homeostasis plays a protective role from the onset of cancer, on the other, it is involved in cancer progression and in therapy resistance. In this review, after a brief report on the physiological role of GSH, we have focused the attention on its role in cancer and refractoriness to anticancer therapy giving an update on the preclinical and clinical studies relied on the compounds targeting GSH system. Based on these considerations, a deeper knowledge of GSH-dependent network can be crucial to identify new strategies for preventing and/or curing cancer.

**Keywords**

- glutathione
- oxidative stress
- cancer
- chemoresistance

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## Introduction

Glutathione (GSH) is mainly present in the cytosol, and its distribution between the intracellular compartments is crucial to regulate redox homeostasis, gene expression, cell signaling and proliferation (Sies 1999). The increase in GSH content is associated with early cell proliferation and is crucial to stimulate the shift from G0 to G1 phase of the cell cycle (Lu & Ge 1992). Moreover, it has been reported that GSH sequestration in the nucleus correlates with a reduced transcription of genes encoding for stress and defense proteins and is involved in the modulation of DNA synthesis (Diaz Vivancos *et al.* 2010). It is also likely that the presence of GSH and GSH-related enzymes in the nucleus (Soboll *et al.* 1995) can contribute to maintain nuclear proteins, such as histones and other chromatin-related proteins, in a reduced state in order to guarantee chromatin stability and cell cycle progression.

In this context, the glutathionylation of histone H3, which increases cell proliferation, has been shown to increase the susceptibility of human breast cancer cells to doxorubicin treatment (de Luca *et al.* 2011).

With regard to cancer development and treatment, a different role of GSH has been described in the literature. In fact, on one hand GSH is involved in the detoxification and elimination of carcinogens (Forman *et al.* 2009), and on the other hand the elevated levels of GSH and of other antioxidants detected in different cancers (i.e. melanoma, hepatocarcinoma, bone marrow, breast, colon, pancreatic and lung cancer) can contribute to neoplastic progression and support the acquisition of drug resistance (Gamcsik *et al.* 2012, Traverso *et al.* 2013).

In fact, the anticancer strategy is often based on the induction of oxidative stress through the administration of pro-oxidant chemotherapeutic drugs or ionizing radiation (Pelicano *et al.* 2004, Holley *et al.* 2014). Unfortunately, these approaches, after an initial success, lead to the onset of chemoresistance due to the increase

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of antioxidant defense (Kim *et al.* 2019, Domenicotti & Marengo 2022).

Then, the antioxidant response in cancer is triggered by the increased production of reactive oxygen species (ROS) as a result of mitochondrial dysfunctions, metabolic alterations or long-term anticancer therapy (Sabharwal & Schumacker 2014) (Fig. 1). It has been demonstrated that GSH and other antioxidants maintain ROS at physiological levels stimulating cell survival and proliferation through the activation of redox signaling pathways (e.g. protein kinase B (Akt), mitogen-activated protein kinase (MAPK) and protein kinase C (PKC)) and suppressing cell death induced by supraphysiological ROS concentrations (Dickinson & Chang 2011, Marengo *et al.* 2016).

## Biosynthesis and physiological role of GSH

GSH is a tripeptide, consisting of glutamic acid, cysteine and glycine. The majority of GSH is represented by the reduced form (GSH), while the oxidized form (glutathione disulfide, GSSG) is found to be less than 1% of the total GSH (Lu 2013). Notably, the GSH/GSSG ratio has been reported to be decreased under some physiological conditions as observed in newborns (full-term and preterm) (Frosali *et al.* 2004) and following anaerobic exercise (Wiecek *et al.* 2015).

GSH is present at millimolar concentrations mainly in the cytosol, reaching over 70% of total intracellular content. The remaining intracellular GSH (30%) is distributed in the mitochondria (Marí *et al.* 2009), in the nucleus (García-Giménez *et al.* 2013) and in the endoplasmic reticulum

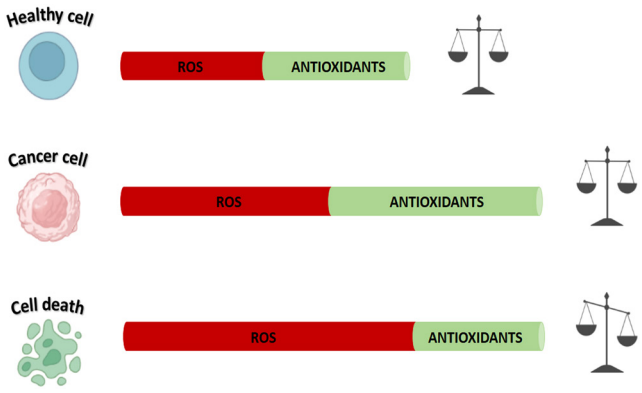


**Figure 1**
Redox balance in healthy and cancer cells. Healthy and cancer cells maintain redox homeostasis by balancing ROS production with adequate levels of antioxidants. Cancer cells produce greater amounts of ROS, due to their high metabolic rate, and consequently have an increased production of antioxidants. In both cells, when redox balance is not maintained, a condition of oxidative stress occurs, leading to cell death.

where GSSG is the predominant form (Hwang *et al.* 1992). The distribution between different intracellular compartments is important to better regulate cell needs and functions. In fact, GSH can act as a substrate for GSH peroxidases (GPXs) and GSH-S-transferases (GSTs) (Forman *et al.* 2009), it takes part in iron and sulfur metabolism (Liochev 1996) and it is involved in maintaining the reduced form of several dehydrogenases, ATPases (Huang & Philbert 1995) and thioredoxin which is required for the activity of ribonucleotide reductase, a critical enzyme in DNA synthesis (Holmgren 1981).

GSH synthesis is strongly influenced by the availability of cysteine and by the activity of glutamate cysteine ligase (GCL), an enzyme consisting of a modifier subunit (GCLM) and a catalytic subunit, which, in the presence of ATP and $Mg^{2+}$, forms γ-glutamylcysteine starting from cysteine and glutamate (Lu 2013). Subsequently, the synthesis is finished through the activity of GSH synthetase (GS) which adds glycine to the dipeptide (Forman *et al.* 2009, Lu 2013). Moreover, the maintenance of intracellular GSH and its aminoacids as well as *de novo* synthesis is guaranteed by the 'GSH or γ-glutamyl cycle' (Fig. 2) (Orlowski & Meister 1970). In fact, in this pathway, GSH is degraded into cysteinyl glycine and 5-oxoproline by the enzyme γ-glutamyltransferase (GGT) which is followed by

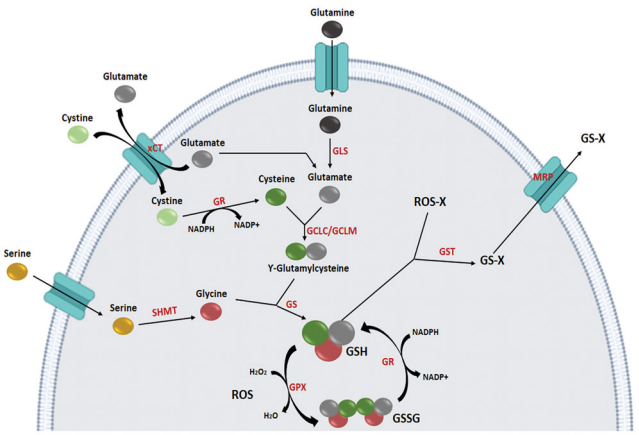


**Figure 2**
GSH cycle. GSH is synthesized within the cytosol from its precursor aminoacids cysteine, glutamate and glycine through two consecutive reactions controlled by the catalytic and modifier subunits of GSH cysteine ligase (GCLC and GCLM) and by GSH synthase (GS). Cystine is imported through xCT, a cystine/glutamate transporter, and converted to cysteine by GSH reductase (GR); glutamate and glycine can be obtained from their precursors, glutamine and serine, through a reaction catalyzed by glutaminase (GLS) and serine hydroxymethyltransferase (SHMT). GSH can be conjugated with other molecules (GS-X) through the activity of GSH-S-transferase (GST) and then exported via multidrug resistance protein (MRP), or it can detoxify ROS by means the activation of glutathione peroxidase (GPX). If GSH is oxidized to GSSG, it can be regenerated via GR and nicotinamide adenine dinucleotide phosphate (NADPH).

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the release of cysteine, glycine and glutamate. Indeed, GSH is the main source of cysteine, controlling and maintaining the pool and allowing its storage in the reduced form, and in the free form it is rapidly oxidized to cystine (Lu 2013, Traverso *et al.* 2013).

GSH plays a key role not only in maintaining intracellular oxidative balance, acting as an antioxidant, but also in driving metabolism (Meister 1995, Sies 1999) and detoxification mechanisms (Lu 2013). In fact, GSH is able to neutralize free radicals and ROS, and it is also crucial for the activity of GPXs, by acting as a substrate (Forman *et al.* 2009). Upon reaction with oxidizing compounds, GSH is converted to GSSG that, being potentially toxic to cells, is extruded or converted to GSH through the action of GSH reductase (GR), whose coenzyme is nicotinamide adenine dinucleotide phosphate (NADPH) (Couto *et al.* 2016). Moreover, GSH prevents lipid peroxidation of membranes through the regeneration of alpha-tocopherol and protects from ferroptosis, an iron-dependent form of cell death characterized by intracellular lipid hydroperoxide accumulation (Ursini & Maiorino 2020).

In addition, GSH can form conjugates with xenobiotic compounds, either directly or via GSTs which catalyze the conjugation of the sulfhydric residue of GSH with the electrophilic residue of xenobiotic compounds inactivating their toxic potential and favoring their export out of the cell (Strange *et al.* 2001). According to substrate specificity and amino acid sequences, GST isoenzymes are classified as alpha (A), pi (P), mu (M), sigma (S), theta (T) and zeta (Z), which are located into the cytosol, and kappa (K) and omega (O) which are detected respectively into mitochondria and associated to membranes (Hayes *et al.* 2005). In addition to the transferase function, GSTs have been shown to form protein–protein interactions with members of the MAPKs involved in cell survival and death signaling. For instance, GSTP1 inhibits the activity of c-Jun N-terminal kinase *in vivo*, blocking apoptosis and favoring cellular transformation (Chatterjee & Gupta 2018).

Notably, GSH is also involved in (i) the transport and metabolism of copper and iron, (ii) in the homeostasis of nitric oxide, (iii) in the metabolism of estrogens, leukotrienes, prostaglandins and nucleotides, (iv) in DNA repair and (v) in the modulation of cell death (Sies 1999, Lu 2013).

Therefore, GSH has several pleiotropic functions that are mainly due to its ability to maintain intracellular proteins (e.g. metabolic enzymes, transcription factors and antioxidant molecules) in a reduced state, supporting and guaranteeing cell metabolism and survival (Aquilano *et al.* 2014) (Fig. 3).

## Role of GSH in cancer

The pathogenesis of several human diseases including cancer is characterized by alterations of intracellular redox homeostasis (Traverso *et al.* 2013, Nitti *et al.* 2022).

As reported in the introduction, ROS production is enhanced in cancer cells (Weinberg *et al.* 2010) that trigger an adaptive response by increasing GSH levels and activating GSH-dependent enzymes (Estrela *et al.* 2006, Gamcsik *et al.* 2012). Also, many anticancer therapies (anthracyclines, alkylating agents, platinum coordination complexes and camptothecins) act by increasing ROS production, and cancer cells defend their survival keeping low ROS thresholds and abolishing senescence or cell death (O'Brien & Tew 1996). However, it has not yet been fully clarified whether the increase in the antioxidant defense, and in particular of GSH content, is the adaptive response triggered by cancer cells as a consequence of the altered redox balance or it is an innate property of cancer cells which makes them 'ready in advance' to counteract any possible increase of ROS generation.

However, an important role is played by the nuclear factor erythroid 2-related factor 2 (NRF2) that is involved in the regulation of several antioxidant genes and, in particular, of those coding for the enzymes involved in GSH synthesis, such as GCL, GR, GPX and GST, which have been found to be overexpressed in several cancers (e.g. pancreatic, lung, breast, ovarian, skin and prostate cancers) (Gorrini *et al.* 2013, Rojo de la Vega *et al.* 2018). Moreover, it has been reported that NRF2 can modulate GSH by promoting the expression of solute carrier family

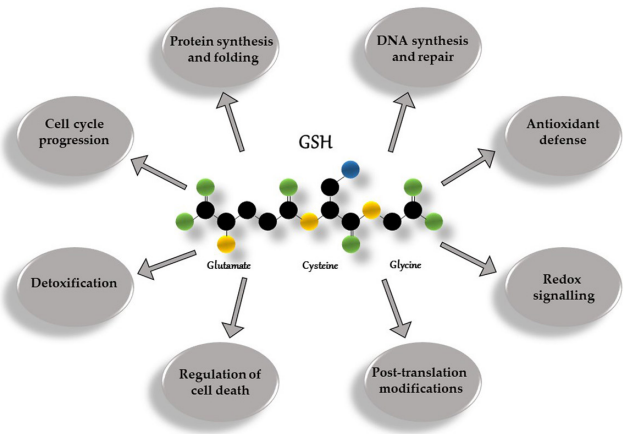


**Figure 3**
The biological functions of GSH. GSH plays pleiotropic functions including antioxidant defense, redox signaling, cell cycle progression, xenobiotic detoxification, regulation of cell death, post-translation modifications, protein synthesis and folding, DNA synthesis and repair.

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7 member 11 (*SLC7A11*) encoding xCT (Sasaki *et al.* 2002), a cysteine/glutamate antiporter, which is overexpressed in many cancers (Jiang *et al.* 2015) and in chemoresistant ones (Monteleone *et al.* 2021).

Interestingly, it has been reported that the expression of GCLM and *SLC7A11* genes are regulated by hypoxia-inducing factor with an upregulation of GSH biosynthesis in hypoxic cancers (Cuperlovic-Culf *et al.* 2016).

As above reported, cells can resynthesize GSH from GSSG via GR activity which requires the presence of NADPH as a substrate (Lu 2013). With this regard, high GSH/GSSG ratios in cancer cells can be due to the activation of pentose phosphate pathway (PPP), leading to NADPH production indispensable for GSH reduction (Li *et al.* 2014, Zhang *et al.* 2016).

PPP activity in cancer cells can be upregulated by the activation of oncogenes (i.e. Ras, mTORC1 and Nrf2) or the inactivation of oncosuppressors (p53) and is increased in response to ionizing radiation or chemotherapy (Patra & Hay 2014). This metabolic response plays a crucial role in helping glycolytic cancer cells to synthesize nucleic acids, coenzymes, ATP and fatty acids and to fight oxidative stress via GSH reduction.

Although cancer cells prefer aerobic glycolysis (Warburg effect), in order to satisfy their energy demands, under pro-oxidant conditions their metabolism can shift toward oxidative phosphorylation (OXPHOS), which is a dynamic and reversible process strongly involved in cancer progression and therapy resistance (Nitti *et al.* 2022). In this connection, our recent study demonstrated that the inhibition of PKCα, by favoring the switch from OXPHOS to aerobic glycolysis, is able to reduce GSH levels and stimulate ferroptosis (see the next paragraph) of chemoresistant neuroblastoma stem cells (Monteleone *et al.* 2021).

## GSH and cancer cell death

GSH plays a crucial role in the induction of apoptosis, autophagy, necroptosis and ferroptosis, and consequently modulation of GSH levels may have important therapeutic implications.

In detail, it has been recognized that the reduction in the GSH/GSSG ratio and the resulting increase in ROS production lead to mitochondrial damage and induce apoptosis (Franco & Cidlowski 2009). However, since GSH loss precedes mitochondrial injury followed by cytochrome c release and caspase activation, the stimulation of GSH synthesis could be a strategy of cancer cells to escape apoptosis.

GSH is also involved in necroptosis, a form of necrosis regulated by receptor interacting protein kinases 1 (RIPK1) and 3 (RIPK3), and characterized by translucent cytoplasm, organelle swelling, increased cell volume and disruption of the plasma membrane (Gong *et al.* 2019). In this regard, it has been reported that dimethyl fumarate and artesunate are used for inducing necroptosis in colon cancer cells and in renal carcinoma cells, respectively (Xie *et al.* 2015, Chauhan *et al.* 2017). Conversely, the use of necrostatin-1, an inhibitor of RIPK1, prevents cell death by preserving them from GSH depletion (Xu *et al.* 2007).

The drastic reduction in GSH levels is also accompanied by ferroptosis, an iron-dependent cell death that can be induced by GPX4 inhibition resulting in lipid peroxidation and ROS accumulation (Ursini & Maiorino 2020). In this regard, it has been shown that RSL3 induces ferroptosis by inactivating GPX4 active site and, consequently, leading to lipid peroxidation and ROS accumulation (Sui *et al.* 2018). Another ferroptosis inducer is erastin, an xCT inhibitor that acts by inducing GSH depletion (Dixon *et al.* 2014). On the contrary, compounds able to inhibit lipid peroxidation, such as liproxstatin-1 and ferrostatin-1, counteract ferroptotic death (Zilka *et al.* 2017).

However, it has been demonstrated that GSH depletion due to xCT inhibition can also lead to autophagy which represents a mechanism of tumor suppression (Jin & White 2008) even though more studies are needed to clarify the relationship between GSH and autophagy.

## GSH and chemoresistance

Chemoresistance is a complex and frequent consequence of cancer treatment, and it is responsible for a poor prognosis for cancer patients. There are several mechanisms underlying cancer cell resistance to chemotherapeutic drugs: (i) drug inactivation, (ii) induction of efflux transporters, (iii) inhibition of apoptosis, (iv) deregulation of cell cycle and checkpoints, (v) enhanced DNA repair and (vi) genetic and epigenetic alterations of cellular oxidative metabolism (Zheng 2017). Indeed, as reported earlier, the increase in GSH levels and the activation of GSH-related enzymes can support tumor growth and counteract the efficacy of therapy, contributing to promote the onset of chemoresistance (Kim *et al.* 2019). In this context, our study demonstrated that chronic treatment with etoposide, a drug which exerts its cytotoxic effect by stimulating ROS overproduction, leads to a selection of multidrug resistant neuroblastoma cells displaying higher levels of GSH with

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respect to parental cells (Colla *et al.* 2016). In addition, the drugs targeting xCT, by limiting the influx of cysteine and GSH biosynthesis, were able to sensitize neuroblastoma stem cells to etoposide counteracting chemoresistance (Monteleone *et al.* 2021).

Notably, the overexpression of GGT in ovarian, colon, liver, prostate, sarcoma, melanoma and breast cancer has been associated with a poor prognosis and resistance to treatment with alkylating agents (e.g. cisplatin and oxaliplatin), which are metabolized and inactivated by GGT (Pompella *et al.* 2006).

Another GSH-dependent enzyme involved in chemoresistance is GST which is highly expressed in many cancers that become chemoresistant (Singh & Reindl 2021). In fact, as reported earlier, GST catalyses the conjugation of GSH with drugs (e.g. cisplatin) and facilitates drug efflux through multidrug resistance proteins (MRPs), which are membrane transporters whose enhanced expression can be associated with the acquisition of chemoresistance (Lautier *et al.* 1996). Also, the overexpression of GR, a key enzyme involved in the GSH cycle, contributes to the development of chemoresistance as observed in temozolomide-resistant glioblastoma cells (Zhu *et al.* 2018).

Furthermore, the overexpression of the enzymes involved in GSH synthesis such as GCL and GS can contribute to chemoresistance by enhancing GSH levels (Backos *et al.* 2012).

An eminent role is played by GPX4, an enzyme involved in the regulation of ferroptosis and that has been found overexpressed in drug-resistant tumors with poor prognosis (Liu *et al.* 2021).

Based on these findings, GSH and GSH-dependent antioxidant pathways are under investigation as potential targets of innovative therapeutic strategies aimed at counteracting cancer progression and therapy resistance.

## GSH-based therapies

Therefore, it is clearly evident that GSH is related to cancer progression and therapy resistance, although many aspects need to be investigated. However, several strategies aimed at targeting GSH system have been proposed, and many of them are currently included in clinical trials (Table 1).

The most used strategy is represented by GSH depletion. In fact, since many anticancer therapies act by stimulating ROS overproduction, their failure is due to scavenging effects of GSH and of GSH-dependent antioxidant system. Therefore, the combination of GSH-depleting agents with a pro-oxidant therapy can be effective to enhance therapeutic sensitivity. In addition, the depletion of GSH, which is involved in drug metabolism, could lead to an increase in the bioavailability of chemotherapeutic drugs, enhancing their efficacy and allowing the clinical use of a lower drug dosage with a decrease in side effects.

The main strategies that can be exploited to induce GSH depletion are the following: (i) to inhibit GSH biosynthesis; (ii) to block precursor amino acids of GSH; (iii) to promote GSH efflux or (iv) to consume intracellular GSH reservoir (Fig. 4).

### GSH biosynthesis inhibitors

In order to inhibit GSH biosynthesis, it is necessary to target the enzymes involved in its synthesis and regeneration. Indeed, GCL is critical for GSH synthesis, and one of its inhibitors mainly used is L-buthionine sulfoximine (BSO, Table 1), which has been shown to induce cancer cell death and to increase chemotherapeutic drug sensitivity of neuroblastoma cells (Domenicotti *et al.* 2003, Marengo *et al.* 2008, Marengo *et al.* 2011, Monteleone *et al.* 2021). However, although these results are encouraging, the clinical use of BSO is restricted due to its short half-life and the non-selective effect of GSH depletion in both healthy and cancer cells. In addition, a further limitation is the difficulty to distinguish and produce the active stereoisomer that might be tumor selective (Sandor *et al.* 1995, Lewis-Wambi *et al.* 2009, Wu & Batist 2013, Hamilton *et al.* 2007).

In order to overcome this limitation, interesting studies have been carried out using polymeric nanoparticles loaded with BSO and other anticancer agents (Cruz *et al.* 2020).

Moreover, also GS could be a therapeutic target. In fact, Wang and coauthors have realized a novel L-cysteine-based poly(disulfideamide) polymer encapsulated with UNC0638 (a histone methyltransferase G9a inhibitor) which is able to inhibit GSH synthesis and to eliminate its intracellular pool (Wang *et al.* 2020). The nanodrug, in comparison with UNC0638 *per se*, had an improved anticancer activity on pancreatic ductal adenocarcinoma and, more importantly, a greater tolerability and absence of toxic effects (Wang *et al.* 2020).

Notably, another possibility to induce GSH depletion is to counteract its regeneration by inhibiting the enzymes involved in the reduction reaction of GSSG. In this context, Xia *et al.* have recently investigated the effects of Stattic, a STAT3 inhibitor, able to inhibit GR and to induce ROS-mediated death of cervical cancer cells (Xia *et al.* 2021).

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**Table 1** Drugs targeting GSH system and currently included in clinical trials.

| Drug | Cancer type | Status | Identifier |
|---|---|---|---|
| Blocking aminoacid precursors | | | |
| Sulfasalazine | | | |
| | Breast cancer | Recruiting | NCT03847311 |
| | Glioblastoma | Recruiting | NCT04205357 |
| Sorafenib | | | |
| | Prostate cancer | Completed | NCT00090545 |
| | Hepatocellular cancer | Completed | NCT00813293 |
| | Breast cancer | Completed | NCT00101400 |
| | Ovarian cancer | Completed | NCT00436215 |
| | Lung cancer | Completed | NCT00609804 |
| | Solid tumors | Completed | NCT00572078 |
| Artemisinin | | | |
| | Breast cancer | Completed | NCT00764036 |
| | Cervical neoplasia | Recruiting | NCT04098744 |
| | Colorectal cancer | Recruiting | NCT03093129 |
| | Ovarian cancer | Recruiting | NCT04805333 |
| Luteolin | | | |
| | Tongue carcinoma | Unknown | NCT03288298 |
| Synthesis inhibitors | | | |
| BSO | | | |
| | Neuroblastoma | Completed | NCT00005835 |
| | Neuroblatoma | Completed | NCT00002730 |
| B-lapachone (ARQ 501) | | | |
| | Advanced solid tumors | Completed | NCT00524524 |
| | Solid tumors | Completed | NCT00099190 |
| | Solid tumors | Completed | NCT01502800 |
| | Head-and-neck cancer | Completed | NCT00358930 |
| | Pancreatic cancer | Completed | NCT00102700 |
| | Pancreatic cancer | Stopped | NCT02514031 |
| Efflux promoters | | | |
| Verapamil | | | |
| | Brain cancer | Completed | NCT00706810 |
| | Gastric cancer | Completed | n.a. |
| | Lymphoma | Active | NCT03013933 |
| | Colorectal cancer | Completed | n.a. |
| Resveratrol | | | |
| | Colon cancer | Completed | NCT00256334 |
| | Colon cancer | Completed | NCT00433576 |
| | Colorectal cancer | Completed | NCT00920803 |
| | Colorectal cancer | Completed | n.a. |
| | Cancer prevention | Completed | NCT00098969 |
| | Multiple myeloma | Completed | NCT00920556 |
| Apigenin | | | |
| | Colorectal cancer | Unknown | NCT00609310 |
| Quercetin | | | |
| | Prostate cancer | Unknown | NCT01538316 |
| | Prostate cancer | Completed | NCT01912820 |
| | Prostate cancer | Completed | NCT03493997 |
| | Childhood cancer | Recruiting | NCT04733534 |
| | Oral cancer | Recruiting | NCT05456022 |
| | Colon cancer | Completed | NCT00003365 |
| | Colorectal cancer | Completed | NCT02195232 |
| | Kidney cancer | Unknown | NCT02446795 |
| | Squamous cell carcinoma | Recruiting | NCT03476330 |
| | Lymphoma | Unknown | NCT00455416 |
| Sulforaphane | | | |
| | Lung cancer | Active | NCT03232138 |
| | Prostate cancer | Completed | NCT01228084 |
| | Breast cancer | Recruiting | NCT03934905 |

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**Table 1** (*continued*)

| Drug | Cancer type | Status | Identifier |
|---|---|---|---|
| | Melanoma | Completed | NCT01568996 |
| Romidepsin | | | |
| | Lung cancer | Completed | NCT01302808 |
| | Solid tumors | Completed | NCT00379639 |
| | Solid tumors | Completed | NCT01537744 |
| | Solid tumors | Completed | NCT00019318 |
| | Prostate cancer | Completed | NCT00106418 |
| | Colorectal cancer | Completed | NCT00077337 |
| | Colorectal cancer | Completed | NCT02512172 |
| | Thyroid cancer | Completed | NCT00098813 |
| | Leukemia | Completed | NCT00042822 |
| Reserve consumption | | | |
| Disulfiram | | | |
| | Breast cancer | Recruiting | NCT04265274 |
| | Breast cancer | Recruiting | NCT03323346 |
| | Prostate cancer | Completed | NCT01118741 |
| | Solid tumors | Completed | NCT00742911 |
| | Multiple myeloma | Recruiting | NCT04521335 |
| | Glioblastoma | Completed | NCT02678975 |

Also, 2-acetylamino-3-(4-(2-acetylamino-2-carboxye thylsulfanylcarbonylamino) phenyl carbamoylsulfanyl) propionic acid has been identified as a GR inhibitor that is able to induce cell cycle arrest of human esophageal cancer cells through a generation of oxidative stress (Li *et al.* 2017).

An additional approach to reduce GSH availability is the inhibition of GGT that, as above reported, is upregulated in many cancers (Pompella *et al.* 2006). Glutamate analogues, such as acivicin, azaserine and boronate derivatives, which have been identified as GGT inhibitors have shown a marked toxicity in patients (Joyce-Brady & Hiratake 2011). Notably, the nanoparticle-encapsulated compound OU749 revealed a greater efficacy than the GGT inhibitor alone, and it has been tested on cisplatin-resistant human non-small cell lung cancer cells (Wang *et al.* 2021).

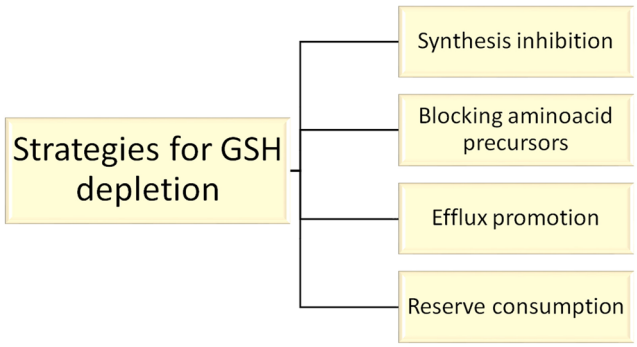


**Figure 4**
Strategies aimed at inducing GSH depletion.

## GSH precursor amino acid uptake inhibitors

Another possibility to limit GSH synthesis is to reduce the availability of cysteine by inhibiting xCT transporter. In this context, erastin and its analogues and sulfasalazine (Table 1) have been found to be effective in depleting GSH by reducing cysteine influx. Consequently, erastin has been found to induce ferroptosis and to counteract the progression of several cancers (Zhao *et al.* 2020). Furthermore, erastin combined with docetaxel reduced the onset of chemoresistance in ovarian cancer (Zhou *et al.* 2019), while an encapsulated form was effective in breast cancer (Yu *et al.* 2019). Notably, two erastin analogues, imidazole ketone erastin and piperazine erastin, due to improvement of solubility, potency and stability, induced ferroptosis in mouse models of fibrosarcoma, lymphoma and hepatocellular carcinoma (Zhang *et al.* 2019). Also, sorafenib acts by inhibiting xCT, and its administration alone or in combination with other drugs has been shown to activate ferroptosis in hepatocellular carcinoma and in kidney cancer (Table 1) (Lachaier *et al.* 2014). Among xCT inhibitors, sulfasalazine, initially identified as an immunosuppressant for chronic inflammatory diseases, has been recognized as ferroptosis inducer in breast cancer, lymphoma, bladder cancer and colon cancer treated in combination with cisplatin (Table 1) (Liu *et al.* 2017). Moreover, it has been recently demonstrated that C2-4, a PKCα inhibitor, analogously to sulfasalazine, sensitizes etoposide-resistant neuroblastoma stem cells by inducing ferroptosis (Monteleone *et al.* 2021).

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Other compounds, such as capsazepine, pseudolaric acid B, artemisinin (Table 1) and its derivatives and metaderin, are able to inhibit xCT (Zhang *et al.* 2021), and among them, artesunate and dihydroartesiminin have shown anticancer properties by inducing ferroptotic death (Zhang *et al.* 2021).

Since xCT overexpression may be due to the increased activity of Nrf2, the compounds targeting this transcription factor have been proposed to treat cancer and/or to counteract the onset of chemoresistance. In this regard, natural compounds such as brusatol, halofuginone luteolin, chrysin and emetine showed anticancer properties (Table 1) (Xiang *et al.* 2018, Panda *et al.* 2022) and a chemosensitizer action (Panieri & Saso 2019).

Notably, the glucocorticoid clobetasol propionate (Choi *et al.* 2017) and antitubercular drugs such as isoniazid and ethionamide, by interfering with Nrf2 nuclear translocation, were able to enhance therapy sensitivity in lung cancer and leukemia cells, respectively (Verma *et al.* 2015, Peng *et al.* 2016).

Also trigonelline, by inhibiting Nrf2, has been demonstrated to increase the sensitivity of lung cancer cells to etoposide and cisplatin (Fouzder *et al.* 2021). Analogously, Zhou *et al.* reported that digoxin, a drug used to treat heart failure, sensitizes chemoresistant pancreatic cancer cells to gemcitabine by inhibiting Nrf2-dependent pathways (Zhou *et al.* 2019). In addition, luteolin, a natural flavonoid, and its derivative apigenin were found to sensitize resistant colorectal cancer and hepatocarcinoma cells to doxorubicin via Nrf2 inhibition (Chian *et al.* 2014, Gao *et al.* 2017).

With regard to synthetic Nrf2 inhibitors, (i) Im3829 has been shown to sensitize lung cancer patients to radiotherapy (Lee *et al.* 2012); (ii) AEM1 sensitized A549 lung cancer cells to various anticancer agents (Bollong *et al.* 2015); (iii) compound f4 counteracted A549 and leukemia cell growth and proliferation (Zhang *et al.* 2014, Zhang *et al.* 2017); (iv) compound ML385 sensitized lung cancer cells to carboplatin (Singh *et al.* 2016) and (v) Stattic, above cited as GR inhibitor, was able to sensitize colon cancer cells to 5-fluorouracil (Tajmohammadi *et al.* 2019).

### GSH efflux promoters

Furthermore, another strategy to induce GSH loss is to promote its efflux from cells (Table 1). In this context, the modulation of MRP1, the main transporter of free and drug-conjugated GSH, may lead to GSH depletion circumventing chemoresistance in cancer cells (Lorendeau *et al.* 2017). In addition, verapamil and derivatives, used in the treatment of cardiovascular disease, and flavonoids such as resveratrol, apigenin, quercetin and aminothienopyrimidine derivatives, have been demonstrated to inhibit MRP1 in preclinical studies (Lorendeau *et al.* 2017). Several of these compounds have been also tested in clinical trials which are still ongoing (Table 1). However, the majority of clinical studies (phase 1) carried out are focused to analyze the toxicity of the drug, and only a little number of them (phase 2) are aimed at analyzing the efficacy of the treatment with results not yet available. Recently, a novel MRP1 inhibitor, YAN, has been identified, and a promising effect was observed in the treatment of multidrug-resistant lung cancer cells (Gao *et al.* 2021).

### GSH-consuming drugs

Interestingly, the binding of GSH to anticancer drugs leading to a consumption of intracellular GSH stores is determinant to induce cancer chemoresistance. Therefore, isothiocyanates, such as β-phenylethyl isothiocyanate and sulforaphane, having affinity for GSH binding, can have a promising antitumor activity (Table 1). In detail, it has been found that sulforaphane conjugated with polymer nanoparticles reduces drug toxicity and is effective in counteracting breast cancer cell survival (Xu *et al.* 2019). Aldehydes or α,β-unsaturated ketones as quinone methide, oridonin and cinnamaldehyde are able to form conjugates with GSH and showed anticancer properties (Luo *et al.* 2018). In particular, romidepsin binds to GSH and has been proposed for the treatment of cutaneous T-cell lymphoma and urothelial carcinoma (Table 1) (Pattarawat *et al.* 2020).

Notably, intracellular GSH levels can be depleted by exposure to pro-oxidant compounds, leading to GSSG formation. They are commonly encapsulated in nanocarriers to avoid systemic toxicity and to obtain a major selectivity. In fact, several metal-based nanomaterial approaches, such as manganese dioxide, metal-organic frameworks based on copper, iron or platinum or complexes of several metals, have shown a marked antitumor activity (Wang *et al.* 2019). In addition, it has been demonstrated that disulfiram is able to convert GSH to GSSG and is effective in the combined treatment of metastatic melanoma (Meraz-Torres *et al.* 2020).

## Conclusions

Cancer cells, due to their increased metabolic rate, produce high levels of ROS that are balanced by the presence of

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efficient antioxidants and in particular of GSH-related system. The maintenance of the redox homeostasis guarantees cancer cell survival and proliferation and drives the adaptation to therapy-induced stress. In fact, several traditional anticancer drugs exert their cytotoxic action by stimulating ROS production, and although the initial treatment is able to kill cancer cells, the long-term treatment leads to a selection of more malignant cells equipped with antioxidant defense and characterized by drug refractoriness. Therefore, a strategy able of overcoming such 'adaptive tolerance threshold' could help to fight chemoresistant cancer cells. In this context, GSH depletion has been proposed as a strategy to counteract cancer progression and therapy resistance, via the inhibition of key enzymes or precursors of GSH synthesis, consumption of its intracellular stores and promotion of its efflux.

These GSH-depleting compounds, used alone or in combination with traditional therapies, not only potentiate the pro-oxidant effect of chemotherapeutic drugs but also enhance their biodisponibility as a consequence of the decrease in GSH conjugation and drug elimination. In addition, since GSH is fundamental for GPX4 activity, it is possible to induce ferroptosis of cancer cells by directly targeting GPX4 (Ursini & Maiorino 2020, Wei *et al.* 2020).

Interestingly, the most innovative strategies are focused to load the anticancer drugs or chemosensitizers on nanoparticles whose administration can limit toxic side effects and favor a more selective release of the compounds.

Furthermore, considering that oxidative state can be highly variable in each tumor and each phase of cancer progression, monitoring GSH levels in patients before and during therapy could be crucial to early identify refractory patients and to direct therapy toward a personalized approach.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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GEV, CD and BM conceived the study. GEV, BT, NT and BM wrote the original draft. CD reviewed the final version. All authors have read and agreed to the published version of the manuscript.

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