Asparagine Synthetase Polymorphisms and Toxicity and Efficacy of Asparaginases

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Running Title: ASNS Isoforms and Asparaginase Toxicity

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
Asparaginases develop innovative ‘tumor starvation’ conditions for all anti-leukemia 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 sub-sets of patients, whereas others don’t. Clinical correlations should be evaluated.
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 post-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++ 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 host 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 ASNS instead of a co-substrate (2). Other investigators demonstrated two 14-bp tandem repeat (2R, wild-type) sequences in the first intron of the ASNS gene isolated from human acute lymphoblastic leukemia (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% ALL samples had three (3R) and four (4R) 14-bp tandem repeats in one allele, respectively. They 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 Tanfous et al., paper 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 remains (that), if the patients with lower host toxicities will have reduced PD contribution by ASNase in the combination regimens, would they also be vulnerable to a higher risk for relapse. In either case, this novel methodology and data provided 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 co-substrate of ASNS; therefore, it is indispensable to investigate the sources of this non-essential AA under physiological conditions. Gln becomes essential in certain pathological 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 receptors-ligands).

Asparagine Synthetase (ASNS)

ASNS consists of ~560 AA with oligo-peptide repeats in various isoforms (MW 62 to 64 KDa). Only the 561 AA isoform has been experimentally confirmed, and it has been found upregulated
in nutrient deprivation mammals. ASNS protein is cytoplasmic, but a small fraction shows nuclear localization. Moreover, mesenchymal cells express and release ASNS in their micro-environment (7). As far back as 1960’s, ASNS had been determined as a mode of resistance to ASNase (8).

Of importance is the fact that the leukemic cells and the liver under the AA deprivation augment ASNS mRNA and stabilize the ASNS protein in order 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 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 (EFS) (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 high-risk ALL patients (9). The Asparaginase PD results from high-risk and standard-risk ALL patients were superimposable (9-12). The percentages of Asn and Gln deamination were predicted by population ASNase activity in patients’ sera. Further PD analyses strongly suggested that >90% deamination of Gln must occur before optimal Asn deamination (>90% deamination, < 3 μM) takes place in vivo at ASNase concentrations of 0.3 IU/ml after IM administration, serum levels at trough times (9, 10). These PD analyses also demonstrated the same beneficial PD affect in antibody positive patients to E. coli ASNase who were then switched to Erwinase treatments (CCG-1961), which has greater glutaminase co-activity 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 anti-leukemic contribution of ASNase, 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 PK-PD sampling and analyses in each patient. Hence, it is not reasonable to be applied in most clinical oncology centers.

Our clinical experience in over 1000 ALL patients, reinforces that moderate to severe host toxicity is associated with better EFS and long-term outcomes (9-11). Thus, it is easily inferred that ALL patients with the triple repeat allele (3R) of ASNS gene may have improved EFS and outcomes, whereas patients with haplotype *1 may have lower PD activity by ASNase (9-12). The clinical PD relationships between ASNase and ASNS, even though well understood, were not fully elucidated until the recent papers on the polymorphisms of ASNS. Therefore, the Tanfous et al. (1) article is a seminal paper directly associating specific ASNS isotypes with severe toxicity and presumably, effective outcome in ALL patients. 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 co-activity (Erwinase), which has been associated with improved EFS (10,11).

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

References


**Figure 1.** A, Diagrammatic mechanism of ASNS catalyzing the *de novo* biosynthesis of Asn from Gln 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 is shown of the enrichment of Asn concentration in blood circulation via the nutrients and *via* the *de novo* biosynthesis catalyzed by ASNS in many tissues. The INPUT and Additive Rate of Asn accumulation in central circulation are calculated by integrating the population pharmacokinetic equation of ASNase elimination in patients fused with the Michaelis-Menten equation. The fused equations are then integrated per minute for 43,200 min (a month), and many iterations, in order to minimize the variability errors thus, yielding best-fit pharmacodynamic values of Asn INPUT and its additive rate values. Of interest is that the INPUT values (µM/ml/min) are similar in most patients populations; however, the Additive Error values (nM/ml/min) are increasing with time and therefore, it becomes highly variable in patients. This is especially correct in the older patients and it is even enhanced in patients who appear not to exhibit moderate to severe host toxicity post Induction of ALL treatment, which includes (PEG-)ASNase administrations. Survival analyses (Kaplan-Meyer) are significantly better in patients with low Additive Error values *vs.* the patients with higher values. It is assumed that the increased activity of Asn biosynthesis, which is mostly by the gradually upregulated
ASNS activity over a month, contributes to a less than optimal treatment event-free survival (EFS).
Brown adipose tissue with blood vessels

**Asparagine synthetase (ASNS), Mg**

Aspartic acid + glutamine → Asparagine

ATP → AMP + PPI

Asparagine

Asparaginase

**B**

Brown adipocyte

Adipocyte

Preadipocyte cell

Mesenchymal cell

Nutrition → Liver

Leukemia tumor burden

Other tissues

GS, Gln

ASNS, for optimal Asn INPUT into the circulation expressed in μM/min + additive rate*

*Additive rate is a measure of estimating the continuous increase of Asn de novo biosynthesis by ASNS over 30 days

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