Asparagine Synthetase Polymorphisms and Toxicity and Efficacy of Asparaginases

Vassilios I. Avramis

Asparaginases develop innovative "tumor starvation" conditions for all antileukemia treatments; however, administrations are limited by the toxicities of this drug. Patients exhibiting moderate toxicity have optimal treatment outcomes. Certain asparagine synthetase polymorphisms may contribute to severe host toxicities in divergent subsets of patients, whereas others do not. Clinical correlations should be evaluated. Clin Cancer Res; 21(2); 230–2. ©2014 AACR. See related article by Tanfous et al., p. 329

In this issue of Clinical Cancer Research, Tanfous and colleagues (1) evaluate certain polymorphisms of asparagine synthetase (ASNS) and host toxicity. Specific ASNS polymorphisms were associated with adverse effects after asparaginase (ASNase) treatment; these innovative findings elucidate the variable toxic effects that are seen in patients with acute lymphoblastic leukemia (ALL).

Combination chemotherapy regimens, including ASNase, are successful against ALL. Repetitive administration of ASNase initiates events in patients by deaminating l-asparagine (Asn) and l-glutamine (Gln). Amino acid (AA) deprivation in serum initiates a response to ASNase. These pharmacodynamic (PD) events plus obesity are obstacles to successful treatment in all patients.

Isozymes of ASNS may differ in AA sequence, but catalyze the same biochemical reaction of the de novo Asn biosynthesis from aspartate, Gln, ATP in the presence of Mg$^{2+}$ ion (Fig. 1A). These investigations validate the polymorphisms of ASNS reported previously (2, 3). Moreover, the authors show that specific isoforms of ASNS are associated with severe toxicities (1).

Earlier work resolved the crystalline resolution of ASNS and showed that altering one AA (Cys-1 to Ala or Ser) eliminated the Gln-dependent activity, leading to lack of Asn biosynthesis by these mutants. In these proteins, Gln became an inhibitor of ASNase instead of a cosubstrate (2). Other investigators demonstrated two 14-bp tandem repeat (2R, wild-type) sequences in the first intron of the ASNS gene isolated from human ALL cells. The 14-bp sequence is similar to the three GC boxes (GC-I, -II, and -III) found in the promoter region of the ASNS gene. Approximately 75% of all samples exhibited the 2R sequence in both alleles; however, 20% and 3% of all samples had three (3R) and four (4R) 14-bp tandem repeats in one allele, respectively. The authors concluded that based on the increased number of tandem repeats, the ASNS gene produces variable Asn biosynthesis activity (1, 3). The authors demonstrated a new insight into the pharmacogenetics of asparaginase-related treatment complications in ALL.

It is imperative to emphasize that the article by Tanfous and colleagues (1) showed that a polymorphism of ASNS (haplotype “1”) was associated with reduced sensitivity to ASNase in vitro (1). However, should this haplotype be linked with lower rates of host toxicity, one may wonder if this is also correlated to poorer clinical PD activity by ASNase. Another question is whether the patients with lower host toxicities will also have reduced PD contribution by ASNS in the combination regimens, would they also be vulnerable to a higher risk for relapse. In either case, this novel methodology and provided data that can be used in individualizing treatments in patients with ALL and lymphomas by selecting an appropriate ASNase with optimal glutaminase coactivity. Such an ASNase can nullify the Asn biosynthesis by ASNS, thus achieving greater Asn depletion strategies.

Glutamine Sources

Glutamine (Gln, Q) is a cosubstrate of ASNS; therefore, it is indispensable to investigate the sources of this nonessential AA under physiologic conditions. Gln becomes essential in certain pathologic tissues (ALL cells); thus, it becomes a "conditionally essential" AA (4). In rapidly growing malignancies, severe burns, stress and trauma, skeletal muscle and the liver are unable to maintain normal plasma Gln concentrations because of intensely increased requirements for Gln by the gastrointestinal tract, immune system, inflammatory, and malignant cells. Also, Gln is essential for healthy cellular function and must be provided in the diet or synthesized via the catalysis by glutamine synthetase (GS) in muscle, adipose tissue, liver, brain, etc. (5, 6). Gln crosses the blood–brain barrier, where it is used as an energy source and a precursor for neurotransmitter substances in the neurons (GABA receptor ligands).

Asparagine Synthetase

ASNS consists of approximately 560 AA with oligo-peptide repeats in various isoforms (molecular weight 62–64 KDa). Only the 561 AA isoform has been experimentally confirmed, and it has been found upregulated in nutrient-deprived mammals. ASNS protein is cytoplasmic, but a small fraction shows nuclear localization. Moreover, mesenchymal cells express and release ASNS in...
A, diagrammatic mechanism of ASNS catalyzing the de novo biosynthesis of Asn from Glu and aspartic acid with the energy provided by ATP hydrolysis in the presence of Mg++. Asn is the target amino acid of ASNase in leukemia treatments. B, diagrammatic depiction of the enrichment of Asn in cell types. GS, Gln ASNs, for optimal Asn INPUT into the circulation expressed in umol/min + additive rate* of Asn biosynthesis by ASNS over 30 days. Asterisk indicates additive rate is a measure of estimating the continuous increase of Asn de novo biosynthesis by ASNS over 30 days.

 Their microenvironment (7). As far back as the 1960s, ASNS had been determined as a mode of resistance to ASNase (9). Of importance is the fact that the leukemic cells and the liver under the AA deprivation augment ASNS mRNA and stabilize the ASNS protein to augment the Asn production. This is expressed as the biochemical parameter INPUT of Asn in the circulation. Similarly, GS is gradually augmented in many cell types, for example, adipose and leukemia cells will further increase Gln concentrations (5). These complex biochemical reactions contribute to a step-wise increase of Asn INPUT, known as additive error (9, 10; Fig. 1B). The latter parameter was highly predictive of long-term event-free survival (ref. 10).

**Clinical Data on Asn Deamination and ASNS**

The biochemical relationships between ASNase enzymatic activity and Asn or Gln levels have been examined in 274 pairs of pre- and post-ASNase serum specimens from 200 patients with high-risk ALL (9). The asparaginase PD results from patients with high-risk and standard-risk ALL were superimposable (9–12). The percentages of Asn and Gln deamination were predicted by population of ASNase activity in patients’ sera. Further PD analyses strongly suggested that >90% deamination of Gln must occur before optimal Asn deamination (>90% deamination, <3 umol/L) takes place in vivo at ASNase concentrations of 0.3 IU/mL after intramuscular administration, serum levels at trough times (9, 10). These PD analyses also demonstrated the same beneficial PD affect in antibody-positive patients to Escherichia coli ASNase who were then switched to Erwinase (currently licensed by the FDA as Erwinaze [asparaginase Erwinia chrysanthemi]) to Jazz Pharmaceuticals treatments (CCG-1961), which has greater glutaminase coactivity and faster kcat value than the E. coli ASNase (11). These findings strongly suggested that greater deamination of Gln must occur first for optimal Asn deamination under similar ASNase serum concentrations (9–11). Taken together, these findings suggested that Gln deamination plays an important role in the antileukemic contribution of ASNS, due to the inhibition of the de novo Asn biosynthesis by mammalian ASNS in leukemia cells in vitro (7, 9, 10). This phenomenon was also observed in vivo (10). Unfortunately, the process of estimating additive error biochemical parameter requires intense pharmacokinetics–PD sampling and analyses in each patient. Hence, it is not reasonable to expect this process to be applied in most clinical oncology centers.

Our clinical experience in over 1,000 patients with ALL reinforces that moderate-to-severe host toxicity is associated with better event-free survival and long-term outcomes (9–11). Thus, it is easily inferred that patients with ALL with the triple repeat allele (3R) of the ASNS gene may have improved event-free survival and outcomes, whereas patients with haplotype ‘1’ may have lower PD activity by ASNase (9–12). The clinical PD relationships between ASNS and ASNS, even though well understood, were not fully elucidated until the recent papers on the polymorphisms of ASNS. Therefore, the article by Tanfous and colleagues (1) is a seminal paper directly associating specific ASNS isotypes with severe toxicity and presumably, effective outcome in patients with ALL. Moreover, if these new polymorphism methods are applied in real time in leukemia treatment centers, they will benefit refractory patients by selecting an appropriate alternative ASNase with greater glutaminase coactivity (Erwinase), which has been associated with improved event-free survival (10, 11).

**Conclusions**

This article shows a groundbreaking and promising genomic methodology in identifying patients with leukemia with ASNS isoforms. This work fulfills an unmet medical need, which may yield improved individualized treatments in patients with ALL.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
References
Asparagine Synthetase Polymorphisms and Toxicity and Efficacy of Asparaginases

Vassilios I. Avramis


Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-14-1714

Cited articles  This article cites 12 articles, 5 of which you can access for free at: http://clincancerres.aacrjournals.org/content/21/2/230.full#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.