Cancer Letters 560 (2023) 216125

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Valproate and lithium: Old drugs for new pharmacological approaches in brain tumors?

Gianfranco Natale [a,b,1], Elisabetta Fini [c,1], Pasquale Fabio Calabrò [c], Marco Carli [a], Marco Scarselli [a], Guido Bocci [c,*]

[a] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy
[b] Museum of Human Anatomy "Filippo Civinini", University of Pisa, Italy
[c] Department of Clinical and Experimental Medicine, University of Pisa, Italy

ARTICLE INFO

Keywords:
Valproate
Valproic acid
Lithium carbonate
Lithium chloride
Brain
Cancer
Preclinical research
Clinical studies
Therapeutic drug monitoring
Adverse drug reactions
Drug repositioning

ABSTRACT

Beyond its use as an antiepileptic drug, over time valproate has been increasingly used for several other therapeutic applications. Among these, the antineoplastic effects of valproate have been assessed in several *in vitro* and *in vivo* preclinical studies, suggesting that this agent significantly inhibits cancer cell proliferation by modulating multiple signaling pathways. During the last years various clinical trials have tried to find out if valproate co-administration could enhance the antineoplastic activity of chemotherapy in glioblastoma patients and in patients suffering from brain metastases, demonstrating that the inclusion of valproate in the therapeutic schedule causes an improved median overall survival in some studies, but not in others. Thus, the effects of the use of concomitant valproate in brain cancer patients are still controversial.

Similarly, lithium has been tested as an anticancer drug in several preclinical studies mainly using the unregistered formulation of lithium chloride salts. Although, there are no data showing that the anticancer effects of lithium chloride are superimposable to the registered lithium carbonate, this formulation has shown preclinical activity in glioblastoma and hepatocellular cancers. However, few but interesting clinical trials have been performed with lithium carbonate on a very small number of cancer patients.

Based on published data, valproate could represent a potential complementary therapeutic approach to enhance the anticancer activity of brain cancer standard chemotherapy. Same advantageous characteristics are less convincing for lithium carbonate. Therefore, the planning of specific phase III studies is necessary to validate the repositioning of these drugs in present and future oncological research.

1. Introduction

The non-recent use of an old or existing or even banned drug for new therapeutic indications other than those for which it was initially marketed, is a growing phenomenon formally and initially referred to as "drug repositioning" (or later also repurposing, reprofiling, retasking, switching) by Ashburn and Thor in 2004 [1]. There are several examples of drug repositioning also in the oncology field [2,3]. The oldest case is represented by acetylsalicylic acid, which was initially introduced as analgesic drug and in more recent times as a drug to prevent colorectal cancer, as well [4]. Banned for its dramatic experience of teratogenicity in 1950s, thalidomide was carefully rehabilitated and repositioned as an effective drug against multiple myeloma for its antiangiogenic and immunomodulatory effects [5]. Thus, antitumor activities have described for several agents widely used for other therapeutic indications. These include, for example, non-aspirin nonsteroidal anti-inflammatory drugs [6], the oral contraceptive ormeloxifene [7], and the natural polyphenol resveratrol [8]. More recently, there is increasing evidence that classic antipsychotic, antidepressant, and mood-stabilizing drugs are endowed with anticancer properties. These compounds can inhibit the progression of glioma by targeting signaling pathways related to cell proliferation, apoptosis, or invasion/migration or by increasing the sensitivity of glioma cells to conventional chemotherapy or radiotherapy [9]. Similar findings have been demonstrated for antiepileptic drugs such as levetiracetam and valproate [10]. In line with this profitable perspective, the present work aims to focus the

* Corresponding author. Department of Clinical and Experimental Medicine, School of Medicine, University of Pisa, Via Roma 55, Pisa, 56126, Italy.
E-mail address: guido.bocci@unipi.it (G. Bocci).
[1] Equal contribution to the manuscript.

https://doi.org/10.1016/j.canlet.2023.216125
Received 4 February 2023; Received in revised form 9 March 2023; Accepted 9 March 2023
Available online 11 March 2023

attention on two old neurological and psychoactive compounds, valproate and lithium, as possible candidate to be the next repositioned drugs assessed as anticancer agents effective against brain tumors.

2. Anticancer activity of valproate

2.1. Preclinical studies of valproate on cancer

The antineoplastic effects of valproate (Fig. 1) were discovered fortuitously, and a new important therapeutic role of this old drug was assessed [11]. In a first experiment, valproate inhibited growth and promoted maturation of HL-60 human promyelocytic leukemia cells at concentrations above the recommended therapeutic blood levels [12]. When tested as possible teratogenic agent, valproate showed antiproliferative activity, inhibiting the mitotic index of mouse neuroblastoma (Neuro-2A) and rat glioma (C6) cells with an approximate IC_{50} of 0.5 and 1.0 mM, respectively [13]. In subsequent studies, valproate was found to reduce cell growth of the rapidly dividing cholinergic neuroblastoma x glioma hybrid cell-line (NG108-15) at therapeutic free drug concentrations. Indeed, it behaves as a pro-differentiating agent, since it increases the activity of choline acetyltransferase, beta-galactosidase, and muscarinic cholinergic receptor binding [14]. Since compounds with antiproliferative and differentiation potential are known to inhibit tumor progression or induce malignant reversion, valproate appeared as a good candidate for anticancer therapy. Accordingly, several *in vitro* and *in vivo* pre-clinical studies suggest that this agent significantly inhibits cancer cell proliferation by modulating multiple signaling pathways [11,15].

In additional studies, Regan and his coworkers found that valproate antiproliferative activity was due to the arrest C6 glioma cell at the G1 phase of cycle [16–18]. Anticancer properties of valproate are mainly attributable to its histone deacetylases (HDACs) inhibitory action, as well as to the ability to affect microRNAs expression [19]. Four classes of HDACs inhibitors with potential anticancer activity have been recognized: hydroxamic acids, short chain fatty acids (including valproate), cyclic peptides, and benzamides. Valproate causes acetylation of the N-terminal tails on histones H3 and H4, and inhibits the activity of HDACs I and II, probably by binding to the catalytic center, and thereby blocking access to the substrate. Since histone deacetylation and DNA methylation are involved in resistance to chemotherapy through the protection of cancer cells via silencing of anti-tumor genes, HDACs inhibitors would stimulate or de-repress silenced tumor inhibitor genes [20]. Nevertheless, the mechanisms of antineoplastic activity and the specificity of HDACs have not been perfectly clarified. More importantly, as a psychoactive agent that crosses the blood-brain barrier, valproate might also adequately defeat metastatic cancer cells in the central nervous system.

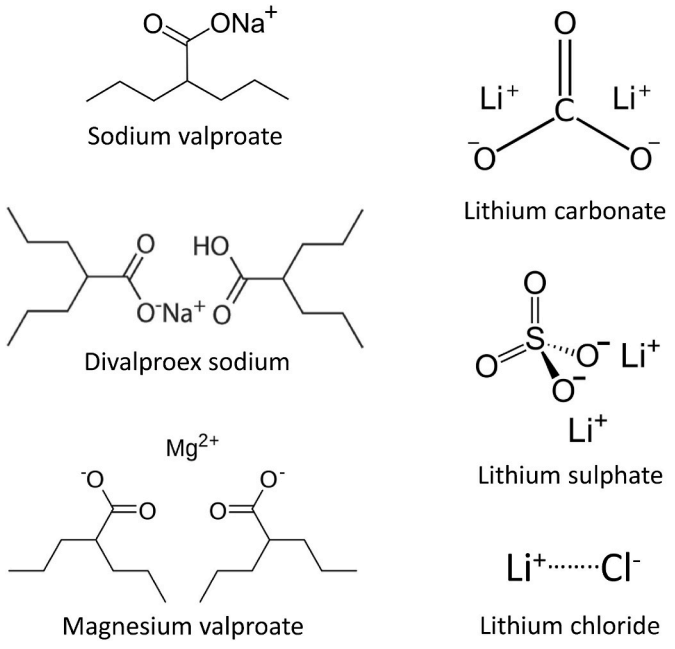

Fig. 1. Chemical structures of valproate and lithium formulations.

Since then, many other works were published, showing the growth inhibition exerted by valproate on several cancer cell lines. These include blood malignancies [21], such as murine B-myeloma (FO) cells and T-lymphoma (AKR-1) cells, as observed in single-cell culture [22], and in other murine and human cell lines of lymphoid origin [20,23]. In a more recent study, valproate induced apoptosis in bone marrow blast cells taken from patients affected by the aggressive acute myeloid leukemia characterized by t(8; 21) chromosomal aberration (AML1/ETO), suggesting the use of this drug to treat this leukemia subtype [24]. According to Schwartz and colleagues, the antiproliferative effect of valproate in different myeloma cell lines (OPM2, RPMI and U266) was not mediated by apoptosis [25].

In line with its hepatotoxicity, in rat hepatome cell line FaO valproate caused cell death through apoptosis, with Fas-ligand and caspase-11 expression, which was increased at the transcriptome level, while caspase-3 was activated at the proteome level during the exposure period [26]. Recent evidence was provided that in human hepatocellular carcinoma (HepG2 cells) cell line valproate induced a significant and dose-dependent cytotoxicity caused by a marked increase in intracellular reactive oxygen species, leading to early and late apoptosis [27].

In a recent study, valproate inhibited cell viability in human prostate cancer PC-3 and LNCaP cells. This effect was due to a decreased lipogenesis and increased apoptosis via the CCAAT-enhancer-binding protein alpha/sterol regulatory element-binding protein 1 (C/EBPα/SREBP-1) pathway [28]. Furthermore, valproate inhibited the metastatic re-activation of PC3 prostate cancer cells due to chronic mammalian target of rapamycin (mTOR) suppression induced by temsirolimus [29].

Valproate appeared beneficial also in the treatment of breast cancer. Promising preclinical findings suggest that it could be considered as a complementary agent in combination with many cytotoxic, hormonal, and immunotherapeutic compounds [30]. Furthermore, in a recent paper it was shown that valproate decreased the expression of pyruvate kinase M2 isoform (PKM2), leading to inhibited cell proliferation and reduced colony formation in breast cancer MCF-7 and MDA-MB-231 cells. This novel mechanism would regulate the Warburg effect, that is the ability of cancer cells to exhibit a unique metabolic way concerning an accelerated glucose consumption, an improved lactate accumulation, and low oxidative phosphorylation even under aerobic conditions. This aspect is essential for developing a successful approach in breast cancer therapy [31].

Valproate proved to be a promising anticancer drug, alone or in combination with classic antitumor agents, in other severe malignancies, including gastric [32], medullary thyroid [33], lung [34], pancreatic [35], cervical cancer cell lines [36], and head and neck squamous carcinoma cell lines [37].

2.2. Preclinical activity of valproate on models of intracranial malignancies

Thanks to its ability to cross the blood-brain barrier, valproate is a good compound for the treatment of intracranial malignancies. Furthermore, considering that some intracranial tumors can induce epileptic activity [38], not surprisingly valproate can be a good candidate to be administered as an anticancer drug, as well [39]. In this respect, in recent years a wide literature considered the importance of valproate as an anticancer compound for the treatment of malignancies of the central nervous system [40–44].

In addition to this, several studies suggested a neuroprotective role for valproate. Repeated administration of both lithium and valproate

increased the expression of brain-derived neurotrophic factor (BDNF), but not of glia-derived neurotrophic factor (GDNF), in the rat frontal cortex and hippocampus [45]. Neuroprotective properties of valproate seem to involve modulation of neurotrophic factors and receptors for melatonin [46]. Furthermore, in different experimental models, valproate is able to reinforce neurons restoration after stroke and brain injury. Finally, valproate was shown to protect dopaminergic neurons in different experimental models of Parkinson's disease [15].

As anticipated, one of the first experiments where valproate disclosed its unexpected antiproliferative activity was just against mouse neuroblastoma (Neuro-2A) and rat glioma (C6) cells [13]. Since the expression of certain HDACs forms differs with glioma tumor grade, valproate can be regarded as an experimental drug in the treatment of these very aggressive malignancies. In particular, valproate induces a reversible hyperacetylation of the N-terminal tails of histones H3 and H4 in glioma cell lines. Another important effect of valproate is its ability to induce DNA hypomethylation, as shown in rat astrocytes, and the modulation of several transcription factors. Thus, valproate induces differentiation of glioma cells, preventing their invasion in brain tissues [47].

Concerning the gene and protein expression mediated by valproate in glioblastoma and other nervous cancers, this drug up-regulates p21, wingless-related integration site (Wnt) 1,2, cyclin D3 (proliferation), glial fibrillary acidic protein (GFAP), BDNF, beta3 tubulin, cluster determinant 133 (cell differentiation), Bax, reactive oxygen species, glutathione (cell death), TIMP metallopeptidase inhibitor 1 (tumor invasion), and down-regulates glycogen synthase kinase-3 (cell proliferation), cluster determinant 44, GDNF (cell differentiation), Bcl 2 (cell death), vascular endothelial growth factor (VEGF; angiogenesis), matrix metalloprotease 2, interleukin 6, NF-κB (tumor invasion) [47,48]. Thus, valproate exhibits different effects on cancer cell proliferation, both through HDACs inhibition and other mechanisms. It can directly alter the acetylation status of p53 and inhibit NF-κB, possibly through HDACs inhibition. At the same time, NF-κB can also be constrained by valproate-mediated HDACs inhibition and subsequent increased expression of cartilage glycoprotein-39, which is an inhibitor of NF-κB. The valproate-mediated cell cycle stop is achieved through p21 increased expression by HDACs inhibition and p53 acetylation, and spindle assembly defects through HDACs inhibition [47]. Direct and indirect inhibition of glycogen synthase kinase-3beta can also induce both tumor proliferation and cell cycle arrest. Furthermore, β-catenin expression appears enhanced by direct and indirect inhibition of GSK-3β [49]. Finally, signal transducer and activator of transcription 3 may still promote oncogenesis, regulated by the epidermal growth factor receptor and phosphatase and tensin homolog mutant status of the tumor [47]. In a recent *in vitro* study valproate enhanced apoptosis by promoting autophagy via Akt/mTOR signaling in two glioma cell lines, U251 and SNB19 [50].

HDACs inhibitors, including valproate, inhibit tumor angiogenesis *in vivo* in xenograft models of medulloblastoma, neuroblastoma, prostatic and colon tumors. Accordingly, in *in vitro* experiments (human glioma cell lines U87-MG, U251 and A172; rat glioma cell line C6), valproate preferentially inhibited endothelial cell proliferation in comparison with glioma cell proliferation. This effect was attributed to a diminution of vascular endothelial growth factor secretion of glioma cells under both normoxic and hypoxic conditions. Valproate was also able to constrain tube formation in the angiogenesis assay. In *in vivo* experiments of female rats inoculated with glioma C6 cells into the right frontal lobe, treatment with valproate combined with irinotecan reduced the number of vessels expressing factor VIII in the brain tumor model. Thus, glioma angiogenesis is suppressed both by a direct way, i.e. inhibition of endothelial cell proliferation and tube formation, and by an indirect way, i.e. decreased secretion of VEGF by glioma cells [51].

Several cell types, especially microglial cells, macrophages, and neural precursor cells, seem to adopt a specific phenotype when attracted to glioblastomas, favoring loss of immunogenicity of cancer cells and tumor invasion. It was suggested that valproate is able to affect the brain tumor microenvironment by modulating gene expression, phenotype and survival of many of these cell populations [47].

A very recent study carried out on human glioma SHG44 and U87 cell lines showed that valproate inhibited glioma invasion and metastasis through the regulation of Smad4 expression. In detail, the drug inhibited the process of conversion of epidermal cells to mesothelial ones by altering the expression level of Small mothers against decapentaplegic homolog 4 (Smad4), which is induced by transforming growth factor beta 1 (TGF-β1) to form a Smad3/4 complex [52].

The remodeling effects induced by valproate on chromatin suggested to include this drug in combination with other chemotherapeutic agents, immunotherapy, and radiation therapy to potentiate their anticancer effects. In this respect, for example valproate synergized with the topoisomerase-II DNA-damaging agent etoposide when tested *in vitro* against glioma cells. This adjuvant role of valproate was proved to be effective with temozolomide, nitrosourea alkylating agents, the natural flavonoid luteolin and the tyrosine kinase inhibitor gefitinib [44]. Again, valproate-induced histone hyperacetylation leads to chromatin de-condensation. This effect sensitizes the DNA to radiation therapy, causing DNA double-strand breaks (DSBs), which initiate apoptotic cell death. Furthermore, valproate may inhibit the dissociation of the DNA repair machinery from the DNA after double-strand breaks repair, leading to apoptosis [48]. Finally, the effects of valproate-adjuvant immunotherapy are also promising. For example, the combination therapy of the oncolytic animal virus equine herpesvirus type 1 (EHV-1) with valproate was evaluated, showing that pretreatment with the drug promoted the infection [47,48]. Accordingly, several *in vivo* and *in vitro* studies have demonstrated that valproate has radio-sensitizing effects for gliomas and radioprotective influence on normal brain tissue or hippocampal neurons [42].

Neuroblastoma is the most common extracranial solid cancer in childhood, and it is originated from the sympathoadrenal progenitors of neural crest [53,54]. The anticancer effects of valproate combined with interferon- α (IFN-α) were examined against two neuroblastoma cell lines (NB-2 and UKF-NB-3) and it was found that drug combination inhibited the growth of UKF-NB-3 xenograft tumors in nude mice synergistically and promoted complete healing in two out of six animals, whereas single therapy only repressed tumor growth [55].

As previously described for breast cancer [31], valproate was shown for the first time to restrain the Warburg effect and tumor progression also in human neuroblastoma cell lines SH-SY5Y and BE(2)C, suggesting a novel therapeutic strategy for this malignancy. More in depth, this anticancer compound curbs the aerobic glycolysis in neuroblastoma cells, acting through a reduction of glucose uptake, of lactate and ATP production, and of E2F transcription factor 1 (E2F1) levels. So, it follows in a diminished expression of glycolytic genes glucose-6-phosphate isomerase and phosphoglycerate pinase 1. In particular, valproate down-regulates E2F1 [56].

Meningioma is one of the most common intracranial tumors. In an *in vitro* experiment, meningioma sphere cells and meningioma adherent cells were found both sensitive to valproate that increased the susceptibility of these cancer cells to irradiation. Furthermore, the expression of the stem cell marker octamer-binding transcription factor 4 (Oct-4) was reduced, especially by combined treatment with irradiation [57].

2.3. Clinical studies of valproate on brain cancer

Based on promising preclinical data, during the last years clinical trials have tried to find out if valproate co-administration could enhance the antineoplastic activity of chemotherapy. Interestingly, randomized phase II studies have confirmed in hematological [58] and solid [59] cancer patients the antineoplastic effect of valproate when concomitantly administered with chemotherapeutic drugs. In 2011, Weller conducted a *post hoc* analysis [60] on glioblastoma patients treated with temozolomide plus radiotherapy, demonstrating that the inclusion of

valproate in the therapeutic schedule caused an improved median overall survival (OS; 17 months) in comparison with the OS of glioblastoma individuals not receiving any antiepileptic drug (AED; 14 months) or the ones treated with an enzyme-inducing AED (EIAED; 14.4 months). Another intriguing clinical finding was related to the period of co-administration of valproate with temozolomide (TMZ). Indeed, Kerkhof and colleagues [61], based on a clinical trial enrolling 108 glioblastoma patients, suggested that the combination of valproate and TMZ for at least 3 months caused a significant enhancement of OS (i.e., a value of 2 months). The antitumor effect of valproate was also demonstrated in patients suffering from brain metastases due to breast cancer, where the simultaneous use of the antiepileptic with whole brain radiotherapy significantly improved the median OS (11 months in patients with valproate vs. 5 months without valproate) [62]. A prospective comparative study in a miscellaneous population of 823 patients both with brain primary or metastatic tumor revealed that patients not treated with antiepileptic drugs had a statistically shorter median OS (45 months) if compared to patients administered with valproate (262 months) [63]. Other small-size clinical trials showed clinical advantages, such as the increase of OS, in the adding of valproate to radio-chemotherapy in glioblastoma patients [64–66], but the data from these trials could only suggest a survival benefit because they were not powered to validate those effects and a wider study was needed. In 2020, a Taiwanese nationwide population-based cohort study based on an administrative database on patients with high-grade gliomas undergoing TMZ showed that the valproate-treated group of patients had a significantly greater OS time compared with the non-valproate group (50.3 vs 42 months, respectively) [67]. Recently, it has been also reported the case of a long-term survival (more than 5 years) of an adolescent patient suffering of glioblastoma prescribed with the promising schedule of vinblastine and valproic acid [68].

However, the effects of concomitant valproate in glioblastoma patients are still controversial. Indeed, Happold and co-workers [69] in a pooled analysis of prospective clinical trials demonstrated that the use of valproate had no significant impact on OS in patients with glioblastoma and also that any therapeutic option for epilepsy did not improve survival. This discouraging data on the use of valproate as an anticancer drug into the clinic of brain tumors were also confirmed by a recent phase 2 study in children with pontine glioma or high-grade gliomas. Children were treated with valproate and radiotherapy and then with a maintenance therapy with valproic acid and bevacizumab, an anti-VEGF monoclonal antibody. The authors concluded that the concomitant treatment of valproate, bevacizumab and radiotherapy, although well-tolerated, did not improve the OS of these pediatric patients [70]. A possible explanation for the discrepancies among the preclinical data and the different clinical results may be related to the valproate plasma concentrations and its antitumor mechanism of action. Indeed, a recent article by Berendsen and colleagues suggest that increased valproate concentrations enhanced the level of acetylated histones *in vitro*, but the same findings were not retrieved in cancer samples obtained from glioblastoma patients using antiepileptic doses of valproate [41].

Antiepileptic drugs may interact with alkylating agents, which are both usually used in patients with brain tumors [71]. Indeed, the therapeutic indication of antiepileptic drugs is widespread because an important percentage of central nervous system tumors will generate seizures [38], impacting on patients' quality of life. An example of possible drug-drug interactions that may modify the effects of antineoplastic therapies is represented by a retrospective study enrolling glioblastoma patients treated with lomustine, where the concomitant P450 enzyme inducing antiepileptic drugs determined a worse median survival (10.8 months) if compared to those patients treated with valproate (13.9 months), a non-enzyme inducing antiepileptic [72]. Lomustine increased efficacy may be determined by the antineoplastic activity of valproate but also by its cytochrome P450 (CYP) inhibitory properties that may as well lead to serious adverse events if the metabolism is already impaired [73]. Indeed, in patients suffering from high-grade gliomas, the up-front regimen of fotemustine, cisplatin, and etoposide followed by whole brain radiotherapy if combined with valproate caused 3-fold higher incidence of bone-marrow toxicities when compared to patients administered with other antiepileptic drugs [74].

3. Anticancer activity of lithium

3.1. New potential therapeutic use of lithium in cancer

Lithium is a monovalent cation, belonging to the family of alkali metals with atomic number 3. In particular, lithium inhibits the coupling of β-adrenergic and muscarinic receptors to G proteins, a process stimulated by magnesium, blocks the activity of inositol monophosphatase, which has magnesium as a cofactor, and inhibits bisphosphate 3-prime-nucleotidase (BPNT1), a magnesium-dependent phosphatase [75,76]. Furthermore, lithium reduces the activity of GSK-3β because it competes against magnesium for an essential binding site for its phosphorylation and transcription. This effect is biologically very relevant since GSK-3β is the kinase with the largest number of known substrates (over 100) [77,78] (Fig. 2). Furthermore, lithium leads to WNT/β-catenin pathway activation, Camp-Response-Element-Binding Protein (CREB) inducement, transducer of regulated CREB activity 1 (TORC1) stimulation, and activated lymphokine killer cell immunomodulation [79,80]. Lithium has been tested as an anticancer drug in several preclinical studies mainly using the formulation of lithium chloride (LiCl) (Fig. 1), a drug not registered for any human use and therefore not present in the clinical practice. To our knowledge, there are no data in the scientific literature where lithium carbonate and lithium chloride activities have been compared *in vivo* and/or *in vitro*, therefore the anticancer effects of the two compounds may be not superimposable. Indeed, it has been shown that some types of cancer might be linked to hyperactivation of GSK-3β [81], therefore lithium, as an inhibitor of this molecular target, could be a possible candidate to a complementary oncological therapy. Duffy and colleagues tested LiCl in six different neuroblastoma cell lines and reported a time and concentration-dependent decrease of cell viability and an increase of cell death through apoptosis. These effects were coupled to the discovery that LiCl caused a reduction of N-myc proto-oncogene protein (MYCN), a marker associated with poor prognosis and a lack of specific therapeutic options, due to the GSK-3β inhibition that reduces the stability of MYCN mRNA [82]. In more recent studies lithium chloride also exhibited activity in acute promyelocytic leukemia cells (i.

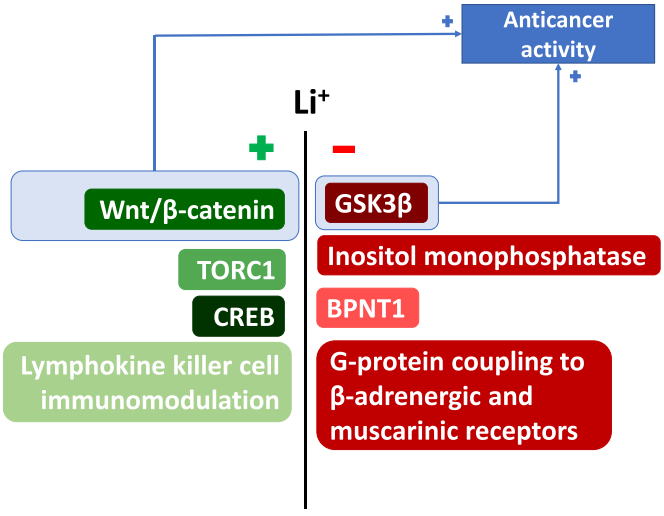

Fig. 2. Molecular mechanisms of action of lithium and their effects on anticancer activity. BPNT1, bisphosphate 3-prime-nucleotidase; CREB, cAMP response element-binding protein; GSK-3β, glycogen synthase kinase-3β; NCLX, mitochondrial sodium calcium exchange channel; TORC1, target of rapamycin complex 1; Wnt, wingless-related integration site.

e., NB4) with an increased rate of apoptosis and G2/M phase arrest in cells treated with 20 mM LiCl for 24 h [83]. Interestingly, the study by Zassadowski and collaborators confirmed the activity of LiCl in two different cell subtypes of acute promyelocytic leukemia (i.e., NB4 and UF-1 cells), showing that LiCl, by inhibiting the GSK-3β pathway, obtained a reduction of proliferation, and an increase in apoptosis in both cell lines. These data were confirmed *in vivo*: mice treated with LiCl alone showed a comparable survival to those treated with retinoic acid alone, while those treated with the combination of lithium chloride and retinoic acid showed a significantly superior survival compared to those treated with retinoic acid only [84]. In medullary thyroid cancer (MTC) excellent results have been reported with LiCl both *in vitro* and *in vivo*. A significant inhibition of MTC cell line growth and a decreased production of neuroendocrine markers occurred with LiCl at concentrations ranging between 10 and 30 mM because of the concomitant inhibition of GSK-3β signaling [85] (Fig. 2).

The antiproliferative and pro-apoptotic activity of LiCl was found also in other tumour cell lines such as prostate cancer [86,87], melanoma [88], ovarian cancer [89] and in follicular thyroid carcinoma [90]. The dysregulation of the Wnt/β-catenin pathway is involved in the tumorigenesis of thyroid carcinomas [91]. Lithium, as reported above, represents an activator of this pathway (Fig. 2) through the inhibition of GSK-3β [80]. In an *in vitro* study performed by Gilbert-Sirieix and collaborators, papillary thyroid carcinoma cells (i.e., TPC-1) were treated with LiCl at different concentrations (1, 5, 10, 20 mM) for 10 min, 24 h and 48 h. The authors demonstrated that the increase in Wnt/β-catenin expression was proportional to the LiCl concentration [92].

3.2. Preclinical studies of lithium carbonate on brain and hepatic cancer

Unlike the numerous LiCl preclinical studies, no comparable studies using lithium carbonate in different cancer models were conducted. A preclinical investigation performed by Furuta and colleagues studied the anticancer activity of cimetidine, lithium, olanzapine and valproic acid alone or in combination (referred to as CLOVA) on three human glioblastoma cell lines (i.e., T98, U87, U251). Of note, all the tested drugs inhibited GSK-3β activity, but CLOVA showed the greatest level of inhibition. In addition, lithium and valproate inhibited the invasion of the extracellular matrix and showed cell proliferation inhibition. In particular, proliferation of glioblastoma cells was suppressed by lithium concentrations ranging between 5 mM and 10 mM [93]. Interestingly, the CLOVA combination was tested also *in vivo*, in a mouse brain model of human glioblastoma. The daily oral administration of the CLOVA cocktail was performed for 2 weeks, and mice showed a significant reduction of diffusely infiltrating cells if compared to temozolomide, whereas the tumors resulted well demarcated from the surrounding tissue [93].

In 2020, Taskaeva and collaborators conducted a study to assess the antineoplastic activity of lithium carbonate on hepatocellular carcinoma cells (i.e., HCC-29). The experiments demonstrated that incubation with lithium 5 mM for 24 h and 48 h increased the accumulation of S-phase and G2/M-phase cells, respectively. Notably, also apoptotic cells increased after incubation with lithium carbonate and signs of autophagy, such as the expression of autophagy marker light chain 3β (LC3β), were found [94]. Interestingly, the same investigators designed an *in vivo* study in mice, xenotranplanting HCC-29 cells and administering 20 mM lithium carbonate every other day by an intramuscular injection. Lithium-treated mice caused an increase of the zones of destruction in the tumor mass if compared to control group of animals [95].

3.3. Clinical studies of lithium carbonate on cancer

Already in 1998 Cohen and colleagues had highlighted retrospectively a lower risk, although non-significant, of developing cancer in patients treated with lithium from 300 to 1800 mg with a modal dose of 900 mg for at least one year than in normal population. Moreover, this risk decreased significantly with the daily dose of lithium [96]. These data were corroborated in 2016 by Martinsson and co-workers who observed a significant reduction in the risk of developing cancer of the digestive tract, respiratory tract, and endocrine glands in patients with bipolar disorder treated with lithium [97]. In the same year, in a retrospective cohort study conducted by Huang et al., the significant protective role of lithium in a dose-dependent manner against the development of cancer was confirmed [98]. Moreover, Pottegard and collaborators examined 36,248 patients diagnosed with colorectal adenocarcinoma on the Danish cancer registry from 2000 to 2012 who were treated with at least 2 or more lithium prescriptions lasting 90 days each. Using a case-control design, the authors concluded that long-term use of lithium did not increas the risk of colorectal adenocarcinoma [99].

However, very few clinical trials have been performed with lithium carbonate on cancer patients and usually enrolling a very small number of subjects, limiting the conclusions on the results. As an example, seven adult patients with recurrent glioblastoma were enrolled in a single-center, single-arm, phase I/II study. Patients were treated with TMZ (200 mg/m^2/day) on the first 5 days of each 4-week cycle and CLOVA (cimetidine 800 mg, lithium 400 mg, olanzapine 10 mg, and valproate 800 mg) administered all days orally. Of note, patients achieved a median OS of 11.2 (95% CI, 3.8–18.6) months, significantly greater than the OS of 4.3 (95% CI, 2.5–6.1) months of the historical control group. The therapy was safe and well tolerated: grade 1/2 somnolence was the most common adverse event. A marked reduction in the size of recurrent tumor was obtained in one patient: this reduction, lasting 9 months, was evaluated as a partial response. Intriguingly, comparing the biopsy performed before the treatment and the autopsy sample, both the inhibition of GSK-3β, and the reduction of nestin and O^6-alkylguanine DNA alkyltransferase (MGMT) levels were found [93].

Another single-center phase I study enrolled 9 patients suffering of non-promyelocytic acute myeloid leukemia, unsuitable for standard intensive induction chemotherapy or with relapsed or refractory disease. Patients were treated with oral lithium carbonate 300 mg 2–3 times a day to reach the target lithium level of 0.6–1.0 mmol/L. All patients had disease progression, but four of them had a period of stable disease (constant circulating blasts) for a period $\geq$4 weeks. The median OS of patients was 106 days (range 60–502). The target lithium plasma level was reached at least once in each patient, but variability in lithium levels required dose adjustments. GSK-3β inhibition correlated positively with plasma lithium concentration and was increased from baseline by 1093. The therapeutic regimen was well tolerated: in fact, only one dose-limiting toxicity-DLT (delirium grade 3 according to National Cancer Institute Common Toxicity Criteria-CTCAE, version 4) was observed [100].

To our knowledge, just a single randomized study was performed with lithium carbonate on cancer patients. Yamazaki and colleagues enrolled 61 patients with low-grade differentiated thyroid carcinoma (DTC) treated by total thyroidectomy. Patients were randomized into two arms: one group received radioiodine therapy, whereas the other group radioiodine therapy (RAI) and an oral dose of 300 mg lithium carbonate every 8 h for 7 days. Noticeably, the lithium-supplemented group had a significantly higher response rate at the whole body scan ($p = 0.017$), improving the efficacy of thyroid remnant ablation [101]. Another cohort study was performed at the National Institute of Health (US) on DTC patients comparing the progression-free and overall survival between a group treated with radioactive iodine therapy and another group of subjects receiving 600 mg of lithium carbonate for 7 days before RAI. Interestingly, although the lithium-RAI group of patients revealed an improved overall survival in unadjusted models, no differences were found if adjustment by clinically relevant factors such as age and disease burden was made. Moreover, no differences were retrieved also in terms of progression-free survival in both group of patients, with no benefit from the adding of lithium carbonate to RAI

[102]. A lack of response after lithium administration was also found in a single-arm, open-label phase II clinical trial involving patients with low-grade neuroendocrine tumors (NETs). Indeed, in 15 patients, the administration of 300 mg lithium chloride orally 3 times daily for 28 days was ineffective at obtaining any radiographic response [103].

4. Conclusions and perspectives

Based on the preclinical and clinical data, valproate could represent a potential complementary therapeutic approach to enhance the anticancer activity of brain cancer standard chemotherapy [104]. In fact, the drug is effective both in preclinical models and in the clinical setting, although clinical data are still preliminary and further studies are strongly required. However, from this analysis it clearly emerges that patients under valproate treatment for other medical conditions, such as epilepsy, bipolar disorder, or migraine, should not suspend this drug if cancer is diagnosed. Furthermore, the use of valproate seems favourable in brain cancer patients with coexisting epilepsy. Adverse drug reactions associated with valproate are usually limited, if maintained in the safety range of concentrations [105] and compared with chemotherapeutic drugs. Indeed, valproate may be a good option for an effective combination for those all-oral chemotherapeutic schedules with low toxicities and multiple mechanisms of actions, such as metronomic chemotherapy [106,107], in different tumour types. Valproate may also represent a good choice for a sort of maintenance therapy because it is well-tolerated, it can be administered orally, it is an off-patent compound, and it has a low cost [108]. However, no data are available on the frequency of monitoring tests in cancer patients, and, above all, which plasma concentrations ranges are compatible with the anticancer activity. Finally, valproate may be used to treat and prevent seizures in brain cancer patients, although other drugs may be preferable for this issue such as levetiracetam [109].

The same advantageous characteristics are less convincing for lithium carbonate. Indeed, both preclinical and clinical studies are very few and the data, although promising in glioblastoma combined with other drugs (such as valproate), do not allow any conclusion on the anticancer activity of this formulation. Although LiCl demonstrated a reproducible activity in various tumor models, the experience with lithium carbonate is circumscribed only to glioblastoma and hepatocellular carcinoma. Furthermore, most of the clinical evidence comes from phase I clinical studies performed in a small number of refractory patients, limiting any conclusion on its actual anticancer effects in humans.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Gianfranco Natale: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Elisabetta Fini**: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Pasquale Fabio Calabrò**: Investigation, Writing – original draft. **Marco Carli**: Investigation, Writing – original draft. **Marco Scarselli**: Investigation, Writing – original draft. **Guido Bocci**: Conceptualization, Supervision, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] T.T. Ashburn, K.B. Thor, Drug repositioning: identifying and developing new uses for existing drugs, Nat. Rev. Drug Discov. 3 (2004) 673–683, https://doi.org/10.1038/NRD1468.
[2] R.G. Armando, D.L.M. Gómez, D.E. Gomez, New drugs are not enough-drug repositioning in oncology: an update, Int. J. Oncol. 56 (2020) 651–684, https://doi.org/10.3892/IJO.2020.4966/HTML.
[3] E. Wu, Discovering new anticancer activities from old drugs, Curr. Med. Chem. 20 (2013) 4093–4094, https://doi.org/10.2174/09298673113209990193.
[4] E. Heer, Y. Ruan, B. Mah, T. Nguyen, H. Lyons, A. Poirier, D.J. Boyne, D. E. O'Sullivan, S.J. Heitman, R.J. Hilsden, N. Forbes, D.R. Brenner, The efficacy of chemopreventive agents on the incidence of colorectal adenomas: a systematic review and network meta-analysis, Prev. Med. 162 (2022), 107169, https://doi.org/10.1016/j.ypmed.2022.107169.
[5] B.A. Costa, T.H. Mouhieddine, J. Richter, What's old is new: the past, present and future role of thalidomide in the modern-day management of multiple myeloma, Target. Oncol. 17 (2022) 383–405, https://doi.org/10.1007/S11523-022-00897-8.
[6] F.Y. Zaman, S.G. Orchard, A. Haydon, J.R. Zalcberg, Non-aspirin non-steroidal anti-inflammatory drugs in colorectal cancer: a review of clinical studies, Br. J. Cancer 127 (2022) 1735–1743, https://doi.org/10.1038/S41416-022-01882-8.
[7] M. Torralba, R. Farra, M. Maddaloni, M. Grassi, B. Dapas, G. Grassi, Drugs repurposing in high-grade serous ovarian cancer, Curr. Med. Chem. 27 (2020) 7222–7233, https://doi.org/10.2174/0929867327666200713190520.
[8] L. Huminiecki, Evidence for multilevel chemopreventive activities of natural phenols from functional genomic studies of curcumin, resveratrol, genistein, quercetin, and luteolin, Int. J. Mol. Sci. 23 (2022), 14957, https://doi.org/10.3390/IJMS232314957.
[9] F. You, C. Zhang, X. Liu, D. Ji, T. Zhang, R. Yu, S. Gao, Drug repositioning: using psychotropic drugs for the treatment of glioma, Cancer Lett. 527 (2022) 140–149, https://doi.org/10.1016/J.CANLET.2021.12.014.
[10] F. Cucchiara, F. Pasqualetti, F.S. Giorgi, R. Danesi, G. Bocci, Epileptogenesis and oncogenesis: an antineoplastic role for antiepileptic drugs in brain tumours? Pharmacol. Res. 156 (2020), 104786 https://doi.org/10.1016/J.PHRS.2020.104786.
[11] R.A. Blaheta, J. Cinatl, Anti-tumor mechanisms of valproate: a novel role for an old drug, Med. Res. Rev. 22 (2002) 492–511, https://doi.org/10.1002/med.10017.
[12] S.A. Fischkoff, E. Walter, Induction of neutrophilic differentiation of human promyelocytic leukemic cells by branched-chain carboxylic acid anticonvulsant drugs, J. Biol. Response Modif. 3 (1984) 132–137.
[13] C.M. Regan, Therapeutic levels of sodium valproate inhibit mitotic indices in cells of neural origin, Brain Res. 347 (1985) 394–398, https://doi.org/10.1016/0006-8993(85)90207-0.
[14] P.A. Slesinger, H.S. Singer, Effects of anticonvulsants on cell growth and enzymatic and receptor binding activity in a neuroblastoma x glioma hybrid cell culture, Epilepsia 28 (1987) 214–221, https://doi.org/10.1111/J.1528-1157.1987.TB04210.X.
[15] D. Singh, S. Gupta, I. Singh, M.A. Morsy, A.B. Nair, A.S.F. Ahmed, Hidden pharmacological activities of valproic acid: a new insight, Biomed. Pharmacother. 142 (2021), 112021, https://doi.org/10.1016/j.biopha.2021.112021.
[16] M.L. Martin, C.M. Regan, The anticonvulsant valproate teratogen restricts the glial cell cycle at a defined point in the mid-G1 phase, Brain Res. 554 (1991) 223–228, https://doi.org/10.1016/0006-8993(91)90193-Y.
[17] C.L. Bacon, H.C. Gallagher, J.C. Haughey, C.M. Regan, Antiproliferative action of valproate is associated with aberrant expression and nuclear translocation of cyclin D3 during the C6 glioma G1 phase, J. Neurochem. 83 (2002) 12–19, https://doi.org/10.1046/j.1471-4159.2002.01081.x.
[18] C.L. Bacon, E. O'Driscoll, C.M. Regan, Valproic acid suppresses G1 phase-dependent sialylation of a 65kDa glycoprotein in the C6 glioma cell cycle, Int. J. Dev. Neurosci. 15 (1997) 777–784, https://doi.org/10.1016/S0736-5748(97)00019-1.
[19] N.S. Gunel, N. Birden, C.C. Kurt, B.G. Bagca, B. Shademan, F. Sogutlu, N. P. Ozates, C.B. Avci, Effect of valproic acid on miRNAs affecting histone deacetylase in a model of anaplastic thyroid cancer, Mol. Biol. Rep. 48 (2021) 6085–6091, https://doi.org/10.1007/S11033-021-06616-2.
[20] M. Göttlicher, S. Minucci, P. Zhu, O.H. Krämer, A. Schimpf, S. Giavara, J. P. Sleeman, F. Lo Coco, C. Nervi, P.G. Pelicci, T. Heinzel, Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells, EMBO J. 20 (2001) 6969–6978, https://doi.org/10.1093/EMBOJ/20.24.6969.
[21] A. Kuendgen, N. Gattermann, Valproic acid for the treatment of myeloid malignancies, Cancer 110 (2007) 943–954, https://doi.org/10.1002/cncr.22891.
[22] T.V. Tittle, B.A. Schaumann, J.E. Rainey, K. Taylor, Segregation of the growth slowing effects of valproic acid from phenytoin and carbamazepine on lymphoid tumor cells, Life Sci. 50 (1992) L79–83, https://doi.org/10.1016/0024-3205(92)90104-W.
[23] T.V. Tittle, B.A. Schaumann, Effect of antiepileptic drugs on growth of murine lymphoid tumor cells in single-cell culture, Epilepsia 33 (1992) 729–735, https://doi.org/10.1111/J.1528-1157.1992.TB02354.X.
[24] M. Zapotocky, E. Mejstrikova, K. Smetana, J. Stary, J. Trka, J. Starkova, Valproic acid triggers differentiation and apoptosis in AML1/ETO-positive leukemic cells specifically, Cancer Lett. 319 (2012) 144–153, https://doi.org/10.1016/J.CANLET.2011.12.041.

[25] C. Schwartz, V. Palissot, N. Aouali, S. Wack, N. Brons, B. Leners, M. Bosseler, G. Berchem, Valproic acid induces non-apoptotic cell death mechanisms in multiple myeloma cell lines, Int. J. Oncol. 30 (2007) 573–582.
[26] A. Phillips, T. Bullock, N. Plant, Sodium valproate induces apoptosis in the rat hepatoma cell line, FaO, Toxicology 192 (2003) 219–227, https://doi.org/10.1016/S0300-483X(03)00331-7.
[27] P. Rithanya, D. Ezhilarasan, Sodium valproate, a histone deacetylase inhibitor, provokes reactive oxygen species-mediated cytotoxicity in human hepatocellular carcinoma cells, J. Gastrointest. Cancer 52 (2021) 138–144, https://doi.org/10.1007/S12029-020-00370-7.
[28] B. Pang, J. Zhang, X. Zhang, J. Yuan, Y. Shi, L. Qiao, Inhibition of lipogenesis and induction of apoptosis by valproic acid in prostate cancer cells via the C/EBPα/SREBP-1 pathway, Acta Biochim. Biophys. Sin. 53 (2021) 354–364, https://doi.org/10.1093/ABBS/GMAB002.
[29] J. Makarević, J. Rutz, E. Juengel, S. Maxeiner, J. Mani, S. Vallo, I. Tsaur, F. Roos, F.K.H. Chun, R.A. Blaheta, HDAC inhibition counteracts metastatic Re-activation of prostate cancer cells induced by chronic mTOR suppression, Cells 7 (2018) 129, https://doi.org/10.3390/CELLS7090129.
[30] A. Wawruszak, M. Halasa, E. Okon, W. Kukula-Koch, A. Stepulak, Valproic acid and breast cancer: state of the art in 2021, Cancers 13 (2021) 3409, https://doi.org/10.3390/CANCERS13143409.
[31] Z. Li, L. Yang, S. Zhang, J. Song, H. Sun, C. Shan, D. Wang, S. Liu, Valproic acid suppresses breast cancer cell growth through triggering pyruvate kinase M2 isoform mediated Warburg effect, 9636897211027524, Cell Transplant. 30 (2021), https://doi.org/10.1177/09636897211027524.
[32] M. Jahani, H. Khanahmad, P. Nikpour, Evaluation of the effects of valproic acid treatment on cell survival and epithelial-mesenchymal transition-related features of human gastric cancer cells, J. Gastrointest. Cancer 52 (2021) 676–681, https://doi.org/10.1007/S12029-019-00332-8.
[33] D.Y. Greenblatt, M.A. Cayo, J.T. Adler, L. Ning, M.R. Haymart, M. Kunnimalaiyaan, H. Chen, Valproic acid activates Notch1 signaling and induces apoptosis in medullary thyroid cancer cells, Ann. Surg. 247 (2008) 1036–1040, https://doi.org/10.1097/SLA.0B013E3181758D0E.
[34] H.K. Park, B.R. Han, W.H. Park, Combination of arsenic trioxide and valproic acid efficiently inhibits growth of lung cancer cells via G2/M-phase arrest and apoptotic cell death, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/IJMS21072649.
[35] H. Li, Z. Zhang, C. Gao, S. Wu, Q. Duan, H. Wu, C. Wang, Q. Shen, T. Yin, Combination chemotherapy of valproic acid (VPA) and gemcitabine regulates STAT3/Bmi1 pathway to differentially potentiate the motility of pancreatic cancer cells, Cell Biosci. 9 (2019), https://doi.org/10.1186/S13578-019-0312-0, 50.
[36] Y. Zhao, W. You, J. Zheng, Y. Chi, W. Tang, R. Du, Valproic acid inhibits the angiogenic potential of cervical cancer cells via HIF-1α/VEGF signals, Clin. Transl. Oncol. 18 (2016) 1123–1130, https://doi.org/10.1007/s12094-016-1494-0.
[37] S.H. Lee, H.J. Nam, H.J. Kang, T.L. Samuels, N. Johnston, Y.C. Lim, Valproic acid suppresses the self-renewal and proliferation of head and neck cancer stem cells, Oncol. Rep. 34 (2015) 2065–2071, https://doi.org/10.3892/OR.2015.4145.
[38] G. Natale, F. Cucchiara, G. Bocci, Historical overview of the "firing" liaison between brain tumors and epilepsy, Neuroscientist 28 (2022) 411–419, https://doi.org/10.1177/1073858421992316.
[39] A.F. Piotrowski, J. Blakeley, Clinical management of seizures in patients with low-grade glioma, Semin. Radiat. Oncol. 25 (2015) 219–224, https://doi.org/10.1016/J.SEMRADONC.2015.02.009.
[40] R.A. Blaheta, J. Cinatl, Anti-tumor mechanisms of valproate: a novel role for an old drug, Med. Res. Rev. 22 (2002) 492–511, https://doi.org/10.1002/MED.10017.
[41] S. Berendsen, E. Frijlink, J. Kroonen, W.G.M. Spliet, W. van Hecke, T. Seute, T. J. Snijders, P.A. Robe, Effects of valproic acid on histone deacetylase inhibition in vitro and in glioblastoma patient samples, Neuro-Oncology Adv. 1 (2019), https://doi.org/10.1093/NOAJNL/VDZ025.
[42] S. Ochiai, Y. Nomoto, Y. Yamashita, Y. Watanabe, Y. Toyomasu, T. Kawamura, A. Takada, N. Ii, S. Kobayashi, H. Sakuma, Roles of valproic acid in improving radiation therapy for glioblastoma: a review of literature focusing on clinical evidence, Asian Pac. J. Cancer Prev. 17 (2016) 463–466, https://doi.org/10.7314/APJCP.2016.17.2.463.
[43] M.E. De Bruin, P.B. Van Der Meer, L. Dirven, M.J.B. Taphoorn, J.A.F. Koekkoek, Efficacy of antiepileptic drugs in glioma patients with epilepsy: a systematic review, Neuro-Oncology Pract. 8 (2021) 501–517, https://doi.org/10.1093/NOP/NPAB030.
[44] W. Han, F. Yu, R. Wang, W. Guan, F. Zhi, Valproic acid sensitizes glioma cells to luteolin through induction of apoptosis and autophagy via Akt signaling, Cell. Mol. Neurobiol. 41 (2021) 1625–1634, https://doi.org/10.1007/S10571-020-00930-2.
[45] T. Fukumoto, S. Morinobu, Y. Okamoto, A. Kagaya, S. Yamawaki, Chronic lithium treatment increases the expression of brain-derived neurotrophic factor in the rat brain, Psychopharmacology (Berl) 158 (2001) 100–106, https://doi.org/10.1007/S002130100871.
[46] L.M.R. Castro, M. Gallant, L.P. Niles, Novel targets for valproic acid: up-regulation of melatonin receptors and neurotrophic factors in C6 glioma cells, J. Neurochem. 95 (2005) 1227–1236, https://doi.org/10.1111/J.1471-4159.2005.03457.X.
[47] S. Berendsen, M. Broekman, T. Seute, T. Snijders, C. Van Es, F. De Vos, L. Regli, P. Robe, Valproic acid for the treatment of malignant gliomas: review of the preclinical rationale and published clinical results, Expet Opin. Invest. Drugs 21 (2012) 1391–1415, https://doi.org/10.1517/13543784.2012.694425.
[48] W. Han, W. Guan, Valproic acid: a promising therapeutic agent in glioma treatment, Front. Oncol. 11 (2021), 687362, https://doi.org/10.3389/FONC.2021.687362.
[49] C. Zhang, S. Liu, X. Yuan, Z. Hu, H. Li, M. Wu, J. Yuan, Z. Zhao, J. Su, X. Wang, Y. Liao, Q. Liu, Valproic acid promotes human glioma U87 cells apoptosis and inhibits glycogen synthase kinase-3β through ERK/Akt signaling, Cell. Physiol. Biochem. 39 (2016) 2173–2185, https://doi.org/10.1159/000447912.
[50] W. Han, F. Yu, J. Cao, B. Dong, W. Guan, J. Shi, Valproic acid enhanced apoptosis by promoting autophagy via akt/mTOR signaling in glioma, Cell Transplant. 29 (2020) 1–10, https://doi.org/10.1177/0963689720981878.
[51] S. Osuka, S. Takano, S. Watanabe, E. Ishikawa, T. Yamamoto, A. Matsumura, Valproic acid inhibits angiogenesis in vitro and glioma angiogenesis in vivo in the brain, Neurol. Med.-Chir. 52 (2012) 186–193, https://doi.org/10.2176/nmc.52.186.
[52] Z.-Y. Yang, X.-H. Wang, Valproic acid inhibits glioma and its mechanisms, J. Healthc. Eng. 2022 (2022) 1–11, https://doi.org/10.1155/2022/4985781.
[53] Q.T. Ostrom, M. Price, K. Ryan, J. Edelson, C. Neff, G. Cioffi, K.A. Waite, C. Kruchko, J.S. Barnholtz-Sloan, CBTRUS statistical report: pediatric brain tumor foundation childhood and adolescent primary brain and other central nervous system tumors diagnosed in the United States in 2014-2018, Neuro Oncol. 24 (2022) iii1–iii38, https://doi.org/10.1093/NEUONC/NOAC161.
[54] B. Qiu, K.K. Matthay, Advancing therapy for neuroblastoma, Nat. Rev. Clin. Oncol. 19 (2022) 515–533, https://doi.org/10.1038/S41571-022-00643-Z.
[55] M. Michaelis, T. Suhan, J. Cinatl, P. Driever, J.J. Cinatl, Valproic acid and interferon-alpha synergistically inhibit neuroblastoma cell growth in vitro and in vivo, Int. J. Oncol. 25 (2004) 1795–1799.
[56] E. Fang, J. Wang, M. Hong, L. Zheng, Q. Tong, Valproic acid suppresses Warburg effect and tumor progression in neuroblastoma, Biochem. Biophys. Res. Commun. 508 (2019) 9–16, https://doi.org/10.1016/J.BBRC.2018.11.103.
[57] H.Y.C. Chiou, W.K. Lai, L.C. Huang, S.M. Huang, S.H. Chueh, H.I. Ma, D. Y. Hueng, Valproic acid promotes radiosensitization in meningioma stem-like cells, Oncotarget 6 (2015) 9959–9969, https://doi.org/10.18632/ONCOTARGET.3692.
[58] M.T. Corsetti, F. Salvi, S. Perticone, A. Baraldi, L. De Paoli, S. Gatto, D. Pietrasanta, M. Pini, V. Primon, F. Zallio, A. Tonso, M.G. Alvaro, G. Ciravegna, A. Levis, Hematologic improvement and response in elderly AML/RAEB patients treated with valproic acid and low-dose Ara-C, Leuk. Res. 35 (2011) 991–997, https://doi.org/10.1016/J.LEUKRES.2011.02.021.
[59] A. Duenas-Gonzalez, M. Candelaria, C. Perez-Plascencia, E. Perez-Cardenas, E. de la Cruz-Hernandez, L.A. Herrera, Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors, Cancer Treat Rev. 34 (2008) 206–222, https://doi.org/10.1016/J.CTRV.2007.11.003.
[60] M. Weller, T. Gorlia, J.G. Cairncross, M.J. Van Den Bent, W. Mason, K. Belanger, A.A. Brandes, U. Bogdahn, D.R. Macdonald, P. Forsyth, A.O. Rossetti, D. Lacombe, R.O. Mirimanoff, C.J. Vecht, R. Stupp, Prolonged survival with valproic acid use in the EORTC/NCIC temozolomide trial for glioblastoma, Neurology 77 (2011) 1156–1164, https://doi.org/10.1212/WNL.0B013E31822F02E1.
[61] M. Kerkhof, J.C.M. Dielemans, M.S. Van Breemen, H. Zwinkels, R. Walchenbach, M.J. Taphoorn, C.J. Vecht, Effect of valproic acid on seizure control and on survival in patients with glioblastoma multiforme, Neuro Oncol. 15 (2013) 961–967, https://doi.org/10.1093/NEUONC/NOT057.
[62] J.P. Reddy, S. Dawood, M. Mitchell, B.G. Debeb, E. Bloom, A.M. Gonzalez-Angulo, E.P. Sulman, T.A. Buchholz, W.A. Woodward, Antiepileptic drug use improves overall survival in breast cancer patients with brain metastases in the setting of whole brain radiotherapy, Radiother. Oncol. 117 (2015) 308–314, https://doi.org/10.1016/J.RADONC.2015.10.009.
[63] B. Cacho-Diaz, D. San-Juan, K. Salmeron, C. Boyzo, N. Lorenzana-Mendoza, Choice of antiepileptic drugs affects the outcome in cancer patients with seizures, Clin. Transl. Oncol. 20 (2018) 1571–1576, https://doi.org/10.1007/S12094-018-1892-6.
[64] C.A. Barker, A.J. Bishop, M. Chang, K. Beal, T.A. Chan, Valproic acid use during radiation therapy for glioblastoma associated with improved survival, Int. J. Radiat. Oncol. Biol. Phys. 86 (2013) 504–509, https://doi.org/10.1016/J.IJROBP.2013.02.012.
[65] A.V. Krauze, S.D. Myrehaug, M.G. Chang, D.J. Holdford, S. Smith, J. Shih, P. J. Tofilon, H.A. Fine, K. Camphausen, A phase 2 study of concurrent radiation therapy, temozolomide, and the histone deacetylase inhibitor valproic acid for patients with glioblastoma, Int. J. Radiat. Oncol. Biol. Phys. 92 (2015) 986–992, https://doi.org/10.1016/J.IJROBP.2015.04.038.
[66] S. Watanabe, Y. Kuwabara, S. Suehiro, D. Yamashita, M. Tanaka, A. Tanaka, S. Ohue, H. Araki, Valproic acid reduces hair loss and improves survival in patients receiving temozolomide-based radiation therapy for high-grade glioma, Eur. J. Clin. Pharmacol. 73 (2017) 357–363, https://doi.org/10.1007/S00228-016-2167-1.
[67] Y.J. Kuo, Y.H. Yang, I.Y. Lee, P.C. Chen, J.T. Yang, T.C. Wang, M.H.C. Lin, W. H. Yang, C.Y. Cheng, K.T. Chen, W.C. Huang, M.H. Lee, Effect of valproic acid on overall survival in patients with high-grade gliomas undergoing temozolomide: a nationwide population-based cohort study in Taiwan, Medicine (Baltim.) 99 (2020), e21147, https://doi.org/10.1097/MD.0000000000021147.
[68] C. Kresbach, A. Bronsema, H. Guerreiro, S. Rutkowski, U. Schüller, B. Winkler, Long-term survival of an adolescent glioblastoma patient under treatment with vinblastine and valproic acid illustrates importance of methylation profiling,

Childs. Nerv. Syst. 38 (2022) 479–483, https://doi.org/10.1007/S00381-021-05278-6.
[69] C. Happold, T. Gorlia, O. Chinot, M.R. Gilbert, L.B. Nabors, W. Wick, S.L. Pugh, M. Hegi, T. Cloughesy, P. Roth, D.A. Reardon, J.R. Perry, M.P. Mehta, R. Stupp, M. Weller, Does valproic acid or levetiracetam improve survival in glioblastoma? A pooled analysis of prospective clinical trials in newly diagnosed glioblastoma, J. Clin. Oncol. 34 (2016) 731–739, https://doi.org/10.1200/JCO.2015.63.6563.
[70] J.M.F. Su, J.C. Murray, R.Y. McNall-Knapp, D.C. Bowers, S. Shah, A.M. Adesina, A.C. Paulino, E. Jo, Q. Mo, P.A. Baxter, S.M. Blaney, A phase 2 study of valproic acid and radiation, followed by maintenance valproic acid and bevacizumab in children with newly diagnosed diffuse intrinsic pontine glioma or high-grade glioma, Pediatr. Blood Cancer 67 (2020), e28283, https://doi.org/10.1002/PBC.28283.
[71] F. Cucchiara, S. Ferraro, G. Luci, G. Bocci, Relevant pharmacological interactions between alkylating agents and antiepileptic drugs: preclinical and clinical data, Pharmacol. Res. 175 (2022) 105976, https://doi.org/10.1016/J.PHRS.2021.105976.
[72] S. Oberndorfer, M. Piribauer, C. Marosi, H. Lahrmann, P. Hitzenberger, W. Grisold, P450 enzyme inducing and non-enzyme inducing antiepileptics in glioblastoma patients treated with standard chemotherapy, J. Neuro Oncol. 72 (2005) 255–260, https://doi.org/10.1007/S11060-004-2338-2.
[73] C.J. Vecht, G.L. Wagner, E.B. Wilms, Interactions between antiepileptic and chemotherapeutic drugs, Lancet Neurol. 2 (2003) 404–409, https://doi.org/10.1016/S1474-4422(03)00435-6.
[74] V. Bourg, C. Lebrun, R.M. Chichmanian, P. Thomas, M. Frenay, Nitroso-urea-cisplatin-based chemotherapy associated with valproate: increase of haematologic toxicity, Ann. Oncol. 12 (2001) 217–219, https://doi.org/10.1023/A:1008331708395.
[75] S. Avissar, D.L. Murphy, G. Schreiber, Magnesium reversal of lithium inhibition of beta-adrenergic and muscarinic receptor coupling to G proteins, Biochem. Pharmacol. 41 (1991) 171–175, https://doi.org/10.1016/0006-2952(91)90473-I.
[76] N. Singh, A.C. Halliday, J.M. Thomas, O. Kuznetsova, R. Baldwin, E.C.Y. Woon, P. K. Aley, I. Antoniadou, T. Sharp, S.R. Vasudevan, G.C. Churchill, A safe lithium mimetic for bipolar disorder, Nat. Commun. 4 (2013) 1332, https://doi.org/10.1038/NCOMMS2320.
[77] W.J. Ryves, A.J. Harwood, Lithium inhibits glycogen synthase kinase-3 by competition for magnesium, Biochem. Biophys. Res. Commun. 280 (2001) 720–725, https://doi.org/10.1006/BBRC.2000.4169.
[78] E. Beurel, S.F. Grieco, R.S. Jope, Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases, Pharmacol. Ther. 148 (2015) 114–131, https://doi.org/10.1016/J.PHARMTHERA.2014.11.016.
[79] Y. Xia, S. Wu, Tissue inhibitor of metalloproteinase 2 inhibits activation of the β-catenin signaling in melanoma cells, Cell Cycle 14 (2015) 1666, https://doi.org/10.1080/15384101.2015.1030557.
[80] S. Thakur, A. Tobey, J. Klubo-Gwiezdzinska, The role of lithium in management of endocrine tumors—a comprehensive review, Front. Oncol. 9 (2019) 1092, https://doi.org/10.3389/fonc.2019.01092.
[81] J.A. Mccubrey, L.S. Steelman, F.E. Bertrand, N.M. Davis, M. Sokolosky, S. L. Abrams, G. Montalto, A.B. D'Assoro, M. Libra, F. Nicoletti, R. Maestro, J. Basecke, D. Rakus, A. Gizak, Z. Demidenko, L. Cocco, A.M. Martelli, M. Cervello, GSK-3 as potential target for therapeutic irvention in cancer, Oncotarget 5 (2014) 2881–2911, https://doi.org/10.18632/oncotarget.2037.
[82] D.J. Duffy, A. Krstic, T. Schwarzl, D.G. Higgins, W. Kolch, GSK3 inhibitors regulate MYCN mRNA levels and reduce neuroblastoma cell viability through multiple mechanisms, including p53 and Wnt signaling, Mol. Cancer Therapeut. 13 (2014) 454–467, https://doi.org/10.1158/1535-7163.MCT-13-0560-T/85400/AM/GSK3-INHIBITORS-REGULATE-MYCN-MRNA-LEVELS-AND.
[83] L. Li, H. Song, L. Zhong, R. Yang, X.Q. Yang, K.L. Jiang, B.Z. Liu, Lithium chloride promotes apoptosis in human leukemia NB4 cells by inhibiting glycogen synthase kinase-3 beta, Int. J. Med. Sci. 12 (2015) 805, https://doi.org/10.7150/IJMS.12429.
[84] F. Zassadowski, K. Pokorna, N. Ferre, F. Guidez, L. Llopis, O. Chourbagi, M. Chopin, J. Poupon, P. Fenaux, R. Ann Padua, M. Pla, C. Chomienne, B. Cassinat, Lithium chloride antileukemic activity in acute promyelocytic leukemia is GSK-3 and MEK/ERK dependent, Leukemia 29 (2015) 2277–2284, https://doi.org/10.1038/LEU.2015.159.
[85] M. Kunnimalaiyaan, A.M. Vaccaro, M.A. Ndiaye, H. Chen, Inactivation of glycogen synthase kinase-3β, a downstream target of the raf-1 pathway, is associated with growth suppression in medullary thyroid cancer cells, Mol. Cancer Therapeut. 6 (2007) 1151–1158, https://doi.org/10.1158/1535-7163.MCT-06-0665.
[86] A. Sun, I. Shanmugam, J. Song, P.F. Terranova, J.B. Thrasher, B. Li, Lithium suppresses cell proliferation by interrupting E2F–DNA interaction and subsequently reducing S–phase gene expression in prostate cancer, Prostate 67 (2007) 976–988, https://doi.org/10.1002/PROS.20586.
[87] Q. Zhu, J. Yang, S. Han, J. Liu, J. Holzbeierlein, J.B. Thrasher, B. Li, Suppression of glycogen synthase kinase 3 activity reduces tumor growth of prostate cancer in vivo, Prostate 71 (2011) 835–845, https://doi.org/10.1002/PROS.21300.
[88] Q. Zhang, Q. Zhang, H. Li, X. Zhao, H. Zhang, LiCl induces apoptosis via CHOP/NOXA/Mcl-1 axis in human choroidal melanoma cells, Cancer Cell Int. 21 (2021) 96, doi:10.1186/S12935-021-01778-2.
[89] Q. Cao, X. Lu, Y.J. Feng, Glycogen synthase kinase-3β positively regulates the proliferation of human ovarian cancer cells, Cell Res. 16 (2006) 671–677, https://doi.org/10.1038/sj.cr.7310078, 2006 167.
[90] C.P. Camacho, F.R.M. Latini, G. Oler, F.C. Hojaij, R.M.B. MacIel, G.J. Riggins, J. M. Cerutti, Down-regulation of NR4A1 in follicular thyroid carcinomas is restored following lithium treatment, Clin. Endocrinol. 70 (2009) 475–483, https://doi.org/10.1111/j.1365-2265.2008.03349.x.
[91] C. García-Jiménez, P. Santisteban, TSH signalling and cancer, Arq. Bras. Endocrinol. Metabol. 51 (2007) 654–671.
[92] M. Gilbert-Sirieix, J. Makoukji, S. Kimura, M. Talbot, B. Caillou, C. Massaad, L. Massaad-Massade, Wnt/β-Catenin signaling pathway is a direct enhancer of thyroid transcription Factor-1 in human papillary thyroid carcinoma cells, PLoS One 6 (2011), e22280, https://doi.org/10.1371/journal.pone.0022280.
[93] T. Furuta, H. Sabit, Y. Dong, K. Miyashita, M. Kinoshita, N. Uchiyama, Y. Hayashi, Y. Hayashi, T. Minamoto, M. Nakada, Biological basis and clinical study of glycogen synthase kinase- 3β-targeted therapy by drug repositioning for glioblastoma, Oncotarget 8 (2017), 22811, https://doi.org/10.18632/ONCOTARGET.15206.
[94] Y.S. Taskaeva, N.P. Bgatova, R.S. Dossymbekova, A.O. Solovieva, S. M. Miroshnichenko, K.O. Sharipov, Z.B. Tungushbaeva, In vitro effects of lithium carbonate on cell cycle, apoptosis, and autophagy in hepatocellular carcinoma-29 cells, Bull. Exp. Biol. Med. 170 (2020) 246–250, https://doi.org/10.1007/S10517-020-05044-9, 2020 1702.
[95] Y.S. Taskaeva, N.P. Bgatova, Cytological characteristics of a heterogeneous population of hepatocellular carcinoma-29 cells after injection of lithium carbonate in the experiment, Bull. Exp. Biol. Med. 167 (2019) 779–783, https://doi.org/10.1007/s10517-019-04621-x.
[96] Y. Cohen, A. Chetrit, Y. Cohen, P. Sirota, B. Modan, Cancer morbidity in psychiatric patients: influence of lithium carbonate treatment, Med. Oncol. 15 (1998) 32–36, https://doi.org/10.1007/BF02787342.
[97] L. Martinsson, J. Westman, J. Hällgren, U. Ösby, L. Backlund, Lithium treatment and cancer incidence in bipolar disorder, Bipolar Disord. 18 (2016) 33–40, https://doi.org/10.1111/BDI.12361.
[98] R.-Y. Huang, K.-P. Hsieh, W.-W. Huang, Y.-H. Yang, Use of lithium and cancer risk in patients with bipolar disorder: population-based cohort study, Br. J. Psychiatry 209 (2016) 393–399, https://doi.org/10.1192/BJP.BP.116.181362.
[99] A. Pottegard, Z.N. Ennis, J. Hallas, B.L. Jensen, K. Madsen, S. Friis, Long-term use of lithium and risk of colorectal adenocarcinoma: a nationwide case–control study, Br. J. Cancer 114 (2016) 571–575, https://doi.org/10.1038/bjc.2016.10, 2016 1145.
[100] M. Ueda, T. Stefan, L. Stetson, J.J. Ignatz-Hoover, B. Tomlinson, R.J. Creger, B. Cooper, H.M. Lazarus, M. de Lima, D.N. Wald, P.F. Caimi, Phase I trial of lithium and tretinoin for treatment of relapsed and refractory non-promyelocytic acute myeloid leukemia, Front. Oncol. 10 (2020) 327, https://doi.org/10.3389/FONC.2020.00327/FULL.
[101] C.A. Yamazaki, R.P. Padovani, R.P.M. Biscolla, E.S. Ikejiri, R.R. Marchetti, M.L. V. Castiglioni, L.K. Matsumura, R.M.D.B. MacIel, R.P. Furlanetto, Lithium as an adjuvant in the postoperative ablation of remnant tissue in low-risk thyroid carcinoma, Thyroid 22 (2012) 1002–1006, https://doi.org/10.1089/thy.2011.0372.
[102] H. Luo, A. Tobey, S. Auh, C. Cochran, M. Zemskova, J. Reynolds, C. Lima, K. Burman, L. Wartofsky, M. Skarulis, E. Kebebew, J. Klubo-Gwiezdzinska, The effect of lithium on the progression-free and overall survival in patients with metastatic differentiated thyroid cancer undergoing radioactive iodine therapy, Clin. Endocrinol. 89 (2018) 481–488, https://doi.org/10.1111/CEN.13806.
[103] S.J. Lubner, M. Kunnimalaiyaan, K.D. Holen, L. Ning, M. Ndiaye, N.K. LoConte, D. L. Mulkerin, W.R. Schelman, H. Chen, A preclinical and clinical study of lithium in low-grade neuroendocrine tumors, Oncol. 16 (2011) 452–457, https://doi.org/10.1634/THEONCOLOGIST.2010-0323.
[104] T. Mezei, D. Mészáros, P. Pollner, A.G. Bagó, I. Fedorcsák, P. Banczerowski, L. Sipos, Supplementary valproate therapy for glioma patients: an alternative opportunity to enhance the efficiency of radio-chemotherapy, Orv. Hetil. 162 (2021) 960–967, https://doi.org/10.1556/650.2021.32110.
[105] D. Bentué-Ferrer, O. Tribut, M.-C. Verdier, Pour le groupe Suivi Thérapeutique Pharmacologique de la Société Française de Pharmacologie et de Thérapeutique, Therapeutic drug monitoring of valproate, Therapie 65 (2010) 233–240, https://doi.org/10.2515/therapie/2010029.
[106] G. Natale, G. Bocci, Does metronomic chemotherapy induce tumor angiogenic dormancy? A review of available preclinical and clinical data, Cancer Lett. 432 (2018) 28–37, https://doi.org/10.1016/J.CANLET.2018.06.002.
[107] T. Di Desidero, P. Xu, S. Man, G. Bocci, R.S. Kerbel, Potent efficacy of metronomic topotecan and pazopanib combination therapy in preclinical models of primary or

late stage metastatic triple-negative breast cancer, Oncotarget 6 (2015) 42396–42410, https://doi.org/10.18632/ONCOTARGET.6377.

[108] A. Marson, G. Burnside, R. Appleton, D. Smith, J.P. Leach, G. Sills, C. Tudur-Smith, C. Plumpton, D.A. Hughes, P. Williamson, G.A. Baker, S. Balabanova, C. Taylor, R. Brown, D. Hindley, S. Howell, M. Maguire, R. Mohanraj, P.E. Smith, K. Lanyon, M. Manford, M. Chitre, A. Parker, N. Swiderska, J. Pauling, A. Hughes, R. Gupta, S. Hanif, M. Awadh, S. Ragunathan, N. Cable, P. Cooper, D. Hindley, K. Rakshi, S. Molloy, M. Reuber, K. Ayonrinde, M. Wilson, S. Saladi, J. Gibb, L. A. Funston, D. Cassidy, J. Boyd, M. Ratnayaka, H. Faza, M. Sadler, H. Al-Moasseb, C. Galtrey, D. Wren, A. Olabi, G. Fuller, M. Khan, C. Kallappa, R. Chinthapalli, B. Aji, R. Davies, K. Foster, N. Hitiris, N. Hussain, S. Dowson, J. Ellison, B. Sharrack, V. Gandhi, R. Powell, P. Tittensor, B. Summers, S. Shashikiran, P. J. Dison, S. Samarasekera, D. McCorry, K. White, K. Nithi, M. Richardson, R. Page, D. Deekollu, S. Slaght, S. Warriner, M. Ahmed, A. Chaudhuri, G. Chow, J. Artal, D. Kucinskiene, H. Sreenivasa, S. Velmurugan, C.S. Zipitis, B. McLean, V. Lal, A. Gregoriou, P. Maddison, T. Pickersgill, J. Anderson, C. Lawthom, G. Whitlingum, W. Rakowicz, L. Kinton, A. McLellan, N. Vora, S. Zuberi, A. Kelso, I. Hughes, J. Martland, H. Emsley, C. de Goede, R.P. Singh, C.C. Moor, J. Aram, K. Sakthivel, S. Nelapatla, C. Rittey, A. Pinto, H. Cock, A. Richardson, E. Houston, C. Cooper, G. Lawson, A. Massarano, C. Burness, U. Wieshmann, I. Dey, P. Sivakumar, L.K. Yeung, P. Smith, H. Bentur, T. Heafield, A. Mathew, D. Smith, P. Jauhari, The SANAD II study of the effectiveness and cost-effectiveness of valproate versus levetiracetam for newly diagnosed generalised and unclassifiable epilepsy: an open-label, non-inferiority, multicentre, phase 4, randomised controlled trial, Lancet (London, England) 397 (2021) 1375–1386, https://doi.org/10.1016/S0140-6736(21)00246-4.

[109] F. Cucchiara, G. Luci, N. Giannini, F.S. Giorgi, P. Orlandi, M. Banchi, A. Di Paolo, F. Pasqualetti, R. Danesi, G. Bocci, Association of plasma levetiracetam concentration, MGMT methylation and sex with survival of chemoradiotherapy-treated glioblastoma patients, Pharmacol. Res. 181 (2022), 106290, https://doi.org/10.1016/j.phrs.2022.106290.