CHAPTER TWO

Targeting amino acid metabolism in cancer

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Abstract

Metabolic rewiring is a characteristic hallmark of cancer cells. This phenomenon sustains uncontrolled proliferation and resistance to apoptosis by increasing nutrients and energy supply. However, reprogramming comes together with vulnerabilities that

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can be used against tumor and can be applied in targeted therapy. In the last years, the genetic background of tumors has been identified thoroughly and new therapies targeting those mutations tested. Nevertheless, we propose that targeting the phenotype of cancer cells could be another way of treatment aiming to avoid drug resistance and non-responsiveness of cancer patients. Amino acid metabolism is part of the altered processes in cancer cells. Amino acids are building blocks and also sensors of signaling pathways regulating main biological processes. In this comprehensive review, we described four amino acids (asparagine, arginine, methionine, and cysteine) which have been actively investigated as potential targets for anti-tumor therapy. Asparagine depletion is successfully used for decades in the treatment of acute lymphoblastic leukemia and there is a strong implication to apply it to other types of tumors. Arginine auxotrophic tumors are great candidates for arginine-starvation therapy. Higher requirement for essential amino acids such as methionine and cysteine point out promising targetable weaknesses of cancer cells.

1. Introduction

One of the druggable targets which have currently receiving increased attention in the field of translational research is cancer metabolism (Hanahan and Weinberg, 2011). Altered cellular metabolism, a cancer hallmark, is one of the key mechanisms that underlie tumorigenesis, tumor progression and chemoresistance (Boroughs and Deberardinis, 2015; Cocetta et al., 2020). Otto Warburg almost a century ago described increased glucose uptake and induced glycolysis in malignant cells, a metabolic alteration which is now considered a general feature of tumor cells (Warburg, 1956). Aerobic glycolysis was regarded as a privileged energetic pathway despite its lower energy production in comparison to oxidative phosphorylation (OXPHOS), the bioenergetic process generally utilized in normal cells. Nevertheless, it is now evident that cancer cells utilize other bioenergetic pathways besides glycolysis. This altered metabolism is critical feature of enhanced adaptive ability (Cordier-Bussat et al., 2018; Starkova et al., 2018). Metabolic plasticity participates in tumorigenesis, tumor progression and helps cancer cells survive and overcome limited conditions, *e.g.,* anti-tumor treatment, developing resistance often leading to recurrence of the disease (Hermanova et al., 2016; Hlozkova et al., 2022; Mostazo et al., 2020).

Herein, we describe metabolism of four amino acids: asparagine, arginine, methionine and cysteine. Due to the different requirements of cancer cells and abnormalities in biosynthetic processes these amino acids gained new characteristics and became conditionally essential. Native and (or) recombinant enzymes depleting these amino acids in cancer cells represent great potential for anti-tumor therapy.

2. Asparagine

Asparagine is a polar amino acid that is, generally, non-essential in humans. It is used for synthesis and N-glycosylation of proteins (Chiu et al., 2020; Larkin and Imperiali, 2011). It also plays a role in nucleotide biosynthesis and ammonium metabolism (Zhu et al., 2017). Recently, it was shown that asparagine plays a role as an amino acid exchange factor, meaning that intracellular asparagine levels regulate uptake of serine, arginine and histidine (Krall et al., 2016). It is also slowly coming into light that asparagine could become essential when glutamine is limited (Pavlova et al., 2018) and that asparagine biosynthesis is fundamental for tumor mitochondrial respiration (Krall et al., 2021). All of the above highlight the importance of asparagine metabolism in both health and disease.

2.1 Asparagine synthetase (ASNS)

2.1.1 Catalytic function and structure of ASNS

Asparagine synthetase (asparagine synthase (glutamine-hydrolysing) or glutamine-dependent asparagine synthetase, E.C. 6.3.5.4) catalyzes the synthesis of asparagine and glutamate from aspartate and glutamine in an ATP-dependent amidotransferase reaction (Fig. 1) (Chiu et al., 2020; Lomelino et al., 2017). *ASNS* gene is located at chromosome 7q21.3 and is 35 kb long with 13 exons. ASNS enzyme consists of 561 amino acid residues with molecular mass of 65 kDa (Lomelino et al., 2017). Two putative truncated ASNS isoforms with no known function are listed in UniProt database. Some prokaryotes express two forms of ASNS characterized by their source of nitrogen donor, either ammonia (AS-A) or glutamine (AS-B)(Richards and Kilberg, 2006). Mammalian cells express only the form that utilizes glutamine as the nitrogen donor (Lomelino et al., 2017). Overall, the aspartic acid is transformed to asparagine *via* three separate reactions that requires magnesium ions and ATP. The transformation begins when the aspartate carboxyl is activated by an ATP-dependent process, forming a β-aspartyl-AMP intermediate. Then, glutamine deamidation releases ammonia. Finally, released ammonia performs a nucleophilic attack on the aspartyl intermediate and asparagine is produced *via* transition state. Glutamate is the second product of the ASNS-catalyzed reaction (Boehlein et al., 1994; Larsen et al., 1999; Lomelino et al., 2017; Tesson et al., 2003). It was also shown that both the β-aspartyl-AMP intermediate and the transition state are tightly bound by the enzyme duringv catalysis (Zhu et al., 2019).

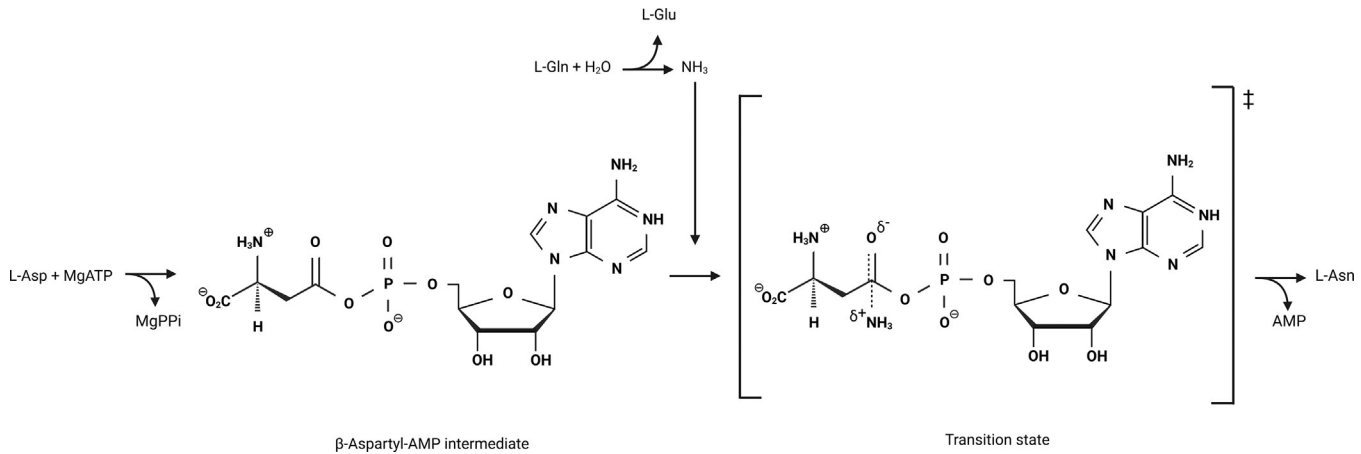

Fig. 1 Catalytic mechanism of human asparagine synthetase.

The crystal structure of *E. coli* AS-B (PDB 1CT9) has been solved for quite a long time (Larsen et al., 1999) in contrast with human ASNS whose crystal structure has not been published until recently (Zhu et al., 2019). Therefore, originally, structural features of human ASNS were based on homology modeling with *E. coli* AS-B structure as a template because they share substantial sequence identity (Lomelino et al., 2017). Human ASNS structure revealed that, indeed, the enzyme is composed of two distinct domains, each containing a separate catalytic site (W. Zhu et al., 2019). C-terminal (residues 203–560) synthetase domain is more conserved than the N-terminal one (residues 1–202). The N-terminal active site catalyzes glutamine hydrolysis to yield glutamate and ammonia and possesses the typical sandwich-like α/β/β/α topology that is observed in the N-terminal amidohydrolase enzyme superfamily (Brannigan et al., 1995; Zhu et al., 2019). Substrate-binding pocket, which mediates substrate recognition and thioester stabilization in the hydrolysis reaction that produces ammonia, is located at the interface of the two domains and is within 5 Å of an absolutely conserved glutamate residue (Glu-414) in the C-terminal domain. Interestingly, there is probably a chloride ion surrounded by Tyr-78, Arg-416, Arg-245, and Val-417 (W. Zhu et al., 2019). The function (if any) of this ion in human ASNS is not yet known but plant ASNSes are known to be activated by chloride (Rognes, 1980).

2.1.2 ASNS deficiency

ASNS deficiency is relatively newly described neurometabolic disorder (Ruzzo et al., 2013). It manifests with intellectual disability, microcephaly, severe developmental delay, intractable seizures, progressive brain atrophy or respiratory deficiency (Gupta et al., 2017;Lomelino et al., 2017;Ruzzo et al., 2013). Currently, ASNS deficiency can only be diagnosed by DNA sequencing. It is because only some of the patients have a measurable decrease of asparagine concentration in serum and/or cerebrospinal fluid, which limits this analysis as a preliminary screen (Lomelino et al., 2017). The disease is caused by homozygous or compound heterozygous mutations in *ASNS* gene. Up to day, the exact mechanism causing the symptoms are not well characterized (N. Gupta et al., 2017). However, performed studies indicate that asparagine synthesis is essential for the development and function of the brain but not for that of other organs (Ruzzo et al., 2013). Whether this dependence is the direct result of asparagine depletion or it is caused by perturbation of one of the other ASNS reactants has not yet been established (Lomelino et al., 2017; Ruzzo et al., 2013).

2.1.3 ASNS regulation

Many times, it has been documented that ASNS is at the center of the cell response to various forms of cellular stress (Chiu et al., 2020). Transcriptionally, *ASNS* gene is regulated *via* two signaling pathways designed to improve cell survival under imbalanced AA availability (= AA response (AAR)) and in the case of increased endoplasmic reticulum stress (= Unfolded Protein Response (UPR)) (Kilberg et al., 2009, 2012). Among the AAR pathway, GCN2-eIF2-ATF4 appears to be the predominant signaling mechanism that activates transcription from the *ASNS* gene during the AAR (Balasubramanian et al., 2013). UPR pathway works *via* PERK-eIF2-ATF4 mechanism (Chiu et al., 2020). Both of the pathways rely on activation of kinases (GCN2 or PERK) which then phosphorylate α-subunit of eIF2. These results in attenuation of global protein synthesis and, at the same time, enhanced translation of select mRNA species, such as transcription factor ATF4. One of ATF4 target genes is *GADD34*, which leads PP1 (protein phosphatase-1) to phosphorylated eIF2 and returns the translation factor to its dephosphorylated state and thus promote the global translation. ATF4-responsive enhancer element, the CARE sequence, lies in the *ASNS* promoter and serves as a target of ATF4-induced transcription of *ASNS* during either AAR or UPR (Balasubramanian et al., 2013; Chiu et al., 2020). Pathria et al. have recently shown that in asparagine-deprived cancer cells, *ASNS* expression is enhanced through translational reprogramming dependent on MNK1 (MAPK-interacting kinase 1) and eIF4E (Pathria et al., 2019). *ASNS* expression is also affected by p53. Interestingly, WT p53 down-regulates the *ASNS* transcription while mutant p53-D281G up-regulates it (Stagliano et al., 2003).

2.2 L-asparaginase (ASNase)

Bacterial L-asparaginases, enzymes that catalyze the hydrolysis of asparagine to aspartate, have been used for over 50 years as therapeutic agents in the treatment of childhood acute lymphoblastic leukemia (ALL) (Aghaiypour et al., 2001). ASNase is closely related to ASNS mainly because ALL cells are known to have low *ASNS* expression. In general, *ASNS* expression is high in pancreatic cells, brain, testes and thyroid cells and low in kidney tissue.

ASNase can be found in many organisms including bacteria, yeasts, plants, birds and mammals, except for human. Mostly, ASNase is a tetramer but can also be found in a form of a hexamer, dimer or monomer.

Therapeutically used ASNases are mainly those from bacteria *Escherichia coli* and *Dickeya dadantii* (previously known as *Erwinia chrysanthemi* or *D. chrysanthemi*), these ASNases have the same 3D structure (Fig. 2) (Lubkowski et al., 2003).

D. dadanti ASNase (ErA) has a tetramer structure. In more detail, it is a dimer of dimers where each dimer consists of two identical monomers. Each monomer has two domains - bigger N-terminal and smaller C-terminal domain (Aghaiypour et al., 2001). Four active sites of ErA are located between the N-terminal and C-terminal domains of two adjacent monomers. The flexible part of the active site, referred to as flexible loop, covers the binding pocket upon substrate binding to the enzyme (Aghaiypour et al., 2001). It has been described that ASNase uses two-step ping-pong mechanism similar to mechanism of serine proteases (Röhm and Van Etten, 1986). Therefore, ASNase-catalyzed enzymatic reaction is a double-displacement mechanism with two nucleophilic attacks. First, threonine

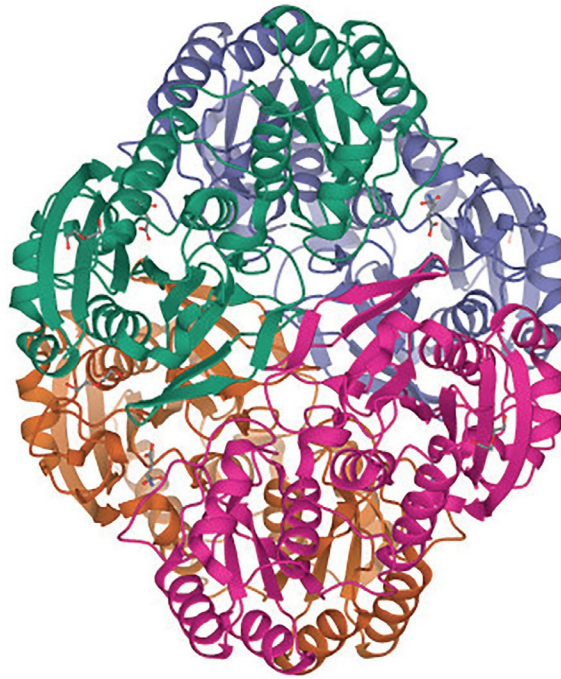

Fig. 2 3D structure of *D. chrysanthemi* ASNase homo-tetramer. Image from the RCSB PDB ID 5F52 (Nguyen et al., 2016).

residue attacks the carboxamide carbon of L-asparagine with subsequent release of ammonia. Because ammonia escapes the active site, this catalytic step is irreversible. The second nucleophilic substitution, which is reversible, is performed by a water molecule and leads to the release of the second product, L-Asp (Lubkowski et al., 2003). ErA can also use D-asparagine, L-glutamine and succinate-NH_2 as substrates. K_M (V_{max}) values for L-asparagine, D-asparagine, L-glutamine and succinate-NH_2 by this enzyme are 0.01 mM (0.923 μM/min/unit), 62 (0.177), 1.1 (0.087) and 15 (0.24), respectively (Howard and Carpenter, 1972).

Gram-negative bacteria *E. coli* synthetize two ASNase isoenzymes - cytosolic, type I ASNase (EcAI) and periplasmatic, type II ASNase (EcAII, EcA) (Campbell et al., 1967). However, EcAII has higher affinity for substrates and shows anti-tumor activity (in contrast with EcAI) (Campbell et al., 1967; Whitecar et al., 1970). Different affinity for substrates is caused by flexibility of the active site flexible loop. When the loop is stabilized by hydrogen bonds, both asparaginase and glutaminase activity of the enzyme are increased (Offman et al., 2011). Furthermore, there are Ser and Glu residue at the active site of EcAII. Glu residue is negatively charged, thus attracting α-amino group of the substrate. On the other hand, EcAI active site contains residues of glutamine and asparagine (Aghaiypour et al., 2001). EcAII has an affinity for the same four substrates as ErA, however, L-glutaminase activity of EcAII is much lower than for ErA (Röhm and Van Etten, 1986).

2.2.1 ASNase in leukemias and lymphomas

ASNases were discovered 50 years ago to have anti-tumor properties and are particularly effective against ALL (Zhang et al., 1995). ASNase therapy targets the leukemia or lymphoma cells, which usually have low *ASNS* expression or do not even express *ASNS* at all. It is probably caused by hypermethylation of CpG islands in *ASNS* promoter (Ding et al., 2005). These low-ASNS cells cannot synthesize enough amount of Asn that is needed for proliferation and basal functions, and are, therefore, auxotrophic for asparagine. Leukemia cells after ASNase treatment inhibit mTOR pathway, thus inhibit proteosynthesis, synthesis of nucleic acids, induce G1-phase cell cycle arrest, activate autophagy and, at the end, apoptosis. ASNase also causes metabolic reprogramming of leukemia cells, specifically, ASNase inhibits glucose uptake and increases FAO and maximal respiration (Hermanova et al., 2016; Leslie et al., 2006; Yu et al., 2012).

ASNase has been used in the clinics since 1970' and it is now the most abundantly used cytostatic enzyme in the clinical oncology. It is routinely used during leukemia and some type of non-Hodgkin lymphoma treatment. More specifically, it is a cornerstone of childhood ALL treatment (Pieters et al., 2011). ASNase can be administered by intramuscular injection or by intravenous infusion. Since ASNase does not cross the blood-brain barrier, it can also be administered intrathecally (Capizzi et al., 1970). In Europe, ASNase is usually administered intravenously whereas in the USA the prevalent administration method is intramuscularly. During ALL-BFM 2000 Protocol, the activity of ErA administered either intravenously or intramuscularly was monitored. It was found out that ErA injected intramuscularly had higher activity compared to ErA administered intravenously (Schrey et al., 2010).

For ASNase treatment, different enzymes can be used. As the frontline treatment, EcA is used. However, when allergic reactions occur, EcA is switched to ErA. The incidence of side effects of ErA is lower than these of EcA, specifically regarding neurotoxicity, pancreatitis and sepsis. However, ErA activity is lower than that of EcA. The chances of anaphylactic shock are similar when comparing EcA and ErA (Duval et al., 2002). *In vivo* half-time of EcA is 1.24 days. That is more than twice longer than the half-time of ErA which is 0.6 days. Hence, ErA needs to be administered more frequently (Müller and Boos, 1998). Because of hypersensitivity reactions, polyethylenglycol-conjugated EcA has become available. During PEGylation, units of monomethoxy PEG are attached to EcA by covalent bonds. PEG-EcA is more stable than native EcA, hence, *in vivo* half-life is longer. PEG-EcA is soluble in polar solvents and the organism produce less antibodies against PEG-EcA compared to native EcA. PEG-EcA has higher anti-tumor activity than the native form of the enzyme (Müller and Boos, 1998). Half-life of PEG-EcA is 5.73 days, in contrast with 1.24 days in the case of native EcA. The difference is 4.49 days. Higher activity and longer half-life allow to decrease the frequency of PEG-EcA administration, therefore, the risk of side effects is lower. In Europe, the first-choice ASNase is native EcA. Only when patient has developed an immune reaction, PEG-EcA or ErA is used. Unfortunately, up to one third of the patients allergic to EcA are also allergic to ErA (Billett et al., 1992; Vrooman et al., 2010). In the USA, the first-choice drug is PEG-ASNase. However, PEG-ASNase can also cause hypersensitivity, the organism starts to produce antibodies against PEG and, therefore, PEG-EcA is inactivated and eliminated.

Sometimes even without observable allergic reactions (Armstrong et al., 2007; van der Sluis et al., 2016). Cross-reactivity of antibodies against PEG-EcA and native EcA can occur. Furthermore, PEGylation has been also used to improve ErA. PEG-ErA has longer half-life than native ErA (Rau et al., 2018; Torres-Obreque et al., 2019). Like in the case of native forms of ASNase, intramuscular administration, compared to intravenous infusion, increases the half-life of PEGylated ASNases. However, it is more painful, the onset is slower and there is quite a big chance of skin hypersensitivity development (Douer et al., 2007).

2.2.2 ASNase in solid tumors

In general, solid tumors express *ASNS* and are able to synthesize asparagine. However, some types of tumors express *ASNS* but its activity is lower, *e.g.*, ovarian cancer (Krall et al., 2016; Purwaha et al., 2014; Story et al., 1993). Unfortunately, although the sensitivity of ovarian cancer cell lines to ASNase has been previously demonstrated by preclinical findings, the clinical trial using PEG-EcA had to be prematurely terminated after excessive toxicity identified in patients (Hays et al., 2013). The use of ASNase in solid tumor treatment is not yet well described and established; nevertheless, new insights are coming into light. *Salmonella typhimurium* was engineered to express *E. coli* ASNase within tumor tissues. Antitumor efficacy of the engineered bacteria was then demonstrated *in vivo* in colon carcinoma, mammary carcinoma and pancreatic tumor. In addition to ASNase efficacy, this result also showed the merit of bacteria as cancer drug delivery vehicles (Kim et al., 2015). Sun *et al.* demonstrated that SLC1A3 plays a role in ASNase resistance in solid tumors (Sun et al., 2019). *ASNS* KO melanoma cells are sensitive to ASNase *in vitro*. However, the same cells are able to rewire multiple pathways *in vivo* to sustain tumor growth. To corroborate this story, clinical trials in melanoma showed only a little efficacy against profound Asn decrease (Apfel et al., 2021).

2.2.3 Glutaminase activity of ASNase

In clinically used ASNases, their glutaminase activity (compared to asparaginase activity) is around 5% (Wriston Jr and Yellin, 1973). However, some ASNases have their glutaminase activity higher than asparaginase one. It seems that glutaminase activity of ASNase significantly helps during tumor treatment. Some tumors have lower glutamine synthetase (*GS*) expression or even do not express *GS* at all, for example ovarian cancer, oligodendroglioma or multiple myeloma. Surprisingly, glutaminase

activity of ASNase also helps with the treatment of the tumors with *GS* expression (Bolzoni et al., 2016; Chiu et al., 2018; Furusawa et al., 2018). Some studies also suggest that glutaminase activity of ASNase is necessary for its success in ALL treatment (Offman et al., 2011; Panosyan et al., 2004).

To better understand the role of glutaminase activity of ASNase, ASNases with different glutaminase activities were developed. Enzymes without glutaminase activity were still effective against tumor cells with no *ASNS* expression (Parmentier et al., 2015). Offman *et al.* showed, that ALL cells without *ASNS* expression were more sensitive to high-glutaminase-activity ASNase than low-glutaminase-activity ASNase (Offman et al., 2011). Assuming that tumor cells with no *ASNS* expression do not use glutamine to produce asparagine but to supplement anaplerosis and therefore to proliferate, glutaminase activity is perquisite. Regarding the cells with *ASNS* expression, glutaminase activity of ASNase depletes extracellular glutamine that is used as an amido-group donor for asparagine and other metabolites synthesis (Ratnikov et al., 2015). Without extracellular glutamine, these cells undergo apoptosis (Krall et al., 2016). There is also a direct positive correlation between cytotoxicity and glutaminase activity of ASNase in *ASNS*-expressing tumor cells (Chan et al., 2014; Parmentier et al., 2015). Considering glutamine as an important amino acid in the organism, its depletion could be a problem. Concerns about metabolic damage caused by glutamine depletion as a result of ASNase therapy have led to an idea of glutamine supplementation. However, although some studies describes the advantage of glutamine supplementation during ALL therapy and chemotherapy (Sands et al., 2017; Tanaka et al., 2016; Vicentini et al., 2016), others do not see the glutamine supplementation beneficial (Moe-Byrne et al., 2012; Tao et al., 2014).

2.2.4 Limits of the ASNase therapy

Although glutaminase activity of ASNase can be an important part of the therapy, it also has some negative aspects. In phase I clinical trial where ASNase with balanced glutaminase activity (sometimes called glutaminase-asparaginase) was used in adult oncology patients, a large quantity of side effects was observed, including hyperglycemia, respiratory alkalosis, nausea, vomiting, fever or chills. Moreover, this therapy also led to asterixis, lethargy and disorientation. These symptoms indicate that CNS could be affected (Warrell et al., 1980). Therefore, phase II has never even started. ErA and EcA have their glutaminase activity in only a few percent units. Nevertheless, both of them can cause many side effects, *e.g.*, hypersensitivity,

anaphylactic reactions, pancreatitis, thrombosis, increased bleeding, stomach ache, fever, infections, hyperglycemia and sepsis (Avramis et al., 2002; Haskell et al., 1969; Oettgen et al., 1970; Plourde et al., 2014; Vrooman et al., 2016). Coagulation problems are caused by affecting the proteosynthesis (Nowak-Göttl et al., 2001). Overall, ASNase treatment is more effective in childhood oncology patient compared to the adult ones. It is because the number of side effects correlates with the age of the patient (Stock et al., 2011).

ASNase with lowered glutaminase activity has started to be engineered (W. K. Chan et al., 2014; Reinert et al., 2006). ErA glutaminase activity can be reduced by amino acid change in the active site of the enzyme. When one of two amino acid residues in the flexible loop is changed to the residue of glutamine, α-amino group of glutamine is strongly repelled by hydrogen bond donors. Asparagine is smaller and it can, therefore, dodge these hydrogen bond donors. Hence, affinity to glutamine is lowered (Nguyen et al., 2016). Mutant ASNase without any glutaminase activity has been produced. This enzyme was effective against tumor cells with *ASNS* expression (Chan et al., 2014). In addition to mutant enzymes, ASNase from other sources than *E. coli* and *D. dadantii* can be used. However, alternative-source ASNases usually have quite low asparaginase activity. Unfortunately, when asparaginase activity is engineered to be higher, glutaminase activity also increases (Sudhir et al., 2016). Nevertheless, alternative-source ASNases could be used after immune reactions against EcA and/or ErA. The alternative sources can be fungi (L. Huang et al., 2014; Mohan Kumar and Manonmani, 2013; Vala et al., 2018), plants (Liu et al., 2019), yeasts (Darvishi et al., 2019) or other bacteria (El-Naggar et al., 2016; Reinert et al., 2006). Because of possible allergic reactions to alternative-source ASNases, human-like ASNase (part human, part guinea pig) has been engineered (Rigouin et al., 2017). Any of the alternative-source ASNase has not yet been tested in clinical trial.

Lower effectivity of ASNase or even resistance against it can be caused by cooperation of tumor cells with adipocytes (gain of glutamine) or mesenchymal stromal cells (gain of asparagine) (Ehsanipour et al., 2013; Iwamoto et al., 2007; Parmentier et al., 2015). Hence, ASNase effectivity also depends on the amount of fat tissue of the patient. This phenomenon was demonstrated in mice where ASNase therapy was not effective in obese individuals. This normal tissue support can lead to insufficient eradication of leukemia cells and consecutive relaps of the disease (Williams, 2007).

Interestingly, ASNase can also be encapsulated into erythrocytes (Kravtzoff et al., 1996; Updike et al., 1976). Asparagine and glutamine

are actively transported through cytoplasmatic membrane of the erythrocytes towards encapsulated ASNase where they are degraded to aspartate and glutamate, respectively. In this case, ASNase is a part of an erythrocyte, therefore it is not attacked by the immune system and half-life of this encapsulated ASNase depends on the viability of an erythrocyte, that is 120 days. Usually, half-life of the encapsulated ASNase is around one month, which is significantly longer than ErA (0.6 days), EcA (1.24 days) or even PEG-EcA (5.73 days) (Halfon-Domenech et al., 2011; Müller and Boos, 1998). ASNase encapsulated in erythrocytes (Eryaspase) was tested in clinical trials for the patients with adenocarcinoma of the pancreas (with low *ASNS* expression) and with ALL. Eryaspase caused fewer coagulation problems and fewer allergic reactions compared to non-encapsulated variants of the enzyme (Bachet et al., 2015; Halfon-Domenech et al., 2011; Hammel et al., 2020; Hunault-Berger et al., 2015).

3. Arginine

Arginine is a semi-essential aminoacid since in normal physiological conditions its endogenous synthesis is sufficient. However during embryogenesis and catabolic stress the organism is not able to synthesize necessary concentrations and arginine is therefore supplemented from a diet. Arginine is an integral component of urea, nitric oxide, citrulline, ornithine, proline, glutamine, creatinine, agmatine and polyamines (Morris, 2007).

3.1 Metabolism of arginine

Arginine is synthetized in a majority of the cells in the two step reaction from citrulline by arginine succinate synthase 1 (ASS1) and argininosuccinate lyase (ASL). The central organs where arginine is synthetized are liver and kidney.

During catabolism of amino acids ammonium ions are produced which are toxic already in a very small concentrations for human organism. Ammonia is therefore carried into the cells of blood plasma in the form of glutamine or alanine and subsequently transported into the liver. Ammonia from glutamine or alanine is cleaved in enterocytes. Carbamoyl phosphate synthetase 1 (CPS1) synthetizes carbamoyl phosphate from ammonia and bicarbonate ions in the matrix of liver mitochondria. Binding of carbamoyl phosphate to ornithine produces citrulline which is catalyzed by ornithine transcarbamoylase (OTC). Citrulline is then transported into the cytoplasm where it reacts with aspartate through ASS1 and arginine succinate is produced. In the next step,

fumarate is cleaved from arginine succinate through ASL reaction and by that arginine is produced. By hydrolytic cleavage of arginine by arginase 1 (ARG1) urea is released and produced ornithine is transported back to matrix of mitochondria by translocase ORNT1. By ornithine being recycled, the whole process can be repeated. This reaction is called urea cycle and is ongoing only in liver (Fig. 3). Renal cortex is the place with highest concentrations of ASL and synthesis of arginine. Citrulline is originated mostly from intestine from which it is transported to liver and transformed to arginine by ASS1 and ASL enzymes. Arginine is then transported by blood stream to distinct parts of body (Crenn et al., 2008).

3.2 Arginine-nitric oxide pathway

Biosynthesis of nitric oxide (NO), a short-lived gas, is dependent on presence of arginine which serves as a starting material in enzymatic reaction of

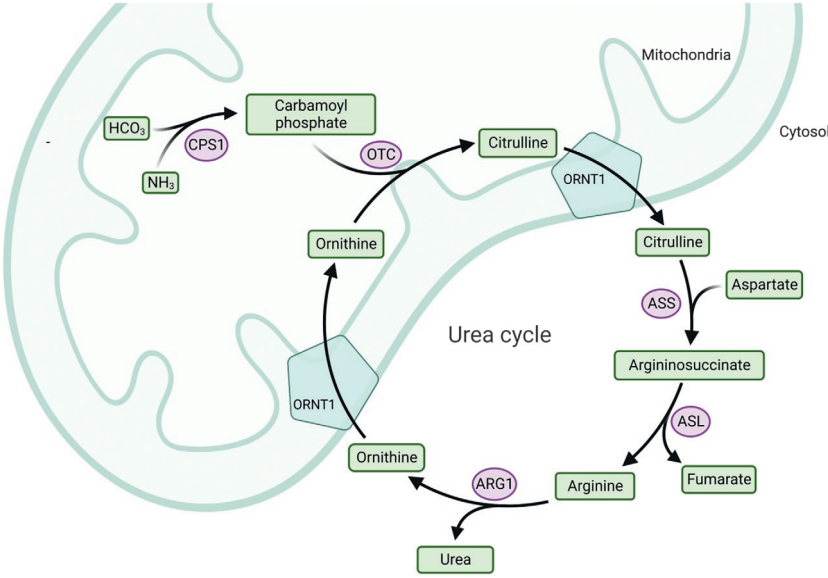

Fig. 3 Urea cycle. The mitochondrial enzyme CPS1 synthesizes carbamoyl phosphate from HCO_3 and NH_3. To form citrulline, carbamoyl phosphate is attached to ornithine by the enzyme OTC. Citrulline is transported from the mitochondria to the cytosol *via* ORNT1. In the cytosol, the enzyme ASS links citrulline to aspartate. Product of ASS reaction is argininosuccinate, which is cleaved into arginine and fumarate by the enzyme ASL in the following reaction. The enzyme ARG1 cleaves urea from arginine to produce ornithine, and ornithine is transported into the mitochondrial matrix by ORNT1; thereby cycle is complete.

nitric oxide synthase (NOS). Together with nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen they produce NO and citrulline in all cell types. Higher concentrations of NO causing toxicity of cells can be prevented by ARG1 reaction which catalyzes arginine to urea and ornithine.

NO is a signaling molecule participating in various physiological processes. However, in case of imbalanced NO synthesis it takes part in formation of pathophysiological events including cancer development. Expression of *NOS* has been detected in various cancers indicating that NO levels contribute to cancer processes (Glynn et al., 2010; Klotz et al., 1998; Zhang et al., 2014). Expression of *NOS* was even shown to predict prognosis of cancer patients (Glynn et al., 2010). Nevertheless, recent studies show that the effect of NO in cancer is an interplay of its activities which can be both, pro- and anti-tumorigenic (Wang et al., 2003; Mocellin et al., 2007). Example can be found in activation of apoptosis. In low concentrations NO prevents cell from apoptosis which can favor cancer progression while in higher concentrations it activates pro-apoptotic activities and by that acts as anti-tumor molecule (Lind, 2004; Oronsky et al., 2014; Ridnour et al., 2008). Another important role of NO which can support cancer growth is supplying the tumor with nutrients by inducing angiogenesis (Jadeski et al., 2000; Vakkala et al., 2000).

3.3 Arginine-ornithine-polyamine pathway

Polyamines is a family of molecules (*i.e.,* putrescine, spermine, spermidine) derived from arginine-ornithine-polyamine axis catalyzed by ARG. Polyamines are essential for the growth and proper function of normal cells which is securely regulated. Multiple aberrations in the control of polyamines metabolism might lead to pathological processes (Moinard et al., 2005). The critical role of polyamines in cell growth has led to the development of a number of agents that interfere with polyamine metabolism.

Example how polyamines derived from arginine-ornithine axis can participate in progression of cancer growth is described in paper of Miska *et al.* Glioblastoma is characterized by the robust infiltration of immunosuppressive tumor-associated myeloid cells. The authors showed that despite acidic environment of tumor, active *de novo* synthesis of highly basic polyamines in myeloid cells maintained normal intracellular pH and cell survival and thus promoted immunosuppression during tumor evolution (Miska et al., 2021). Another study presented how mutation KRAS-G12D in patients with ALL reprogrammed arginine and methionine metabolism

and by that supported anabolism of polyamines (Xu et al., 2022). In triple-negative breast cancer (TNBC) which lacks effective targeted therapy the reduction of rate-limiting polyamine biosynthetic enzyme, ornithine decarboxylase ODC, was found to be a suitable targetable vulnerability. Difluoromethylornithine, an ODC inhibitor, sensitized TNBC cells to chemotherapy in a subtype specific manner (Geck et al., 2020).

3.4 Suitable candidates for arginine-depleting enzyme therapies

ASS1 catalyzes synthesis of argininossuccinate from aspartate and citrulline. In some cancers such as hepatocellular carcinoma, ovarian cancer, mesothelioma and acute myeloid leukemia (AML) expression of *ASS1* gene is decreased (Dillon et al., 2004; Gupta et al., 2018; Miraki-Moud et al., 2015; Nicholson et al., 2009; Szlosarek et al., 2006; Tan et al., 2014). Hypermethylation of *ASS1* promoter was determined in myxofibrosarcoma which is also substantially dependent on arginine supplementation from extracellular environment (Huang et al., 2013). These observations led to investigations of experimental treatment which would deplete arginine from blood stream in order to starve the tumors of this amino acid. Existence of five enzymes catabolizing arginine was established; ARG, NOS, arginine: glycine amidinotransferase (AGAT), arginine decarboxylase (ADC), and arginine deiminase (ADI). However, ADC is not suitable for therapy since the reaction of agmatine is not reversible and it is toxic for tissues. Therapeutic effect is studied so far in ARG and ADI.

3.5 Arginine deiminase

Arginine deiminase is an enzyme produced by some eukaryotes and bacteria. For therapy purposes ADI is isolated from *Mycoplasma arginini* (Ensor et al., 2002; Takaku et al., 1992, 1995). ADI catalases reaction from which citrulline and ammonia is produced. First, the enzyme binds arginine by polar interaction, after that nucleophilic attack is performed on Cζ arginine, following cleavage of ammonia and thus arginine is transformed to citrulline (Das et al., 2004). ADI is the first enzyme of so called ADI pathway. The next reaction is catalyzed by OTC which transforms citrulline to ornithine and carbamoyl phosphate. In the last step of ADI pathway carbamoyl phosphate is metabolized to ammonia and carbon dioxide by carbamate kinase which also phosphorylates ADP to ATP.

3.5.1 Arginine deiminase-based therapy

The best target for arginine-deprivation therapy are arginine auxotrophic tumors. There are several types of cancers which have typically low levels of urea cycle enzymes such as colorectal carcinoma, hepatocellular carcinoma, ovarian cancer, breast cancer or glioblastoma. ADI as a therapeutical strategy was tested in all those cancers both *in vitro* and *in vivo*, also in clinical trials as monotherapy or in combination with standard chemotherapeutics.

Recent implementation of PEGylated form of ADI (ADI-PEG20) was developed to moderate immune responses and prolong biological half-life. After *in vitro* incubation of cells with ADI-PEG20, both normal and AML cells increased *ASS1* expression, indicating the effort of both cell types to adapt to an arginine-deficient environment. By increasing the expression of *ASS1*, the tumor cells achieved a certain degree of resistance, but compared to tumor growth with sufficient arginine, tumor growth in arginine deficiency was noticeably slower (Miraki-Moud et al., 2015).

Treatment using ADI-PEG20 has passed I. and II. phase of clinical testing (Izzo et al., 2004; Tsai et al., 2017) and was also tested in III. phase of clinical testing on patients with hepatocellular carcinoma. In patients with advanced hepatocellular carcinoma who did not respond to previous treatment, administration of ADI-PEG20 did not prolong overall survival, but it was increased in other patients (Abou-Alfa et al., 2018).

ADI-PEG20 is suitable for use with other cytostatic agents, since when ADI-PEG20 is administered alone, tumor cell resistance to treatment may occur due to restoration of *ASS1* expression or activation of a pathway causing resistance to apoptosis (Tsai et al., 2009).

Phase I trial of ADI-PEG20 and liposomal doxorubicin in patients with metastatic solid tumors was completed. Preclinical data demonstrated that depletion of arginine by ADI-PEG20 enhanced liposomal doxorubicin cytotoxicity in cancer cells with ASS1 deficiency (Yao et al., 2022).

SpyADI (ADI isolated from *Streptococcus pyogenes*) was also administered to patient-derived glioblastoma multiforme models *in vitro* and *in vivo* and their susceptibility was confirmed (Fiedler et al., 2015; Schwarz et al., 2022). Moreover, they monitored the combination of cyclin-dependent kinases (CDK) inhibition and ADI. CDKi/SpyADI combination yielded synergistic antitumoral effects. Study highlights the strong antitumoral potential of a combined arginine deprivation and CDK inhibition approach *via* effects on mitochondrial malfunction, invasiveness as well as DNA-damage response (Riess et al., 2021).

Arginine-degrading enzymes were effective anticancer drugs also in colorectal cancers (CRCs) which exhibit low expression of OTC and, in some cases, ASS1 proteins. CRC cells failed to grow in arginine-free medium and dietary arginine deprivation slowed growth of cancer cells in immunocompromised mice (Alexandrou et al., 2018).

In vascular endothelial cells of several models, including human umbilical vein cells, ADI has been found to have anti-angiogenic effects, which may be due to its ability to degrade arginine, a precursor of pro-angiogenic NO, or to an as-yet-unidentified pathway (Beloussow et al., 2002; Park et al., 2003).

The success of arginine deiminase therapy is attributed precisely to its effect on NO production, ADI therefore remains a great hope for cancer diseases such as breast cancer, which is dependent on its ability to induce angiogenesis.

3.5.2 Limitations of arginine deiminase therapy

From the enzymes used in various therapies, the forms obtained from human sources are the best tolerated. Since ADI is derived from bacteria, it is more likely that its presence in the body will trigger an unwanted immune response when used. The level of enzyme concentration in the plasma is therefore balanced between the concentration, when sufficiently unfavorable conditions for tumor cells are induced, and the concentration, which is not yet toxic for other cells of the body. Another limit of ADI therapy may be the biological half-life, which is 4 h, and affects the effectiveness of the treatment (Holtsberg et al., 2002). In a recent study, erythrocyte-encapsulated ADI was used in mouse models with various ASS1-deficient tumor cell lines, and no hypersensitivity was observed (Gay et al., 2016).

3.6 Arginase

The enzyme arginase (ARG) belongs to the enzymes of the urea cycle. ARG ensures the hydrolytic cleavage of urea from arginine to form ornithine. It seems that ARG is originally a bacterial enzyme, which began to be expressed in eukaryotes only after the emergence of mitochondria (Dzik, 2014). Most eukaryotes express only *arginase 2 (ARG2)*, which is found in mitochondria (Morris et al., 1997; Samson, 2000). Some eukaryotes that use urea as a means of excreting excess ammonia also express a cytosolic isoform, *ARG1*, which is found mainly in the kidney (Kepka-Lenhart et al., 2000). The isoforms have a number of common properties: they consist of three identical subunits, although the subunits differ slightly between the isoforms; they have the same reaction mechanism, for which a cofactor

in the form of manganese cations is important; and both require the same starting substances and generate the same products (Jenkinson et al., 1996; Kepka-Lenhart et al., 2000; Morris et al., 1997; Stone et al., 2010a). An increase in ARG1 activity results in excessive arginine depletion and reduced NOS function (Kepka-Lenhart et al., 2000). Conversely, reduced ARG1 activity leads to structural changes in the vasculature, and thus to increased tumor growth (Caldwell et al., 2018).

3.6.1 Arginase therapy

Another promising arginine-depleting enzyme is ARG1. Using PEGylated recombinant human ARG1 *in vitro*, it was found to inhibit the proliferation of tumor cells deficient in ASS1 or OTC synthesis (Ensor et al., 2002). The advantages of arginase therapy lie mainly in its human origin, but even then it struggled with a not very long half-life and low catalytic activity. In order to increase the catalytic activity, a variant was developed that uses cobalt cations instead of manganese cations as a cofactor (Stone et al., 2010b). The modified enzyme in higher doses caused bone marrow necrosis leading to death in mouse models, which limited its potential clinical use (Agrawal et al., 2012; Mauldin et al., 2012). Further research will thus focus on balancing the effectiveness and toxicity of this variant of the enzyme.

Several phase I/II clinical trials with arginase or its pegylated form is being launched in arginine auxotrophic solid tumors such as melanoma, prostate, liver cancer (De Santo et al., 2018; Chan et al., 2021). Also Phase I trial of pegylated recombinant arginase (PEG-BCT-100) in combination with systemic chemotherapy in advanced hepatocellular carcinoma was completed with promising results (Yau et al., 2022). Results showed anti-tumor activity with survival advantage for patients with advanced hepatocellular carcinoma.

3.6.2 Limitations of arginase therapy

One of the main limits of arginase therapy is resistance development. The short-term resistance of tumor cells to arginase therapy is characterized by the activation of the autophagy process, when the cells try to replace the missing arginine from their own sources. However, this process is not sustainable in the long term, and after the depletion of intracellular stores, the process of apoptosis is triggered (Lin et al., 2015; Zeng et al., 2013). Cancer cells can resist the lack of arginine in the plasma even in the long term. They take missing nutrients, including amino acids, from stromal cells, which can cope with their deficiency (Kwong-Lam and Chi-Fung, 2013).

The ability of tumor cells to obtain missing nutrients from the stroma is essential for their dynamic growth, but reduces the effectiveness of therapy. This problem can be solved by administering ARG with another cytotoxic agent.

4. Methionine

Methionine is one of the few amino acids that contains sulfur. It is one of the essential proteinogenic amino acids. Humans cannot synthesize it *de novo*, thus they must obtaine it from diet or from microbes living in their gut. Methionine is a precursor for succinyl-CoA, S-adenosylmethionine (SAM), cysteine, glutathione (GSH) and polyamines such as spermine and spermidine. SAM is a major methylation agent- the methyl group is transferred from SAM to DNA, RNA and proteins including histones, and thus methionine, as a precursor of SAM, has an effect on genome stability and epigenetic processes. The antioxidant GSH helps to protect cells from damage caused by reactive oxygen species (ROS), with particularly severe consequences arising from DNA damage. Methionine thus also plays a role in maintaining redox balance. Last but not least, it is involved in proteosynthesis and serves as a sulfur donor. Even from this brief list of functions of methionine, its importance for the proper functioning of cellular processes is obvious.

4.1 Methionine metabolism

In the middle of methionine metabolism is the methionine cycle. In the first of the four reactions of the cycle, SAM, the methyl group donor, is synthesized from methionine and ATP by methionine adenosyltransferase 2A (MAT2A). In the second reaction, methyltransferase (MT) removes the methyl group from SAM to form S-adenosylhomocysteine (SAH). The third reaction is catalyzed by adenosylhomocysteinase (AHCY), here SAH is converted to homocysteine. The last reaction is catalyzed by the enzyme methionine synthase (MS), whose corrector is vitamin B12. The methyl group of 5-methyltetrahydrofolate (5-MTHF) is transferred to homocysteine, which leads to the formation of methionine and folate. This reaction is the reason why the methionine cycle is linked to the folate cycle. In addition to MS, there is another enzyme that can synthesize methionine from homocysteine, betaine homocysteine methyltransferase (BHMT), which is expressed in the kidney cortex and liver (Sunden et al., 1997). BHMT transfers the methyl group of betaine to homocysteine.

Beside folate cycle, the methionine salvage cycle and transsulfuration pathway are also connected to the methionine cycle. The methionine

salvage cycle allows methionine to be produced again from the SAM formed in the first reaction of the methionine cycle. The methionine salvage pathway is important for the synthesis of polyamines and purines. The transsulfuration pathway involves only two reactions. The starting substance is homocysteine and the product is cysteine, which is a precursor of GSH (more in the paragraph about cysteine).

4.2 The Hoffman effect

The fact that cancer cells are dependent on methionine supply from the exogenous environment was observed more than 60 years ago (Sugimura et al., 1959). The dependence of cancer cells on methionine was intensively investigated in the following two decades; cancer and non-cancer cells were cultured in medium that was free of methionine but contained its precursor homocysteine; normal cells were not significantly affected, while almost no cell lines of various types of cancer were able to proliferate under these conditions (Halpern et al., 1974; Hoffman and Erbe, 1976; Mecham et al., 1983; Stern et al., 1984). Interestingly, when methionine is deficient, the cell cycle of cancer cells stops in the late S/G2 phase (Hoffman and Jacobsen, 1980; Stern and Hoffman, 1986; Yano et al., 2014). A hypothesis of deficiency or malfunction of the MS enzyme has suggest itself as an answer to the question of why cancer cells were unable to proliferate in that methionine free medium; however, this hypothesis has not been confirmed (Ashe et al., 1974; Hoffman and Erbe, 1976; Hoffman et al., 1978). Results of recent study have shown that cancer cells have MS levels comparable to normal cells and synthesize methionine in adequate amounts or even greater amounts than normal cells, thus nowadays hypothesis is that cancer cells are dependent on methionine from the extracellular environment because their metabolism consumes it in excessive amounts (Hoffman et al., 2019; Stern et al., 1984). Human cancer cell lines were tested and it was found that they all have impaired methionine metabolism in some way. The higher consumption is enabled by upregulation of MAT2A, an enzyme of the methionine cycle (Wang et al., 2019b). And deletion of methylthioadenosine phosphorylase (MTAP), an enzyme of the methionine salvage pathway is also common (Behrmann et al., 2003; Beroukhim et al., 2010). Thus, the dependence of cancer cells on methionine has become another hallmark of cancer metabolism and is now known as the "Hofmann effect" after Dr. Robert M. Hoffman. The Hoffman effect is thought to be as or more significant than the Warburg effect, since it is now the only known metabolic change that all cancer cells suffer from.

4.3 Methioninase

Based on the findings of the Hoffman effect, research has focused on restrictive diets (Durando et al., 2008; Hoshiya et al., 1996; Tan et al., 1997a) and the enzyme L-Methionine-γ-lyase known as methioninase (METase). One of the reactions that METase catalyzes is the conversion of methionine to α-ketobutyrate, methanethiol and ammonia (Kreis and Hession, 1973). METase is dependent on the presence of pyridoxal phosphate (PLP), which serves as its cofactor. The first methioninase that was used in tumor treatment was purified from *Clostridium sporogenes*; after treatment, tumor growth was inhibited and there was no concomitant weight loss orthotopic in rats as it sometimes happened during methionine free dieting (Kreis and Hession, 1973). However, a recombinant form of METase (rMETase), which was isolated from *Pseudomonas putida*, had better properties and subsequent recombination resulted in higher stability and prolonged half-life of the enzyme; higher yields were achieved through transformation into *E. coli* (Tan et al., 1997b).

4.3.1 Methioninase therapy

Excellent results have been obtained with rMETase used for the treatment of melanomas, sarcomas and osteosarcomas in patient-derived xenograft (PDX) and patient-derived orthotopic xenograft (PDOX) mouse models of cancer. The mice were administered rMETase into the abdominal cavity, *via* the peritoneum into the bloodstream where direct methionine depletion occurred. The rMETase effectively inhibited tumor growth, the tumors had low methionine concentrations and grew smaller in size (Igarashi et al., 2017; Kawaguchi et al., 2018b; Murakami et al., 2017; Tan et al., 1996a). rMETase achieves even better results when administered orally (o-rMETase), the efficacy is probably due to the fact that it depletes methionine already in the gastrointestinal tract; that was observed in PDOX mouse models of melanoma (Kawaguchi et al., 2018a). Other studies describe successful methionine depletion when o-rMETase is administered to PDOX models with pancreatic cancer, which usually has a very poor prognosis (Igarashi et al., 2018; Maisonneuve, 2019; Zhu et al., 2018).

In the 1990s, there were two phase I clinical trials of rMETase in cancer patients, specifically patients with breast cancer, lymphoma, lung cancer or renal cancer. All patients experienced a reduction in serum methionine levels, while no adverse side effects, including rMETase toxicity, were observed in either of these two studies (Tan et al., 1996b;). Further clinical

trials investigating the effect of METase on cancer proliferation were conducted after more than 20 long years later. Even in the following clinical trial, no toxicities were observed and methionine levels began to decrease within 30 min of application (Hoffman et al., 2019). In a recent clinical trial, the effects of o-rMETase were investigated in prostate cancer patients, where methionine was again successfully depleted (Han and Hoffman, 2021). Cell cycle arrest in the late S/G2 phase when administering rMETase makes cancer cells much more sensitive to the use of chemotherapy, thus rMETase making chemotherapy more effective (Kawaguchi et al., 2017; Stern and Hoffman, 1986; Yano et al., 2014).

To determine potential toxicity, rMETase was administered to primates, specifically male *Macaca fascicularis*. Food intake and weight loss were observed after application (Yang et al., 2004c). In an effort to eliminate side effects and improve the therapeutic properties of rMETase, its PEGylated form (PEG-rMETase) was developed. PEG-rMETase has twice as long a half-life as rMETase (Tan et al., 1998). The same year, just a few months later, a publication by an almost identical team was published on its use again in male *M. fascicularis*. When compared with the results of the previous study, it was concluded that the PEG-rMETase was preferable as it did not elicit any immune response and also the biological half-life of apoenzyme activity was increased by PEGylation, specifically from 2.5 h to 143 h (Yang et al., 2004b). Moreover, PEG-rMETase was shown to kill cultured cancer cells *in vitro* more reliably than rMETase (Tan et al., 2010). Potential toxicity could be alleviated by co-administration of homocysteine, vitamin B12 and folate, which promotes methionine synthesis in non-malignant cells (Epner et al., 2002). Another substance whose co-administration enhances the properties of METase is its cofactor PLP, which increases the half-life of holoenzyme (Yang et al., 2004a). An innovative solution to the immunogenicity and short circulating time of the enzyme in the body is the injection of METase-encapsulated erythrocytes, erymethionase. Erythrocytes have already been injected into mouse models bearing breast cancer, gastric adenocarcinoma and glioblastoma cells with the result prolonged enzyme activity and dramatic inhibition of tumor proliferation (Gay et al., 2017; Machover et al., 2019; Sénéchal et al., 2019).

5. Cysteine

Cysteine, as well as the aforementioned asparagine, arginine and glutamine, are conditionally essential amino acids. In the extracellular space,

disulfide bridges are formed between cysteins by the binding of its sulfanyl groups. This disulfide is called cystine (CSSC). Cysteine is an indispensable component of almost all of proteins; a precursor for important metabolites such as the antioxidant GSH, coenzyme A, sulfate, and taurine; and because of GSH it is important protection against oxidative stress.

5.1 Cysteine metabolism

Cysteine metabolism is closely linked to methionine metabolism. Cysteine is synthesized from methionine in four steps - the first two steps are part of the methionine cycle and the second two are part of the transsulfuration pathway. As described above in the methionine cycle, methionine gives rise to SAM, from which homocysteine is subsequently synthesized (Fig. 4). Homocysteine is coupled to serine in the first reaction of the transsulfuration pathway by the enzyme cystathionine-β-synthase (CBS) to form cystathionine. In the next reaction, cystathionine is cleaved by the enzyme cystathion-γ-lyase (CGL) into cysteine, 2-oxobutanoate and ammonia.

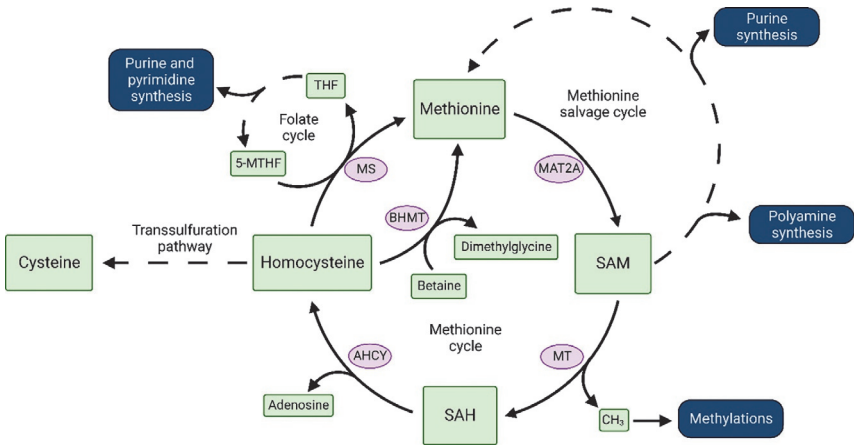

Fig. 4 Methionine metabolism. At the center of methionine metabolism is the methionine cycle. Methionine is sequentially converted to SAM, SAH and homocysteine. First, methionine is converted to SAM by the MAT2A enzyme. Then SAH is synthesized from SAM, which is catalyzed by the enzyme MT. The enzyme AHCY can form homocysteine from SAH. Homocysteine is methylated by either the enzyme MS or the enzyme BHMT. MS transfers the methyl group of 5-MRHF to methionine, making 5-MTHF into THF, while BHMT transfers the methyl group of betaine, making dimethylglycine from betaine. The demethylation of 5-MTHF to THF is part of the folate cycle, thus methionine contributing to the synthesis of both purines and pyrimidines. Methionine can be re-created from SAM *via* the methionine salvage cycle and polyamines and purines can be synthesized at the same time. Homocysteine is further processed *via* the transsulfuration pathway into the amino acid cysteine.

Two more reactions are required to produce the GSH tripeptide from cysteine. First, the enzyme γ-glutamylcysteine synthetase (γGSC) combines cysteine with glutamate to form γ-glutamylcysteine. In a second reaction catalyzed by the enzyme glutathione synthetase (GSS), GSH is formed from γ-glutamylcysteine and glycine. GSH is able to oxidize, thereby helping to maintain redox homeostasis. In a reducing environment, such as in the presence of reactive oxygen species (ROS), a disulfide bridge is formed between the two GSH. By linking the two GSHs, a glutathione disulfide (GSSG) is formed.

Due to genetic and metabolic changes, rapid proliferation, abnormal growth, and an inflammatory microenvironment, cancer cells are subjected to high oxidative stress (Olsen et al., 2013; Trachootham et al., 2009). In order for cancer cells to reduce ROS levels, they need more of the antioxidant GSH, and thus more cysteine, to synthesize GSH (Trachootham et al., 2009). If cancer cells do not obtain sufficient amounts of cysteine from the extracellular environment, they face high oxidative stress, which can be fatal to them, as cancer cells are unable to cover their own antioxidant consumption (Trachootham et al., 2009; Zhang et al., 2012). This means that cancer cells depend on cysteine import from the extracellular environment. The import is mainly facilitated by the xCT(-) antiporter, through which cysteine enters the cells in the form of its dimer CSSC and glutamate is secreted into the extracellular environment (Doxsee et al., 2007; Shiozaki et al., 2014; Takeuchi et al., 2013). The dependence of some cancer cells on cysteine from the extracellular environment is caused by the fact that they have low expression of enzymes of the transsulfuration pathway (You et al., 2011; Zhao et al., 2012).

5.2 Cyst(*e*)inase

The knowledge of the dependence of cancer cells on cysteine from the extracellular environment reveals another Achilles heel of cancer cells that is suitable for targeted therapy. The research team of Cramer focused on the use of CGL as a therapeutic enzyme (Cramer et al., 2017). The CGL enzyme catalyzes the conversion of cystathionine to cysteine, 2-oxobutanoate and ammonia within the transsulfur pathway (Fig. 5). However, CGL can also catalyze two other reactions - firstly, it cleaves CSSC to thiocysteine, pyruvate and ammonia; and secondly, it cleaves cysteine to form pyruvate, sulphate and ammonia. However, the enzymatic kinetics of these two reactions are so low that it would not make sense to use CGL in targeted therapy. Upon substitution of two glutamic acids (Glu59 and Glu339) within the active site of

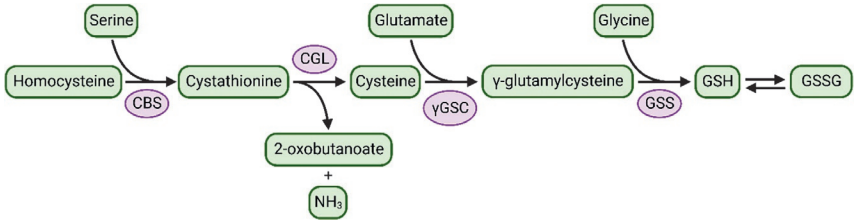

Fig. 5 Cysteine metabolism. The enzyme CBS synthesizes cystathionine from homocysteine and serine. Cystathionine is cleaved by the enzyme CGL into cysteine, 2-oxobutanoate and ammonia. The enzyme γ-GSC forms γ-glutamylcysteine from cysteine and glutamate. The reaction of GSH synthesis from γ-glutamylcysteine and glycine is catalyzed by the enzyme GSS. GSSG is a dimer composed of two GSHs that are linked by a disulfide bridge.

CGL, there was a fifty-fold increase in the catalytic efficiency of CGL in the reaction with CSSC, and the catalytic efficiency of the reaction of CGL with cysteine was increased twenty-fivefold. The version of the modified CGL to which the methoxy PEG succinimidyl carboxymethyl ester was attached was named cyst(*e*)inase.

5.2.1 Cyst(e)inase therapy

To test the functionality of cyst(*e*)inase in primates, cyst(*e*)inase was administered to two cynomolgus monkeys. Cyst(*e*)inase in primates successfully depleted cysteine and CSSC in the serum without any toxic effect of the enzyme being observed (Cramer et al., 2017). An *in vitro* experiment on prostate cancer cells showed that after cyst(*e*)inase treatment there was a decrease in GSH, an increase in ROS, an increase in AMP kinase (AMPK), a decrease in phosphorylated mechanistic target of rapamycin (mTOR), and an increase in the frequency of autophagy of these cells (Cramer et al., 2017). An interesting experiment was to investigate the effects of cyst(*e*)inase and curcumin on tumor growth in mice. When cyst(*e*)inase or curcumin was used alone, only mild tumor inhibition occurred, whereas when these agents were used together, much more pronounced growth inhibition occurred, which was consistent with the use of cyst(*e*)inase alone at a higher dose (Cramer et al., 2017).

It is known that cancer cells are often nourished by cells of the tumor microenvironment, which greatly complicates therapy and can lead to relapses. For example, nourishment of chronic lymphocytic leukemia (CLL) cells with low levels of *xCT(-)* expression by stromal cells with high levels of *xCT(-)* expression in a medium which is depleted in cysteine but

CSSCs rich goes to that CLL cells successfully surviving in this medium, as the stromal cells imported CSSCs and subsequently exported the cysteine that the CLL cells imported (Zhang et al., 2012). In conjunction with this observation, (Cramer et al., 2017) tested cyst(*e*)inase on a similar coculture with excellent results, giving hope for the treatment of previously resistant cancer lines with low *xCT(-)* expression. In cancer cells, therefore, the level of *xCT* antiporter expression may serve as a good indicator of the suitability of deploying cyst(*e*)inase therapy, as well as high ROS levels (Shiozaki et al., 2014; Takeuchi et al., 2013). A good sign of cyst(*e*)inase therapy is that, in addition to cancer cells undergoing ferroptosis after cyst(*e*)inase treatment, the therapy also increases T cell-mediated anti-tumor immunity (Wang et al., 2019a).

The main limitations of therapies are the resistance of cells to treatment and the toxicity of the agents. Reduction of resistance to cyst(*e*)inase therapy can be achieved by the use of aurafonin, a thioredoxin reductase inhibitor. Resistant pancreatic cancer cells exhibited high levels of thioredoxin 1 and good mitochondrial fitness, and were significantly more sensitive to the effects of cyst(*e*)inase therapy *in vitro* and *in vivo* after the use of auranofin, without toxic manifestations (Kshattry et al., 2019). To achieve a reduction in cyst(*e*)inase cytotoxicity, it is recommended to use the enzyme together with buthionine sulfoximine (BSO), which also interferes with the GSH synthesis pathway, and together they are more effective and much less toxic than when acting alone (Cramer et al., 2017). However, cyst(*e*)inase is still a new player in the field, and it is important that further experiments are conducted.

6. Concluding remarks

Demand for amino acids in cancer cells differs from that of normal cells. In this pathological environment, some non-essential amino acids become (conditionally) essential and a permanent extracellular supply is required. The difference between normal and tumor cells is being used for therapeutic purposes which specifically target cancer cells.

Asparagine, a non-essential amino acid, plays an essential part in different cell metabolism processes. It is biosynthesized *via* a single enzyme, asparagine synthetase, often deficient in leukemias and sometimes in other tumors. This feature makes the cells sensitive to asparagine deprivation which can be relatively easily achieved by treating cells with L-asparaginase.

Arginine is an essential amino acid in tumors in which urea cycle enzymes are not expressed or only at low levels. These tumors are susceptible for arginine depletion which can be reached by so far two most tested therapeutic approaches; using arginine deiminase or arginase. Ongoing clinical trials with these agents bring promising results and hopefully will improve the survival of cancer patients, particularly those with dismal prognoses.

So far, all cancer cells investigated have altered methionine metabolism in some way. Methioninase takes advantage of the higher consumption of methionine by tumor cells and elegantly transforms it into their weakness. By depleting extracellular methionine, it selectively disadvantages cancer cells, which are then less likely to proliferate, while normal cells are not affected by these conditions.

As cancer cells face induced oxidative stress (Renaudin, 2021), they need high concentrations of antioxidants. The most abundant antioxidant is glutathione, the precursor of which is, among other things, cysteine. Cyst(*e*)inase in serum depletes not only cysteine but also its dimer cystine. For cancer cells, in the absence of cysteine or cystine, their metabolism becomes their enemy; by producing ROS in high amount, cancer cells destroy themselves.

There are other amino acids that are metabolism is dysregulated in cancer cells as well. An example of an amino acid which we did not discuss here despite its importance is serine. Serine is part of one-carbon metabolism, including folate and methionine cycle. Recent studies show that both exogenous and endogenous sources of serine are required for tumor progression and contribute to treatment resistance (Montrose et al., 2021; Tajan et al., 2021). Serine metabolism affects multiple metabolic pathways, which can increase the survival of cancer cells (Maddocks et al., 2013, 2016). Deprivation-based therapy is therefore desired to target both, extracellular pool and *de novo* synthesis of serine.

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References

Abou-Alfa, G.K., Qin, S., Ryoo, B.Y., Lu, S.N., Yen, C.J., Feng, Y.H., Lim, H.Y., et al., 2018. Phase III randomized study of second line ADI-PEG 20 plus best supportive care versus placebo plus best supportive care in patients with advanced hepatocellular carcinoma. Ann. Oncol. 29 (6), 1402–1408. https://doi.org/10.1093/annonc/mdy101.

Aghaiypour, K., Wlodawer, A., Lubkowski, J., 2001. Structural basis for the activity and substrate specificity of Erwinia chrysanthemi L-asparaginase. Biochemistry 40 (19), 5655–5664. https://doi.org/10.1021/bi0029595.

Agrawal, V., Woo, J.H., Mauldin, J.P., Jo, C., Stone, E.M., Georgiou, G., Frankel, A.E., 2012. Cytotoxicity of human recombinant arginase i (co)-PEG5000 in the presence of supplemental L-citrulline is dependent on decreased Argininosuccinate Synthetase expression in human cells. Anticancer Drugs 23 (1), 51–64. https://doi.org/10.1097/CAD.0b013e32834ae42b.

Alexandrou, C., Al-Aqbi, S.S., Higgins, J.A., Boyle, W., Karmokar, A., Andreadi, C., Luo, J.L., et al., 2018. Sensitivity of colorectal cancer to arginine deprivation therapy is shaped by differential expression of urea cycle enzymes. Sci. Rep. 8 (1), 12096. https://doi.org/10.1038/s41598-018-30591-7.

Apfel, V., Begue, D., Cordo', V., Holzer, L., Martinuzzi, L., Buhles, A., Kerr, G., et al., 2021. Therapeutic assessment of targeting ASNS combined with l -Asparaginase treatment in solid tumors and investigation of resistance mechanisms. ACS Pharmacol. Transl. Sci. 4 (1), 327–337. https://doi.org/10.1021/ACSPTSCI.0C00196/SUPPL_FILE/PT0C00196_SI_001.PDF.

Armstrong, J.K., Hempel, G., Koling, S., Chan, L.S., Fisher, T., Meiselman, H.J., Garratty, G., 2007. Antibody against poly(ethylene glycol) adversely affects PEG-Asparaginase therapy in acute lymphoblastic leukemia patients. Cancer 110 (1), 103–111. https://doi.org/10.1002/cncr.22739.

Ashe, H., Clark, B.R., Chu, F., Hardy, D.N., Halpern, B.C., Halpern, R.M., Smith, R.A., 1974. N5-Methyltetrahydrofolate: homocysteine methyltransferase activity in extracts from Normal, malignant and embryonic tissue culture cells. Biochem. Biophys. Res. Commun. 57 (2), 417–425. https://doi.org/10.1016/0006-291X(74)90947-4.

Avramis, V.I., Sencer, S., Periclou, A.P., Sather, H., Bostrom, B.C., Cohen, L.J., Ettinger, A.G., et al., 2002. A randomized comparison of native Escherichia Coli Asparaginase and polyethylene glycol conjugated Asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: A Children's cancer group study. Blood 99 (6), 1986–1994. https://doi.org/10.1182/blood.V99.6.1986.

Bachet, J.B., Gay, F., Maréchal, R., Galais, M.P., Adenis, A., Salako, D., Cros, J., et al., 2015. Asparagine Synthetase expression and phase i study with L-Asparaginase encapsulated in red blood cells in patients with pancreatic adenocarcinoma. Pancreas 44 (7), 1141–1147. https://doi.org/10.1097/MPA.0000000000000394.

Balasubramanian, M.N., Butterworth, E.A., Kilberg, M.S., 2013. Asparagine Synthetase: regulation by cell stress and involvement in tumor biology. Am. J. Physiol. Endocrinol. Metab. 304 (8). https://doi.org/10.1152/AJPENDO.00015.2013/ASSET/IMAGES/LARGE/ZH10071367980004.JPEG.

Behrmann, I., Wallner, S., Komyod, W., Heinrich, P.C., Schuierer, M., Buettner, R., Bosserhoff, A.-K., 2003. Characterization of Methylthioadenosin phosphorylase (MTAP) expression in malignant melanoma. Am. J. Pathol. 163 (2), 683–690. https://doi.org/10.1016/S0002-9440(10)63695-4.

Beloussow, K., Wang, L., Jun, W., Ann, D., Shen, W.C., 2002. Recombinant arginine deiminase as a potential anti-Angiogenic agent. Cancer Lett. 183 (2), 155–162. https://doi.org/10.1016/S0304-3835(01)00793-5.

Beroukhim, R., Mermel, C.H., Porter, D., Wei, G., Raychaudhuri, S., Donovan, J., Barretina, J., et al., 2010. The landscape of somatic copy-number alteration across human cancers. Nature 463 (7283), 899–905. https://doi.org/10.1038/nature08822.

Billett, A.L., Sallan, S.E., Carls, A., Gelber, R.D., 1992. Allergic reactions to Erwinia Asparaginase in children with acute lymphoblastic leukemia who had previous allergic reactions to Escherichia Coli Asparaginase. Cancer 70 (1), 201–206. https://doi.org/10.1002/1097-0142(19920701)70:1<201::AID-CNCR2820700131>3.0.CO;2-M.

Boehlein, S.K., Richards, N.G.J., Schuster, S.M., 1994. Glutamine-dependent nitrogen transfer in Escherichia Coli asparagine Synthetase B. searching for the catalytic triad. J. Biol. Chem. 269 (10), 7450–7457. https://doi.org/10.1016/s0021-9258(17)37307-6.

Bolzoni, M., Chiu, M., Accardi, F., Vescovini, R., Airoldi, I., Storti, P., Todoerti, K., et al., 2016. Dependence on glutamine uptake and glutamine addiction characterize myeloma cells: A new attractive target. Blood 128 (5), 667–679. https://doi.org/10.1182/blood-2016-01-690743.

Boroughs, L.K., Deberardinis, R.J., 2015. Metabolic pathways promoting cancer cell survival and growth. Nat. Cell Biol. 17 (4), 351–359. https://doi.org/10.1038/NCB3124.

Brannigan, J.A., Dodson, G., Duggleby, H.J., Moody, P.C.E., Smith, J.L., Tomchick, D.R., Murzin, A.G., 1995. A protein catalytic framework with an N-terminal nucleophile is capable of self-activation. Nature 378 (6555), 413–416. https://doi.org/10.1038/378416a0.

Caldwell, R.W., Rodriguez, P.C., Toque, H.A., Priya Narayanan, S., Caldwell, R.B., 2018. Arginase: A multifaceted enzyme important in health and disease. Physiol. Rev. 98 (2), 641–665. https://doi.org/10.1152/physrev.00037.2016.

Campbell, H.A., Mashburn, L.T., Boyse, E.A., Old, L.J., 1967. Two L-Asparaginases from Escherichia Coli B. their separation, purification, and antitumor activity*. Biochemistry 6 (3), 721–730. https://doi.org/10.1021/bi00855a011.

Capizzi, R.L., Bertino, J.R., Handschumacher, R.E., 1970. L-Asparaginase. Annu. Rev. Med. https://doi.org/10.1146/annurev.me.21.020170.002245.

Chan, S.L., Cheng, P.N.M., Liu, A.M., Chan, L.L., Li, L., Chu, C.M., Chong, C.C.N., et al., 2021. A phase II clinical study on the efficacy and predictive biomarker of Pegylated recombinant arginase on hepatocellular carcinoma. Invest. New Drugs 39 (5), 1375–1382. https://doi.org/10.1007/s10637-021-01111-8.

Chan, W.K., Lorenzi, P.L., Anishkin, A., Purwaha, P., Rogers, D.M., Sukharev, S., Rempe, S.B., Weinstein, J.N., 2014. The glutaminase activity of L- asparaginase is not required for anticancer activity against ASNS-negative cells. Blood 123 (23), 3596–3606. https://doi.org/10.1182/blood-2013-10-535112.

Chiu, M., Taurino, G., Bianchi, M.G., Ottaviani, L., Andreoli, R., Ciociola, T., Lagrasta, C.A.M., Tardito, S., Bussolati, O., 2018. Oligodendroglioma cells lack glutamine Synthetase and are auxotrophic for glutamine, but do not depend on glutamine Anaplerosis for growth. Int. J. Mol. Sci. 19 (4). https://doi.org/10.3390/ijms19041099.

Chiu, M., Taurino, G., Bianchi, M.G., Kilberg, M.S., Bussolati, O., 2020. Asparagine Synthetase in cancer: beyond acute lymphoblastic leukemia. Front. Oncol. https://doi.org/10.3389/fonc.2019.01480. Frontiers Media S.A.

Cocetta, V., Ragazzi, E., Montopoli, M., 2020. Links between cancer metabolism and cisplatin resistance. Int. Rev. Cell Mol. Biol. 354 (January), 107–164. https://doi.org/10.1016/BS.IRCMB.2020.01.005.

Cordier-Bussat, M., Thibert, C., Sujobert, P., Genestier, L., Fontaine, É., Billaud, M., 2018. Even the Warburg effect can be oxidized: metabolic cooperation and tumor development. Med. Sci. 34 (8–9), 701–708. https://doi.org/10.1051/MEDSCI/20183408017.

Cramer, S.L., Saha, A., Liu, J., Tadi, S., Tiziani, S., Yan, W., Triplett, K., et al., 2017. Systemic depletion of L-cyst(e)Ine with cyst(e)Inase increases reactive oxygen species and suppresses tumor growth. Nat. Med. 23 (1), 120–127. https://doi.org/10.1038/nm.4232.

Crenn, P., Messing, B., Cynober, L., 2008. Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. Clin. Nutr. 27 (3), 328–339. https://doi.org/10.1016/j.clnu.2008.02.005.

Darvishi, F., Faraji, N., Shamsi, F., 2019. Production and structural modeling of a novel Asparaginase in Yarrowia Lipolytica. Int. J. Biol. Macromol. 125 (March), 955–961. https://doi.org/10.1016/j.ijbiomac.2018.12.162.

Das, K., Butler, G.H., Kwiatkowski, V., Clark, A.D., Yadav, P., Arnold, E., 2004. Crystal structures of arginine deiminase with covalent reaction intermediates: implications for catalytic mechanism. Structure 12 (4), 657–667. https://doi.org/10.1016/j.str.2004.02.017.

Santo, C.D., Cheng, P., Beggs, A., Egan, S., Bessudo, A., Mussai, F., 2018. Metabolic therapy with PEG-arginase induces a sustained complete remission in immunotherapy-resistant melanoma. J. Hematol. Oncol. 11 (1). https://doi.org/10.1186/s13045-018-0612-6.

Dillon, B.J., Prieto, V.G., Curley, S.A., Mark Ensor, C., Holtsberg, F.W., Bomalaski, J.S., Clark, M.A., 2004. Incidence and distribution of Argininosuccinate Synthetase deficiency in human cancers: A method for identifying cancers sensitive to arginine deprivation. Cancer 100 (4), 826–833. https://doi.org/10.1002/cncr.20057.

Ding, Y., Li, Z., Broome, J.D., 2005. Epigenetic changes in the repression and induction of asparagine Synthetase in human leukemic cell lines. Leukemia 19 (3), 420–426. https://doi.org/10.1038/sj.leu.2403639.

Douer, D., Yampolsky, H., Cohen, L.J., Watkins, K., Levine, A.M., Periclou, A.P., Avramis, V.I., 2007. Pharmacodynamics and safety of intravenous Pegaspargase during remission induction in adults aged 55 years or younger with newly diagnosed acute lymphoblastic leukemia. Blood 109 (7), 2744–2750. https://doi.org/10.1182/blood-2006-07-035006.

Doxsee, D.W., Gout, P.W., Kurita, T., Lo, M., Buckley, A.R., Wang, Y., Xue, H., et al., 2007. Sulfasalazine-induced Cystine starvation: potential use for prostate cancer therapy. Prostate 67 (2), 162–171. https://doi.org/10.1002/pros.20508.

Durando, X., Thivat, E., Farges, M.C., Cellarier, E., D'Incan, M., Demidem, A., Vasson, M.P., Barthomeuf, C., Chollet, P., 2008. Optimal methionine-free diet duration for Nitrourea treatment: A phase I clinical trial. Nutr. Cancer 60 (1), 23–30. https://doi.org/10.1080/01635580701525877.

Duval, M., Suciu, S., Ferster, A., Rialland, X., Nelken, B., Lutz, P., Benoit, Y., et al., 2002. Comparison of Escherichia coli-asparaginase with Erwinia-Asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized european organisation for research and treatment of cancer - Children's leukemia group phase 3 trial. Blood 99 (8), 2734–2739. https://doi.org/10.1182/blood.V99.8.2734.

Dzik, J.M., 2014. Evolutionary roots of arginase expression and regulation. Front. Immunol. 5 (NOV), 1–12. https://doi.org/10.3389/fimmu.2014.00544.

Ehsanipour, E.A., Sheng, X., Behan, J.W., Wang, X., Butturini, A., Avramis, V.I., Mittelman, S.D., 2013. Adipocytes cause leukemia cell resistance to L-Asparaginase via release of glutamine. Cancer Res. 73 (10), 2998–3006. https://doi.org/10.1158/0008-5472.CAN-12-4402.

El-Naggar, N.E.A., Deraz, S.F., Soliman, H.M., El-Deeb, N.M., El-Ewasy, S.M., 2016. Purification, characterization, cytotoxicity and anticancer activities of L-Asparaginase, anti-Colon cancer protein, from the newly isolated Alkaliphilic Streptomyces Fradiae NEAE-82. Sci. Rep. 6 (September). https://doi.org/10.1038/srep32926.

Ensor, C.M., Holtsberg, F.W., Bomalaski, J.S., Clark, M.A., 2002. Pegylated arginine deiminase (ADI-SS PEG20,000 mw) inhibits human melanomas and hepatocellular carcinomas in vitro and in vivo. Cancer Res. 62 (19), 5443–5450.

Epner, D.E., Morrow, S., Wilcox, M., Houghton, J.L., 2002. Nutrient intake and nutritional indexes in adults with metastatic cancer on a phase I clinical trial of dietary methionine restriction. Nutr. Cancer 42 (2), 158–166. https://doi.org/10.1207/S15327914NC422_2.

Fiedler, T., Strauss, M., Hering, S., Redanz, U., William, D., Rosche, Y., Classen, C.F., Kreikemeyer, B., Linnebacher, M., Maletzki, C., 2015. Arginine deprivation by arginine deiminase of *streptococcus pyogenes* controls primary glioblastoma growth *in vitro* and *in vivo*. Cancer Biol. Ther. 16 (7), 1047–1055. https://doi.org/10.1080/15384047.2015.1026478.

Furusawa, A., Miyamoto, M., Takano, M., Tsuda, H., Song, Y.S., Aoki, D., Miyasaka, N., Inazawa, J., Inoue, J., 2018. Ovarian cancer therapeutic potential of glutamine depletion based on GS expression. Carcinogenesis 39 (6), 758–766. https://doi.org/10.1093/carcin/bgy033.

Gay, F., Aguera, K., Senechal, K., Bes, J., Chevrier, A.-M., Gallix, F., Guicher, C., et al., 2016. Abstract 4812: arginine deiminase loaded in erythrocytes: A promising formulation for L-arginine deprivation therapy in cancers. Cancer Res. 76, 4812. American Association for Cancer Research (AACR). https://doi.org/10.1158/1538-7445.am2016-4812.

Gay, F., Aguera, K., Sénéchal, K., Tainturier, A., Berlier, W., Maucort-Boulch, D., Honnorat, J., Horand, F., Godfrin, Y., Bourgeaux, V., 2017. Methionine tumor starvation by erythrocyte-encapsulated methionine gamma-Lyase activity controlled with per Os vitamin B6. Cancer Med. 6 (6), 1437–1452. https://doi.org/10.1002/cam4.1086.

Geck, R.C., Foley, J.R., Stewart, T.M., Asara, J.M., Casero, R.A., Toker, A., 2020. Inhibition of the polyamine synthesis enzyme ornithine decarboxylase sensitizes triple-negative breast cancer cells to cytotoxic chemotherapy. J. Biol. Chem. https://doi.org/10.1074/jbc.RA119.012376. American Society for Biochemistry and Molecular Biology Inc.

Glynn, S.A., Boersma, B.J., Dorsey, T.H., Yi, M., Yfantis, H.G., Ridnour, L.A., Martin, D.N., et al., 2010. Increased NOS2 predicts poor survival in estrogen receptor-negative breast cancer patients. J. Clin. Investig. 120 (11), 3843–3854. https://doi.org/10.1172/JCI42059.

Gupta, N., Tewari, V.V., Kumar, M., Langeh, N., Gupta, A., Mishra, P., Kaur, P., et al., 2017. Asparagine Synthetase deficiency-report of a novel mutation and review of literature. Metab. Brain Dis. 32 (6), 1889–1900. https://doi.org/10.1007/S11011-017-0073-6.

Gupta, S., Sahu, D., Bomalaski, J.S., Frank, I., Boorjian, S.A., Thapa, P., Cheville, J.C., Hansel, D.E., 2018. Argininosuccinate Synthetase-1 (ASS1) loss in high-grade neuroendocrine carcinomas of the urinary bladder: implications for targeted therapy with ADI-PEG 20. Endocr. Pathol. 29 (3), 236–241. https://doi.org/10.1007/s12022-018-9516-9.

Halfon-Domenech, C., Thomas, X., Chabaud, S., Baruchel, A., Gueyffier, F., Mazingue, F., Auvrignon, A., et al., 2011. L-Asparaginase loaded red blood cells in refractory or relapsing acute lymphoblastic Leukaemia in children and adults: results of the GRASPALL 2005-01 randomized trial. Br. J. Haematol. 153 (1), 58–65. https://doi.org/10.1111/j.1365-2141.2011.08588.x.

Halpern, B.C., Clark, B.R., Hardy, D.N., Halpern, R.M., Smith, R.A., 1974. The effect of replacement of methionine by Homocystine on survival of malignant and Normal adult mammalian cells in culture. Proc. Natl. Acad. Sci. U. S. A. 71 (4), 1133–1136. https://doi.org/10.1073/pnas.71.4.1133.

Hammel, P., Fabienne, P., Mineur, L., Metges, J.P., Andre, T., De La Fouchardiere, C., Louvet, C., et al., 2020. Erythrocyte-encapsulated Asparaginase (Eryaspase) combined with chemotherapy in second-line treatment of advanced pancreatic cancer: An open-label, randomized phase IIb trial. Eur. J. Cancer 124 (January), 91–101. https://doi.org/10.1016/j.ejca.2019.10.020.

Han, Q., Hoffman, R.M., 2021. Lowering and stabilizing PSA levels in advanced-prostate Cancer patients with Oral Methioninase. Anticancer Res 41 (4), 1921–1926. https://doi.org/10.21873/anticanres.14958.

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. Cell. https://doi.org/10.1016/j.cell.2011.02.013.

Haskell, C.M., Canellos, G.P., Leventhal, B.G., Carbone, P.P., Block, J.B., Serpick, A.A., Selawry, O.S., 1969. L-Asparaginase: therapeutic and toxic effects in patients with neoplastic disease. N. Engl. J. Med. 281 (19), 1028–1034. https://doi.org/10.1056/NEJM196911062811902.

Hays, J.L., Kim, G., Walker, A., Annuziata, C.M., Lee, J.-M., Squires, J., Houston, N., Steinberg, S.M., Kohn, E.C., 2013. A phase II clinical trial of polyethylene glycol-conjugated L-Asparaginase in patients with advanced ovarian cancer: early closure for safety. Mol. Clin. Oncol. 1 (3), 565–569. https://doi.org/10.3892/mco.2013.99.

Hermanova, I., Arruabarrena-Aristorena, A., Valis, K., Nuskova, H., Alberich-Jorda, M., Fiser, K., Fernandez-Ruiz, S., et al., 2016. Pharmacological inhibition of fatty-acid oxidation synergistically enhances the effect of l-Asparaginase in childhood ALL cells. Leukemia 30 (1), 209–218. https://doi.org/10.1038/leu.2015.213.

Hlozkova, K., Hermanova, I., Safrhansova, L., Alquezar-Artieda, N., Kuzilkova, D., Vavrova, A., Sperkova, K., et al., 2022. PTEN/PI3K/Akt pathway alters sensitivity of T-cell acute lymphoblastic leukemia to L-Asparaginase. Sci. Rep. 12 (1). https://doi.org/10.1038/S41598-022-08049-8.

Hoffman, R.M., Erbe, R.W., 1976. High in vivo rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine. Proc. Natl. Acad. Sci. U. S. A. 73 (5), 1523–1527. https://doi.org/10.1073/pnas.73.5.1523.

Hoffman, R.M., Jacobsen, S.J., 1980. Reversible growth arrest in simian virus 40-transformed human fibroblasts. Proc. Natl. Acad. Sci. U. S. A. 77 (12 II), 7306–7310. https://doi.org/10.1073/pnas.77.12.7306.

Hoffman, R.M., Jacobsen, S.J., Erbe, R.W., 1978. Reversion to methionine Independence by malignant rat and SV40-transformed human fibroblasts. Biochem. Biophys. Res. Commun. 82 (1), 228–234. https://doi.org/10.1016/0006-291X(78)90600-9.

Hoffman, R.M., Tan, Y., Li, S., Han, Q., Zavala, J., Zavala, J., 2019. Pilot phase I clinical trial of Methioninase on high-stage cancer patients: rapid depletion of circulating methionine. Methods Mol. Biol., 231–242. https://doi.org/10.1007/978-1-4939-8796-2_17.

Holtsberg, F.W., Ensor, C.M., Steiner, M.R., Bomalaski, J.S., Clark, M.A., 2002. Poly(ethylene glycol) (PEG) conjugated arginine deiminase: effects of PEG formulations on its pharmacological properties. J. Control. Release 80 (1–3), 259–271. https://doi.org/10.1016/S0168-3659(02)00042-1.

Hoshiya, Y., Kubota, T., Matsuzaki, S.W., Kitajima, M., Hoffman, R.M., 1996. Methionine starvation modulates the efficacy of cisplatin on human breast cancer in nude mice. Anticancer Res 16 (6B), 3515–3517.

Howard, J.B., Carpenter, F.H., 1972. L-Asparaginase from Erwinia Carotovora: SUBSTRATE SPECIFICITY AND ENZYMATIC PROPERTIES. J. Biol. Chem. 247 (4), 1020–1030. https://doi.org/10.1016/S0021-9258(19)45610-X.

Huang, H.Y., Wen Ren, W., Wang, Y.H., Wang, J.W., Fang, F.M., Tsai, J.W., Li, S.H., et al., 2013. ASS1 as a novel tumor suppressor gene in myxofibrosarcomas: aberrant loss via epigenetic DNA methylation confers aggressive phenotypes, negative prognostic impact, and therapeutic relevance. Clin. Cancer Res. 19 (11), 2861–2872. https://doi.org/10.1158/1078-0432.CCR-12-2641.

Huang, L., Liu, Y., Sun, Y., Yan, Q., Jiang, Z., 2014. Biochemical characterization of a novel L-Asparaginase with low Glutaminase activity from Rhizomucor Miehei and its application in food safety and leukemia treatment. Appl. Environ. Microbiol. 80 (5), 1561–1569. https://doi.org/10.1128/AEM.03523-13.

Hunault-Berger, M., Leguay, T., Huguet, F., Leprêtre, S., Deconinck, E., Ojeda-Uribe, M., Bonmati, C., et al., 2015. A phase 2 study of L-Asparaginase encapsulated in erythrocytes in elderly patients with Philadelphia chromosome negative acute lymphoblastic leukemia: the GRASPALL/GRAALL-SA2-2008 study. Am. J. Hematol. 90 (9), 811–818. https://doi.org/10.1002/ajh.24093.

Igarashi, K., Kawaguchi, K., Kiyuna, T., Miyake, K., Murakami, T., Yamamoto, N., Hayashi, K., et al., 2017. Effective metabolic targeting of human osteosarcoma cells in vitro and in Orthotopic nude-mouse models with recombinant Methioninase. Anticancer Res 37 (9), 4807–4812. https://doi.org/10.21873/anticanres.11887.

Igarashi, K., Kawaguchi, K., Li, S., Han, Q., Tan, Y., Gainor, E., Kiyuna, T., et al., 2018. Recombinant Methioninase combined with doxorubicin (DOX) regresses a DOX-resistant synovial sarcoma in a patient-derived Orthotopic xenograft (PDOX) mouse model. Oncotarget 9 (27), 19263–19272. https://doi.org/10.18632/oncotarget.24996.

Iwamoto, S., Mihara, K., Downing, J.R., Pui, C.H., Campana, D., 2007. Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to Asparaginase. J. Clin. Investig. 117 (4), 1049–1057. https://doi.org/10.1172/JCI30235.

Izzo, F., Marra, P., Beneduce, G., Castello, G., Vallone, P., De Rosa, V., Cremona, F., et al., 2004. Pegylated arginine deiminase treatment of patients with Unresectable hepatocellular carcinoma: results from phase I/II studies. J. Clin. Oncol. 22 (10), 1815–1822. https://doi.org/10.1200/JCO.2004.11.120.

Jadeski, L.C., Hum, K.O., Chakraborty, C., Lala, P.K., 2000. Nitric oxide promotes murine mammary tumour growth and metastasis by stimulating tumour cell migration, invasiveness and angiogenesis. Int. J. Cancer 86 (1), 30–39. https://doi.org/10.1002/(SICI)1097-0215(20000401)86:1<30::AID-IJC5>3.0.CO;2-I.

Jenkinson, C.P., Grody, W.W., Cederbaum, S.D., 1996. Comparative properties of arginases. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 114 (1), 107–132. https://doi.org/10.1016/0305-0491(95)02138-8.

Kawaguchi, K., Igarashi, K., Li, S., Han, Q., Tan, Y., Kiyuna, T., Miyake, K., et al., 2017. Combination treatment with recombinant Methioninase enables Temozolomide to arrest a BRAF V600E melanoma in a patient-derived Orthotopic xenograft (PDOX) mouse model. Oncotarget 8 (49), 85516–85525. https://doi.org/10.18632/oncotarget.20231.

Kawaguchi, K., Han, Q., Li, S., Tan, Y., Igarashi, K., Kiyuna, T., Miyake, K., et al., 2018a. Targeting methionine with Oral recombinant Methioninase (o-RMETase) arrests a patient-derived Orthotopic xenograft (PDOX) model of BRAF-V600E mutant melanoma: implications for chronic clinical cancer therapy and prevention. Cell Cycle 17 (3), 356–361. https://doi.org/10.1080/15384101.2017.1405195.

Kawaguchi, K., Igarashi, K., Li, S., Han, Q., Tan, Y., Miyake, K., Kiyuna, T., et al., 2018b. Recombinant Methioninase (RMETase) is an effective therapeutic for BRAF-V600E-negative as well as-positive melanoma in patient-derived Orthotopic xenograft (PDOX) mouse models. Oncotarget 9. www.impactjournals.com/oncotarget.

Kepka-Lenhart, D., Mistry, S.K., Guoyao, W., Morris, S.M., 2000. Arginase I: A limiting factor for nitric oxide and polyamine synthesis by activated macrophages? Am. J. Physiol. Regul. Integr. Comp. Physiol. 279 (6 48-6), 2237–2242. https://doi.org/10.1152/ajpregu.2000.279.6.r2237.

Kilberg, M.S., Shan, J., Su., N., 2009. ATF4-dependent transcription mediates signaling of amino acid limitation. Trends. Endocrinol. Metab. 20 (9), 436–443. https://doi.org/10.1016/J.TEM.2009.05.008.

Kilberg, M.S., Balasubramanian, M., Lingchen, F., Shan, J., 2012. The transcription factor network associated with the amino acid response in mammalian cells. Adv. Nutr. 3 (3), 295–306. https://doi.org/10.3945/AN.112.001891.

Kim, K., Jeong, J.H., Lim, D., Hong, Y., Lim, H.J., Kim, G.J., Shin, S.R., et al., 2015. L-Asparaginase delivered by salmonella typhimurium suppresses solid tumors. Mol. Ther. Oncolytics. 2 (June), 15007. https://doi.org/10.1038/MTO.2015.7.

Klotz, T., Bloch, W., Volberg, C., Engelmann, U., Addicks, K., 1998. Selective expression of inducible nitric oxide synthase in human prostate carcinoma. Cancer 82 (10), 1897–1903. https://doi.org/10.1002/(SICI)1097-0142(19980515)82:10<1897::AID-CNCR12>3.0.CO;2-O.

Krall, A.S., Shili, X., Graeber, T.G., Braas, D., Christofk, H.R., 2016. Asparagine promotes cancer cell proliferation through use as an amino acid exchange factor. Nat. Commun. 7. https://doi.org/10.1038/ncomms11457.

Krall, A.S., Mullen, P.J., Surjono, F., Momcilovic, M., Schmid, E.W., Halbrook, C.J., Thambundit, A., et al., 2021. Asparagine couples mitochondrial respiration to ATF4 activity and tumor growth. Cell Metab. 33 (5), 1013–1026.e6. https://doi.org/10.1016/j.cmet.2021.02.001.

Kravtzoff, R., Desbois, I., Lamagnere, J.P., Muh, J.P., Ch Valat, M., Colombat, C.,.P., Ropars, C., 1996. Improved pharmacodynamics of L-Asparaginase-loaded in human red blood cells. Eur. J. Clin. Pharmacol. 49 (6), 465–470. https://doi.org/10.1007/BF00195932.

Kreis, W., Hession, C., 1973. Isolation and purification of L-methionine-alpha-Deamino-gamma-Mercaptomethane-Lyase (L-Methioninase) from clostridium Sporogenes. Cancer Res. 33 (8), 1862–1865.

Kshattry, S., Saha, A., Gries, P., Tiziani, S., Stone, E., Georgiou, G., DiGiovanni, J., 2019. Enzyme-mediated depletion of l-cyst(e)Ine synergizes with Thioredoxin reductase inhibition for suppression of pancreatic tumor growth. NPJ Precis. Oncol. 3 (1). https://doi.org/10.1038/s41698-019-0088-z.

Kwong-Lam, F., Chi-Fung, C.G., 2013. Vincristine could partly suppress stromal support to T-ALL blasts during Pegylated arginase I treatment. Exp. Hematol. Oncol. 2 (1). https://doi.org/10.1186/2162-3619-2-11.

Larkin, A., Imperiali, B., 2011. The expanding horizons of asparagine-linked glycosylation. Biochemistry. https://doi.org/10.1021/bi200346n.

Larsen, T.M., Boehlein, S.K., Schuster, S.M., Richards, N.G.J., Thoden, J.B., Holden, H.M., Rayment, I., 1999. Three-dimensional structure of Escherichia Coli asparagine Synthetase B: A short journey from substrate to product. Biochemistry 38 (49), 16146–16157. https://doi.org/10.1021/bi9915768.

Leslie, M., Case, M.C., Hall, A.G., Coulthard, S.A., 2006. Expression levels of asparagine Synthetase in blasts from children and adults with acute lymphoblastic Leukaemia. Br. J. Haematol. 132 (6), 740–742. https://doi.org/10.1111/j.1365-2141.2005.05945.x.

Lin, C., Wang, Z., Li, L., He, Y., Fan, J., Liu, Z., Zhao, S., Dianwen, J., 2015. The role of autophagy in the cytotoxicity induced by recombinant human arginase in laryngeal squamous cell carcinoma. Appl. Microbiol. Biotechnol. 99 (20), 8487–8494. https://doi.org/10.1007/s00253-015-6565-6.

Lind, D.S., 2004. Arginine and cancer. J. Nutr. 134 (10 SUPPL), 2837–2841. https://doi.org/10.1093/jn/134.10.2837s.

Liu, C., Luo, L., Lin, Q., 2019. Antitumor activity and ability to prevent acrylamide formation in fried foods of Asparaginase from soybean root nodules. J. Food Biochem. 43 (3). https://doi.org/10.1111/jfbc.12756.

Lomelino, C.L., Andring, J.T., McKenna, R., Kilberg, M.S., 2017. Asparagine Synthetase: function, structure, and role in disease. J. Biol. Chem. https://doi.org/10.1074/jbc.R117.819060. American Society for Biochemistry and Molecular Biology Inc.

Lubkowski, J., Dauter, M., Aghaiypour, K., Wlodawer, A., Dauter, Z., 2003. Atomic resolution structure of Erwinia Chrysanthemi L-Asparaginase. Acta Crystallogr. D 59 (1), 84–92. https://doi.org/10.1107/S0907444902019443.

Machover, D., Rossi, L., Hamelin, J., Desterke, C., Goldschmidt, E., Chadefaux-Vekemans, B., Bonnarme, P., et al., 2019. Effects in cancer cells of the recombinant L-methionine gamma-Lyase from Brevibacterium aurantiacum. Encapsulation in human erythrocytes for sustained l-methionine elimination. J. Pharmacol. Exp. Ther. 369 (3), 489–502. https://doi.org/10.1124/jpet.119.256537.

Maddocks, O.D.K., Berkers, C.R., Mason, S.M., Zheng, L., Blyth, K., Gottlieb, E., Vousden, K.H., 2013. Serine starvation induces stress and P53-dependent metabolic remodelling in cancer cells. Nature 493 (7433), 542–546. https://doi.org/10.1038/NATURE11743.

Maddocks, O.D.K., Labuschagne, C.F., Adams, P.D., Vousden, K.H., 2016. Serine metabolism supports the methionine cycle and DNA/RNA methylation through De novo ATP synthesis in cancer cells. Mol. Cell 61 (2), 210–221. https://doi.org/10.1016/J.MOLCEL.2015.12.014.

Maisonneuve, P., 2019. Epidemiology and burden of pancreatic cancer. Presse Med. 48 (3P2), e113–e123. https://doi.org/10.1016/j.lpm.2019.02.030.

Mauldin, J.P., Zeinali, I., Kleypas, K., Woo, J.H., Blackwood, R.S., Jo, C.H., Stone, E.M., Georgiou, G., Frankel, A.E., 2012. Recombinant human arginase toxicity in mice is reduced by Citrulline supplementation. Transl. Oncol. 5 (1), 26–31. https://doi.org/10.1593/tlo.11262.

Mecham, J.O., David Rowitch, C., Wallace, D., Stern, P.H., Hoffman, R.M., 1983. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. Biochem. Biophys. Res. Commun. 117 (2), 429–434. https://doi.org/10.1016/0006-291X(83)91218-4.

Miraki-Moud, F., Ghazaly, E., Ariza-McNaughton, L., Hodby, K.A., Clear, A., Anjos-Afonso, F., Liapis, K., et al., 2015. Arginine deprivation using Pegylated arginine deiminase has activity against primary acute myeloid leukemia cells in vivo. Blood 125 (26), 4060–4068. https://doi.org/10.1182/blood-2014-10-608133.

Miska, J., Rashidi, A., Lee-Chang, C., Gao, P., Lopez-Rosas, A., Zhang, P., Burga, R., et al., 2021. Polyamines drive myeloid cell survival by buffering intracellular PH to promote immunosuppression in glioblastoma. Sci. Adv. 7 (8), 8929–8946. https://doi.org/10.1126/sciadv.abc8929.

Mocellin, S., Bronte, V., Nitti, D., 2007. Nitric oxide, a double edged sword in cancer biology: searching for therapeutic opportunities. Med. Res. Rev. 27 (3), 317–352. https://doi.org/10.1002/med.20092.

Moe-Byrne, T., Wagner, J.V.E., McGuire, W., 2012. Glutamine supplementation to prevent morbidity and mortality in preterm infants. Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.cd001457.pub4. John Wiley & Sons, Ltd.

Mohan Kumar, N.S., Manonmani, H.K., 2013. Purification, characterization and kinetic properties of extracellular l-Asparaginase produced by Cladosporium Sp. World J. Microbiol. Biotechnol. 29 (4), 577–587. https://doi.org/10.1007/s11274-012-1213-0.

Moinard, C., Cynober, L., de Bandt, J.P., 2005. Polyamines: metabolism and implications in human diseases. Clin. Nutr. https://doi.org/10.1016/j.clnu.2004.11.001.

Montrose, D.C., Saha, S., Foronda, M., McNally, E.M., Chen, J., Zhou, X.K., Ha, T., et al., 2021. Exogenous and endogenous sources of serine contribute to colon cancer metabolism, growth, and resistance to 5-fluorouracil. Cancer Res. 81 (9), 2275–2288. https://doi.org/10.1158/0008-5472.CAN-20-1541.

Morris, S.M., 2007. Arginine metabolism: boundaries of our knowledge. J. Nutr. 137 (6). https://doi.org/10.1093/jn/137.6.1602s.

Morris, S.M., Bhamidipati, D., Kepka-Lenhart, D., 1997. Human type II arginase: sequence analysis and tissue-specific expression. Gene 193 (2), 157–161. https://doi.org/10.1016/S0378-1119(97)00099-1.

Mostazo, C., Miriam, G., Kurrle, N., Casado, M., Fuhrmann, D., Alshamleh, I., Häupl, B., Martín-Sanz, P., et al., 2020. Metabolic plasticity is an essential requirement of acquired tyrosine kinase inhibitor resistance in chronic myeloid leukemia. Cancer 12 (11), 1–26. https://doi.org/10.3390/CANCERS12113443.

Müller, H.J., Boos, J., 1998. Use of L-Asparaginase in childhood ALL. Crit. Rev. Oncol. Hematol. 28 (2), 97–113. https://doi.org/10.1016/s1040-8428(98)00015-8.

Murakami, T., Li, S., Han, Q., Tan, Y., Kiyuna, T., Igarashi, K., Kawaguchi, K., et al., 2017. Recombinant Methioninase effectively targets a Ewing's sarcoma in a patient-derived Orthotopic xenograft (PDOX) nude-mouse model. Oncotarget 8 (22), 35630–35638. https://doi.org/10.18632/oncotarget.15823.

Nguyen, H.A., Su, Y., Lavie, A., 2016. Design and characterization of Erwinia Chrysanthemi L-Asparaginase variants with diminished L-Glutaminase activity. J. Biol. Chem. 291 (34), 17664–17676. https://doi.org/10.1074/jbc.M116.728485.

Nicholson, L.J., Smith, P.R., Hiller, L., Szlosarek, P.W., Kimberley, C., Sehouli, J., Koensgen, D., Mustea, A., Schmid, P., Crook, T., 2009. Epigenetic silencing of Argininosuccinate Synthetase confers resistance to platinum-induced cell death but collateral sensitivity to arginine Auxotrophy in ovarian cancer. Int. J. Cancer 125 (6), 1454–1463. https://doi.org/10.1002/ijc.24546.

Nowak-Göttl, U., Heinecke, A., Von Kries, R., Nürnberger, W., Münchow, N., Junker, R., 2001. Thrombotic events revisited in children with acute lymphoblastic leukemia - impact of concomitant Escherichia Coli Asparaginase/prednisone administration. Thromb. Res. 103 (3), 165–172. https://doi.org/10.1016/S0049-3848(01)00286-9.

Oettgen, H.F., Stephenson, P.A., Schwartz, M.K., Leeper, R.D., Tallal, L., Tan, C.C., Clarkson, B.D., et al., 1970. Toxicity of E. Coli L-asparaginase in man. Cancer 25 (2), 253–278. https://doi.org/10.1002/1097-0142(197002)25:2<253::AID-CNCR2820250204>3.0.CO;2-U.

Offman, M.N., Krol, M., Patel, N., Krishnan, S., Liu, J.Z., Saha, V., Bates, P.A., 2011. Rational engineering of L-Asparaginase reveals importance of dual activity for cancer cell toxicity. Blood 117 (5), 1614–1621. https://doi.org/10.1182/blood-2010-07-298422.

Olsen, L.F., Issinger, O.-G., Guerra, B., 2013. The yin and Yang of redox regulation. Redox Rep. 18 (6), 245–252. https://doi.org/10.1179/1351000213Y.0000000059.

Oronsky, B., Oronsky, N., Knox, S., Fanger, G., Scicinski, J., 2014. Episensitization: therapeutic tumor Resensitization by epigenetic agents: A review and reassessment. Anticancer Agents Med Chem. 14 (8), 1121–1127. https://doi.org/10.2174/1871520614666140418144610.

Panosyan, E.H., Grigoryan, R.S., Avramis, I.A., Seibel, N.L., Gaynon, P.S., Siegel, S.E., Fingert, H.J., Avramis, V.I., 2004. Deamination of glutamine is a prerequisite for optimal asparagine deamination by Asparaginases in vivo (CCG-1961). Anticancer Res 24 (2C), 1121–1125.

Park, I.S., Kang, S.W., Shin, Y.J., Chae, K.Y., Park, M.O., Kim, M.Y., Wheatley, D.N., Min, B.H., 2003. Arginine deiminase: A potential inhibitor of angiogenesis and tumour growth. Br. J. Cancer 89 (5), 907–914. https://doi.org/10.1038/sj.bjc.6601181.

Parmentier, J.H., Maggi, M., Tarasco, E., Scotti, C., Avramis, V.I., Mittelman, S.D., 2015. Glutaminase activity determines cytotoxicity of L-Asparaginases on Most leukemia cell lines. Leuk. Res. 39 (7), 757–762. https://doi.org/10.1016/j.leukres.2015.04.008.

Pathria, G., Lee, J.S., Hasnis, E., Tandoc, K., Scott, D.A., Verma, S., Feng, Y., et al., 2019. Translational reprogramming Marks adaptation to asparagine restriction in cancer. Nat. Cell Biol. 21 (12), 1590–1603. https://doi.org/10.1038/S41556-019-0415-1.

Pavlova, N.N., Hui, S., Ghergurovich, J.M., Fan, J., Intlekofer, A.M., White, R.M., Rabinowitz, J.D., Thompson, C.B., Zhang, J., 2018. As Extracellular Glutamine Levels Decline, Asparagine Becomes an Essential Amino Acid. Cell Metab. 27 (2), 428–438.e5. https://doi.org/10.1016/j.cmet.2017.12.006.

Pieters, R., Hunger, S.P., Boos, J., Rizzari, C., Silverman, L., Baruchel, A., Goekbuget, N., Schrappe, M., Pui, C.H., 2011. L-Asparaginase Treatment in Acute Lymphoblastic Leukemia. Cancer. https://doi.org/10.1002/cncr.25489.

Plourde, P.V., Jeha, S., Hijiya, N., Keller, F.G., Silverman, L.B., Rheingold, S.R., Dreyer, Z.E., et al., 2014. Safety profile of Asparaginase Erwinia Chrysanthemi in a large compassionate-use trial. Pediatr. Blood Cancer. 61 (7), 1232–1238. https://doi.org/10.1002/pbc.24938.

Purwaha, P., Lorenzi, P.L., Silva, L.P., Hawke, D.H., Weinstein, J.N., 2014. Targeted Metabolomic analysis of amino acid response to L-Asparaginase in adherent cells. Metabolomics 10 (5), 909–919. https://doi.org/10.1007/s11306-014-0634-1.

Ratnikov, B., Aza-Blanc, P., Ronai, Z.'e.A., Smith, J.W., Osterman, A.L., Scott, D.A., 2015. Glutamate and asparagine cataplerosis underlie glutamine addiction in melanoma. Oncotarget 6 (10), 7379–7389. https://doi.org/10.18632/oncotarget.3132.

Rau, R.E., Dreyer, Z.A., Choi, M.R., Liang, W., Skowronski, R., Allamneni, K.P., Devidas, M., et al., 2018. Outcome of pediatric patients with acute lymphoblastic leukemia/lymphoblastic lymphoma with hypersensitivity to Pegaspargase treated with PEGylated Erwinia Asparaginase, Pegcrisantaspase: A report from the Children's oncology group. Pediatr. Blood Cancer. 65 (3). https://doi.org/10.1002/pbc.26873.

Reinert, R.B., Morgan Oberle, L., Wek, S.A., Bunpo, P., Xue, P.W., Mileva, I., Goodwin, L.O., et al., 2006. Role of glutamine depletion in directing tissue-specific nutrient stress responses to L-Asparaginase. J. Biol. Chem. 281 (42), 31222–31233. https://doi.org/10.1074/jbc.M604511200.

Renaudin, X., 2021. Reactive oxygen species and DNA damage response in cancer. Int. Rev. Cell Mol. Biol. 364 (January), 139–161. https://doi.org/10.1016/BS.IRCMB.2021.04.001.

Richards, N.G.J., Kilberg, M.S., 2006. Asparagine Synthetase chemotherapy. Annu. Rev. Biochem. https://doi.org/10.1146/annurev.biochem.75.103004.142520.

Ridnour, L.A., Thomas, D.D., Switzer, C., Flores-Santana, W., Isenberg, J.S., Ambs, S., Roberts, D.D., Wink, D.A., 2008. Molecular mechanisms for discrete nitric oxide levels in cancer. Nitric Oxide - Biology and Chemistry. https://doi.org/10.1016/j.niox.2008.04.006. Academic Press.

Riess, C., Irmscher, N., Salewski, I., Strüder, D., Classen, C.F., Große-Thie, C., Junghanss, C., Maletzki, C., 2021. Cyclin-dependent kinase inhibitors in head and neck Cancer and glioblastoma—backbone or add-on in immune-oncology? Cancer Metastasis Rev. https://doi.org/10.1007/s10555-020-09940-4.

Rigouin, C., Nguyen, H.A., Schalk, A.M., Lavie, A., 2017. Discovery of human-like L-Asparaginases with potential clinical use by directed evolution. Sci. Rep. 7 (1). https://doi.org/10.1038/s41598-017-10758-4.

Rognes, S.E., 1980. Anion regulation of Lupin asparagine Synthetase: chloride activation of the glutamine-utilizing reactions. Phytochemistry 19 (11), 2287–2293. https://doi.org/10.1016/S0031-9422(00)91013-6.

Röhm, K.H., Van Etten, R.L., 1986. The 18O isotope effect in 13C nuclear magnetic resonance spectroscopy: mechanistic studies on Asparaginase from Escherichia Coli. Arch. Biochem. Biophys. 244 (1), 128–136. https://doi.org/10.1016/0003-9861(86)90101-3.

Ruzzo, E.K., Capo-Chichi, J.M., Ben-Zeev, B., Chitayat, D., Mao, H., Pappas, A.L., Hitomi, Y., et al., 2013. Deficiency of asparagine Synthetase causes congenital microcephaly and a progressive form of encephalopathy. Neuron 80 (2), 429–441. https://doi.org/10.1016/J.NEURON.2013.08.013/ATTACHMENT/E1EE256F-439E-4B3D-A529-35F8DBF42C82/MMC1.PDF.

Samson, M.L., 2000. Drosophila arginase is produced from a nonvital Gene that contains the Elav locus within its third intron. J. Biol. Chem. 275 (40), 31107–31114. https://doi.org/10.1074/jbc.M001346200.

Sands, S., Ladas, E.J., Kelly, K.M., Weiner, M., Lin, M., Ndao, D.H., Dave, A., Vahdat, L.T., Bender, J.G., 2017. Glutamine for the treatment of vincristine-induced neuropathy in children and adolescents with cancer. Support. Care Cancer 25 (3), 701–708. https://doi.org/10.1007/s00520-016-3441-6.

Schrey, D., Borghorst, S., Lanvers-Kaminsky, C., Hempel, G., Gerß, J., Möricke, A., Schrappe, M., Boos, J., 2010. Therapeutic drug monitoring of Asparaginase in the ALL-BFM 2000 protocol between 2000 and 2007. Pediatr. Blood Cancer 54 (7), 952–958. https://doi.org/10.1002/pbc.22417.

Schwarz, R., Zitzow, E., Fiebig, A., Hering, S., Humboldt, Y., Schoenwaelder, N., Kämpfer, N., et al., 2022. PEGylation increases Antitumoral activity of arginine deiminase of streptococcus pyogenes. Appl. Microbiol. Biotechnol. 106 (1), 261–271. https://doi.org/10.1007/s00253-021-11728-7.

Sénéchal, K., Maubant, S., Leblanc, M., Ciré, S., Gallix, F., Andrivon, A., Duchamp, O., et al., 2019. Abstract 2258: Erymethionase (methionine-gamma-Lyase encapsulated into red blood cells) potentiates anti-PD-1 therapy in TNBC syngeneic mouse model. Cancer Res. 79, 2258. American Association for Cancer Research (AACR) https://doi.org/10.1158/1538-7445.am2019-2258.

Shiozaki, A., Iitaka, D., Ichikawa, D., Nakashima, S., Fujiwara, H., Okamoto, K., Kubota, T., et al., 2014. XCT, component of cysteine/glutamate transporter, as an independent prognostic factor in human esophageal squamous cell carcinoma. J. Gastroenterol. 49 (5), 853–863. https://doi.org/10.1007/s00535-013-0847-5.

Stagliano, K.E.R., Carchman, E., Deb, S., 2003. Real-time polymerase chain reaction quantitation of relative expression of genes modulated by P53 using SYBR green I. Methods Mol. Biol. 234, 73–91. https://doi.org/10.1385/1-59259-408-5:73.

Starkova, J., Hermanova, I., Hlozkova, K., Hararova, A., Trka, J., 2018. Altered metabolism of leukemic cells: new therapeutic opportunity. Int. Rev. Cell Mol. Biol. 336 (January), 93–147. https://doi.org/10.1016/BS.IRCMB.2017.07.012.

Stern, P.H., Hoffman, R.M., 1986. Enhanced in vitro selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic Defect2. J. Natl. Cancer Inst. 76 (4), 629–639. https://doi.org/10.1093/jnci/76.4.629.

Stern, P.H., Wallace, C.D., Hoffman, R.M., 1984. Altered methionine metabolism occurs in all members of a set of diverse human tumor cell lines. J. Cell. Physiol. 119 (1), 29–34. https://doi.org/10.1002/jcp.1041190106.

Stock, W., Douer, D., Deangelo, D.J., Arellano, M., Advani, A., Damon, L., Kovacsovics, T., et al., 2011. Prevention and Management of Asparaginase/Pegasparaginase-associated toxicities in adults and older adolescents: recommendations of an expert panel. Leuk Lymphoma. 52 (12), 2237–2253. https://doi.org/10.3109/10428194.2011.596963.

Stone, E.M., Chantranupong, L., Georgiou, G., 2010a. The second-Shell metal ligands of human arginase affect coordination of the nucleophile and substrate. Biochemistry 49 (49), 10582–10588. https://doi.org/10.1021/bi101542t.

Stone, E.M., Glazer, V.S., Chantranupong, L., Cherukuri, P., Breece, R.M., Tierney, D.L., Curley, S.A., Iverson, B.L., Georgiou, G., 2010b. Replacing Mn2+ with Co2+ in human arginase i enhances cytotoxicity toward L-arginine auxotrophic cancer cell lines. ACS Chem. Biol. 5 (3), 333–342. https://doi.org/10.1021/cb900267j.

Story, M.D., Voehringer, D.W., Clifton Stephens, L., Meyn, R.E., 1993. L-Asparaginase kills lymphoma cells by apoptosis. Cancer Chemother. Pharmacol. 32 (2), 129–133. https://doi.org/10.1007/BF00685615.

Sudhir, A.P., Agarwaal, V.V., Dave, B.R., Patel, D.H., Subramanian, R.B., 2016. Enhanced catalysis of L-Asparaginase from bacillus Licheniformis by a rational redesign. Enzyme Microb. Technol. 86 (May), 1–6. https://doi.org/10.1016/j.enzmictec.2015.11.010.

Sugimura, T., Birnbaum, S.M., Winitz, M., Greenstein, J.P., 1959. Quantitative nutritional studies with water-soluble, chemically defined diets. VIII. The forced feeding of diets each lacking in one essential amino acid. Arch. Biochem. Biophys. 81 (2), 448–455. https://doi.org/10.1016/0003-9861(59)90225-5.

Sun, J., Nagel, R., Zaal, E.A., Ugalde, A.P., Han, R., Proost, N., Song, J.-Y., et al., 2019. SLC1A3 contributes to L-Asparaginase resistance in solid tumors. EMBO J. 38 (21), e102147. https://doi.org/10.15252/EMBJ.2019102147.

Sunden, S.L.F., Renduchintala, M.S., Park, E.I., Miklasz, S.D., Garrow, T.A., 1997. Betaine-homocysteine methyltransferase expression in porcine and human tissues and chromosomal localization of the human gene 1. Arch. Biochem. Biophys. 345.

Szlosarek, P.W., Klabatsa, A., Pallaska, A., Sheaff, M., Smith, P., Crook, T., Grimshaw, M.J., et al., 2006. In vivo loss of expression of Argininosuccinate Synthetase in malignant pleural mesothelioma is a biomarker for susceptibility to arginine depletion. Clin. Cancer Res. 12 (23), 7126–7131. https://doi.org/10.1158/1078-0432.CCR-06-1101.

Tajan, M., Hennequart, M., Cheung, E.C., Zani, F., Hock, A.K., Legrave, N., Maddocks, O.D.K., et al., 2021. Serine synthesis pathway inhibition cooperates with dietary serine and glycine limitation for cancer therapy. *Nat.* Commun. 12 (1). https://doi.org/10.1038/S41467-020-20223-Y.

Takaku, H., Takase, M., Abe, S.-I., Hayashi, H., Miyazaki, K., 1992. In vivo anti-tumor activity of arginine deiminase purified FromMycoplasma Arginini. Int. J. Cancer 51 (2), 244–249. https://doi.org/10.1002/ijc.2910510213.

Takaku, H., Matsumoto, M., Misawa, S., Miyazaki, K., 1995. Anti-tumor activity of arginine deiminase from *mycoplasma Arginini* and its growth-inhibitory mechanism. Jpn. J. Cancer Res. 86 (9), 840–846. https://doi.org/10.1111/j.1349-7006.1995.tb03094.x.

Takeuchi, S., Wada, K., Toyooka, T., Shinomiya, N., Shimazaki, H., Nakanishi, K., Nagatani, K., et al., 2013. Increased XCT expression correlates with tumor invasion and outcome in patients with glioblastomas. Neurosurgery 72 (1), 33–41. https://doi.org/10.1227/NEU.0b013e318276b2de.

Tan, G.S., Lim, K.H., Tan, H.T., Khoo, M.L., Tan, S.H., Toh, H.C., Chung, M.C.M., 2014. Novel proteomic biomarker panel for prediction of aggressive metastatic hepatocellular carcinoma relapse in surgically Resectable patients. J. Proteome Res. 13 (11), 4833–4846. https://doi.org/10.1021/pr500229n.

Tan, Y., Xu, M., Guo, H., Sun, X., Kubota, T., Hoffman, R.M., 1996a. Anticancer efficacy of Methioninase in vivo. Anticancer Res 16 (6C), 3931–3936.

Tan, Y., Sr Zavala, J., Xu, M., Jr Zavala, J., Hoffman, R.M., 1996b. Serum methionine depletion without side effects by Methioninase in metastatic breast Cancer patients. Anticancer Res 16 (6C), 3937–3942.

Tan, Y., Zavala, J., Han, Q., Xu, M., Sun, X., Tan, X., Tan, X., Magana, R., Geller, J., Hoffman, R.M., 1997a. Recombinant Methioninase infusion reduces the biochemical endpoint of serum methionine with minimal toxicity in high-stage cancer patients. Anticancer Res 17 (5B), 3857–3860.

Tan, Y., Mingxu, X., Tan, X., Tan, X., Wang, X., Saikawa, Y., Nagahama, T., Sun, X., Lenz, M., Hoffman, R.M., 1997b. Overexpression and large-scale production of Recombinantl-methionine-α-Deamino-γ-Mercaptomethane-Lyase for novel anticancer therapy. Protein Expr. Purif. 9 (2), 233–245. https://doi.org/10.1006/prep.1996.0700.

Tan, Y., Sun, X., Mingxu, X., An, Z., Tan, X., Tan, X., Han, Q., Miljkovic, D.A., Yang, M., Hoffman, R.M., 1998. Polyethylene glycol conjugation of recombinant Methioninase for cancer therapy. Protein Expr. Purif. 12 (1), 45–52. https://doi.org/10.1006/prep.1997.0805.

Tan, Y., Xu, M., Hoffman, R.M., 2010. Broad selective efficacy of recombinant Methioninase and polyethylene glycol-modified recombinant Methioninase on cancer cells in vitro. Anticancer Res 30 (4), 1041–1046.

Tanaka, Y., Takahashi, T., Yamaguchi, K., Osada, S., Shimokawa, T., Yoshida, K., 2016. Elemental diet plus glutamine for the prevention of mucositis in esophageal cancer patients receiving chemotherapy: A feasibility study. Support. Care Cancer 24 (2), 933–941. https://doi.org/10.1007/s00520-015-2864-9.

Tao, K.M., Li, X.Q., Yang, L.Q., Wei Feng, Y., Zhi Jie, L., Sun, Y.M., Fei Xiang, W., 2014. Glutamine Supplementation for Critically Ill Adults. Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.CD010050.pub2. John Wiley and Sons Ltd.

Tesson, A.R., Soper, T.S., Ciustea, M., Richards, N.G.J., 2003. Revisiting the steady state kinetic mechanism of glutamine-dependent asparagine synthetase from Escherichia coli. Arch. Biochem. Biophys. 413 (1), 23–31. https://doi.org/10.1016/S0003-9861(03)00118-8.

Torres-Obreque, K., Meneguetti, G.P., Custódio, D., Monteiro, G., Pessoa-Junior, A., de Oliveira, C., Rangel-Yagui., 2019. Production of a novel N-terminal PEGylated Crisantaspase. Biotechnol. Appl. Biochem. 66 (3), 281–289. https://doi.org/10.1002/bab.1723.

Trachootham, D., Alexandre, J., Huang, P., 2009. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? Nat. Rev. Drug Discov. 8 (7), 579–591. https://doi.org/10.1038/nrd2803.

Tsai, H.J., Jiang, S.S., Hung, W.C., Borthakur, G., Lin, S.F., Pemmaraju, N., Jabbour, E., et al., 2017. A phase II study of arginine deiminase (ADI-PEG20) in relapsed/refractory or poor-risk acute myeloid leukemia patients. Sci. Rep. 7 (1), 1–10. https://doi.org/10.1038/s41598-017-10542-4.

Tsai, W.B., Aiba, I., Lee, S.Y., Feun, L., Savaraj, N., Kuo, M.T., 2009. Resistance to arginine deiminase treatment in melanoma cells is associated with induced Argininosuccinate Synthetase expression involving C-Myc/HIF-1α/Sp4. Mol. Cancer Ther. 8 (12), 3223–3233. https://doi.org/10.1158/1535-7163.MCT-09-0794.

Updike, S.J., Wakamiya, R.T., Lightfoot, E.N., 1976. Asparaginase entrapped in red blood cells: action and survival. Science 193 (4254), 681–683. https://doi.org/10.1126/science.821145.

Vakkala, M., Kahlos, K., Lakari, E., Pääkkö, P., Kinnula, V., Soini, Y., Medicine, I., 2000. Inducible nitric oxide synthase expression, apoptosis, and angiogenesis in in situ and invasive breast carcinomas 1. AACR. https://aacrjournals.org/clincancerres/article-abstract/6/6/2408/288405.

Vala, A.K., Sachaniya, B., Dudhagara, D., Panseriya, H.Z., Gosai, H., Rawal, R., Dave, B.P., 2018. Characterization of L-Asparaginase from marine-derived aspergillus Niger AKV-MKBU, its Antiproliferative activity and bench scale production using industrial waste. Int. J. Biol. Macromol. 108 (March), 41–46. https://doi.org/10.1016/j.ijbiomac.2017.11.114.

Sluis, I.M.v.d., Vrooman, L.M., Pieters, R., Baruchel, A., Escherich, G., Goulden, N., Mondelaers, V., et al., 2016. Consensus expert recommendations for identification and Management of Asparaginase Hypersensitivity and Silent Inactivation. Haematologica 101 (3), 279–285. https://doi.org/10.3324/haematol.2015.137380.

Vicentini, G.E., Fracaro, L., De Souza, S.R.G., Martins, H.A., Guarnier, F.A., Zanoni, J.N., 2016. Experimental cancer cachexia changes Neuron numbers and peptide levels in the intestine: partial protective effects after dietary supplementation with l-glutamine. PLoS One 11 (9). https://doi.org/10.1371/journal.pone.0162998.

Vrooman, L.M., Supko, J.G., Neuberg, D.S., Asselin, B.L., Athale, U.H., Clavell, L., Kelly, K.M., et al., 2010. Erwinia Asparaginase after allergy to E. Coli Asparaginase in children with acute lymphoblastic leukemia. Pediatr. Blood Cancer. 54 (2), 199–205. https://doi.org/10.1002/pbc.22225.

Vrooman, L.M., Kirov, I.I., Zo, A.E., Dreyer, M.K., Hijiya, N., Brown, P., Drachtman, R.A., et al., 2016. Activity and toxicity of intravenous Erwinia Asparaginase following allergy to E. Coli-derived Asparaginase in children and adolescents with acute lymphoblastic leukemia. Pediatr. Blood Cancer. 63 (2), 228–233. https://doi.org/10.1002/pbc.25757.

Wang, B., Wei, D., Crum, V.E., Richardson, E.L., Xiong, H.H., Luo, Y., Huang, S., Abbruzzese, J.L., Xie, K., 2003. A novel model system for studying the double-edged roles of nitric oxide production in pancreatic cancer growth and metastasis. Oncogene 22 (12), 1771–1782. https://doi.org/10.1038/sj.onc.1206386.

Wang, W., Green, M., Choi, J.E., Gijón, M., Kennedy, P.D., Johnson, J.K., Liao, P., et al., 2019a. CD8+ T cells regulate tumour Ferroptosis during Cancer immunotherapy. Nature 569 (7755), 270–274. https://doi.org/10.1038/s41586-019-1170-y.

Wang, Z., Yip, L.Y., Lee, J.H.J., Zhengwei, W., Chew, H.Y., Chong, P.K.W., Teo, C.C., et al., 2019b. Methionine is a metabolic dependency of tumor-initiating cells. Nat. Med. 25 (5), 825–837. https://doi.org/10.1038/s41591-019-0423-5.

Warburg, O., 1956. On respiratory impairment in cancer cells. Science vol. 124 (3215), 269–270. https://pubmed.ncbi.nlm.nih.gov/13351639/.

Warrell, R.P., Chou, T.C., Gordon, C., Tan, C., Roberts, J., Sternberg, S.S., Philips, F.S., Young, C.W., 1980. Phase I evaluation of Succinylated Acinetobacter Glutaminase-Asparaginase in adults. Cancer Res. 40 (12), 4546–4551. https://pubmed.ncbi.nlm.nih.gov/7438089/.

Whitecar, J.P., Bodey, G.P., Harris, J.E., Freireich, E.J., 1970. L-Asparaginase. N. Engl. J. Med. 282 (13), 732–734. https://doi.org/10.1056/NEJM197003262821307.

Williams, D.A., 2007. A new mechanism of leukemia drug resistance? N. Engl. J. Med. 357 (1), 77–78. https://doi.org/10.1056/nejmcibr072412.

Wriston Jr., J.C., Yellin, T.O., 1973. L-Asparaginase: A Review. Adv. Enzymol. Relat. Areas Mol. Biol. 39, 185–248. https://doi.org/10.1002/9780470122846.ch3.

Xu, Y., Fang, H., Chen, Y., Tang, Y., Sun, H., Kong, Z., Yang, F., et al., 2022. The KRAS-G12D mutation induces metabolic vulnerability in B-cell acute lymphoblastic leukemia. IScience 25 (3). https://doi.org/10.1016/j.isci.2022.103881.

Yang, Z., Sun, X., Li, S., Tan, Y., Wang, X., Zhang, N., Yagi, S., et al., 2004a. Circulating half-life of PEGylated recombinant Methioninase holoenzyme is highly dose dependent on cofactor Pyridoxal-5′-phosphate. Cancer Res. 64 (16), 5775–5778. https://doi.org/10.1158/0008-5472.CAN-04-1406.

Yang, Z., Wang, J., Lu, Q., Jinbao, X., Kobayashi, Y., Takakura, T., Takimoto, A., et al., 2004b. PEGylation confers greatly extended half-life and attenuated immunogenicity to recombinant Methioninase in Primates. Cancer Res. 64 (18), 6673–6678. https://doi.org/10.1158/0008-5472.CAN-04-1822.

Yang, Z., Wang, J., Yoshioka, T., Li, B., Quan, L., Li, S., Sun, X., et al., 2004c. Pharmacokinetics, methionine depletion, and antigenicity of recombinant Methioninase in Primates. Clin. Cancer Res. 10 (6), 2131–2138. https://doi.org/10.1158/1078-0432.CCR-03-0068.

Yano, S., Li, S., Han, Q., Tan, Y., Bouvet, M., Fujiwara, T., Hoffman, R.M., 2014. Selective Methioninase-induced trap of Cancer cells in S/G2 phase visualized by FUCCI imaging confers Chemosensitivity. Oncotarget 5 (18), 8729–8736. https://doi.org/10.18632/oncotarget.2369.

Yao, S., Janku, F., Koenig, K., Tsimberidou, A.M., Piha-Paul, S.A., Shi, N., Stewart, J., et al., 2022. Phase 1 trial of ADI-PEG 20 and liposomal doxorubicin in patients with metastatic solid tumors. Cancer Med. 11 (2), 340–347. https://doi.org/10.1002/cam4.4446.

Yau, T., Cheng, P.N.M., Chiu, J., Kwok, G.G.W., Leung, R., Liu, A.M., Tan To Cheung, Ng, C.T., 2022. A phase 1 study of Pegylated recombinant arginase (PEG-BCT-100) in combination with systemic chemotherapy (Capecitabine and Oxaliplatin)[PACOX] in advanced hepatocellular carcinoma patients. Invest. New Drugs 40 (2), 314–321. https://doi.org/10.1007/s10637-021-01178-3.

You, X.-J., Chen, X., Jian-Qiang, L., Xiao-Yan Zhu, L., Gao, X.-R.C., Li, Y., Hang, G., Ni, X., 2011. Expression of cystathionine β-synthase and cystathionine γ-Lyase in human pregnant myometrium and their roles in the control of uterine contractility. PloS One 6 (8), e23788. https://doi.org/10.1371/journal.pone.0023788.

Yu, M., Henning, R., Walker, A., Kim, G., Perroy, A., Alessandro, R., Virador, V., Kohn, E.C., 2012. L-Asparaginase inhibits invasive and Angiogenic activity and induces autophagy in ovarian cancer. J. Cell. Mol. Med. 16 (10), 2369–2378. https://doi.org/10.1111/j.1582-4934.2012.01547.x.

Zeng, X., Li, Y., Fan, J., Zhao, H., Xian, Z., Sun, Y., Wang, Z., Wang, S., Zhang, G., Ju, D., 2013. Recombinant human arginase induced caspase-dependent apoptosis and autophagy in non-Hodgkin's lymphoma cells. Cell Death Dis. 4 (10). https://doi.org/10.1038/cddis.2013.359.

Zhang, L., Liu, J., Wang, X., Li, Z., Zhang, X., Cao, P., She, X., Dai, Q., Tang, J., Liu, Z., 2014. Upregulation of cytoskeleton protein and extracellular matrix protein induced by stromal-derived nitric oxide promotes lung cancer invasion and metastasis. Curr. Mol. Med. 14 (6), 762–771. https://doi.org/10.2174/1566524014666140724103147.

Zhang, N., Clarke, F., Di Trapani, G., Keough, D., Beacham, I., 1995. Guinea pig serum L-Asparaginase: purification, and immunological relationship to liver l-Asparaginase and serum l-Asparaginases in other mammals. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 112 (4), 607–612. https://doi.org/10.1016/0305-0491(95)00106-9.

Zhang, W., Trachootham, D., Liu, J., Chen, G., Pelicano, H., Garcia-Prieto, C., Weiqin, L., et al., 2012. Stromal control of Cystine metabolism promotes cancer cell survival in chronic lymphocytic Leukaemia. Nat. Cell Biol. 14 (3), 276–286. https://doi.org/10.1038/ncb2432.

Zhao, H., Li, Q., Wang, J., Xianwei, S., Ng, K.M., Qiu, T., Shan, L., et al., 2012. Frequent epigenetic silencing of the folate-Metabolising gene cystathionine-Beta-synthase in gastrointestinal cancer. PLoS One 7 (11), 3–8. https://doi.org/10.1371/journal.pone.0049683.

Zhu, H., Li, T., Yiqi, D., Li, M., 2018. Pancreatic cancer: challenges and opportunities. BMC Med. 16 (1), 18–20. https://doi.org/10.1186/s12916-018-1215-3.

Zhu, W., Radadiya, A., Bisson, C., Wenzel, S., Nordin, B.E., Martínez-Márquez, F., Imasaki, T., et al., 2019. High-resolution crystal structure of human asparagine Synthetase enables analysis of inhibitor binding and selectivity. Commun. Biol. 2 (1). https://doi.org/10.1038/s42003-019-0587-z.

Zhu, Y., Li, T., Da Silva, S.R., Lee, J.J., Lu, C., Eoh, H., Jung, J.U., Gao, S.J., 2017. A critical role of glutamine and asparagine γ-nitrogen in nucleotide biosynthesis in cancer cells hijacked by an oncogenic virus. MBio 8 (4). https://doi.org/10.1128/mBio.01179-17.