Improving the Treatment of Acute Lymphoblastic Leukemia

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ABSTRACT: L-Asparaginase (EC 3.5.1.1) was first used as a component of combination drug therapies to treat acute lymphoblastic leukemia (ALL), a cancer of the blood and bone marrow, almost 50 years ago. Administering this enzyme to reduce asparagine levels in the blood is a cornerstone of modern clinical protocols for ALL; indeed, this remains the only successful example of a therapy targeted against a specific metabolic weakness in any form of cancer. Three problems, however, constrain the clinical use of L-asparaginase. First, a type II bacterial variant of L-asparaginase is administered to patients, the majority of whom are children, which produces an immune response thereby limiting the time over which the enzyme can be tolerated. Second, L-asparaginase is subject to proteolytic degradation in the blood. Third, toxic side effects are observed, which may be correlated with the L-glutaminase activity of the enzyme. This Perspective will outline how asparagine depletion negatively impacts the growth of leukemic blasts, discuss the structure and mechanism of L-asparaginase, and briefly describe the clinical use of chemically modified forms of clinically useful L-asparaginases, such as Aspelas, which was recently given FDA approval for use in children (babies to young adults) as part of multidrug treatments for ALL. Finally, we review ongoing efforts to engineer L-asparaginase variants with improved therapeutic properties and briefly detail emerging, alternate strategies for the treatment of forms of ALL that are resistant to asparagine depletion.

Acute lymphoblastic leukemia (ALL) primarily affects young children (2–4 years of age), although it is also seen in adolescents and young adults. In this disease, too many stem cells differentiate into lymphoblasts leading to the excessive overproduction of leukocytes, which cannot fight infections, and a decreased number of circulating, healthy white and red blood cells. A serendipitous observation led to the discovery that L-asparaginase, which catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonia, is the component of guinea pig serum that prevents lymphoma proliferation. Over the 60 intervening years, this finding has been exploited in the development of clinical protocols to treat ALL that include injection of L-asparaginase together with the administration of other anticancer agents, which now result in a survival rate for childhood ALL of >90% in the United States. On the other hand, serious side effects associated with L-asparaginase continue to drive studies of engineered and chemically modified variants of the enzyme with lower immunogenicity, higher catalytic activity, and extended half-lives. These efforts have led to new forms of the enzyme, such as Aspelas (calaspargase pegol-mknl), which received FDA approval in December 2018. In this Perspective, we will discuss (i) the molecular mechanisms by which asparagine depletion is thought to impact the growth of leukemic blasts, (ii) the structure and mechanism of L-asparaginase, and (iii) chemically modified forms of type II Escherichia coli L-asparaginases that are used in the clinic. We also review ongoing efforts to develop L-asparaginase variants with improved pharmacokinetic properties of the enzyme.

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Humans possess a type III (Ntn-amidohydrolase) L-asparaginase, which requires an N-terminal threonine residue for catalytic activity and is produced by autoprotoelytic activation in a process that is stimulated by glycine. However, the native human L-asparaginase is poorly efficient and is comparatively easy to produce. Wild type (WT) type II L-asparaginases have a strong preference for L-asparagine as a substrate ($K_M = 11 \mu M$) and exhibit a low glutaminase side activity (2–10%). In contrast, the type I L-asparaginases of bacterial origin hydrolyze L-asparagine ($K_M = 1 \text{mM}$) and L-glutamine with similar catalytic efficiencies. Some evidence suggests that glutaminase activity is correlated with toxic side effects, including hyperglycemia, pancreatitis, and neurological seizures. Type I bacterial L-asparaginases are therefore not used in the clinic.

**STRUCTURE AND CATALYTIC MECHANISM OF TYPE II L-ASPARAGINASES**

Numerous X-ray crystal structures have been reported for bacterial type II L-asparaginases, as both the free enzymes and complexes with small molecules. Enzymes in this family are homotetramers in their biologically active form with each L-asparaginase monomer being composed of two domains (Figure 2a). The four active sites in the tetramer are located between the N- and C-domains of adjacent monomers and contain a catalytic triad comprising residues Thr12, Tyr25, and Asp90 (E. coli numbering) (Figure 2b). Although alternate residues were originally proposed as defining a catalytic triad, the geometrical constraints imposed by the need for any nucleophile to attack the carbonyl amide in the L-asparagine side chain at an angle of 107° (the Bürgi–Dunitz angle) relative to the plane of the C–O σ-bond support the view that Thr12 plays a critical role in catalysis. The ability of the Thr12 side chain to adopt the correct orientation relative to the bound substrate is facilitated by its location on a flexible loop of the enzyme. In addition, the X-ray crystal structure of the complex between L-aspartate and the T89V variant of the type II L-asparaginase shows a covalent bond between the side chain of Thr12 and the carboxylate group of the amino acid (Figure 2c).
Remarkably, given the plethora of structural data, the catalytic mechanism employed by type II bacterial L-asparaginases remains the subject of debate. By analogy with serine proteases, and in light of the acyl-enzyme intermediate observed by crystallography (Figure 2c), it is generally accepted that hydrolysis proceeds by a double-displacement mechanism (Figure 3). This is an interesting finding in light of the reduced nucleophilicity of secondary alcohols but is supported by 18O/16O exchange experiments when β-protonated L-aspartic acid is incubated with the enzyme. In addition, threonine is known to be the active site nucleophile in the proteasome. A network of hydrogen bonds among Tyr25, Asp90, and active site water molecules mediates activation of Thr12 for nucleophilic attack (Figure 2b). Recent experimental and computational work, however, has challenged this mechanistic orthodoxy. Thus, it has been proposed that hydrolysis occurs via direct attack of water, which is activated by a catalytic triad consisting of Thr12, Lys162, and Asp90 in the E. coli L-asparaginase. The resulting tetrahedral intermediate is thought to be stabilized by a second triad (Thr89, Tyr25, and Glu283) before breaking down to yield ammonia and L-aspartate. This contentious proposal is inconsistent with the crystallographic observation of an acyl-enzyme intermediate in the T89V E. coli L-asparaginase variant but is weakly supported by studies of WT guinea pig L-asparaginase for which an acyl-enzyme intermediate was not detected during turnover.

PEGYLATED L-ASPARAGINASES: ADVANTAGES AND DISADVANTAGES

As outlined in the introduction, using WT bacterial type II enzymes in clinical protocols has a number of disadvantages. Some of these problems can be mitigated, however, by attaching polyethylene glycol (PEG) polymers onto surface lysines of the protein using linkers in a process that is termed PEGylation. The resulting PEGylated proteins are highly hydrated, with two or three waters solvating each ethylene glycol unit, increasing both the size and the hydrophilicity. As a result, the proteins are less likely to aggregate. PEGylation also boosts the half-life of L-asparaginase in blood, presumably because binding to proteases is precluded by the steric bulk of the biocconjugate associated with the external layer of PEG molecules. Clinical studies of Oncaspar, a PEGylated E. coli L-asparaginase in which the PEG polymers are conjugated by a succinimidyl-succinate linker (Figure 4b), have shown that this form of the enzyme has a half-life in blood that is 5-fold longer than that of the WT enzyme. Oncaspar also appears to be less immunogenic than unmodified WT E. coli L-asparaginase. Of course, this chemical modification strategy does have drawbacks. For example, multiple lysine residues adorn the surface of the enzyme, and attachment of the linker—PEG conjugate proceeds in a random fashion. As a result, PEGylation often yields polydisperse L-asparaginase preparations, which can lead to batch-dependent variations in catalytic activity.
combined use of nuclear magnetic resonance and X-ray crystallography offers a strategy for understanding how to control the extent and location of the modified protein surface.63,64 “Shedding” PEG molecules from the circulating PEGylated enzyme due to uncatalyzed hydrolysis of the ester in the succinimidyl-succinate linker is also a problem.32 Moreover, increasing the half-life of L-asparaginase may raise blood ammonia levels leading to toxic side effects.

As a relatively new drug developed only for the complicated multidrug protocols for the treatment of pediatric ALL, it is reasonable to ask whether Asparlas has significant clinical advantages compared with other PEGylated forms of L-asparaginase, such as Oncaspar.65,66 At the time of writing, the answer seems to depend on the exact clinical protocol that is being considered. In the protocol developed at the Dana Farber Cancer Institute,66 the increased hydrolytic stability of Asparlas, because a succinimidyl-carbamate linker is employed in PEGylation (Figure 4b),67 means that the drug has to be administered intravenously only every 21 days rather than the 14-day regimen required for Oncaspar. The more stable linker in Asparlas also gives this form of PEGylated L-asparaginase a longer shelf life. On the contrary, inferior outcomes were observed for Asparlas in an alternate protocol,68 developed by the Children’s Oncology Group. In addition, the incidence of hypersensitivity to Asparlas in the combined drug protocol was >20%, identical to that seen for Oncaspar. Minor side effects, such as hyperglycemia and hyperbilirubinemia, were also stronger when Asparlas was given to patients.68

L-ASPARAGINASE RESISTANCE

A number of resistance mechanisms have been identified in clinical studies that limit the effectiveness of L-asparaginase as an anticancer drug. In addition to the production of neutralizing antibodies targeted against the bacterial enzymes and/or the linkers in PEGylated variants, leukemic lymphoblasts can degrade L-asparaginase thereby potentiating antigen processing and an immune response.69 Alternatively, depleting circulating L-asparaginase may lead to upregulation of Asns expression in lymphoblasts, which leads to drug resistance.31,70 Other adaptive metabolic changes must also occur, however, to ensure that adequate amounts of glutamine and aspartate are available for asparagine synthesis,71 resulting in complex phenotypes. For example, overexpression of the ASNS gene must be correlated with the coordinated translation of genes encoding the glutamate/aspartate transporter, glutamine synthetase, and aspartate transaminase to permit increased levels of asparagine synthesis (Figure 5).71,72 Protein degradation may also contribute to the levels of endogenous asparagine needed for cell growth and proliferation.73 Other resistance mechanisms have been reported, including L-asparagine secretion by mesenchymal stromal cells (MSCs) in the cancer microenvironment. MSCs in bone marrow are resistant to many drugs but can be killed by vincristine,74 which inhibits microtubule assembly.75 Thus, treating MSCs with vincristine decreases the L-asparaginase concentration in the microenvironment,76 explaining the reported synergistic effect of this drug and L-asparaginase in clinical protocols.77,78

ENGINEERING THE THERAPEUTIC PROPERTIES OF L-ASPARAGINASE

With regard to the future, there is substantial current interest in characterizing the properties of L-asparaginases from all kingdoms of life to identify those that combine stability and high catalytic efficiencies that are non-immunogenic and have reduced glutaminase side activities.44,47,79,80 Structure-based engineering strategies are therefore being pursued to obtain human L-asparaginase variants with clinically viable activity and substantially lower ability to use glutamine as a substrate.

The molecular origin of the asparaginase/glutaminase selectivity exhibited by type II bacterial L-asparaginases remains under investigation.46 X-ray crystal structures of D. chrysanthemi L-asparaginase bound to L-aspartate show that this bacterial enzyme has an active site loop that exists in disordered, “open”, and “closed” conformations.51,82 Thus, as first proposed by Lubkowski and co-workers,81 substrate binding gives rise to a conformation in which Thr15 (equivalent to Thr12 in the E. coli enzyme) is correctly positioned for nucleophilic attack on the side chain amide. In this model, substrate selectivity is therefore a consequence of differences in the conformational preferences of this loop after the enzyme binds to either of the amino acid substrates. Structures of L-asparaginase bound to L-glutamate or L-glutamate in a catalytically competent complex that could test this model, however, have yet to be reported.

Four residues (Ala31, Glu63, Pro123, and Ser254) thought to mediate changes in loop conformation in the D. chrysanthemi L-asparaginase were identified on the basis of X-ray crystallography. A series of single, double, and triple variants were prepared by site-directed mutagenesis and characterized for substrate selectivity.80 Although many of these variants do indeed exhibit lower L-glutaminase activity, their ability to catalyze the conversion of L-asparagine to L-aspartate is also considerably impaired. The E63Q D. chrysanthemi variant, however, shows a 95% decrease in L-glutaminase activity while retaining 90% of WT L-asparaginase activity.

In unrelated work, molecular dynamics (MD) simulations on the E. coli L-asparaginase showed the importance of enzyme–substrate contacts in facilitating a conformational change to the active form of the enzyme.83 These calculations imply that L-glutamine has fewer contacts with every active site residue when compared to L-asparagine, except Gln59 (equivalent to Glu59 in the Dickeya homologue), Thr89, and Lys162. The interaction of the α-carboxylate of L-glutamine with Gln59 has the effect of moving the side chain amide away from the active site of the enzyme.83
from Thr12 in the E. coli L-asparaginase. Replacing Gln59 with a leucine residue gives a Q59L L-asparaginase variant, which is capable of hydrolyzing L-asparagine with 80% of WT enzyme activity while exhibiting almost undetectable glutaminase activity.83 Cell-based assays showed that the Q59L L-asparaginase variant was cytotoxic against cells lacking the ability to biosynthesize L-asparagine. Subsequent studies using the Q59L L-asparaginase variant to treat xenografts of the Sup-B15 leukemia cell line, which does not express ASNS, in NOD/SCID gamma mice suggested that L-asparaginase activity alone only delays growth.84 This finding supports the hypothesis that durable in vivo anticancer activity requires a low level of L-glutaminase side activity when the enzyme is administered without other drugs in a microenvironment of noncancerous cells. Unambiguously resolving the importance of L-glutaminase activity in the treatment of ALL remains an important problem in this research area.

OUTLOOK

The clinical use of L-asparaginase in treatment protocols is an important factor in the very successful outcomes experienced by ALL patients. Developments in linker design and stability, coupled with more consistent bioconjugation procedures, will likely improve the solubility and other pharmacokinetic properties of PEGylated forms of L-asparaginase.8 Evidence that shows the general importance of L-asparaginase in the growth and proliferation of cancers, such as sarcoma20 and those of the lung, breast,21,86 and prostate,87 is also accumulating. It therefore seems reasonable to expect that strategies that target ASNS are therefore being discussed.15,88 These results, however, will not overcome resistance due to upregulation of ASNS expression or be viable for tumors that do not require a supply of endogenous asparagine. Alternate strategies that target ASNS are therefore being discussed.15,88 Unfortunately, although sulfoximine-based inhibitors have been reported that exhibit high affinities for human ASNS89–91 these compounds are poorly bioavailable, and hence, this therapeutic strategy remains to be validated in animal models.

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Notes

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ABBREVIATIONS

ALL, acute lymphoblastic leukemia; Asparlas, calaspargase pegol-mknl; ASNS, asparagine synthetase; MD, molecular dynamics; PDB, Protein Data Bank; PEG, polyethylene glycol; WT, wild type; MSC, mesenchymal stromal cell.

REFERENCES

(1) Inaba, H., Greaves, M., and Mullighan, C. G. (2013) Acute lymphoblastic leukemia. Lancet 381, 1943–1955.
(2) Pui, C., Relling, M. V., and Downing, J. R. (2004) Mechanisms of disease: Acute lymphoblastic leukemia. N. Engl. J. Med. 350, 1535–1548.
(3) Ramya, L. N., Doble, M., Rekha, V. P. B., and Pulicherla, K. K. (2012) L-Asparaginase as potent anti-leukemia agent and its significance of having reduced glutaminase side activity for better treatment of acute lymphoblastic leukaemia. Appl. Biochem. Biotechnol. 167, 2144–2159.
(4) Kidd, J. G. (1953) Regression of transplanted lymphomas induced in vivo by means of normal guinea pig serum. II. Studies on the nature of the active serum constituent histological mechanism of the regression: tests for effects of guinea pig serum in vitro: discussion. J. Exp. Med. 98, 583–606.
(5) Ehrman, M., Cedar, H., and Schwartz, J. H. (1971) L-Asparaginase II of Escherichia coli: Studies of the enzymatic mechanism of action. J. Biol. Chem. 246, 88–94.
(6) Broome, J. D. (1961) Evidence that the L-asparaginase activity of guinea pig serum is responsible for its anti-lymphoma effects. Nature 191, 1114–1115.
(7) Tabe, Y., Lorenzi, P. L., and Konopleva, M. (2019) Amino acid metabolism in hematologic malignancies and the era of targeted therapy. Blood 134, 1014–1023.
(8) Brumano, L. P., da Silva, F. V. S., Costa-Silva, T. A., Apolinário, A. C., Santos, J. H. P. M., Kleingesinds, E. K., Monteiro, G., de Oliveira Rangel-Yagui, C., Benyahia, B., and Junior, A. P. (2019) Development of L-asparaginase biobetters: Current research status and review of the desirable quality profiles. Front. Bioeng. Biotechnol. 6, 212.
(9) Wek, R. C., and Staszke, K. A. (2010) How do tumors react to nutritional stress. EMBO J. 29, 1946–1947.
(10) Swain, A. L., Jaskolski, M., Housset, D., Rao, J. K. M., and Wlodawer, A. (1993) Crystal structure of Escherichia coli L-asparaginase, an enzyme used in cancer therapy. Proc. Natl. Acad. Sci. U. S. A. 90, 1474–1478.
(11) Cedar, H., and Schwartz, J. H. (1968) Production of L-asparaginase II by Escherichia coli. J. Bacteriol. 96, 2043–2048.
(12) Beckett, A., and Gervais, D. (2019) What makes a good new therapeutic L-asparaginase? World J. Microbiol. Biotechnol. 35, 152.
(13) Richards, N. G. J., and Kilberg, M. S. (2006) Asparagine synthetase chemotherapy. Annu. Rev. Biochem. 75, 629–654.
(14) Galluzzi, L., Kepp, O., Vander Heiden, M., and Kroemer, G. (2013) Metabolic targets for cancer therapy. Nat. Rev. Drug Discovery 12, 829–846.
and Ronai, Z. A. (2019) Translational reprogramming marks human L-asparaginase autocleavage and substrate hydrolysis. Biochemistry pubs.acs.org/biochemistry

Kilberg, M. S., Pan, X.-Y., Chen, H., and Leung-Pineda, V. (2005) Nutritional control of gene expression: how mammalian cells respond to amino acid limitation. Annu. Rev. Nutr. 25, 59–85.

Baird, T. D., and Wek, R. C. (2012) Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism. Adv. Nutr. 3, 307–321.

Pathria, G., Lee, J.-S., Hasnis, E., Tandoc, K., Scott, D. A., Verma, S., Feng, Y., Larue, L., Sahu, A. D., Topisirovic, I., Ruppin, E., and Ronai, Z. A. (2019) Translational reprogramming marks adaptation to asparagine restriction in cancer. Nat. Cell Biol. 21, 1590–1603.

Kilberg, M. S., Shan, J., and Su, N. (2009) ATF4-dependent transcription mediates signaling of amino acid limitation. Trends Endocrinol. Metab. 20, 436–443.

Hettmer, S., Schinzel, A. C., Tchessalova, D., Schneider, M., Paton, R. C., Lefkowitz, R. J., Richards, N. G. J., Hahn, W. C., and Wagers, A. J. (2015) Functional genomic screening reveals asparagine dependence as a metabolic vulnerability in sarcoma. eLife 4, e04936.

Knott, S. R. V., Wagenblast, E., Khan, S., Kim, S. Y., Soto, M., Wagner, M., Turgeon, M.-O., Fish, L., Erard, N., Gable, A. L., Maceli, A. R., Dickkopf, S., Papachristou, E. K., D’Santos, C. S., Carey, L. A., Wilkinson, J. E., Harrell, J. C., Perou, C. M., Goodarzi, H., Poulougiannis, G., and Hannon, G. J. (2018) Asparagine availability governs metastasis in a model of breast cancer. Nature 554, 378–381.

Krall, A. S., Xu, S., Graeber, T. G., Braas, D., and Christofk, H. R. (2016) Asparagine promotes cancer cell proliferation through use as an amino acid exchange factor. Nat. Commun. 7, 11457.

Pieters, R., Hunger, S. P., Boos, J., Rizzari, C., Silverman, K., Baruchel, A., Goekebuet, N., Schrappe, M., and Pui, C.-H. (2011) L-Asparaginase treatment in acute lymphoblastic leukemia: A focus on Erwinia asparaginase. Cancer 117, 238–249.

Avramis, V. I., Sencer, S., Periclou, A. P., Sather, H., Bostrom, B. C., Cohen, L. J., Ettinger, A. G., Ettinger, L. J., Franklin, J., Gaynon, P. S., Hildén, J. M., Lange, B., Majlessipour, F., Mathew, P., Needle, M., Neglia, J., Reaman, G., and Holcenberg, J. S. (2002) A protease-resistant asparaginase mutant T89V with covalently bound aspartate. FEBS Lett. 390, 211–216.

Lubkowski, J., Palm, G. J., Gilliland, G. L., Derst, C., Röhm, K.-H., and Wlodawer, A. (1996) The asparaginase from Escherichia coli, mutant T89V with covalently bound aspartate. FEBS Lett. 390, 211–216.

Lubkowski, J., Palm, G. J., Gilliland, G. L., Derst, C., Röhm, K.-H., and Wlodawer, A. (1996) Crystal structure and amino acid sequence of Wolinella succinogenes L-asparaginase. Eur. J. Biochem. 241, 201–207.

Agihaypour, K., Wlodawer, A., and Lubkowski, J. (2001) Structural basis for the activity and substrate specificity of Erwinia chrysanthemi L-asparaginase. Biochemistry 40, 5655–5664.

Agihaypour, K., Wlodawer, A., and Lubkowski, J. (2001) Do bacterial L-asparaginase utilize a catalytic triad Thr-Tyr-Glu. Biochim. Biophys. Acta. Protein Struct. Mol. Enzymol. 1550, 117–128.

Jakovski, M., Kozak, M., Lubkowski, J., Palm, G., and Wlodawer, A. (2001) Structures of two highly homologous bacterial L-asparaginases: a case of enantiomorphic space groups. Acta Crystallogr., Sect. D: Biol. Crystallogr. 57, 369–377.

Lubkowski, J., Dauter, M., Agihaypour, K., Wlodawer, A., and Dauter, Z. (2003) Atomic resolution structure of Erwinia chrysanthemi L-asparaginase. Acta Crystallogr., Sect. D: Biol. Crystallogr. 59, 84–92.

Sanches, M., Barbosa, J. A. R. G., de Oliveira, R. T., Abrahão Neto, J., and Polikarpov, I. (2003) Structural comparison of Ershchichia coli L-asparaginase in two monoclinic space groups. Acta Crystallogr., Sect. D: Biol. Crystallogr. 59, 416–422.

Kravchenko, O. V., Kislitsin, Y. A., Popov, A. N., Nikonov, S. V., and Kuznetsova, L. P. (2008) Three-dimensional structures of L-asparaginase from Erwinia carotovora complexed with aspartate and glutamate. Acta Crystallogr., Sect. D: Biol. Crystallogr. 64, 248–256.

Dhavala, P., and Papageorgiou, A. C. (2009) Structure of Helicobacter pylori L-asparaginase at 1.4 Å resolution. Acta Crystallogr., Sect. D: Biol. Crystallogr. 65, 1253–1261.

Borek, D., Kozak, M., Pei, J., and Jaksoloki, M. (2014) Crystal structure of active site mutant of antileukemic L-asparaginase reveals a conserved zinc-binding site. FEBS J. 281, 4097–4111.

Tomar, R., Sharma, P., Srivastava, A., Bansal, S., Ashish, and Kundra, B. (2014) Structural and functional insights into an archaeal L-asparaginase variants with outstanding stability and enhanced anti-leukaemic activity in vitro. Sci. Rep. 7, 14479.
(49) Lubkowski, J., and Wlodawer, A. (2019) Geometric considerations support the double-displacement catalytic mechanism of L-asparaginase. Protein Sci. 28, 1850−1864.

(50) Lubkowski, J., Vanegas, J., Chan, W.-K., Lorenzi, P. L., Weinstein, J. N., Sukharev, S., Fushman, D., Rempe, S., Anishkin, A., and Wlodawer, A. (2020) Mechanism of catalysis by L-asparaginase. Biochemistry 59, 1927−1945.

(51) Bürgi, H. B., Dunitz, J. D., Lehn, J.-M., and Wipf, G. (1974) Stereochemistry of reaction paths at carbonyl centers. Tetrahedron 30, 1563−1572.

(52) Dodson, G., and Wlodawer, A. (1998) Catalytic triads and their relatives. Trends Biochem. Sci. 23, 347−352.

(53) Rühl, K. H., and Van Etten, R. L. (1986) The 1H isotope effect in 13C nuclear magnetic resonance spectroscopy: Mechanistic studies on asparaginase from Escherichia coli. Arch. Biochem. Biophys. 244, 128−136.

(54) Seemueller, E., Lupas, A., Stock, D., Lüwe, J., Huber, R., and Baumeister, W. (1995) Proteasome from Thermoamphilicus acidophilicus: A threonine protease. Science 268, 579−582.

(55) Schalk, A. M., Antansijevic, A., Caffrey, M., and Lavie, A. (2016) Experimental data in support of a direct displacement mechanism for type I/II L-asparaginas. J. Biol. Chem. 291, 5088−5100.

(56) Gesto, D. S., Cerqueira, N. M., Fernandes, P. A., and Ramos, M. J. (2013) Unraveling the enigmatic mechanism of L-asparaginase II with QM/QM calculations. J. Am. Chem. Soc. 135, 7146−7158.

(57) Harris, J. M., and Chess, R. B. (2003) Effect of pegylation on nonclinical safety information of approved drugs. Curr. Opin. Chem. Biol. 7A, 128−136.

(58) Turecek, P. L., Bossard, M. J., Schoetens, F., and Ivens, I. A. (2016) PEGylation of biopharmaceuticals: A review of chemistry and structural basis for the regulation of tubulin by vinblastine. Nature 435, 519−522.

(59) Han, R., Proost, N., Luchinat, C., Mileti, A., Cuccia, A., and Gutierrez, A. (2019) Synthetic lethality of Wnt pathway activation and asparaginase in drug-resistant leukemias. Cancer Cell 35, 664−676.

(60) Li, J., Law, H. K., Lau, Y. L., and Chan, G. C. (2004) Differential damage and recovery of human mesenchymal stem cells after exposure to chemotherapeutic agents. Br. J. Haematol. 127, 326−334.

(61) Gigant, B., Wang, C., Ravelli, R. B. G., Roussi, F., Steinmetz, M. O., Curmi, P. A., Sobé, A., and Knossow, M. (2005) Structural basis of the regulation of tubulin by vinblastine. Nature 435, 519−522.

(62) Vettore, L., Westbrook, R. L., and Tennant, D. A. (2020) New aspects of amino acid metabolism in cancer. Br. J. Cancer 122, 150−156.

(63) Schalk, A. M., and Lavie, A. (2014) Structural and kinetic characterization of guinea pig L-asparaginase type III. Biochemistry 53, 2318−2328.

(64) Chan, W. K., Lorenzi, P. L., Anishkin, A., Purwaha, P., Rogers, D. M., Sukharev, S., Rempe, S. B., and Weinstein, J. N. (2014) The glutaminase activity of L-asparaginase is not required for anticancer activity against acute lymphoblastic leukemia. Mol. Cancer Ther. 13, 1587−1592.

(65) Marini, B. L., Perissinotti, A. J., Biax, D. L., Brown, J., and Burke, P. W. (2017) Catalyzing improvements in ALL therapy with asparaginase. Blood Rev. 31, 328−338.

(66) Angiolillo, A. L., Schore, R. J., Devidas, M., Borowitz, M. J., Carroll, A. J., Gastier-Foster, J. M., Heerema, N. A., Keilani, T., Lane, A. R., Loh, M. L., Reaman, G. H., Adamson, P. C., Wood, B., Wood, C., Zheng, H. W., Raetz, E. A., Winick, N. J., Carroll, W. L., and Hunger, S. P. (2014) Pharmacokinetic and pharmacodynamic properties of calaspargase pegol Escherichia coli L-asparaginase in the treatment of patients with acute lymphoblastic leukemia: results from Children's Oncology Group study AALL07PA. J. Clin. Oncol. 32, 3874−3882.

(67) Patel, N., Krishnan, S., Offman, M. N., Krol, M., Moss, C. X., Leighton, C., van Delft, F. W., Holland, M., Liu, J., Alexander, S., et al. (2009) A dyad of lymphoblastic lysosomal cysteine proteases degrades the antileukemic drug L-asparaginase. J. Clin. Invest. 119, 1964−1973.

(68) Vettore, L., Westbrook, R. L., and Tennant, D. A. (2020) New aspects of amino acid metabolism in cancer. Br. J. Cancer 122, 150−156.

(69) Li, J., Law, H. K., Lau, Y. L., and Chan, G. C. (2004) Differential damage and recovery of human mesenchymal stem cells after exposure to chemotherapeutic agents. Br. J. Haematol. 127, 326−334.

(70) Gigant, B., Wang, C., Ravelli, R. B. G., Roussi, F., Steinmetz, M. O., Curmi, P. A., Sobé, A., and Knossow, M. (2005) Structural basis of the regulation of tubulin by vinblastine. Nature 435, 519−522.

(71) Vettore, L., Westbrook, R. L., and Tennant, D. A. (2020) New aspects of amino acid metabolism in cancer. Br. J. Cancer 122, 150−156.

(72) Schalk, A. M., and Lavie, A. (2014) Structural and kinetic characterization of guinea pig L-asparaginase type III. Biochemistry 53, 2318−2328.

(73) Chan, W. K., Lorenzi, P. L., Anishkin, A., Purwaha, P., Rogers, D. M., Sukharev, S., Rempe, S. B., and Weinstein, J. N. (2014) The glutaminase activity of L-asparaginase is not required for anticancer activity against ASNS-negative cells. Blood 123, 3595−3606.

(74) Chan, W.-K., Horvath, T. D., Tan, L., Link, T., Harutyunyan, K. G., Pountos, M. A., Anishkin, A., Du, D., Martin, L. A., Yin, E., Rempe, S. B., Sukharev, S., Konopleva, M., Weinstein, J. N., and Lorenzi, P. L. (2019) Glutaminase activity of L-asparaginase contributes to durable preclinical activity against acute lymphoblastic leukemia. Mol. Cancer Ther. 18, 1587−1592.

(75) Marini, B. L., Perissinotti, A. J., Biax, D. L., Brown, J., and Burke, P. W. (2017) Catalyzing improvements in ALL therapy with asparaginase. Blood Rev. 31, 328−338.

(76) Angiolillo, A. L., Schore, R. J., Devidas, M., Borowitz, M. J., Carroll, A. J., Gastier-Foster, J. M., Heerema, N. A., Keilani, T., Lane, A. R., Loh, M. L., Reaman, G. H., Adamson, P. C., Wood, B., Wood, C., Zheng, H. W., Raetz, E. A., Winick, N. J., Carroll, W. L., and Hunger, S. P. (2014) Pharmacokinetic and pharmacodynamic properties of calaspargase pegol Escherichia coli L-asparaginase in the treatment of patients with acute lymphoblastic leukemia: results from Children’s Oncology Group study AALL07PA. J. Clin. Oncol. 32, 3874−3882.
arrest and inhibits cell proliferation of breast cancer. Chem. Biol. Drug Des. 84, 578–584.

(87) Sircar, K., Huang, H., Hu, L., Cogdell, D., Dhillon, J., Tzelepi, V., Efstathiou, E., Koumakpayi, I. H., Saad, F., Luo, D., Bismar, T. A., Aparicio, A., Troncoso, P., Navone, N., and Zhang, W. (2012) Integrative molecular profiling reveals asparagine synthetase is a target in castration-resistant prostate cancer. Am. J. Pathol. 180, 895–903.

(88) Chiu, M., Taurino, G., Bianchi, M. G., Kilberg, M. S., and Bussolati, O. (2020) Asparagine synthetase in cancer: Beyond acute lymphoblastic leukemia. Front. Oncol. 9, 1480.

(89) Ikeuchi, H., Ahn, Y.-M., Otokawa, T., Watanabe, B., Hegazy, L., Hiratake, J., and Richards, N. G. J. (2012) A sulfoximine-based inhibitor of human asparagine synthetase kills L-asparaginase-resistant leukemia cells. Bioorg. Med. Chem. 20, 5915–5927.

(90) Ikeuchi, H., Meyer, M. E., Ding, Y., Hiratake, J., and Richards, N. G. J. (2009) A critical electrostatic interaction mediates inhibitor recognition by human asparagine synthetase. Bioorg. Med. Chem. 17, 6641–6650.

(91) Gutierrez, J. A., Pan, X.-Y., Koroniak, L., Hiratake, J., Kilberg, M. S., and Richards, N. G. J. (2006) An inhibitor of human asparagine synthetase suppresses proliferation of an L-asparaginase-resistant leukemia cell line. Chem. Biol. 13, 1339–1347.